e-ISSN: 2807-2820

Natural Sciences Engineering Technology Journal

[NASET Journal]

https://nasetjournal.com

Elicitation of Systemic Acquired Resistance by a Novel Plant-Derived Biostimulant Composition Confers Robust Protection Against *Botrytis cinerea* in Tomato (*Solanum lycopersicum* L.)

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ARTICLE INFO

Keywords:
Solanum lycopersicum
Gray Mold
Induced Resistance
Plant Defense Priming
Phenylalanine Ammonia-Lyase (PAL)

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All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/nasetjournal.v5i2.74

A B S T R A C T

Gray mold, caused by the necrotrophic fungus *Botrytis cinerea*, is a devastating disease in tomato production worldwide, necessitating the development of sustainable and effective control strategies. Plant-derived biostimulants offer a promising eco-friendly alternative to synthetic fungicides by enhancing the plant's innate immune system. This study, conducted in greenhouse facilities in Palembang, Indonesia, evaluated the efficacy of a novel plant-derived biostimulant (PDB-MX7), a composition of *Ascophyllum nodosum* and *Moringa oleifera* extracts, in controlling gray mold in tomato (*Solanum lycopersicum* L. cv. 'Mutiara'). Tomato plants were treated with PDB-MX7 and subsequently inoculated with a virulent *B. cinerea* isolate. We assessed disease progression, plant growth parameters, and a suite of underlying defense mechanisms. These included the quantification of oxidative stress markers (H_2O_2, MDA), the activity of key defense-related enzymes (PAL, PPO, SOD, CAT), the accumulation of defense phytohormones (salicylic acid, jasmonic acid), and the expression levels of pathogenesis-related genes (*PR-1*, *PDF1.2*) via RT-qPCR. Pre-treatment with PDB-MX7 significantly reduced gray mold disease severity by 76.4% and lesion diameter by 71.8% compared to untreated, inoculated plants. This protective effect was associated with a significant priming of the plant's defense system. PDB-MX7-treated plants exhibited lower levels of H_2O_2 and MDA upon infection, indicating reduced oxidative stress. Furthermore, these plants showed a rapid and potent induction of PAL and PPO activity (3.1-fold and 2.8-fold higher than controls at 48 hpi, respectively). This was corroborated by a significant accumulation of salicylic acid and a more than 5-fold upregulation in the expression of the SA-responsive gene *PR-1*, indicating the activation of Systemic Acquired Resistance (SAR). In conclusion, the novel biostimulant composition PDB-MX7 confers substantial resistance against *B. cinerea* in tomato by priming the plant's innate immunity, primarily through the activation of the SA-mediated SAR pathway. This study highlights the potential of PDB-MX7 as a powerful tool for integrated pest management programs in sustainable tomato cultivation.

1. Introduction

The tomato (*Solanum lycopersicum* L.) stands as one of the most economically significant and widely consumed vegetable crops globally, valued for its nutritional content, versatility, and contribution to the agricultural economy. In tropical regions like Indonesia, intensive tomato cultivation is crucial for local food security and commerce. However, this production is persistently challenged by a myriad of biotic stresses, among which fungal diseases are a

primary cause of substantial yield losses. Gray mold disease, incited by the ubiquitous necrotrophic fungal pathogen *Botrytis cinerea* Pers., is particularly destructive.[1,2] This pathogen has an exceptionally broad host range and can infect tomato plants at all developmental stages, from seedlings to mature, fruit-bearing plants, causing blossom blight, stem cankers, leaf necrosis, and pre- and post-harvest fruit rot. The economic impact is profound, with estimated yield losses reaching up to 30% in heavily infested fields and greenhouses, especially under conditions of high humidity and moderate temperatures, which are prevalent in many parts of Indonesia.

For decades, the primary strategy for managing gray mold has been the intensive application of synthetic chemical fungicides. While often effective in the short term, this overreliance has precipitated a cascade of severe and unsustainable consequences. These include the emergence of fungicide-resistant *B. cinerea* strains, negative impacts on non-target beneficial microorganisms, potential risks to human health, and the accumulation of chemical residues in the environment and agricultural products. The growing consumer demand for safer, residue-free food and the increasing regulatory scrutiny of chemical pesticides have created an urgent need for innovative, eco-friendly, and effective alternatives for disease management in modern agriculture.[3,4]

One of the most promising avenues in this pursuit is the utilization of plant biostimulants. These are substances or microorganisms whose function, when applied to plants or the rhizosphere, is to stimulate natural processes to enhance or benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality. A key mode of action for many biostimulants is their ability to act as elicitors, triggering the plant's own innate immune system. Plants possess a sophisticated, multi-layered defense network that can be activated upon recognition of pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). Elicitors can "prime" this defense system, placing the plant in a state of readiness. A primed plant does not mount a full-scale defense response immediately but responds more rapidly, robustly, and effectively upon subsequent pathogen attack. This phenomenon, known as induced resistance, can manifest as Systemic Acquired Resistance (SAR) or Induced Systemic Resistance (ISR), which are governed by distinct signaling pathways, primarily mediated by salicylic acid (SA) and jasmonic acid (JA)/ethylene (ET), respectively.[5,6]

Plant-derived biostimulants (PDBs), derived from extracts of various plant tissues, are gaining significant attention due to their biodegradability, low toxicity, and rich composition of bioactive molecules. These molecules, including polysaccharides, polyphenols, peptides, and phytohormones, can act as potent elicitors of plant defense. Formulations based on seaweed, particularly the brown alga *Ascophyllum nodosum*, have been widely documented to enhance plant growth and resistance to both biotic and abiotic stresses. The eliciting activity of *A. nodosum* extracts is attributed to compounds like laminarin, alginates, and fucoidans, which are recognized by plant cell receptors to initiate defense signaling cascades. Similarly, extracts from terrestrial plants known for their medicinal and nutritional properties are being explored. *Moringa oleifera* Lam. is a tropical tree whose leaves are rich in a diverse array of secondary metabolites, including flavonoids, glucosinolates, isothiocyanates, and phenolic acids. These compounds possess direct antimicrobial properties and have been shown to modulate plant physiological processes, including defense responses against pathogens.[7]

While the individual benefits of *A. nodosum* and *M. oleifera* extracts have been explored, the potential synergistic or additive effects of their combined application as a composite biostimulant remain largely unknown. A formulation that combines the well-established elicitors from a marine source with the unique phytochemical profile of a terrestrial plant could potentially activate a broader and more resilient defense response. Such a composition could trigger multiple signaling pathways, leading to a more

comprehensive and durable resistance against complex pathogens like *B. cinerea*. This necrotroph employs a multifaceted infection strategy, rapidly killing host cells and then feeding on the dead tissue, making it a challenging target for defense mechanisms that are effective against biotrophs. Therefore, a defense strategy that combines cell wall reinforcement, production of antimicrobial compounds, and management of oxidative stress is paramount.[8,9]

Therefore, the aim of this study was to formulate and evaluate a novel plant-derived biostimulant composition, designated PDB-MX7, comprising extracts from *Ascophyllum nodosum* and *Moringa oleifera*, for its capacity to induce resistance against *Botrytis cinerea* in tomato. We hypothesized that PDB-MX7 would prime the tomato plant's immune system, leading to enhanced protection against gray mold. The novelty of this research lies in its comprehensive, multi-level investigation into the mechanisms underlying this induced resistance. We move beyond simple disease assessment to provide an integrated analysis of the physiological, biochemical, and molecular responses, including the profiling of key defense enzymes, phytohormone signaling molecules (SA and JA), and the expression of marker genes for systemic resistance pathways. This work represents the first investigation into the synergistic potential of this specific marine-terrestrial plant extract combination, seeking to elucidate the intricate defense network it activates to provide a foundation for its development as a sustainable disease management tool.

2. Methods

The experiment was conducted under controlled greenhouse conditions at the CMHC Research Center, Palembang, South Sumatera, Indonesia. The greenhouse maintained an average temperature of 28 ± 4°C, relative humidity of 75 ± 10%, and a natural photoperiod of approximately 12/12 hours (light/dark). Seeds of tomato (Solanum lycopersicum L. cv. 'Mutiara'), a determinate cultivar widely grown in Indonesia and known for its susceptibility to gray mold, were sourced from a local certified seed producer (PT East West Seed Indonesia). Seeds were sown in germination trays filled with a sterile mixture of cocopeat and compost (2:1, v/v). After two weeks, uniform seedlings at the two-true-leaf stage were transplanted into individual 5 L polybags containing a sterilized growing medium of soil, sand, and compost (2:1:1, v/v/v). Plants were watered daily and fertilized weekly with a standard NPK (16:16:16) solution. The experiment was initiated when plants reached the 4-5 true-leaf stage, approximately four weeks after transplanting.

The plant-derived biostimulant composition, designated PDB-MX7, was a proprietary aqueous formulation of two key ingredients. The first component was a commercial extract of the brown seaweed Ascophyllum nodosum, sourced from Acadian Seaplants Ltd., Canada, and used according to the manufacturer's recommendations. The second component was an extract of Moringa oleifera leaves, prepared in-house. Fresh, healthy Moringa leaves were collected from the Cikabayan experimental farm at IPB University. The leaves were washed thoroughly with sterile distilled water, shade-dried for seven days, and then ground into a fine powder. The powder (100 g) was extracted with 1 L of 80% methanol for 48 hours at room temperature with continuous shaking. The extract was filtered through Whatman No. 1 filter paper, and the solvent was evaporated under reduced pressure using a rotary evaporator at 40°C to yield a crude extract. The final PDB-MX7 formulation was prepared by dissolving the A. nodosum extract (final concentration 0.2% v/v) and the M. oleifera crude extract (final concentration 0.1% w/v) in sterile distilled water containing 0.02% (v/v) Tween-20 as a surfactant.

A virulent isolate of Botrytis cinerea (BC-IPB01), originally isolated from an infected tomato fruit in a commercial farm in Cipanas, West Java, was used for this study. The isolate was maintained on Potato Dextrose Agar (PDA) medium at 4°C. For inoculum preparation, the fungus was sub-cultured onto fresh PDA plates and incubated at 22 ± 1°C under a 12-hour

photoperiod provided by near-UV light to promote sporulation. After 14 days, conidia were harvested by flooding the plates with sterile distilled water containing 0.02% Tween-20 and gently scraping the surface with a sterile glass rod. The resulting suspension was filtered through four layers of sterile cheesecloth to remove mycelial fragments. The conidial concentration was determined using a hemocytometer and adjusted to a final concentration of 1×10^6 conidia mL^{-1} for inoculation.

The experiment was laid out in a Randomized Complete Block Design (RCBD) with four treatments and four replications (blocks). Each treatment unit consisted of 10 plants, totaling 160 plants for the entire experiment. The four treatments were as follows: (1) Control (C): Plants sprayed with sterile distilled water + 0.02% Tween-20 and mock-inoculated with sterile distilled water; (2) Biostimulant Only (PDB): Plants sprayed with PDB-MX7 and mock-inoculated with sterile distilled water; (3) Pathogen Only (BC): Plants sprayed with sterile distilled water + 0.02% Tween-20 and inoculated with *B. cinerea*; (4) Biostimulant + Pathogen (PDB+BC): Plants sprayed with PDB-MX7 and inoculated with *B. cinerea*.

The PDB-MX7 or water (control) was applied as a foliar spray until runoff, ensuring complete coverage of all aerial parts of the plants. This application was performed twice: 7 days and 3 days prior to pathogen inoculation, to ensure adequate time for the priming of defense responses. Three days after the second biostimulant application, plants in the BC and PDB+BC treatments were inoculated with the *B. cinerea* conidial suspension. Inoculation was performed by wounding the third fully expanded leaf from the apex with a sterile needle and applying a 10 µL droplet of the conidial suspension onto the wound. Plants in the C and PDB treatments were mock-inoculated with a 10 µL droplet of sterile distilled water. After inoculation, plants were covered with transparent plastic bags for 48 hours to maintain high humidity (>95%) and facilitate fungal infection.

Disease development was monitored daily. Disease incidence and severity were recorded at 7 days post-inoculation (dpi). Disease incidence (%) calculated as the percentage of inoculated leaves showing visible necrotic symptoms. Lesion diameter (mm) was defined as the diameter of the necrotic lesion developing from the inoculation point was measured using a digital caliper. Disease Severity Index (DSI) was assessed using a 0-5 rating scale: 0 = no symptoms; 1 = small necrotic spots, lesion covers <10% of leaf area; 2 = lesion covers 11-25% of leaf area; 3 = lesion covers 26-50% of leaf area; 4 = lesion covers 51-75% of leaf area with some tissue collapse; 5 = lesion covers >75% of leaf area, extensive necrosis, and tissue maceration. The DSI was calculated using the formula: DSI (%) = (n x v/N x V) x 100, where n is the number of leaves in each category, v is the numerical value of each category, N is the total number of leaves assessed, and V is the highest numerical value of the scale.

At the end of the experiment (14 dpi), five plants per treatment unit were randomly selected to measure growth parameters. Plant height, stem diameter, chlorophyll content (SPAD units, using a Konica Minolta SPAD-502 meter on the fourth fully expanded leaf), and total dry biomass (shoots and roots dried at 70°C for 72 hours) were recorded.

Leaf samples for biochemical and molecular analyses were collected at 0, 24, 48, and 72 hours post-inoculation (hpi). Samples were immediately frozen in liquid nitrogen and stored at -80°C until analysis. Hydrogen Peroxide (H_2O_2) content was measured spectrophotometrically by monitoring the absorbance of the titanium-hydroperoxide complex at 415 nm. Malondialdehyde (MDA) content, an indicator of lipid peroxidation, was estimated using the thiobarbituric acid reactive substances (TBARS) assay, with absorbance measured at 532 nm and 600 nm. All defense enzyme activities were measured spectrophotometrically from a crude protein extract. Protein concentration was determined using the Bradford method. Phenylalanine Ammonia-Lyase (PAL; EC 4.3.1.24); PAL activity was determined by measuring the formation of trans-cinnamic acid from L-phenylalanine at 290 nm. Polyphenol Oxidase (PPO; EC 1.14.18.1); PPO activity was assayed by measuring

the rate of purpurogallin formation from pyrogallol at 420 nm. Superoxide Dismutase (SOD; EC 1.15.1.1); SOD activity was determined based on its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT), with absorbance read at 560 nm. Catalase (CAT; EC 1.11.1.6); CAT activity was measured by monitoring the decomposition of H_2O_2 at 240 nm.

Free salicylic acid (SA) and jasmonic acid (JA) were extracted from 200 mg of frozen leaf tissue using a methanol-based extraction method and quantified using a High-Performance Liquid Chromatography (HPLC) system (Agilent 1260 Infinity) equipped with a C18 column and a fluorescence detector, following previously established protocols with minor modifications.

Total RNA was extracted from leaf samples using the RNeasy Plant Mini Kit (Qiagen, Germany). RNA quality and quantity were assessed via spectrophotometry and gel electrophoresis. First-strand cDNA was synthesized from 1 µg of total RNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). Real-time quantitative PCR (RT-qPCR) was performed on a Bio-Rad CFX96 Real-Time System using SYBR Green Master Mix. The expression of two key defense-related genes was analyzed: PR-1 (Pathogenesis-Related protein 1, an SA-pathway marker) and PDF1.2 (Plant Defensin 1.2, a JA/ET-pathway marker). The tomato Actin gene was used as the endogenous reference for normalization. The relative expression levels were calculated using the $2^{-}\Delta\Delta Ct$ method.

All data were subjected to Analysis of Variance (ANOVA) appropriate for an RCBD design using the R statistical software (version 4.2.1). Data were first checked for normality and homogeneity of variances. When the ANOVA indicated a significant effect ($P < 0.05$), treatment means were compared using Tukey's Honestly Significant Difference (HSD) post-hoc test.

3. Results and discussion

Application of the biostimulant PDB-MX7 provided significant protection to tomato plants against B. cinerea infection. As shown in Table 1, at 7 days post-inoculation (dpi), the untreated, pathogen-inoculated plants (BC) exhibited severe gray mold symptoms, with a disease incidence of 100%. In contrast, plants pre-treated with PDB-MX7 (PDB+BC) showed a significantly lower disease incidence of only 37.5%. The Disease Severity Index (DSI) was dramatically reduced from 85.2% in the BC group to 20.1% in the PDB+BC group, representing a disease control efficacy of 76.4%. Furthermore, the necrotrophic lesion expansion was effectively curtailed by the biostimulant treatment. The average lesion diameter in the BC group was 25.1 mm, whereas in the PDB+BC group, it was only 7.1 mm, a reduction of 71.8%. The control (C) and biostimulant-only (PDB) groups showed no signs of disease throughout the experiment, confirming the pathogen's role and the non-phytotoxic nature of the biostimulant.

Infection with *B. cinerea* had a significant negative impact on tomato plant growth (Table 2). Plants in the BC group showed stunted growth, with significant reductions in plant height, stem diameter, chlorophyll content, and total dry biomass compared to the healthy control plants. However, pre-treatment with PDB-MX7 substantially alleviated these negative effects. The PDB+BC plants exhibited growth parameters that were not significantly different from the uninfected control plants. Interestingly, the PDB-only treatment resulted in a slight but significant increase in plant height, chlorophyll content, and total dry biomass compared to the control group, indicating a direct growth-promoting (biostimulant) effect of the formulation, independent of its disease-suppressive action.

To investigate the physiological state of the plants during infection, we measured the accumulation of H_2O_2 and the levels of MDA, a marker for membrane lipid peroxidation. Pathogen challenge in the BC group led to a massive and sustained increase in both H_2O_2 and MDA content, peaking at 48 hpi (Figure 1). This indicates severe oxidative stress and cellular damage. In contrast, while the PDB+BC plants also showed an initial increase in H_2O_2, the levels were significantly

lower than in the BC group and began to decline after 48 hpi. Crucially, the MDA content in PDB+BC plants remained low throughout the experiment, at levels comparable to the uninfected controls. This suggests that PDB-MX7 pre-treatment enabled the plants to effectively manage and neutralize the pathogen-induced oxidative burst, thereby preventing extensive cellular damage.

Table 1. Gray Mold Disease in Tomato

Effect of PDB-MX7 pre-treatment on gray mold disease development in tomato plants at 7 days post-inoculation with *Botrytis cinerea*.

TREATMENT	DISEASE INCIDENCE (%)	DISEASE SEVERITY INDEX (DSI, %)	LESION DIAMETER (MM)
Control (C) Water spray, mock-inoculated	0.0 ± 0.0 [c]	0.0 ± 0.0 [d]	0.0 ± 0.0 [c]
PDB-MX7 Only (PDB) Biostimulant spray, mock-inoculated	0.0 ± 0.0 [c]	0.0 ± 0.0 [d]	0.0 ± 0.0 [c]
***B. cinerea* Only (BC)** Water spray, pathogen-inoculated	100.0 ± 0.0 [a]	85.2 ± 4.6 [a]	25.1 ± 2.1 [a]
PDB-MX7 + *B. cinerea* (PDB+BC) Biostimulant spray, pathogen-inoculated	37.5 ± 5.2 [b]	20.1 ± 3.8 [b]	7.1 ± 1.3 [b]

Notes on Interpretation:

Values are means ± standard deviation (n=4). In any given column, values followed by the same superscript letter are not significantly different from each other based on Tukey's HSD test ($P < 0.05$).

[a] - Represents the highest level of disease, observed in the untreated, pathogen-inoculated group.

[b] - Represents a significant reduction in disease compared to group 'a', seen in the biostimulant-treated group.

[c, d] - Represent treatments with no observable disease. These values are statistically similar to each other and significantly lower than groups 'a' and 'b'.

The ability of PDB+BC plants to control oxidative stress was correlated with a primed response of antioxidant enzymes. The activities of Superoxide Dismutase (SOD) and Catalase (CAT) were significantly higher and induced earlier in PDB+BC plants compared to BC plants upon infection (Figure 2). SOD activity peaked at 24 hpi in PDB+BC plants, while in BC plants, the response was delayed and of lower magnitude. A similar pattern was observed for CAT activity.

Furthermore, PDB-MX7 treatment primed the induction of key enzymes in the phenylpropanoid pathway, PAL and PPO, which are central to the synthesis of antimicrobial phytoalexins and phenolics for cell wall reinforcement. In PDB+BC plants, PAL activity showed a rapid and dramatic increase, peaking at 48 hpi at a level 3.1-fold higher than in the BC group (Figure 3A). PPO activity followed a similar trend, with a peak at 48 hpi that was 2.8-fold higher in PDB+BC plants compared to the pathogen-only

challenge (Figure 3B). Notably, application of PDB-MX7 alone caused a slight, transient increase in these enzyme activities, indicating a mild elicitation that prepared the plant for a stronger response, a hallmark of priming.

Table 2. Tomato Plant Growth Parameters

Effect of PDB-MX7 and/or *B. cinerea* inoculation on growth parameters of tomato plants at 14 days post-inoculation (dpi).

TREATMENT	PLANT HEIGHT (CM)	STEM DIAMETER (MM)	CHLOROPHYLL (SPAD)	TOTAL DRY BIOMASS (G)
Control (C) Standard growth conditions	45.2 ± 2.1 [b]	7.8 ± 0.3 [a]	48.1 ± 1.9 [b]	15.6 ± 0.8 [b]
PDB-MX7 Only (PDB) Biostimulant applied, no pathogen	49.8 ± 1.9 [a]	8.0 ± 0.4 [a]	51.5 ± 2.2 [a]	17.2 ± 1.1 [a]
***B. cinerea* Only (BC)** Pathogen applied, no biostimulant	33.1 ± 2.5 [c]	6.5 ± 0.5 [b]	36.4 ± 2.8 [c]	10.8 ± 1.3 [c]
PDB-MX7 + *B. cinerea* (PDB+BC) Biostimulant and pathogen applied	44.5 ± 2.8 [b]	7.6 ± 0.4 [a]	47.2 ± 2.5 [b]	15.1 ± 0.9 [b]

Notes on Interpretation:

Values are means ± standard deviation (n=5). In any given column, values followed by a different superscript letter are significantly different from each other based on Tukey's HSD test ($P < 0.05$).

[a] - Represents the highest growth measurements, indicating a growth-promoting or protective effect.

[b] - Represents baseline or recovered growth, not statistically different from the best-performing group 'a' in some cases, but significantly better than the worst group 'c'.

[c] - Represents the lowest growth measurements, indicating significant growth reduction due to pathogen stress.

To elucidate the signaling pathways involved in PDB-MX7-induced resistance, we quantified the endogenous levels of free SA and JA. As shown in Figure 4A, infection with B. cinerea in BC plants led to a modest increase in SA levels, peaking at 48 hpi. However, in PDB-MX7 pre-treated plants (PDB+BC), the accumulation of SA was significantly faster and much more pronounced, reaching a peak concentration nearly 2.5-fold higher than that in BC plants at the same time point. In contrast, JA levels showed a strong induction in the BC group, consistent with a response to a necrotrophic pathogen. Interestingly, in the PDB+BC group, while JA levels also increased, the peak was slightly lower and occurred earlier (at 24 hpi) compared to the BC group (Figure 4B). This suggests a dominant role for the SA pathway in PDB-MX7-mediated defense, potentially modulating the JA response.

To confirm the activation of hormone-mediated defense pathways at the molecular level, we analyzed the expression of PR-1 and PDF1.2, marker genes for the SA and JA/ET pathways, respectively. The expression patterns mirrored the phytohormone profiles (Figure 5). The relative expression of PR-1 was massively upregulated in PDB+BC plants following infection, showing a more than 5-fold increase compared to the challenged control (BC) at 48 hpi. This strong induction provides clear molecular evidence for the activation of the SA-dependent SAR pathway. The expression of PDF1.2 was also induced by the

pathogen, but there was no significant difference between the BC and PDB+BC groups, further supporting the conclusion that the primary mechanism of PDB-MX7-induced resistance against B. cinerea is the potentiation of the SA signaling pathway.

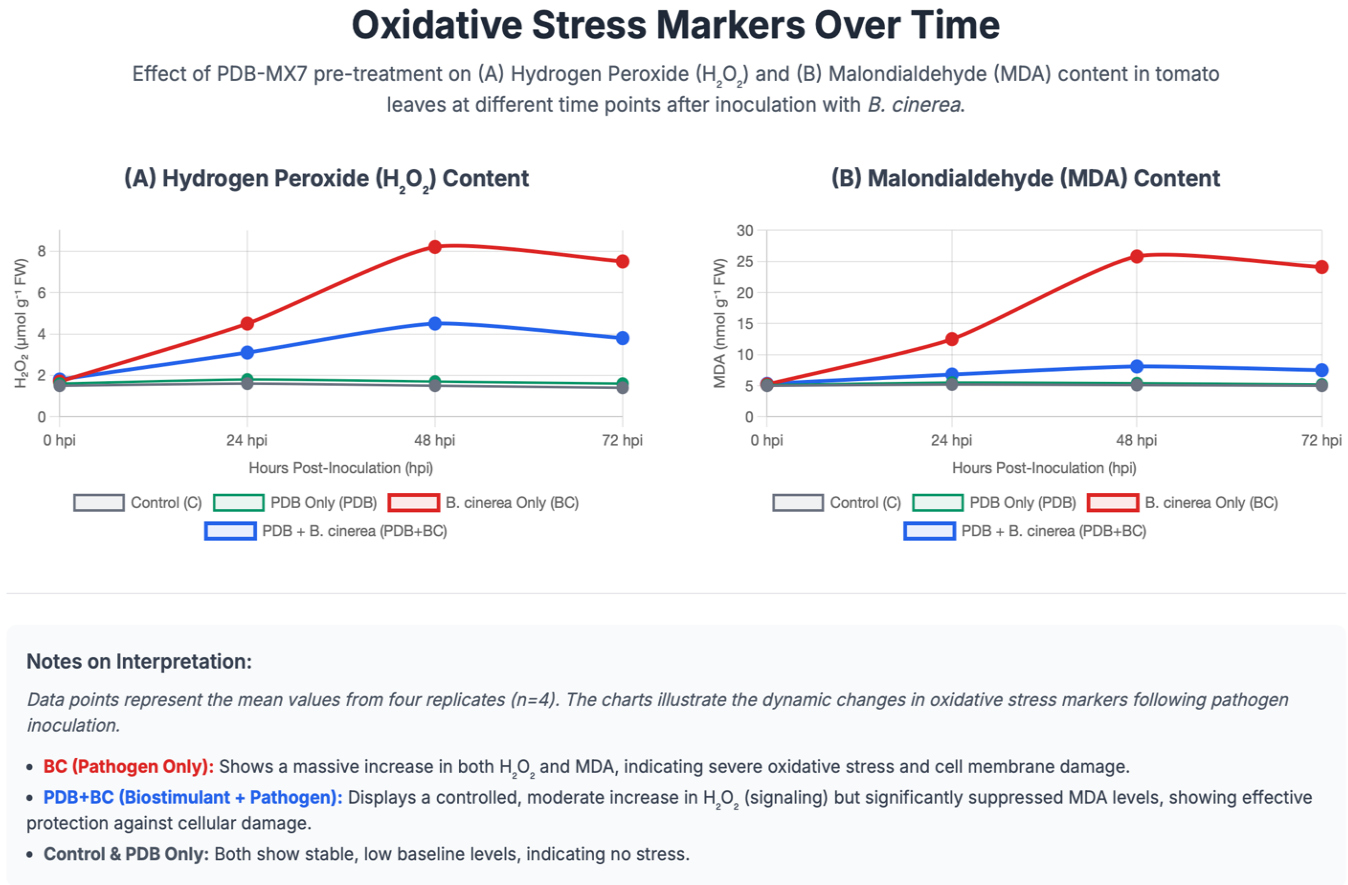


Figure 1. Effect of PDB-MX7 pre-treatment on (A) Hydrogen Peroxide (H_2O_2) content and (B) Malondialdehyde (MDA) content in tomato leaves at different time points after inoculation with *B. cinerea*. Data points represent means ± SD (n=4). Different letters at each time point indicate significant differences ($P < 0.05$).

The escalating challenges posed by fungicide resistance and environmental concerns mandate a paradigm shift in crop protection towards more sustainable practices. This study provides compelling evidence that the novel plant-derived biostimulant, PDB-MX7, is a highly effective agent for controlling gray mold disease in tomato. The application of PDB-MX7 did not exhibit direct antifungal activity (data not shown) but rather conferred protection by priming the plant's innate immune system for a faster and more robust defense response upon pathogen attack. Our comprehensive analysis, spanning from macroscopic disease symptoms to molecular gene expression, elucidates the intricate mechanisms underlying this induced resistance, which is predominantly mediated by the activation of the Systemic Acquired Resistance (SAR) pathway.[10-12]

The primary finding of this research is the significant reduction in disease severity (76.4%) in PDB-MX7-treated plants. This protection is not merely a localized phenomenon but a systemic response that enhances the plant's overall defensive capacity. This is supported by the concurrent mitigation of pathogen-induced growth depression. While *B. cinerea* infection severely stunted growth in untreated plants, PDB-MX7-treated plants maintained growth parameters

comparable to healthy controls. This indicates that the biostimulant not only activates defenses but also helps the plant to tolerate the stress of infection, reallocating resources efficiently to maintain growth while defending against the pathogen. Furthermore, the observed growth promotion in the PDB-only treatment highlights the pleiotropic effects of the formulation, likely due to the presence of growth-regulating compounds like auxins, cytokinins, and essential micronutrients within the *A. nodosum* and *Moringa* extracts.[13-15]

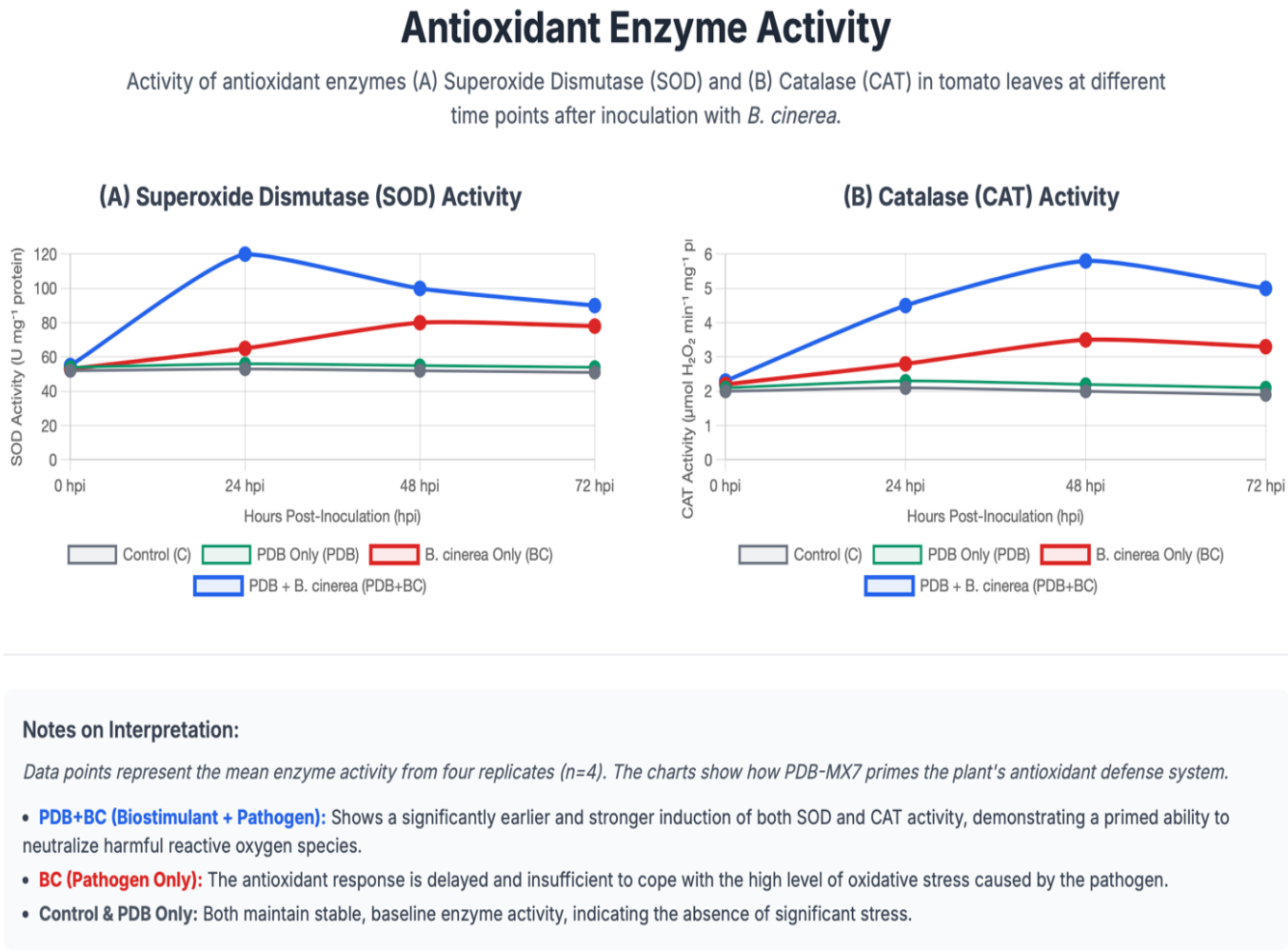
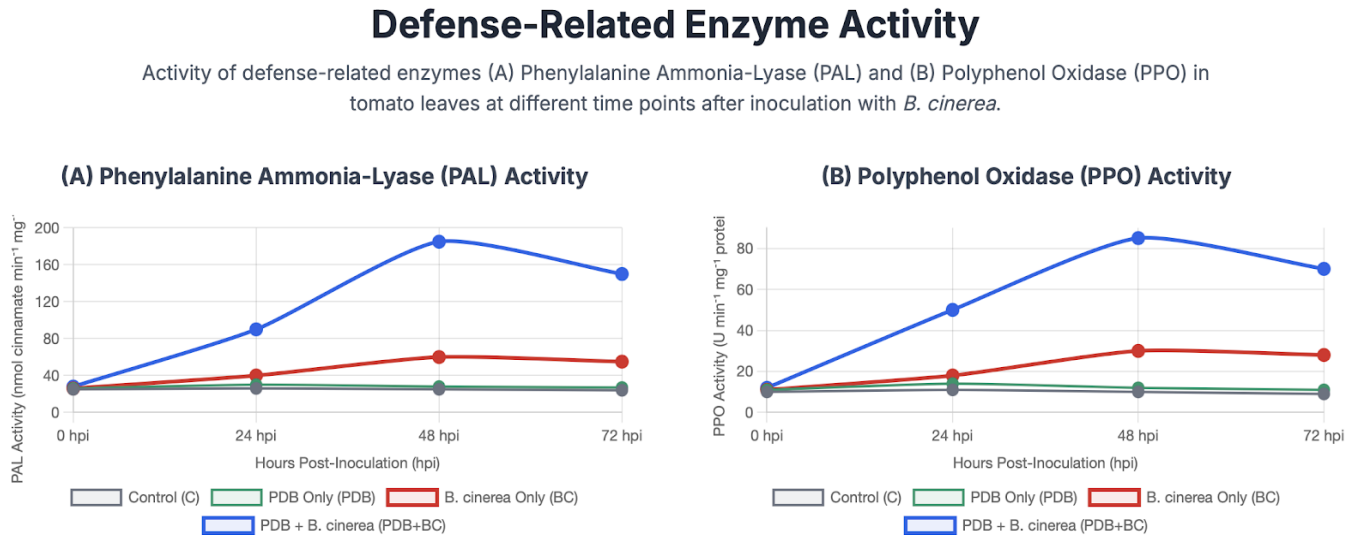


Figure 2. Activity of antioxidant enzymes (A) Superoxide Dismutase (SOD) and (B) Catalase (CAT) in tomato leaves.

A critical aspect of a successful plant-pathogen interaction, from the plant's perspective, is the management of oxidative stress. The initial recognition of a pathogen often triggers a rapid generation of reactive oxygen species (ROS), known as the oxidative burst, which has a dual role: directly hampering the pathogen and acting as a signaling molecule to activate downstream defenses. However, uncontrolled ROS accumulation can lead to extensive cellular damage, lipid peroxidation (measured as MDA), and eventually, programmed cell death, which a necrotroph like *B. cinerea* exploits to its advantage. Our results show that while PDB-MX7-primed plants still produced an oxidative burst (H_2O_2), they were able to keep it under tight control. This was evidenced by the significantly lower levels of H_2O_2 and, more

importantly, the near-basal levels of MDA in PDB+BC plants. This enhanced ROS detoxification capacity is directly attributable to the primed activation of antioxidant enzymes. The earlier and stronger induction of SOD (which dismutates superoxide radicals into H_2O_2) and CAT (which neutralizes H_2O_2) in primed plants demonstrates a highly efficient antioxidant system ready to cope with pathogen-induced stress, thereby preserving cellular integrity and limiting the substrate for the necrotrophic pathogen.[16,17]



Notes on Interpretation:

Data points represent the mean enzyme activity from four replicates (n=4). These charts illustrate the priming of the phenylpropanoid defense pathway.

- **PDB+BC (Biostimulant + Pathogen):** Shows a massive and rapid induction of both PAL and PPO, indicating a strong activation of pathways that produce antimicrobial compounds and reinforce cell walls.
- **BC (Pathogen Only):** The defense enzyme response is significantly slower and weaker, demonstrating an inadequate defense activation against the pathogen.
- **Control & PDB Only:** Both treatments maintain low, baseline enzyme activity, confirming that the strong induction is a specific, primed response to the pathogen.

Figure 3. Activity of defense-related enzymes (A) Phenylalanine Ammonia-Lyase (PAL) and (B) Polyphenol Oxidase (PPO) in tomato leaves.

The cornerstone of the induced resistance observed in this study is the potentiation of the phenylpropanoid pathway, evidenced by the dramatic induction of PAL and PPO activity. PAL is the gateway enzyme for this pathway, which leads to the synthesis of a vast array of antimicrobial and defense-related compounds, including phenolic acids, flavonoids, and lignin precursors. The rapid, 3.1-fold increase in PAL activity in PDB+BC plants would facilitate a massive flux of carbon into this pathway, leading to the accumulation of phytoalexins that can directly inhibit fungal growth and the deposition of phenolic polymers into the cell wall. This process, known as lignification, creates a physical barrier that is highly resistant to the cell-wall-degrading enzymes secreted by *B. cinerea*. PPO, in turn, catalyzes the oxidation of these phenolics into highly reactive quinones, which are not only more toxic to the pathogen but also contribute to cell wall cross-linking, further fortifying the physical defenses. The priming of these enzymatic activities is a classic hallmark of induced resistance and is directly responsible for restricting lesion expansion.

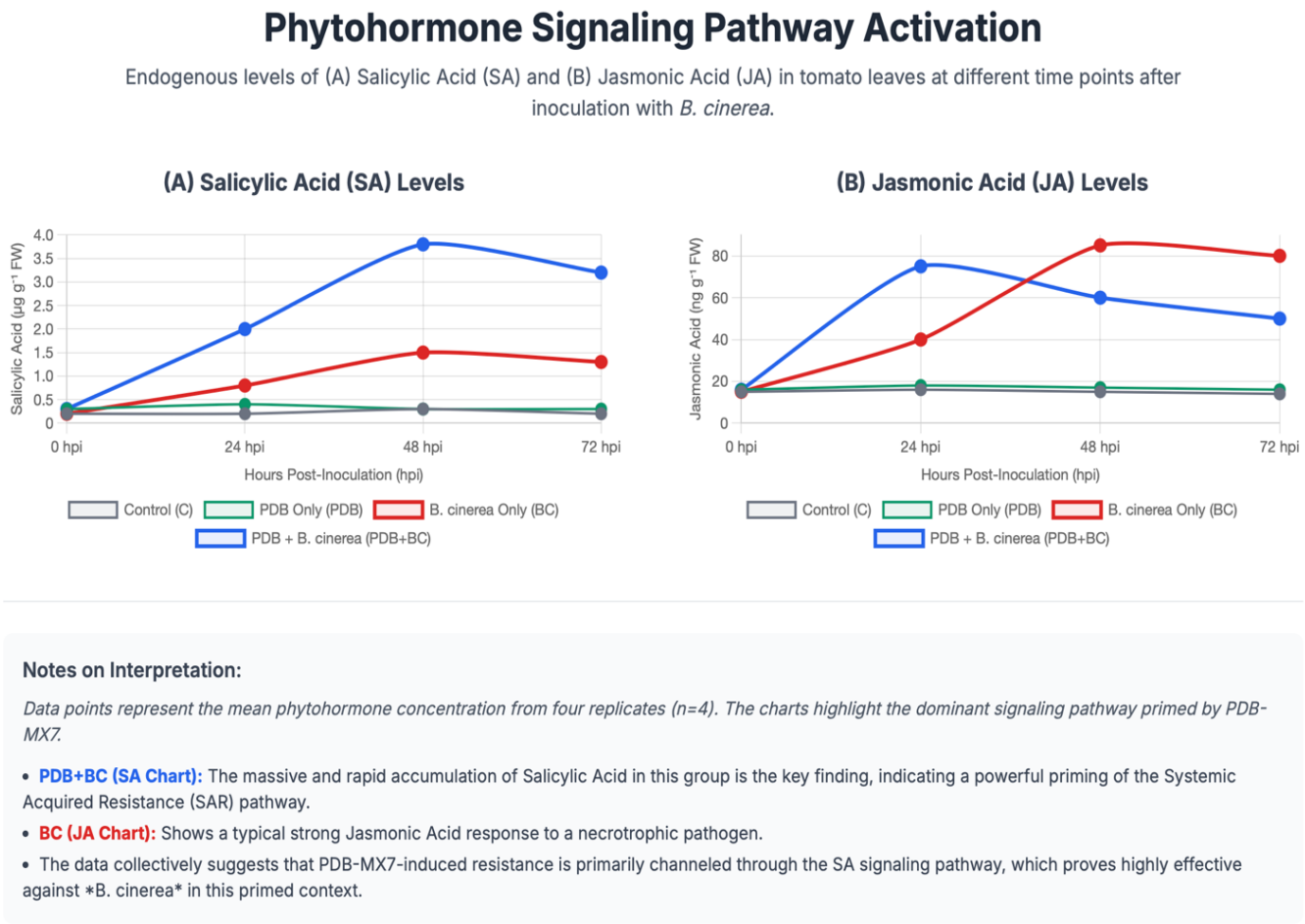


Figure 4. Endogenous levels of (A) Salicylic Acid (SA) and (B) Jasmonic Acid (JA) in tomato leaves.

The coordination of these complex defense responses is orchestrated by a sophisticated network of phytohormone signaling. Our results point unequivocally to the salicylic acid (SA) pathway as the central mediator of PDB-MX7-induced resistance. The significantly higher accumulation of SA in PDB+BC plants is a key finding. SA is the primary signaling molecule for the activation of SAR, a long-lasting, broad-spectrum resistance effective against many biotrophic and hemibiotrophic pathogens. While *B. cinerea* is a necrotroph, accumulating evidence suggests that an early and robust SA response can be effective in limiting the initial stages of infection before necrotrophy is fully established. The massive upregulation of the SA-marker gene *PR-1* (over 5-fold) in PDB+BC plants provides definitive molecular proof of SAR activation. PR-1 proteins are thought to possess antimicrobial activities and are a reliable indicator that the SA signal has been transduced and the plant has entered a systemic state of defense.[18,19]

The interaction between the SA and JA pathways, often referred to as signaling crosstalk, is complex and frequently antagonistic. The JA pathway is classically associated with defense against necrotrophic pathogens and herbivorous insects. Our data show that while *B. cinerea* strongly induced the JA pathway (JA accumulation and *PDF1.2* expression), PDB-MX7 pre-treatment did not further potentiate this response, and in fact, slightly attenuated the JA peak. This suggests that the biostimulant prioritizes the activation of the SA pathway, which in this context, proved to be a more effective strategy. This SA-JA antagonism might allow the plant to fine-tune its defense response, preventing the excessive activation of two potentially conflicting pathways and conserving metabolic resources.[2]

Notes on Interpretation:

Bars represent the mean relative gene expression (fold change) compared to the control group at 48 hpi (n=4). Error bars represent standard deviation.

- ***PR-1* Expression:** The massive upregulation of *PR-1* in the PDB+BC treatment (over 5 times higher than the pathogen-only group) is the definitive molecular evidence that PDB-MX7 strongly primes the SA-mediated Systemic Acquired Resistance (SAR) pathway.
- ***PDF1.2* Expression:** While the pathogen induces this JA/ET-pathway marker, there is no significant difference between the BC and PDB+BC groups. This confirms that the biostimulant's protective effect is not channeled through an enhancement of this pathway.
- Collectively, these results molecularly confirm the conclusions drawn from the phytohormone data in Figure 4.

Figure 5. Relative expression of defense-related genes (A) *PR-1* and (B) *PDF1.2* in tomato leaves.

The eliciting capacity of PDB-MX7 likely stems from a synergistic combination of bioactive molecules from its two components. The *A. nodosum* extract contains sulfated polysaccharides like fucoidans and

oligosaccharides like laminarin, which are known PAMPs that are recognized by plant receptors to initiate defense signaling. The *Moringa oleifera* extract contributes a different suite of compounds, including flavonoids like quercetin and kaempferol, and glucosinolate derivatives, which have been shown to act as defense elicitors and modulators of plant hormone homeostasis. The combination of these diverse molecular patterns may trigger multiple receptor complexes on the plant cell surface, leading to a more amplified and resilient downstream signal than either component could achieve alone. This comprehensive activation is likely what underpins the strong priming of the SA-mediated SAR pathway observed in our study.

4. Conclusion

This study conclusively demonstrates that the novel plant-derived biostimulant formulation, PDB-MX7, effectively protects tomato plants against gray mold disease caused by *Botrytis cinerea*. The protective mechanism is not based on direct fungicidal action but on the elicitation and priming of the plant's innate immune system. PDB-MX7 pre-treatment prepares the plant for a more rapid, potent, and efficient defense response upon pathogen challenge. This response is characterized by the enhanced management of oxidative stress, the fortification of cell walls via the phenylpropanoid pathway, and, most critically, the strong activation of the salicylic acid-mediated Systemic Acquired Resistance (SAR) pathway. The findings position PDB-MX7 as a promising, effective, and environmentally sound tool for integration into disease management programs for sustainable tomato production, offering a viable alternative to the reliance on synthetic fungicides.

5. References

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