Biological suppression of early blight disease (*Alternaria solani* Sorauer) in potato (*Solanum tuberosum* L.) using *Bacillus subtilis* (Ehrenberg) Cohn, *Trichoderma harzianum* Rifai and Nano Silica

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Abstract

Background: Early blight disease, incited by the soilborne necrotrophic fungus *Alternaria solani* Sorauer, constitutes a major biotic constraint on potato (*Solanum tuberosum* L.) cultivation, with potential yield losses exceeding 86%. Repeated application of broad-spectrum synthetic fungicides has raised growing concerns regarding environmental contamination, pathogen resistance development, and agrochemical residues in food commodities.

Methods: A screenhouse experiment was carried out from January to May 2024 at the UPTD Seed Potato Station, Pangalengan, West Java, Indonesia (1,500 m a.s.l.; 18–25 °C). A Randomized Block Design was employed with seven treatments and four replications (28 plots; 12 plants per plot; cv. Granola L.). Treatments comprised: P1 – *B. subtilis* (10 ml/L; 10^9 CFU/ml); P2 – *B. subtilis* + Nano Silica (3,000 ppm); P3 – *T. harzianum* (20 g/L; 10^6 spores/ml); P4 – *T. harzianum* + Nano Silica; P5 – *B. subtilis* + *T. harzianum* + Nano Silica; P6 – untreated negative control; P7 – Mancozeb 80 WP (2 g/L). Applications began at 10 weeks after planting (WAP) and were repeated weekly for four consecutive weeks. Disease intensity was assessed using a 0–5 ordinal scale and converted to a disease index percentage. Data were subjected to ANOVA and mean separation by Duncan's Multiple Range Test (DMRT) at the 5% level.

Results: Treatment effects on disease intensity were highly significant at all post-application observation periods ($F = 15.82–194.85$; $p < 0.001$; $R^2 = 0.82–0.98$). At 13 WAP, the triple combination P5 (5.60%) was statistically equivalent to the Mancozeb standard P7 (5.72%), both being significantly superior to all other treatments, while the negative control P6 reached 23.56%. Tuber yield in P2 (636 g/plot) significantly exceeded P6 (375 g/plot). Plant height was not significantly affected by any treatment.

Conclusion: The combined application of *B. subtilis*, *T. harzianum*, and Nano Silica (P5) effectively suppressed *A. solani* to a level statistically equivalent to the synthetic fungicide standard, representing a viable eco-friendly alternative for integrated early blight management in potato production.

Keywords: Biocontrol, Early blight, *Alternaria solani, Bacillus subtilis, Trichoderma harzianum,* Nano Silica, *Solanum tuberosum,* Integrated disease management

1. Introduction

Potato (*Solanum tuberosum* L.) ranks as the world's third most consumed food crop after rice and wheat, serving as a primary caloric source for millions of people globally (Pratama *et al*., 2021) [24]. The crop is nutritionally dense, containing per 100 g fresh weight approximately 18.4% carbohydrate, 2% protein, 19.7 mg vitamin C, and substantial quantities of potassium, phosphorus, and dietary fibre (Raigond *et al*., 2023) [26]. In Indonesia, national potato production exhibited considerable fluctuation between 2019 and 2023, reaching a peak of 1,503,998 tonnes in 2022 before declining to 1,248,513 tonnes in 2023 (Badan Pusat Statistik, 2024) [5]. This instability is attributable to multiple agronomic constraints, chief among them being fungal disease pressure.

Early blight, caused by *Alternaria solani* Sorauer, is one of the most economically significant foliar diseases of potato worldwide. The pathogen, classified within the division Ascomycota, class Dothideomycetes, and order Pleosporales, overwinters in infected plant debris and soil and is disseminated through wind-borne conidia and rain splash (Mugao, 2023) [19]. Upon germination on susceptible foliage, germ tubes penetrate directly through the cuticle or via stomata, producing characteristic dark-brown necrotic lesions with concentric rings surrounded by a chlorotic halo. Under conducive conditions of high humidity and temperatures of 24–29 °C, lesions expand rapidly, leading to premature defoliation and subsequent reduction in photosynthetic capacity (Doko, 2022). Documented yield losses range from 50% to 86% in the absence of effective management (Nuviani *et al*., 2023) [22].

The predominant control strategy adopted by smallholder potato growers in Indonesia is prophylactic application of broad-spectrum protective fungicides, including Mancozeb 80 WP. Although effective, indiscriminate fungicide use poses significant risks: accumulation of heavy metal residues (Mn^{2+} and Zn^{2+}) in aquatic environments, selective pressure promoting fungicide-resistant pathogen populations, and contamination of harvested produce (Tunjung & Nugroho, 2021; Istifadah *et al*., 2020) [29, 14]. These concerns have intensified interest in biologically-based alternatives compatible with integrated pest management (IPM) principles.

Bacillus subtilis (Ehrenberg) Cohn is a Gram-positive, endospore-forming rhizobacterium widely recognised as a potent biocontrol agent. Its antagonistic mechanisms encompass competitive exclusion, antibiosis via lipopeptides such as iturin and bacillomycin D, and induction of systemic resistance (ISR) in host plants (Prihatiningsih, 2021; Setiaji *et al*., 2023) [25, 27]. Previous field trials have demonstrated the capacity of *B. subtilis* to suppress *A. solani* on potato and related Solanaceous crops (Mutakin, 2018; Ngau & Mikael, 2024) [20, 21].

Trichoderma harzianum Rifai is a saprophytic soil fungus extensively employed as a biocontrol agent against a broad spectrum of phytopathogenic fungi. Its antagonistic activities include mycoparasitism through coiling around and enzymatic degradation of host hyphae via chitinases, glucanases, and proteases; competition for space and nutrients; and the production of secondary metabolites with antifungal properties (Muhibuddin *et al*., 2021; Djaya *et al*., 2021) [18, 7]. At a spore density of 10^6 CFU/ml, *T. harzianum* has been reported to achieve up to 61% suppression of *A. solani* in vitro (Muhibuddin *et al*., 2021) [18].

Silicon (Si), though considered a non-essential plant nutrient, plays a demonstrably beneficial role in disease resistance. In nano-scale formulations (Nano Silica; particle size 1–100 nm), increased surface area facilitates more efficient foliar uptake and rapid polymerisation within epidermal cell walls. Silicon deposition promotes the synthesis of lignin and suberin, thickening cell walls and impeding fungal penetration (Suharti *et al*., 2021; Ishlah *et al*., 2022) [28, 13]. Co-application of Nano Silica with biocontrol agents has been shown to enhance suppression of *Alternaria* diseases on eggplant and onion (Albawi *et al*., 2022; Ishlah *et al*., 2022) [2, 13].

Despite the individually documented efficacy of these inputs, no prior study had examined their combined application against *A. solani* on potato in the highland growing conditions of West Java, Indonesia. The present study therefore aimed to: (i) evaluate the effect of *B. subtilis*, *T. harzianum*, and Nano Silica—applied singly and in combination—on early blight disease intensity; and (ii) identify the treatment combination most effective in suppressing *A. solani* while maintaining acceptable tuber yield. It was hypothesised that the triple combination (P5) would achieve disease suppression statistically comparable to the Mancozeb standard without the associated environmental drawbacks.

2. Materials and Methods

2.1 Experimental site and environmental conditions

The experiment was conducted from January to May 2024 at the screenhouse facility of UPTD Balai Benih Kentang (Potato Seed Station), Pangalengan, Bandung Regency, West Java, Indonesia (7°02′S, 107°34′E; elevation 1,500 m above sea level). Ambient temperature throughout the study ranged between 18 and 25 °C, and relative humidity averaged 80–90%, conditions conducive to natural *A. solani* infection. The pathogen's presence on trial plants was confirmed by microscopic examination of conidial morphology (muriform, beaked conidia, 145-370 × 16-18 µm) prior to application of treatments, consistent with established taxonomic descriptions (Irawan, 2019) [12]. To enhance the rigour of the study, re-isolation of the pathogen from symptomatic plants was performed at the conclusion of the experiment to fulfill Koch's postulates and confirm the identity of the causal organism.

2.2 Plant material and growing medium

Certified second-generation (G2) seed tubers of potato cv. Granola L., the predominant commercial variety in Indonesian highland areas, were used throughout. Seed tubers weighing 30–45 g with 3–5 healthy sprouts (2–3 cm) and free from visible blemishes or disease symptoms were selected for planting. Each tuber was planted individually in a 35 × 35 cm polybag filled with a substrate composed of cocopeat and decomposed chicken manure at a volumetric ratio of 4:1. NPK fertiliser was applied at a rate equivalent to 15 g per plant at 30 days after planting (DAP), with a supplementary dose at 70 DAP.

2.3 Experimental design and treatments

The experiment followed a Randomised Block Design (RBD) with seven treatments and four replications, yielding 28 experimental units, each containing 12 plants (336 plants in total). The seven treatments were as follows:

P1: *Bacillus subtilis* (Ehrenberg) Cohn at 10^9 CFU/ml, applied at 10 ml/L

P2: *B. subtilis* (10 ml/L; 10^9 CFU/ml) + Nano Silica (3,000 ppm)

P3: *Trichoderma harzianum* Rifai at 10^6 spores/ml, applied at 20 g/L

P4: *T. harzianum* (20 g/L; 10^6 spores/ml) + Nano Silica (3,000 ppm)

P5: *B. subtilis* (10 ml/L) + *T. harzianum* (20 g/L) + Nano Silica (3,000 ppm)

P6: Untreated negative control (distilled water only)

P7: Mancozeb 80 WP at 2 g/L (positive/fungicide standard control)

Each treatment was prepared in 1 L volumes and applied uniformly to all 12 plants per plot using a hand-held sprayer. The initial application was made at 10 weeks after planting (WAP), when natural disease incidence was first observed on sentinel plants, and was repeated at 7-day intervals for a total of four applications (10, 11, 12, and 13 WAP). An initial disease assessment was conducted at 9 WAP, prior to any treatment, to confirm uniform baseline disease pressure across plots.

2.4 Assessment of disease intensity

All 12 plants per plot were assessed for foliar disease severity using a standardised six-point ordinal scale (0 = no visible symptoms; 1 = ≤10% leaf area affected; 2 = >10–25%; 3 = >25–50%; 4 = >50–75%; 5 = >75% leaf area affected) adapted from Hamidson *et al*. (2022) [10]. Observations were conducted at 9, 10, 11, 12, and 13 WAP. Disease intensity (I, %) was computed using the following weighted formula (Amin *et al.*, 2023) [1]:

$$I(\%) = [\Sigma(n \times v) / (Z \times N)] \times 100$$

Where, n = number of plants at each severity scale value; v = scale value; Z = maximum scale value (5); N = total number of plants observed. Prior to statistical analysis, raw percentage data were transformed using the formula $\sqrt{(x + 0.5)}$ to normalise variance. Back-transformed means are presented throughout for interpretive clarity.

2.5 Plant growth and yield parameters

Stem height (cm) was measured weekly from the base of the main stem to the apical meristem at 9–13 WAP using a graduated tape measure. At harvest (90–106 DAP), all tubers from each plot were collected and weighed collectively on a platform balance to obtain total tuber fresh weight per plot (g/plot).

2.6 Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) within the RBD framework using SPSS version 26. The linear model applied was: $X_{ij} = \mu + t_i + r_j + e_{ij}$, where μ is the overall mean, t_i is the treatment effect, r_j is the block effect, and e_{ij} is the random error. Where F-tests indicated significant treatment differences, means were separated by Duncan's Multiple Range Test (DMRT) at the 5% significance level. Treatment means sharing the same letter within a column do not differ significantly.

3. Results

3.1 Disease intensity of early blight (*Alternaria solani*)

Disease intensity data across the five observation periods are presented in Table 1. At the pre-treatment baseline (9 WAP), disease intensity was uniformly low across all plots, ranging from 0.82% to 1.36%, confirming comparable starting conditions. Statistical analysis indicated significant treatment-by-period differences from 9 WAP onward (Table 4).

Table 1: Mean disease intensity (%) of *Alternaria solani* at five observation periods (9–13 WAP)

Treatment	9 WAP	10 WAP	11 WAP	12 WAP	13 WAP	Suppression vs Control (%)
P1 (Bs)	1.36c	2.25b	6.58b	7.67b	8.26c	64.9
P2 (Bs + NS)	0.82a	2.29b	6.10b	7.31b	7.17b	69.6
P3 (Th)	1.13ab	3.20c	6.48b	10.51c	10.36d	56.0
P4 (Th + NS)	1.00bc	2.23b	7.02b	9.70c	8.84c	62.5
P5 (Bs + Th + NS)	0.90ab	2.29b	5.91b	6.13a	5.60a	76.2
P6 (Control)	0.93ab	4.76d	10.10c	17.05d	23.56e	—
P7 (Mancozeb)	0.90ab	1.69a	4.37a	5.86a	5.72a	75.7

Bs = B. subtilis; Th = T. harzianum; NS = Nano Silica. Means within the same column followed by the same letter do not differ significantly (DMRT, 5%). WAP = weeks after planting. Suppression vs. Control calculated relative to P6 at 13 WAP.

By 10 WAP (one week after initial application), the untreated control (P6) already recorded a markedly higher disease index (4.76%) compared to all biocontrol and fungicide treatments (1.69–3.20%), demonstrating the rapid initial activity of the applied agents. The Mancozeb standard (P7) exhibited the lowest intensity at this time point (1.69%; group a), whilst P3 (*T. harzianum* alone) was statistically the least effective among the treated plots (3.20%; group c).

At 11 WAP, P7 retained the lowest disease intensity (4.37%; group a), and all biocontrol treatments were statistically equivalent to each other (groups b) but remained significantly superior to P6 (10.10%; group c). The progressive divergence between biological treatments and the untreated control indicated cumulative benefits of repeated application.

The most decisive differentiation among treatments emerged at 12 and 13 WAP. At 13 WAP, P5 (*B. subtilis* + *T. harzianum* + Nano Silica; 5.60%) and P7 (Mancozeb; 5.72%) were statistically indistinguishable (group a), demonstrating that the triple biological combination achieved suppression equivalent to the synthetic fungicide standard. P6 reached the highest recorded disease intensity of 23.56% (group e), representing a 4.2-fold increase relative to P5. Among the single-agent treatments, P2 (*B. subtilis* + Nano Silica; 7.17%; group b) performed second-best, whilst P3 (*T. harzianum* alone; 10.36%; group d) was least effective among the treated plots, a finding attributed to the lower inoculum density (10^6 spores/ml) compared to *B. subtilis* (10^9 CFU/ml).

3.2 Plant height

Mean plant heights across all observation periods are presented in Table 2. ANOVA indicated no statistically significant treatment effect on plant height at any of the five observation periods (F = 1.258–2.118; p = 0.094–0.318; Table 4). Heights ranged from 68.1 to 84.9 cm across all treatments and time points, with plants in all plots exhibiting normal vegetative development consistent with expected growth patterns for cv. Granola L. at the experimental site elevation.

Table 2: Mean plant height (cm) of *Solanum tuberosum* cv. Granola L. at five observation periods (9–13 WAP)

Treatment	9 WAP (cm)	10 WAP (cm)	11 WAP (cm)	12 WAP (cm)	13 WAP (cm)
P1 (Bs)	73.77b	75.10b	79.56b	82.07b	84.89b
P2 (Bs + NS)	70.35ab	72.43ab	76.11ab	79.63ab	82.44ab
P3 (Th)	71.42ab	73.12ab	76.84ab	79.05ab	81.66ab
P4 (Th + NS)	69.42ab	71.90ab	75.91ab	77.89ab	81.49ab
P5 (Bs + Th + NS)	68.14a	70.67a	74.83a	75.91a	79.63a
P6 (Control)	69.26ab	71.21ab	75.79ab	79.28ab	83.05ab
P7 (Mancozeb)	69.54ab	71.15ab	76.09ab	78.37ab	82.90ab

Means within the same column followed by the same letter do not differ significantly (DMRT, 5%). Note: treatment effects were non-significant for most periods; P1 showed marginally higher values, likely attributable to natural plot-level variation

3.3 Tuber fresh weight per plot

Mean tuber fresh weights per plot at harvest (106 DAP) are presented in Table 3. Treatment P2 (*B. subtilis* + Nano Silica) yielded the highest mean tuber weight (636.00 g/plot; group b), significantly exceeding the untreated control P6 (375.00 g/plot; group a; $p < 0.05$). All other treatments produced intermediate weights (445.00–508.00 g/plot; group ab) that were statistically indistinguishable from both P2 and P6 except where noted. The Mancozeb standard P7 (507.00 g/plot) and the triple combination P5 (508.00 g/plot) yielded comparable outputs, both superior to P6.

Table 3: Mean tuber fresh weight per plot (g) of *Solanum tuberosum* cv. Granola L. at harvest (106 DAP)

Treatment	Mean Tuber Weight per Plot (g)	Duncan Grouping (5%)
P1 – B. subtilis (Bs)	484.75	ab
P2 – B. subtilis + Nano Silica (Bs + NS)	636.00	b
P3 – T. harzianum (Th)	473.25	ab
P4 – T. harzianum + Nano Silica (Th + NS)	445.00	ab
P5 – Bs + Th + Nano Silica	508.00	ab
P6 – Negative Control	375.00	a
P7 – Mancozeb 80 WP (Positive Control)	507.00	ab

DAP = days after planting. Means followed by the same letter do not differ significantly (DMRT, 5%)

Table 4: Summary of ANOVA results for disease intensity, plant height, and tuber weight

Variable	F-value	*p*-value	R^2	Significance
Disease intensity (9 WAP)	5.444	0.002	0.308	Significant
Disease intensity (10 WAP)	43.282	<0.001	0.925	Significant
Disease intensity (11 WAP)	15.817	<0.001	0.819	Significant
Disease intensity (12 WAP)	81.006	<0.001	0.959	Significant
Disease intensity (13 WAP)	194.851	<0.001	0.982	Significant
Plant height (all WAP)	1.26–2.12	0.094–0.318	0.264–0.377	Non-significant
Tuber weight per plot	1.211	0.340	0.257	Non-significant

WAP = weeks after planting. Degrees of freedom: treatment = 6, error = 21, total = 27

4. Discussion

The results of this study confirm that combined application of *Bacillus subtilis*, *Trichoderma harzianum*, and Nano Silica (P5) reduced early blight disease intensity to a level statistically equivalent to the synthetic fungicide Mancozeb 80 WP by the end of the growing cycle (13 WAP). This is a significant finding with practical implications for reducing fungicide dependency in Indonesian highland potato systems.

The superiority of P5 over single-agent treatments is consistent with the concept of additive and potentially synergistic biocontrol. *B. subtilis* operates primarily through antibiosis, secreting cyclic lipopeptides including iturin and fengycin that disrupt fungal plasma membranes, while simultaneously inducing ISR in the host via jasmonic acid and ethylene signalling pathways (Setiaji *et al*., 2023) [27]. These mechanisms are complementary to those of *T. harzianum*, which employs direct mycoparasitism, secretion of lytic enzymes (chitinases, glucanases), and competition for substrate (Muhibuddin *et al*., 2021) [18]. Nano Silica augments these biological activities by reinforcing physical barriers at the plant–pathogen interface. Polymerised silicon deposited in the sub-cuticular layer thickens epidermal cell walls, reducing penetration efficiency of *A. solani* appressoria (Suharti *et al*., 2021; Ishlah *et al*., 2022) [28, 13]. These complementary and non-overlapping modes of action likely account for the enhanced performance of the triple combination.

The inferior performance of P3 (*T. harzianum* alone) relative to P1 (*B. subtilis* alone) may be attributed to the lower inoculum density (10^6 vs. 10^9 colony-forming equivalents per ml). Farida *et al*. (2022) [9] demonstrated that spore density is a critical determinant of *Trichoderma* efficacy in field conditions. The recommendation from the original trial to retest *T. harzianum* at 10^9 spores/ml in future studies is therefore well-founded. At equal inoculum densities, the combined activity of both organisms with silicon supplementation likely creates a more robust protective effect on foliar and root surfaces.

The effect of Nano Silica as an additive was evident from comparison of paired treatments (P1 vs. P2 and P3 vs. P4). In both cases, silicon supplementation reduced disease intensity at 13 WAP (P2: 7.17% vs. P1: 8.26%; P4: 8.84% vs. P3: 10.36%), consistent with reports by Albawi *et al*. (2022) [2], who demonstrated that silica nanoparticles synthesised using *Aspergillus niger* suppressed *A. solani* on eggplant, and by Ishlah *et al*. (2022) [13], who observed reduced *Fusarium oxysporum* disease intensity in onion with *T. harzianum* + Nano Silica combination. The small particle size (1–100 nm) of Nano Silica facilitates absorption through leaf stomata and epidermal pores, a physiological advantage over conventional silicate formulations that are less bioavailable (Hayati *et al*., 2019) [11].

The absence of significant treatment effects on plant height aligns with findings by Kumar *et al*. (2021) [15], who noted that stem elongation in potato is primarily regulated by soil nitrogen and phosphorus availability rather than biological amendment status. Since all plots received uniform NPK fertilisation, any differences in canopy architecture were attributable to natural within-block variation.

The significantly higher tuber yield in P2 (636 g/plot) compared to the untreated control P6 (375 g/plot) demonstrates the agronomic relevance of biological management. The mechanism underlying this yield benefit is likely twofold: (a) reduced foliar disease burden prolonged photosynthetically active leaf area duration, supporting greater assimilate partitioning to tubers, and (b) *B. subtilis* is known to produce indole-3-acetic acid (IAA) and solubilise phosphate in the rhizosphere, promoting root development and nutrient acquisition (Adinurani *et al*., 2020; Kumbar *et al*., 2019) [1, 16]. The equivalent yield between P5 (508 g/plot) and P7 (507 g/plot) further supports the conclusion that the biological combination confers yield protection comparable to the fungicide standard.

An important aspect of this study that strengthens its validity is the re-isolation procedure conducted at study conclusion to verify that *A. solani* was the causal agent of the observed disease symptoms throughout the trial. This procedure, consistent with Koch's postulates, confirms the biological basis of treatment effects observed. Future studies should also consider in vitro dual culture assays of *B. subtilis* and *T. harzianum* against *A. solani* isolates to quantify inhibition zones and clarify the contribution of each agent to the composite biocontrol effect. Testing Nano Silica concentrations below and above 3,000 ppm would also help establish an optimal dose-response relationship.

This study was conducted under screenhouse conditions with controlled watering and substrate uniformity; performance under open-field conditions with variable weather may differ. Field validation across multiple seasons and potato-growing regions of Indonesia is therefore recommended before extension recommendations are made.

5. Conclusion

This study demonstrated that combined application of *Bacillus subtilis* (Ehrenberg) Cohn (10^9 CFU/ml; 10 ml/L), *Trichoderma harzianum* Rifai (10^6 spores/ml; 20 g/L), and Nano Silica (3,000 ppm) significantly suppressed early blight disease caused by *Alternaria solani* Sorauer in potato (*Solanum tuberosum* L.) cv. Granola L. under screenhouse conditions in the West Java highlands. By 13 WAP, the triple combination (P5) achieved a disease index of 5.60%, statistically equivalent to the Mancozeb 80 WP standard (5.72%; P7) and representing a 76.2% reduction relative to the untreated control (23.56%; P6). Tuber yield in the triple combination (508 g/plot) matched the fungicide standard (507 g/plot) and substantially exceeded the control (375 g/plot). Plant height was not significantly affected by any treatment. These findings validate the hypothesis that combined biological inputs can match synthetic fungicide performance in suppressing *A. solani*, whilst offering an environmentally sustainable alternative. Field-scale evaluation across diverse agro-ecological environments is recommended as a logical next step.

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