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Field Efficacy of *Bacillus subtilis* and *Pseudomonas fluorescens* against *Rhizoctonia solani* in Pertiwi Exotic F1 Sweet Corn

Abdul Harist[1], Deden[2*]

[1]Agrotechnology Program, Faculty of Agriculture, Swadaya Gunung Jati University, Cirebon, Jl. Pemuda Raya No. 32, Kesambi District, Cirebon City, West Java, **Indonesia**
[2]Faculty of Agriculture, Swadaya Gunung Jati University, Cirebon, Jl. Pemuda Raya No. 32, Kesambi District, Cirebon City, West Java, **Indonesia**

[*]*Coresponding author:* deden.ugj@gmail.com

ABSTRACT

Rhizoctonia solani is a soil-borne pathogen associated with sheath blight in sweet corn. This field study evaluated Bacillus subtilis and Pseudomonas fluorescens as biological control agents and compared two application concentrations. The experiment was conducted in Katileng Hamlet, Luwung Village, Mundu District, Cirebon Regency, using a randomized complete block design with five treatments and five blocks. The treatments comprised an untreated control, B. subtilis at 25 and 50 mL L−1, and P. fluorescens at 25 and 50 mL L−1. Biological-agent application significantly influenced disease incidence, damage severity, and cob weight with and without husks. The 50 mL L−1 B. subtilis treatment produced 51% calculated efficacy at the final efficacy assessment and the highest reported cob weight without husk (284.40 g plant−1). Nevertheless, the assessment dates reported for disease variables and efficacy are inconsistent and must be checked against the original field records before submission. Claims concerning vegetative growth should also be retained only if the relevant methods and results are supplied.

Keywords: Bacillus subtilis, biological control, Pseudomonas fluorescens, Rhizoctonia solani, sweet corn

INTRODUCTION

Sweet corn (Zea mays L. saccharata) is an economically important horticultural crop in Indonesia, and consumer demand has continued to rise (Deden *et al*., 2023; Sari *et al*., 2016). When domestic output does not keep pace with demand, dependence on imports may increase. Statistics cited in this study indicate that national production declined from 119,206.00 tons in 2019 to 111,903.00 tons in 2020 and 58,661.55 tons in 2021 (Central Statistics Agency, 2022). Production is constrained by several factors, including crop susceptibility to pests and diseases that reduce both yield and marketable quality.

Sheath blight caused by the soil-borne fungus Rhizoctonia solani Kühn is an important disease of corn and may cause substantial losses in susceptible cultivars

(Soenartiningsih *et al*., 2015). Infection can occur at several growth stages. Initial symptoms commonly appear as gray, reddish, or brown lesions on the leaf sheath and may extend upward toward leaves and cobs (Juliana *et al*., 2024). The pathogen persists in soil as sclerotia and develops readily under humid conditions. Management in farmers' fields still frequently depends on synthetic fungicides, although prolonged and inappropriate use can contribute to environmental contamination, chemical residues, and selection pressure for less-sensitive pathogen populations (Setiaji *et al*., 2023). These concerns support the development of biologically based alternatives.

Plant growth-promoting rhizobacteria provide one potential approach because some strains can both antagonize pathogens and support plant growth. Bacillus subtilis and Pseudomonas fluorescens are among the most widely studied candidates. B. subtilis has suppressed R. solani in corn, although successful establishment in the rhizosphere may depend on environmental conditions and biofilm formation (Hashem *et al*., 2019; Sholeha & Masnilah, 2022). P. fluorescens can inhibit several soil-borne pathogens, including Fusarium species, but its non-spore-forming cells may lose viability during extended storage (Bejarano *et al*., 2018; Chrisnawati *et al*., 2017). Their contrasting biological characteristics justify comparison under local field conditions.

Accordingly, this study compared two concentrations of B. subtilis and P. fluorescens for reducing R. solani disease on Pertiwi Exotic F1 sweet corn and examined their association with cob yield.

MATERIALS AND METHODS

Study Site and Period

The trial was conducted from February to May 2026 in Katileng Hamlet, Luwung Village, Mundu District, Cirebon Regency. The site is approximately 4 m above sea level, with reported temperatures of 27–33 °C, and has a history of disease associated with R. solani.

Materials and Equipment

Field equipment included hoes, stationery, plot labels, a 1-L sprayer, scales, calipers, measuring tapes, a camera, watering cans, hoses, and raffia. The principal materials were Pertiwi Exotic F1 sweet corn seed, NPK fertilizer, and ready-to-use liquid formulations containing commercial isolates of *Bacillus subtilis* and *Pseudomonas fluorescens*. The biological agents were obtained from Simpang Lima Farm (Indonesia). Prior to application, the products were stored at room temperature in a dark, dry, and cool cabinet to maintain cell viability. The viable-cell concentration for the products was 1×10^8 CFU/mL. The microbial suspensions were prepared at concentrations of 25 and 50 mL L^{-1} and applied at a spray volume of 500 L ha^{-1}. Applications were scheduled at 7, 14, and 21 days after planting (DAP).

Experimental Design

The experiment followed a randomized complete block design with five treatments and five blocks, resulting in 25 plots. To account for potential field variability, the blocking factor was established based on the presumed soil fertility and moisture gradient of the land. The experimental unit was a single plot measuring 4 m × 1 m, containing a population of 20 sweet corn plants. Seeds were sown with a plant spacing of 40 cm × 30 cm. To prevent cross-contamination from spray drift or surface runoff, buffer zones of 50 cm were maintained between adjacent plots and between blocks. Treatments were randomly assigned to the plots within each block using a manual lottery method.

The treatments were:
K0 = Control (without application of biological agents)
B1 = *Bacillus subtilis* at 25 mL L−1
B2 = *Bacillus subtilis* at 50 mL L−1
P1 = *Pseudomonas fluorescens* at 25 mL L−1
P2 = *Pseudomonas fluorescens* at 50 mL L−1

Measured Variables

Disease incidence was assessed visually on 20 plants in each plot, giving 500 plant observations across the experiment. Plants were examined for water-soaked lesions on leaf sheaths, expansion into gray or brown banded lesions, and the presence of brown-to-black sclerotia on infected tissue. The authors should clarify whether the same plants were evaluated at every date and whether observers were blinded to treatment.

Disease incidence (IS) was calculated using the equation attributed to Agrios (2005):

$$IS = \frac{a}{a+b} \times 100\%$$

Where:
IS: disease incidence (%)
a: Number of plants attacked
b: Number of plants that are not attacked

Damage severity was estimated from visual ratings of sheath-blight symptoms using a 0 to 4 scale based on the percentage of the affected area (Agrios, 2005). The five score categories used to make the assessment reproducible were defined as follows.

0	**:**	**No symptoms of attack (Healthy Plant).**
1	**:**	**Very mild infestation. Spots only on the lower leaf sheaths, with an infestation area of <25%.**
2	:	Mild attack. Spots spread to the leaf sheaths above, with an attack area of 25% - 50%.
3	:	Moderate attack. Spots affect the middle leaves and petioles, with an attack area of 50% - 75%.
4	:	Severe attack. Spots reach the upper leaves, attack area > 75%.

Damage severity (IK) was calculated with the Townsend and Heuberger (1943) equation:

$$IK = \frac{\sum(n \times v)}{N \times Z} \times 100\%$$

Where:
IK: damage severity (%)
n: Number of plants or plant parts with a certain score value
v: Score value (attack category)
N: Total number of plants observed
Z: The highest score on the scale

The efficacy of each microbial treatment relative to the untreated control was estimated using the equation attributed to Abbott (1925):

$$E = \frac{Is\ Kontrol - Is\ Perlakuan}{Is\ Kontrol} \text{X } 100\%$$

Information:
E: efficacy of the biological-agent treatment (%)
IS: mean disease incidence in the relevant treatment

Data Analysis

Data were analyzed using a randomized complete block design model that included both treatment and block as fixed factors. To address repeated observations on the same plots, the data for 35, 42, and 49 DAP were analyzed separately for each time point. Percentage data for disease incidence and damage severity were analyzed in their original scale without transformation. The assumption of homogeneity of variances was evaluated prior to the analysis. Analysis of variance (ANOVA) was performed using IBM SPSS Statistics version 26.0. When treatment effects were significant, means were separated using Duncan's Multiple Range Test at $\alpha = 0.05$. Exact p-values and data variability (standard errors) are reported alongside the treatment means in the results.

RESULTS AND DISCUSSION

Disease Incidence (%)

Disease incidence was assessed at 35, 42, and 49 days after planting (DAP). Analysis of variance indicated a significant treatment effect at each assessment (Table 1). Because the same plots appear to have been observed repeatedly, the analysis should account for within-plot correlation over time.

At 35 DAP, the untreated control had the highest disease incidence (37.00%). Both B. subtilis treatments recorded 13.00%, which was lower than the control and the two P. fluorescens treatments according to the reported mean separation.

Disease incidence increased as the crop developed. At 49 DAP, the untreated control reached 53.00% and did not differ statistically from P. fluorescens at 25 mL L−1 (45.00%), but it exceeded the remaining treatments. A denser canopy near the generative stage may restrict air movement and increase humidity around the lower sheaths, conditions that favor sclerotial germination and mycelial spread (Budiarti & Bintang, 2022). At the same assessment, B. subtilis and P. fluorescens at 50 mL L−1 produced incidences of 26.00% and 31.00%, respectively, and were not significantly different from one another. Their lower disease levels may reflect antagonistic activity against pathogen growth or infection processes (Mugiastuti *et al*., 2022).

Damage Intensity (%)

Damage severity was recorded at 35, 42, and 49 DAP to follow the progression of tissue injury. Treatment significantly affected the reported severity at all three dates (Table 2).

At 35 DAP, the control showed the greatest damage severity (9.25%). The two B. subtilis treatments each recorded 3.25% and formed the lowest statistical group at this assessment.

Severity increased between 35 and 49 DAP, consistent with progressive disease under the more humid microclimate created by a closing canopy (Budiarti & Bintang, 2022). At 49 DAP, the control reached 15.25%. The lowest reported values occurred with P. fluorescens at 50 mL L−1 (8.00%) and B. subtilis at 50 mL L−1 (7.75%), both of which differed from the control. Antagonistic bacteria may restrict R. solani through competition for nutrients and colonization sites, antibiosis, or other interactions that inhibit mycelial growth (Mugiastuti *et al*., 2022). These mechanisms were not measured directly in this experiment and should therefore be discussed as plausible explanations rather than demonstrated causes.

Cob Weight with Husk per Plant and per Plot (g)

Analysis of variance detected a treatment effect on cob weight with husk at both the plant and plot levels. Treatment means are presented in Table 3.

The untreated control produced the lowest reported cob weight with husk, 292.52

g plant−1 and 1,462.60 g plot−1. These means differed from those obtained with B. subtilis and P. fluorescens at 50 mL L−1, but not from the corresponding 25 mL L−1 treatments.

Lower cob weight in the control coincided with greater disease incidence and damage severity. Infection of sheath and associated tissues may impair plant function and reduce the supply of assimilates available for cob filling (Juliana *et al*., 2024). In comparison, P. fluorescens and B. subtilis at 50 mL L−1 produced 391.12 and 390.76 g plant−1, equivalent to 1,955.60 and 1,953.80 g plot−1. The association between disease suppression and yield is consistent with the results, although vascular disruption and assimilate movement were not measured directly.

In addition to pathogen antagonism, some strains of B. subtilis and P. fluorescens function as plant growth-promoting rhizobacteria. Reported mechanisms include phytohormone production, phosphate solubilization, and siderophore production, which may improve root development and nutrient acquisition (Setiaji *et al*., 2023). Such mechanisms could have contributed to the higher cob weights observed in treated plots, but they were not directly quantified in this trial (Vicky *et al*., 2019).

Table 1. Disease Incidence (%)

No	Treatment	Attack Intensity (%)		
		35 DAP	42 DAP	49 DAP
1	K0 control	37.00 ± 1.29 a	51.00 ± 3.37 a	53.00 ± 4.45 a
2	P1 *P. fluorescens at 25 mL L−1*	23.00 ± 1.29 c	40.00 ± 3.37 b	45.00 ± 4.45 ab
3	P2 *P . fluorescens at 50 mL L−1*	27.00 ± 1.29 b	27.00 ± 3.37 c	31.00 ± 4.45 c
4	B1 *B. subtilis at 25 mL L−1*	13.00 ± 1.29 d	30.00 ± 3.37 bc	39.00 ± 4.45 bc
5	B2 *B. subtilis at 50 mL L−1*	13.00 ± 1.29 d	26.00 ± 3.37 c	26.00 ± 4.45 c

Note: Data are presented as mean ± standard error. Means followed by the same letter within a column are not significantly different according to Duncan's Multiple Range Test at α = 0.05. Exact p-values for the treatment effects at 35, 42, and 49 DAP were $p < 0.001$, $p < 0.001$, and $p = 0.004$, respectively.

Table 2. Effect of Biological Agents on R. solani Damage Severity in Sweet Corn (%)

No	Treatment	Damage Intensity (%)		
		35 DAP	42 DAP	49 DAP
1	K0 control	9.25 ± 0.32 a	13.75 ± 0.53 a	15.25 ± 1.13 a
2	P1 *P. fluorescens at 25 mL L−1*	5.75 ± 0.32 c	12.25 ± 0.53 a	13.50 ± 1.13 a
3	P2 *P . fluorescens at 50 mL L−1*	6.75 ± 0.32 b	7.25 ± 0.53 b	8.00 ± 1.13 b
4	B1 *B. subtilis at 25 mL L−1*	3.25 ± 0.32 d	7.75 ± 0.53 b	11.75 ± 1.13 a
5	B2 *B. subtilis at 50 mL L−1*	3.25 ± 0.32 d	6.50 ± 0.53 b	7.75 ± 1.13 b

Note: Data are presented as mean ± standard error. Means followed by the same letter within a column are not significantly different according to Duncan's Multiple Range Test at α = 0.05. Exact p-values for the treatment effects at 35, 42, and 49 DAP were $p < 0.001$, $p < 0.001$, and $p = 0.002$, respectively.

Table 3. Effect of Biological-Agent Treatment on Cob Weight with Husk (g)

NO	Treatment	Weight of Cob with Husk (g)	
		per plant	per plot
1	K0 control	292.52 ± 0.54 a	1462.60 ± 2.70 a
2	P1 *P. fluorescens at 25 mL L−1*	298.20 ± 0.54 b	1491.00 ± 2.70 b
3	P2 *P . fluorescens at 50 mL L−1*	391.12 ± 0.54 c	1955.60 ± 2.70 c
4	B1 *B. subtilis at 25 mL L−1*	297.40 ± 0.54 b	1487.00 ± 2.70 b
5	B2 *B. subtilis at 50 mL L−1*	390.76 ± 0.54 c	1953.80 ± 2.70 c

Note: Data are presented as mean ± standard error. Means followed by the same letter within a column are not significantly different according to Duncan's Multiple Range Test at $\alpha = 0.05$. The exact p-value for the treatment effect was $p < 0.001$ for both per plant and per plot observations.

Cob Weight without Husk per Plant and per Plot (g)

Treatment significantly affected cob weight without husk at both the plant and plot levels. The means are reported in Table 4.

The control had the lowest cob weight without husk, at 211.12 g plant−1 and 1,055.60 g plot−1. According to the reported Duncan test, these values were lower than those of every biological-agent treatment.

At the final disease assessment, the low yield of the control coincided with disease incidence of 53.00% and severity of 15.25%. Greater injury to the sheath and foliage may restrict water and nutrient transport or reduce photosynthetic capacity, thereby limiting dry-matter allocation to the cob (Juliana *et al*., 2024). These physiological processes were inferred from previous research and were not measured in the present experiment.

The 50 mL L−1 treatments produced the largest cob weights without husk. B. subtilis yielded 284.40 g plant−1 and 1,422.00 g plot−1, whereas P. fluorescens yielded 284.16 g plant−1 and 1,420.80 g plot−1. Both exceeded the corresponding 25 mL L−1 treatments and the untreated control according to the reported mean comparisons.

The yield response at the higher concentrations may reflect the combined effects of pathogen suppression and rhizosphere activity. B. subtilis and P. fluorescens can compete with pathogens, produce antimicrobial metabolites, and contribute to induced resistance or nutrient acquisition. Siderophore production may also alter iron availability in the rhizosphere (Apastambh, 2025). Because these processes were not measured, the results demonstrate field performance rather than the underlying mechanism.

Healthier roots and less-damaged foliage may support water uptake, nutrient acquisition, photosynthesis, and assimilate allocation during grain filling. This interpretation is consistent with the higher cob weights in the biological-agent treatments, but direct physiological measurements would be needed to confirm it (Vicky *et al*., 2019).

Effectiveness of Biological Agents (%)

Treatment efficacy was calculated to describe the reduction in disease incidence relative to the untreated control. The manuscript adopts a value above 50% as the criterion for effective performance, in accordance with the standard biological efficacy testing guidelines for biopesticides (Komisi Pestisida, 2006). Calculated efficacy values are summarized in Table 5.

At 35 DAP, both B. subtilis concentrations produced a calculated efficacy of 65%, exceeding the stated 50% criterion. The comparatively strong early response may be associated with antimicrobial lipopeptides such as surfactin, iturin, and fengycin, which

are reported for B. subtilis and can inhibit fungal membranes or growth (Setiaji *et al*., 2023). These compounds were not assayed in the present study.

At 42 DAP, the efficacy of all treatments declined below the 50% threshold, with *B. subtilis* at 50 mL L^{-1} and *P. fluorescens* at 50 mL L^{-1} recording the highest values at 49.00% and 47.00%, respectively. Although falling slightly below the criterion, the relative performance of *P. fluorescens* may still be associated with its ability to colonize roots and compete for space and nutrients, or produce metabolites and siderophores that limit pathogen growth (Apastambh, 2025). However, as the experiment did not quantify colonization or metabolite production, these processes remain explanatory hypotheses.

At 49 DAP, B. subtilis at 50 mL L−1 retained an efficacy of 51%, while the remaining treatments were below 50%. Endospore formation can improve the environmental persistence of Bacillus species, whereas non-spore-forming P. fluorescens may be more sensitive to declining viability (Bejarano *et al*., 2018; Miljaković *et al*., 2020). However, microbial survival was not measured in this study. More importantly, the efficacy dates (35, 42, and 49 DAP) conflict with the disease-assessment dates (35, 42, and 49 DAP); the values and dates must be verified against the raw records before the treatment is recommended.

Table 4. Effect of Biological-Agent Treatment on Cob Weight without Husk (g)

NO	Treatment	Weight of Corn Without Husk (g)	
		per plant	per plot
1	K0 control	211.12 ± 1.11 a	1055.60 ± 5.53 a
2	P1 *P. fluorescens at 25 mL L−1*	216.44 ± 1.11 b	1082.20 ± 5.53 b
3	P2 *P. fluorescens at 50 mL L−1*	284.16 ± 1.11 c	1420.80 ± 5.53 c
4	B1 *B. subtilis at 25 mL L−1*	215.88 ± 1.11 b	1079.40 ± 5.53 b
5	B2 *B. subtilis at 50 mL L−1*	284.40 ± 1.11 c	1422.00 ± 5.53 c

Note: Data are presented as mean ± standard error. Means followed by the same letter within a column are not significantly different according to Duncan's Multiple Range Test at $\alpha = 0.05$. The exact p-value for the treatment effect was $p < 0.001$ for both per plant and per plot observations.

Table 5. Calculated Efficacy of Biological Agents against R. solani (%)

No	Treatment	Effectiveness of Biological Agents (%)		
		35 DAP	42 DAP	49 DAP
1	P1 *P. fluorescens at 25 mL L−1*	38.00	22.00	15.00
2	P2 *P . fluorescens at 50 mL L−1*	27.00	47.00	42.00
3	B1 *B. subtilis at 25 mL L−1*	65.00	41.00	26.00
4	B2 *B. subtilis at 50 mL L−1*	65.00	49.00	51.00

CONCLUSION

Application of B. subtilis and P. fluorescens influenced R. solani disease incidence, damage severity, and cob weight in Pertiwi Exotic F1 sweet corn. At the final efficacy assessment, B. subtilis at 50 mL L−1 produced a calculated efficacy of 51% and the highest cob weight without husk (284.40 g plant−1). P. fluorescens at 50 mL L−1

produced 42% efficacy and 284.16 g plant−1. The reported vegetative-growth conclusion should be included only if plant height and leaf-number methods and results are presented. The conflicting observation schedules must be resolved from the original records before journal submission.

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