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Biocontrol Efficacy of *Trichoderma harzianum* and *Pistacia atlantica* Extract in Managing *Pectobacterium carotovorum* Soft Rot Disease in Potato (*Solanum tuberosum* L.) CV. El Mundo

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Abstract: The synergistic interaction between *Trichoderma harzianum* and *Pistacia atlantica* extract was evaluated as a sustainable intervention against potato soft rot (*Pectobacterium carotovorum*). By assessing antioxidant dynamics (SOD, POD, CAT, PPO) and lipid peroxidation under greenhouse conditions, the study demonstrated that combined and preventive applications significantly attenuated biotic stress. Initial H2O2 levels were lowest in the combined treatment (37.27 µmol/g FW), correlating with enhanced enzymatic defense and total protein content. While infected controls suffered significant growth inhibition (31.83 cm), plants receiving the bio-synergy preserved their developmental trajectory (avg. 45.75 cm). This research confirms that co-applying these biological elicitors induces systemic resistance, providing a highly effective framework for soft rot control in "El Mundo" cultivars.

Keywords: *Solanum tuberosum*, *Pectobacterium carotovorum*, Biocontrol, *Trichoderma harzianum*, *Pistacia atlantica*, Oxidative stress.

فعالية المكافحة الحيوية للفطر ***Trichoderma harzianum*** ومستخلص ***Pistacia atlantica*** في إدارة مرض العفن الطري المتسبب عن بكتيريا ***Pectobacterium carotovorum*** في البطاطا صنف إلموندو

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المستخلص: تم تقييم التفاعل التآزري بين الفطر *Trichoderma harzianum* ومستخلص نبات *Pistacia atlantica* كتدخل مستدام لمكافحة التعفن الطري في البطاطس (*Pectobacterium carotovorum*). ومن خلال تقييم ديناميكيات مضادات الأكسدة (SOD، POD، CAT، PPO) وبيروكسيد الدهون تحت ظروف الصوبة، أظهرت الدراسة أن التطبيقات المشتركة والوقائية خفّضت بشكل ملحوظ من الإجهاد الحيوي. وكانت مستويات بيروكسيد الهيدروجين (H_2O_2) الأولية في أدنى مستوياتها في المعاملة المشتركة (37.27 ميكرو مول/جرام وزن طازج)، وهو ما ارتبط بتعزيز الدفاعات الإنزيمية ومحتوى البروتين الكلي. وبينما عانت نباتات الشاهد المصاب من تثبيط نمو كبير (31.83 سم)، حافظت النباتات التي تلقت المعاملة الحيوية التآزرية على مسارها النمائي (متوسط 45.75 سم). ويؤكد هذه البحث أن التطبيق المشترك لهذين المحفزين الحيويين يحفز المقاومة الجهازية في النبات، مما يوفر إطارًا عالي الفعالية للتحكم في مرض التعفن الطري في أصناف البطاطس "إل موندو" (El Mundo).

الكلمات المفتاحية: البطاطس(*Solanum tuberosum*) ؛ العفن الطري(*Pectobacterium carotovorum*) ؛ المكافحة الحيوية؛ *Trichoderma harzianum* ؛ البطوم الأطلسي(*Pistacia atlantica*) ؛ الإجهاد التأكسدي

Introduction

Among global food staples, the potato (*Solanum tuberosum* L.) remains a cornerstone of international food security and agricultural economies (FAO, 2022). Despite its critical value, cultivation is perpetually threatened by a spectrum of biotic constraints. Prominent among these is bacterial soft rot, primarily orchestrated by *Pectobacterium carotovorum*, a formidable pathogen responsible for extensive tissue liquefaction both in the field and during post-harvest storage. This bacterium's virulence is largely driven by the secretion of a potent

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suite of plant cell wall-degrading enzymes (PCWDEs), which facilitate rapid maceration of the host parenchyma (Charkowski, 2018). Moreover, contemporary genomic insights emphasize the remarkable evolutionary plasticity and broad ecological niche of *P. carotovorum*, a factor that continues to challenge the development of stable management protocols in modern agroecosystems (Perfileva *et al*., 2025).

Traditional control paradigms, primarily dependent on synthetic antimicrobial agents and antibiotics, are facing scrutiny due to ecological degradation and the selective pressure leading to pathogen resistance (Buttimer et al., 2017; Gill *et al*., 2023). This shift has catalyzed an urgent demand for sustainable, biorational alternatives. A prime example is the diminishing efficacy of streptomycin—formerly a gold standard for managing soft rot. The therapeutic utility of this antibiotic is being systematically eroded by the adaptive evolution of Pectobacterium populations. Recent toxicological assessments reveal a troubling trend: isolates characterized after 2021 exhibit a doubling in their minimum inhibitory concentrations (MICs) relative to historical baselines (Shen *et al*., 2025). This escalating resistance profile highlights the inadequacy of conventional chemical frameworks and reinforces the necessity for multifaceted, eco-friendly defense strategies.

Furthermore, regulatory frameworks in the European Union have progressively restricted agricultural antibiotic applications since 2022, prohibiting routine and prophylactic uses to mitigate antimicrobial resistance risks and safeguard food safety (Verhaegen *et al*., 2024)

Copper-based bactericides, another cornerstone of conventional chemical control, present additional limitations. While effective as prophylactic agents, copper compounds exhibit limited curative activity once infection is established and may accumulate in soils, posing risks to non-target microorganisms and ecosystem health (PNW Handbooks, 2024).

Moreover, atmospheric dispersion of copper-based formulations has been implicated in potential environmental side effects, including contributions to ozone layer depletion, underscoring the need for more sustainable alternatives (Sanders, R, 2022).

Biological control agents (BCAs) have gained prominence as sustainable alternatives. Species of the genus *Trichoderma* are ubiquitous soil fungi known for their microparasitic activity, competition for nutrients, and ability to induce systemic resistance in plants against a broad spectrum of pathogens (Alfiky & Weisskopf, 2021). Recent studies have demonstrated that *Trichoderma* spp. can elicit plant defense mechanisms by modulating the activity of antioxidant enzymes such as peroxidase (POD), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL), thereby reinforcing cell walls against bacterial invasion (Sood *et al*., 2020).

Concurrently, plant extracts rich in bioactive secondary metabolites offer another avenue for disease management. *Pistacia atlantica* Desf. (Anacardiaceae) has been documented to possess significant antimicrobial, antifungal, and antioxidase properties attributed to its rich content of phenolic compounds, flavonoids, and terpenoids (Benhammou *et al*., 2018; Ghalem & Mohamed, 2020). The application of such plant extracts can directly inhibit pathogen growth or act synergistically with BCAs to enhance plant health. Importantly, recent evidence indicates that phenolic compounds such as carvacrol can potentiate the efficacy of reduced-dose antibiotics against streptomycin-resistant *P. carotovorum*, offering a promising strategy to circumvent resistance while minimizing chemical inputs (Shen *et al*., 2025).

Despite the individual potential of these agents, there remains a significant knowledge gap regarding the integrated deployment of *Trichoderma* species alongside *Pistacia atlantica* extracts within a unified management framework against P. carotovorum in potato systems. This research sought to bridge this gap by investigating the synergistic and solitary impacts of *Trichoderma harzianum* and *P. atlantica* foliar extracts on the suppression of soft rot in the "El Mundo" cultivar. Our analysis focused on the biochemical orchestration of host defenses, specifically quantifying the induction of antioxidant enzymes—including catalase (CAT), guaiacol peroxidase (POD), polyphenol oxidase (PPO), and superoxide dismutase (SOD). Furthermore, the study scrutinized the attenuation of oxidative damage markers, such as malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), and their subsequent influence on vegetative development under controlled greenhouse environments.

Material and Methods

Plant Procurement and Controlled Environment Cultivation

Tubers of the soft rot-susceptible potato cultivar ‘El Mundo’ underwent a rigorous surface decontamination protocol involving immersion in a 1% sodium hypochlorite (NaClO) solution for a 5-minute duration. Following this, the specimens were subjected to triple-rinsing with deionized water to eliminate residual ions. The prepared tubers were then sown into plastic vessels filled with autoclaved sandy-loam substrate. All experimental units were maintained within a controlled greenhouse facility, where the thermal regime was stabilized at $25\pm2\ ^{O}C$. To simulate optimal growth conditions, a consistent 16-hour diurnal light cycle and an 8-hour nocturnal dark period were established.

Microbial Inoculum Standardization

A highly pathogenic isolate of *Pectobacterium carotovorum* was reactivated on Nutrient Agar (NA) and incubated at $28\ ^{O}C$ for a 24-hour duration. The resulting bacterial biomass was harvested and diluted in sterile saline to achieve a standardized density of 10^8 CFU/ml colony-forming units (CFU) per ml. For the fungal component, *Trichoderma harzianum*—sourced from the Microbial Vaccine Centre at Ain Shams University (Cairo, Egypt)—was propagated on Potato Dextrose Agar (PDA). After a 7-day incubation period, conidia were collected to formulate a suspension calibrated to 107 conidial/ml using a hemocytometer.

Botanical Extraction of Pistacia atlantica

Foliar samples of *P. atlantica* were initially dehydrated under ambient, shaded conditions for four weeks, followed by a 48-hour thermal stabilization phase at 40°C to reach a constant dry weight. To isolate the polar metabolic fraction, the desiccated tissues were macerated and extracted using a 75% methanolic solvent system. The crude extract underwent filtration and was subsequently standardized to a working concentration of 1 mg/mL. The preparation of these bio-active materials was executed by adapting and refining the methodologies previously established by Malkhan *et al*. (2012) and Rahman *et al*. (2012).

Botanical specimen

Plant material of *Pistacia atlantica* was collected and subjected to shade-drying at ambient temperature for a period of four weeks. Subsequently, the dried samples were transferred to a drying oven and maintained at 40°C for 48 hours to ensure complete desiccation. For the extraction process, 75% methanol was employed as the solvent for the extraction of polar metabolites was based on previous studies conducted by Gurjar *et al*. (2012) and Rahman *et al*. (2012).

Experimental Design and Treatments

The experiment was arranged in a completely randomized design (CRD) with five treatments and three replicates per treatment, with three plants per replicate. The treatments were applied as follows:

- T1: Control (untreated plants irrigated with sterile water).
- T2: Irrigation with *P. carotovorum* suspension (Pathogen control).
- T3: Irrigation with *Trichoderma* suspension, followed by *P. carotovorum* irrigation after 2 days.
- T4: Irrigation with *P. carotovorum*, followed by *Trichoderma* irrigation after 2 days, then spraying with *P. atlantica* extract after another 2 days (Curative approach).

- T5: Spraying with *P. atlantica* extract, followed by *P. carotovorum* irrigation after 2 days, then *Trichoderma* irrigation after another 2 days (Preventive/Integrated approach).

Disease Severity Assessment

Disease severity was assessed using a 0–5 visual rating scale as described by Bdliya and Langerfeld (2005), where 0 = no symptoms and 5 = ≥61% tuber rot. The Disease Severity Index (DSI) was then calculated according to the formula: $DSI\ (\%) = [\Sigma(n \times v) / (N \times G)] \times 100$.

Where:

Σ = Summation total

n = Number of tubers assigned to a specific disease severity rating

v = Numerical value of the severity rating (ranging from 0 to 5)

N = Total number of tubers evaluated

G = Highest possible severity rating on the scale (i.e., 5)

Biochemical Analyses

Leaf samples were collected at two intervals: 3 days and 12 days after the final treatment application. Samples were homogenized in 50 mM phosphate buffer (pH 7.0) to extract enzymes.

Enzyme Assays: Catalase (CAT), Peroxidase (POD), Polyphenol oxidase (PPO), and Superoxide dismutase (SOD) activities were determined according to established spectrophotometric methods (Aebi, 1984; Chance & Maehly, 1955).

Oxidative Stress Markers: Malondialdehyde (MDA) content, an indicator of lipid peroxidation, and Hydrogen Peroxide (H_2O_2) levels were quantified to assess oxidative stress (Velikova *et al*., 2000). Total chlorophyll content (SPAD units) was measured using a SPAD-502 meter.

Growth Parameters

At the end of the experiment, plants were harvested to measure fresh and dry weights of shoots and roots (g), as well as shoot and root lengths (cm).

Statistical Analysis

Data were subjected to Analysis of Variance (ANOVA). Means were compared using the Least Significant Difference (LSD) test at $P \leq 0.05$ using CoStat statistical software (version 6.4).

Results

Biochemical Responses 3 Days Post-Treatment

At 3 days post-treatment (Table 1), the early biochemical responses indicated differential activation of the antioxidant system. Catalase activity was highest in T1 (211.9 U/mg Protein), while POD activity peaked in T4 (294.1 U/mg Protein). Lipid peroxidation (MDA) remained at basal levels across all treatments (0.08–0.47 µmol/g FW), with no significant differences detected ($P > 0.05$), suggesting that membrane damage had not yet manifested at this early stage. Similarly, Total Protein content showed minimal variation among treatments (21.25–22.37 mg/g FW), indicating stable protein metabolism during initial stress exposure.

By day 12 (Table 2), the long-term effects of the treatments became clearly evident. The control plants (T1) exhibited the highest catalase activity (401.2 U/mg Protein) but also accumulated significantly elevated levels of H_2O_2 (57.6 µmol/g FW), reflecting a severe oxidative burst. In contrast, the combined treatments (T3, T4, and T5) maintained substantially lower H_2O_2 levels (37.3–50.7 µmol/g FW), indicating sustained and efficient scavenging of reactive oxygen species.

Lipid peroxidation, measured as MDA content, was significantly lowest in T1 (0.80 µmol/g FW) and significantly highest in T2 (4.74 µmol/g FW), demonstrating that the pathogen-only treatment induced the most severe membrane damage. The combined treatments (T3–T5) showed intermediate MDA values (1.57–2.43 µmol/g FW), reflecting partial but meaningful protection of membrane integrity.

Total Phenolic Compounds (TCP) were highest in the control group (19.18 mg GAE/g FW), likely representing a massive defensive phenolic accumulation in response to unmitigated stress. Notably, TCP levels were significantly preserved in T4 (10.82 mg GAE/g FW) and T5 (9.44 mg GAE/g FW) compared to the pathogen-only treatment T2 (5.53 mg GAE/g FW). This retention of phenolic compounds in the combined treatments suggests robust activation of the non-enzymatic antioxidant system, contributing to enhanced cellular protection and overall plant resilience under prolonged stress conditions.

Table 1: Effect of treatments on enzymatic activity and biochemical markers in potato plants 3 days after the last treatment.

Treat	Catalase U/mg Protein	POD U/mg Protein	PPO U/mg Protein	SOD U/mg Protein	MDA µmol/g FW	Total Protein mg/g FW	H2O2 µmol/g FW
T1	211.9^{a} ± 1.96	217.0^{e} ± 0.37	1.19^{e} ± 0.00	172.3^{e} ± 0.87	0.08^{a} ± 0.00	21.84^{ab} ± 0.00	57.61^{a} ± 0.12
T2	200.7^{b} ± 0.42	281.2^{c} ± 0.23	1.59^{a} ± 0.00	213.9^{c} ± 2.38	0.47^{a} ± 0.00	21.32^{bc} ± 0.04	48.00^{b} ± 0.12
T3	185.0^{d} ± 0.69	240.5^{d} ± 0.41	1.38^{d} ± 0.00	216.7^{b} ± 1.94	0.16^{a} ± 0.00	21.66^{bc} ± 0.04	41.59^{c} ± 0.00
T4	181.3^{e} ± 0.67	294.1^{a} ± 0.94	1.47^{b} ± 0.00	247.6^{a} ± 2.59	0.17^{a} ± 0.00	22.37^{a} ± 0.04	37.27^{d} ± 0.03
T5	194.1c ± 0.70	290.1^{b} ± 0.38	1.46^{c} ± 0.00	196.4^{d} ± 2.35	0.24^{a} ± 0.00	21.25^{c} ± 0.04	50.71^{b} ± 0.06
LSD (0.05)	0.331	0.539	0.006	1.430	0.720	0.549	3.539

*Values are means ± standard deviation Different letters alongside data values in each column mean the values differed.

** Values are means ± standard deviation. Means followed by the same lowercase letter in each
column are not significantly different according to the Least Significant Difference (LSD) test at P ≤ 0.05.

*** U: Unit, FW: Fresh Weight

Biochemical Responses 12 Days Post-Treatment

By day 12 (Table 2), the long-term effects of the treatments became evident. The control plants (T1) exhibited high catalase activity (401.2 U/mg Protein) but also accumulated significantly elevated levels of H_2O_2 (57.6 µmol/g FW). In contrast, the combined treatments (T3, T4, and T5) maintained substantially lower H_2O_2 levels (ranging from 37.3 to 50.7 µmol/g FW), indicating sustained stress mitigation and efficient reactive oxygen species scavenging.

Lipid peroxidation, measured as MDA content, remained significantly lower in T3 (1.57 µmol/g FW) and T5 (2.43 µmol/g FW) compared to T4 (1.72 µmol/g FW); however, all treated plants demonstrated better membrane stability than observed during the early infection stage. Total Phenolic Compounds (TCP) were highest in the control group (19.18 mg GAE/g FW), likely reflecting a massive defensive phenolic accumulation in response to severe stress. Notably, TCP levels were significantly preserved in T4 (10.82 mg GAE/g FW) and T5 (9.44 mg GAE/g FW) compared to the pathogen-only treatment T2 (5.53 mg GAE/g FW). This retention of phenolic compounds in the combined treatments suggests a robust activation of the non-enzymatic antioxidant system, contributing to enhanced cellular protection and overall plant resilience under prolonged stress conditions.

Table 2: Effect of treatments on enzymatic activity and biochemical markers in potato plants 12 days after the last treatment.

Treat	Catalase U/mg Protein	POD U/mg Protein	PPO U/mg Protein	SOD U/mg Protein	MDA µmol/g FW	Total Protein mg/g FW	H2O2 µmol/g FW	TCP mg GAE/g FW
T1	401.2^{a} ± 10.0	216.2^{e} ± 1.6	1.18^{e} ± 0.00	171.5^{e} ± 1.0	0.80^{d} ± 0.08	21.9^{a} ± 0.0	57.^{6a} ± 0.1	19.18^{a} ± 0.00
T2	250.6^{c} ± 2.5	282.3^{c} ± 0.0	1.59^{a} ± 0.00	214.4^{c} ± 1.3	4.74^{a} ± 0.00	21.4^{a} ± 0.0	48.0^{c} ± 0.0	5.53^{c} ± 0.00
T3	274.4^{b} ± 5.3	240.9^{d} ± 0.3	1.38^{d} ± 0.00	216.7^{b} ± 1.1	1.57^{c} ± 0.06	21.6^{a} ± 0.0	41.6^{d} ± 0.0	6.07^{c} ± 0.00
T4	210.8^{d} ± 1.7	293.7^{a} ± 0.4	1.47^{b} ± 0.00	247.6^{a} ± 2.8	1.72^{c} ± 0.06	22.3^{a} ± 0.0	37.3^{e} ± 0.0	10.82^{b} ± 0.00
T5	194.2^{e} ± 0.4	290.3^{b} ± 0.4	1.46^{c} ± 0.00	196.4^{d} ± 11.9	2.43^{b} ± 0.06	21.3^{a} ± 0.0	50.7^{b} ± 0.1	9.44^{b} ± 0.00
LSD (0.05)	1.08	0.88	0.008	1.23	0.70	3.22	2.66	1.61

*Values are means ± standard deviation Different letters alongside data values in each column mean the values differed.

** Values are means ± standard deviation. Means followed by the same lowercase letter in each column are not significantly different according to the Least Significant Difference (LSD) test at P ≤ 0.05.

*** U: Unit, FW: Fresh Weight

Modulation of Biomass and Growth Dynamics

The experimental data (Table 3) illustrates that the integrated biocontrol strategies exerted a substantial regulatory influence on the developmental profile of potato plants under pathogen pressure. The synergistic regime involving T4 (Pathogen + *Trichoderma* + Extract) elicited the most favorable biomass accumulation, with shoot fresh and dry weights peaking at 14.33 g and 1.21 g, respectively. This performance represented a statistically superior outcome compared to both the healthy controls (T1) and the disease-stressed T2 cohort. Regarding vertical elongation, both the prophylactic (T5) and integrated (T4) treatments effectively neutralized the growth-inhibitory effects of *P. carotovorum*, sustaining shoot lengths at 46.33 cm and 45.17 cm, respectively. In contrast, the T2 group exhibited a pronounced stunting effect, with lengths averaging only 31.83 cm. Subterranean development remained robust across the study, though T4 mirrored the high root biomass levels observed in the absolute control (T1).

Physiological Vigor and Chlorophyll Retention

Photosynthetic health, assessed via SPAD-indexed chlorophyll density, further validated the efficacy of the bio-synergy. Plants subjected to T5 (32.58) and T4 (31.58) maintained significantly higher pigment concentrations relative to the untreated infected group (24.87). This preservation of chlorophyll content strongly correlates with the observed improvements in vegetative vigor, suggesting that the dual application of *Trichoderma* and *Pistacia* extract effectively shields the host's photosynthetic machinery from the degradative stress typical of soft rot pathogenesis.

Table 3: Effect of various treatments on Potato Plants growth parameters under greenhouse conditions.

Treat	**Fresh weight Shoots (g)**	**Fresh weight Roots (g)**	**Dry weight Shoots (g)**	**Dry weight Roots (g)**	**Length Shoots (cm)**	**Length Roots (cm)**	**SPAD unit**
T1	8.83_{c} ±0.29	16_{a} ±7.81	0.63_{ab} ±0.09	1.62_{a} ±0.90	33.58_{b} ±5.79	20.67_{ab} ±1.15	31.06_{ab} ±3.61
T2	10_{b} ±3.97	8.33_{a} ±5.51	0.67_{abl} ±0.18	1.05_{a} ±0.60	31.83_{bc} ±4.62	17.33_{ab} ±3.79	24.87_{b} ±3.60
T3	5.83_{c} ±0.76	8.67_{a} ±2.08	0.42_{b} ±0.07	0.94_{a} ±0.44	22_{c} ±3.50	14.33_{ab} ±2.08	29.74_{ab} ±5.48
T4	14.33_{a} ±0.29	12.33_{a} ±2.89	1.21_{a} ±0.23	1.68_{a} ±0.86	45.17_{a} ±1.26	13.33_{b} ±3.51	31.58_{a} ±1.98
T5	10.83_{ab} ±2.02	8.67_{a} ±0.58	0.89_{ab} ±0.82	1_{a} ±0.16	46.33_{a} ±9.36	21.33_{a} ±7.57	32.58_{a} ±2.24
LSD (0.05)	3.69	8.31	0.72	1.19	10.17	7.70	6.56

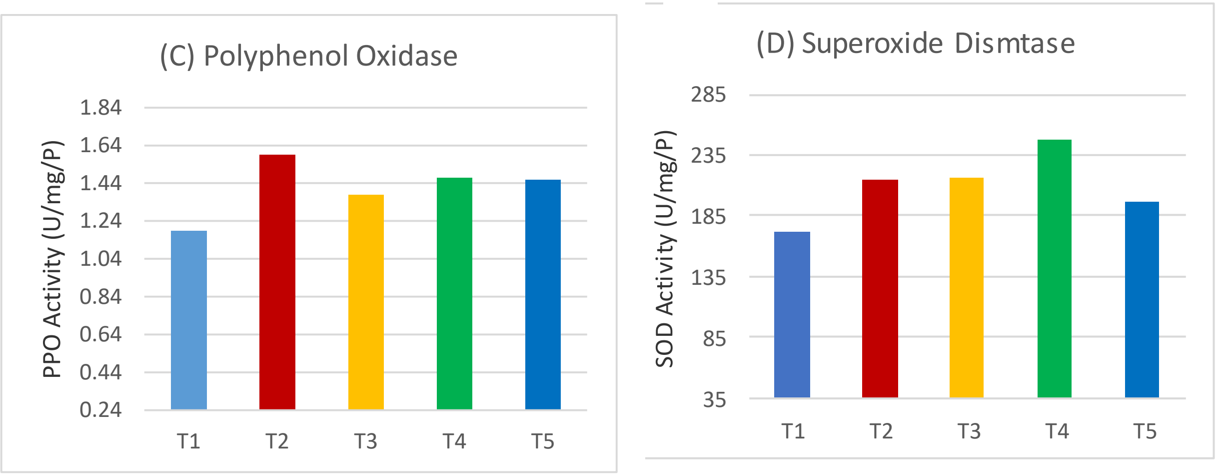
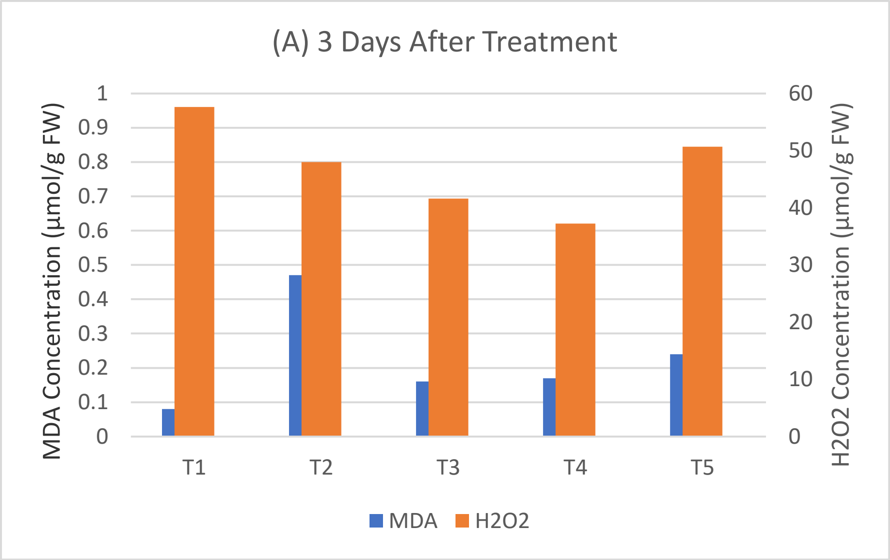


Figure 1: Defense enzyme activities (Catalase, POD, PPO, SOD) in potato cv. El Mundo plants 3 days after treatment.

Values represent means ± standard error. Different letters indicate significant differences at $P \leq 0.05$ (LSD test).



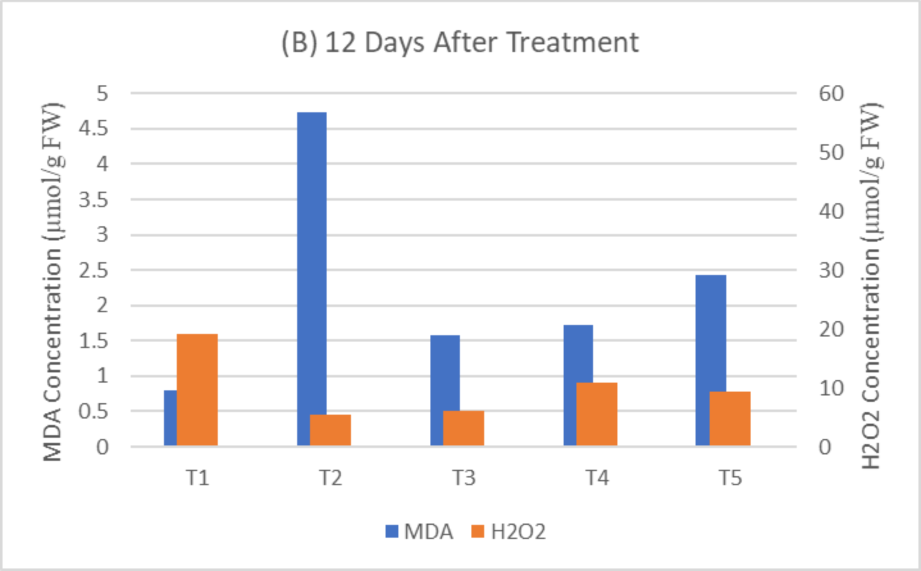


Figure 2: Oxidative Stress Markers (MDA and H_2O_2) in potato cv. El Mundo plants at 3 days (A) and 12 days (B) after final treatment. Dual y-axis shows MDA concentration (left, red bars) and H_2O_2 concentration (right,

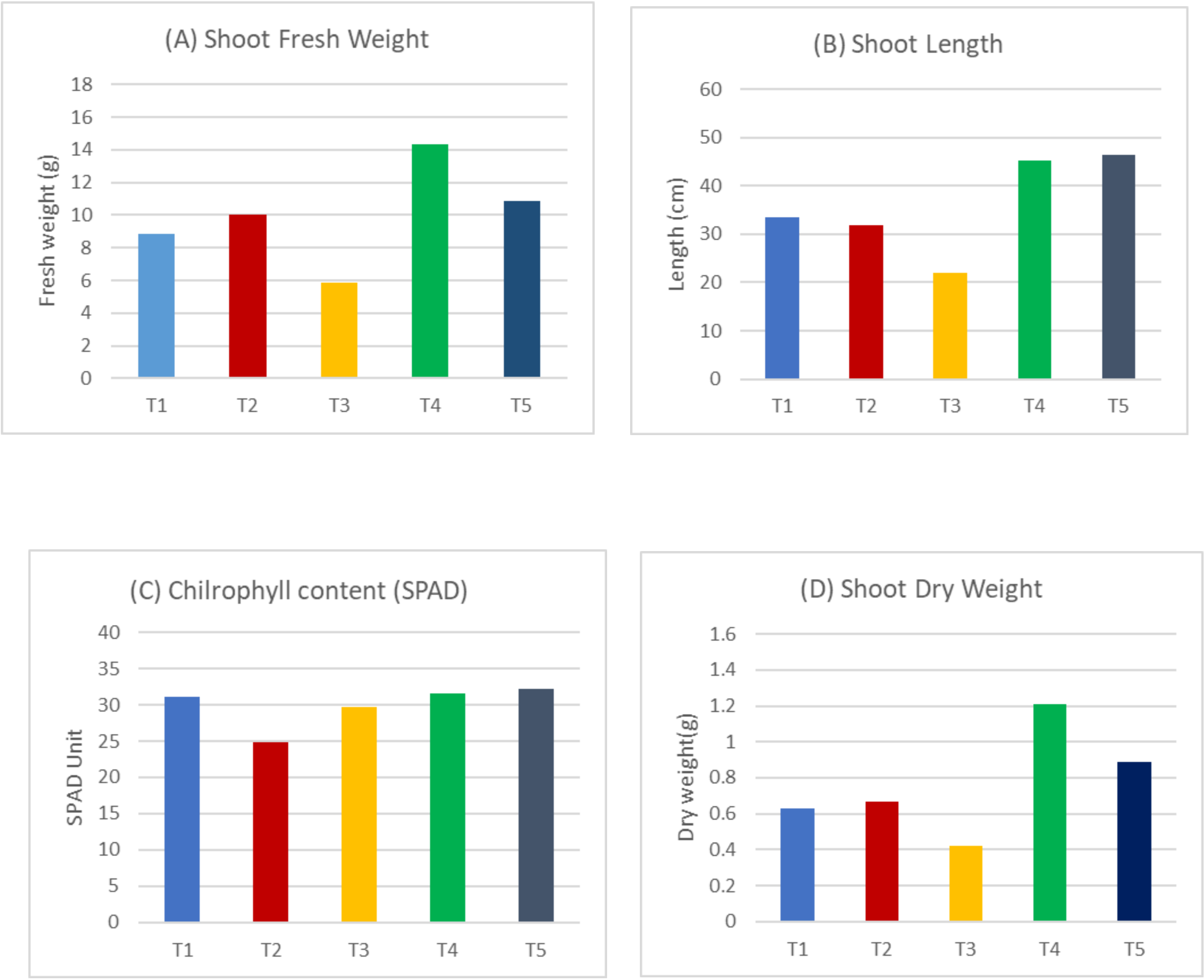


Figure 3: Effect of various treatments on potato cv. El Mundo growth parameters under greenhouse conditions. (A) Shoot fresh weight, (B) Shoot length, (C) Chlorophyll content (SPAD unit), (D) Shoot dry weight. Error bars

Discussion

Pathogen-Induced Redox Imbalance and Cellular Damage

The experimental outcomes highlight the tactical efficacy of combining microbial antagonists with phytochemical elicitors to counteract *P. carotovorum*-mediated soft rot. The pronounced virulence of the T2 cohort (pathogen only) was marked by severe oxidative disequilibrium, manifested through escalated lipid peroxidation—quantified via malondialdehyde (MDA) accumulation—and suppressed developmental vigor. These observations corroborate the findings of Cui *et al.* (2019), illustrating that soft rot pathogenesis initiates a rapid surge of reactive oxygen species (ROS), which precipitates systemic tissue liquefaction. From a physiological standpoint, this early-stage ROS generation transcended simple cellular injury; it acted as a fundamental, albeit overwhelmed, signaling event. The recognition of pathogen-associated molecular patterns (PAMPs) by host pattern recognition receptors (PRRs) typically stimulates mitogen-activated protein kinase (MAPK) pathways and respiratory burst oxidase homologs (RBOHs), leading to the liberation of superoxide (O_2^-) and hydrogen peroxide (H_2O_2). While this oxidative flux is an intrinsic component of the plant's innate immune architecture, its unregulated escalation past a physiological tipping point triggers catastrophic lipid peroxidation and programmed cell death. This metabolic failure was clearly evidenced in our untreated infected controls, where the host's primary defenses were unable to sequester the excess ROS.

Phytochemical and Microbial Elicitation of Systemic Resistance

The integration of *Trichoderma harzianum* and *Pistacia atlantica* extracts, specifically within the T4 and T5 experimental regimes, exerted a profound regulatory influence on the host's biochemical landscape, effectively reinstating cellular redox homeostasis. The fundamental physiological advantage observed was not the absolute eradication of reactive oxygen species (ROS), but rather the sophisticated calibration of the cellular reduction-oxidation equilibrium. The intensified activities of peroxidase (POD) and polyphenol oxidase (PPO) in these cohorts point toward a robust, elicitor-mediated defense activation. It is highly probable that fungal structural components, such as chitinous oligomers and glucans, acted in concert with *P. atlantica's* secondary metabolites—notably phenolic and flavonoid fractions—to function as exogenous elicitors. These molecules likely triggered calcium-dependent protein kinase (CDPK) signaling pathways, which govern the transcriptional upregulation and post-translational modification of the plant's antioxidant machinery.From a structural perspective, the elevated titers of POD and PPO are critical for the biosynthesis of lignin and the conversion of phenols into antimicrobial quinones. This process establishes a formidable physico-chemical barrier that restricts the systemic ingress of *P. carotovorum* (Moghaddam *et al.*, 2022; Zhang *et al.*, 2020). Furthermore, the early-phase surge in superoxide dismutase (SOD) activity recorded in the T4 treatment (day 3) suggests an accelerated quenching of superoxide radicals (O_2^-) , By facilitating the dismutation of these radicals into hydrogen peroxide H_2O_2 , which is subsequently neutralized by catalase and POD, the synergistic treatment effectively shielded the host's cellular architecture from oxidative disintegration during the critical initial hours of pathogenesis.

Attenuation of Lipid Peroxidation and Hormonal Integration

A pivotal observation in this study was the distinct divergence in lipid peroxidation kinetics between the infected controls and the treated cohorts. While the diseased T2 group exhibited a sharp escalation in malondialdehyde (MDA) concentrations, the application of T3 and T4 effectively buffered the host against such oxidative damage over the experimental duration. This trend is consistent with the metabolic framework proposed by Elsherbiny et al. (2021) and reinforces contemporary findings from 2024, which suggest that *Trichoderma* species mitigate biotic stress by orchestrating a comprehensive reprogramming of the host's metabolic network and upregulating endogenous antioxidant defenses. The progressive decline in H_2O_2 titers observed by the 12th day in treated plants substantiates the premise that the integration of biocontrol agents and botanical extracts accelerates the restoration of a homeostatic state—a recovery period that remains significantly delayed in untreated diseased plants. Recent analytical reviews (2025) indicate that this regulatory efficiency is likely governed by an intricate crosstalk between reactive oxygen species (ROS) signaling and phytohormone pathways. Specifically, the synergy between Jasmonic acid (JA) and ethylene (ET) signaling appears to be a critical factor. These hormones synergistically modulate the expression of defense-responsive genes, allowing the host to mount a robust resistance against *P. carotovorum* without crossing the metabolic threshold into excessive, unprogrammed cell death. Consequently, the treatments used in this study act not only as physical and chemical barriers but also as biochemical fine-tuners of the plant's internal defense circuitry.

The growth promotion observed in T4 and T5, characterized by increased shoot length and fresh weight, can be attributed to the dual role of *Trichoderma* as a growth promoter and the biostimulant properties of Pistacia extracts. *Trichoderma harzianum* is known to solubilize nutrients and produce auxins, enhancing root and shoot development (Vinale *et al.*, 2018).

Moreover, *Trichoderma* improves the uptake of micronutrients (e.g., Fe, Zn, Mn), which serve as essential cofactors for antioxidant enzymes (e.g., Fe-SOD, Cu/Zn-SOD), thereby enhancing their catalytic efficiency under stress conditions. Furthermore, the preservation of chlorophyll content (SPAD units) in T4 and T5 suggests that the treatments protected the photosynthetic apparatus from pathogen-induced damage observed in T2. Excessive H_2O_2 can inactivate Photosystem II (PSII) reaction centers and promote singlet oxygen ($^1O_2$) formation, which destroys chlorophyll; thus, effective ROS scavenging preserves photosynthetic efficiency and biomass production during disease pressure (Gouda *et al*., 2020).

The superior performance of T4 (curative application: Pathogen followed by *Trichoderma* and Extract) suggests that applying these agents after infection onset can effectively halt disease progression and stimulate recovery. This phenomenon may be explained by the concept of "plant immune priming." The initial pathogen attack creates a "preparedness state," inducing epigenetic modifications that facilitate a faster and stronger defense response upon subsequent exposure to the biocontrol agents. Conversely, T5 (preventive application) also showed robust growth, indicating that priming the plant with extracts and beneficial fungi prepares the defense machinery against subsequent infections (Mauch-Mani *et al*., 2017). However, the data suggest that the curative timing in T4 allowed for a more targeted activation of defense pathways when the threat was already recognized by the plant system.

This investigation establishes that the potency of integrated biocontrol frameworks transcends simple antimicrobial action, acting instead through the comprehensive physiological recalibration of the host. By fortifying the plant’s antioxidant architecture, safeguarding photosynthetic stability, and stabilizing redox equilibrium, the consortium of *Trichoderma harzianum* and *P. atlantica* foliar extracts emerges as a resilient, ecologically sound paradigm for the mitigation of bacterial soft rot. The observed bio-synergy suggests a multi-layered defense strategy that facilitates host survival under high pathogen pressure. Moving forward, the molecular landscape of this interaction warrants deeper exploration. Specifically, future inquiries should prioritize the quantification of transcriptomic signatures—such as RBOHD and PR1—alongside the deployment of high-resolution spatio-temporal imaging to map reactive oxygen species (ROS) flux. Such advancements will be instrumental in decoding the intricate molecular circuitry that governs this synergistic immune elicitation.

Conclusion

This research validates the exceptional biocontrol efficacy of combining *Trichoderma harzianum* with *Pistacia atlantica* foliar extracts to counteract *Pectobacterium carotovorum* in the ‘El Mundo’ potato cultivar. Specifically, the T4 and T5 treatment regimes proved instrumental in modulating host physiology, effectively quenching oxidative bursts and amplifying the titers of key antioxidant enzymes such as POD and SOD. From a mechanistic standpoint, the marked attenuation of malondialdehyde (MDA) and H_2O_2 levels, integrated with an upregulated enzymatic scavenging system, confirms that this dual-agent approach reinstates cellular redox equilibrium. Such stabilization is vital for safeguarding membrane structural integrity and maintaining photosynthetic throughput during pathogenesis. The observed bio-synergy—merging *Trichoderma*-driven systemic acquired resistance with the potent antimicrobial and elicitation properties of *P. atlantica*—highlights a resilient, multi-phasic strategy for enhancing crop vigor. By reducing the chemical footprint of potato cultivation without compromising yield, this integrated framework offers a viable, sustainable alternative to conventional bactericides. Future investigations should prioritize multi-locational field trials to refine application parameters and validate these greenhouse-based physiological outcomes.

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