Vol. 15, No. 2 (2026): 821 - 831
http://dx.doi.org/10.23960/jtepl.v15i2.821-831

Agricultural Engineering
UNIVERSITY OF LAMPUNG

JURNAL TEKNIK PERTANIAN LAMPUNG

ISSN 2302-559X (print) / 2549-0818 (online)
Journal homepage : https://jurnal.fp.unila.ac.id/index.php/JTP

Effect of *Serratia* sp. Application Rate on Frost Damage, Growth, and Yield of Potato (*Solanum tuberosum* L.) Under Field Conditions

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Article History:

Received : 24 November 2025
Revised : 31 January 2026
Accepted : 4 February 2026

Keywords:

Dieng plateau,
Frost,
PGPR,
Potato,
Serratia sp..

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ABSTRACT

Frost damage poses a major constraint to potato (Solanum tuberosum L.) production in the Dieng Plateau, leading to significant yield loss. This study aimed to evaluate the effectiveness of Serratia sp. in mitigating the physical impacts of natural frost and improving plant growth and yield. The experiment was arranged in a Randomized Complete Block Design (RCBD) with four treatments of bacterial suspension application volume (density of 1×10^7 *CFU/ml): control (0 ml/plant), 20 ml/plant, 40 ml/plant, and 60 ml/plant. A natural frost event occurred at a minimum temperature of -2 °C, causing minor damage. The results showed that the application of 60 ml/plant provided the best protection, with the lowest incidence of damage (<0.5%). The 40 and 60 ml/plant treatments consistently showed the highest plant height and leaf area, indicating accelerated vegetative recovery post-stress. In terms of yield, the 60 ml/plant treatment produced the highest tuber weight per plant (1,363 g). Further analysis confirmed that application volumes of 40 and 60 ml/plant significantly increased tuber size compared to the control. It was concluded that the application of Serratia* sp. *at a volume of 60 ml/plant was the best treatment in this study for stabilizing plant growth and maintaining potato productivity under low-temperature stress conditions in highland environments.*

1. INTRODUCTION

The Dieng Plateau, situated in Banjarnegara and Wonosobo Regencies, is a pivotal region for potato cultivation in Central Java, encompassing a planting area of 17,212 hectares (BPS Jateng, 2020). Banjarnegara Regency accounts 36.88% of the province's total potato planted area, covering 6,347 hectares, followed by Wonosobo with 3,461 hectares (20.11%). However, potato productivity in this area faces significant challenges due to the frost phenomenon, locally referred as "*embun upas*", which is characterized by extreme subfreezing temperatures that induce the formation of ice crystals within plant tissues

The economic repercussions of frost on potato farming are significant. Arsyadah (2018) demonstrated that during frost events, approximately 61% of agricultural land fails to yield marketable potatoes, primarily due to the prevalence of small green tubers and a high incidence of rotting tubers resulting from premature plant death. The magnitude of loss is heavily contingent on the plant growth phase at the time of the frost event, with plants younger than 60 days being particularly vulnerable to severe damage (Stegner *et al.*, 2022). This phenomenon not only diminishes the yield quantity but also degrades tuber quality, thereby adversely affecting market value (Arsyadah, 2018).

Low temperatures can reduce plant chlorophyll content, inhibit plant growth and development, and induce a range of physical symptoms such as leaf curling, stem damage, and stunted growth (Chen *et al.*, 2024). This temperature stress significantly constrains plant morphology, and exposure to low temperatures (e.g., 10 °C) has been shown to markedly inhibit plant height and leaf area expansion compared to optimal conditions. This inhibition during the vegetative phase directly impacts the yield formation phase. Potato seedlings are known to exhibit slow growth at temperatures below 7 °C, and tuber development ceases entirely if temperatures fall below -2 °C. Furthermore, plants subjected to this stress often fail to initiate tuberization or produce only small, unmarketable tubers, ultimately leading to drastic yield reductions or even total crop failure (Li *et al.*, 2021).

Frost-induced damage is initiated by the formation of ice crystals within plant tissues, which compromises their structural integrity (Nabati *et al.*, 2023). Agronomically, this cellular disruption manifests as rapidly expanding necrosis or tissue death in the leaves. Consequently, the active photosynthetic green leaf area is significantly diminished, thereby impeding the supply of assimilates necessary for tuber formation and enlargement (Chen *et al.*, 2024).In the absence of sufficient plant vigor for recovery, physical damage results in a substantial reduction in crop yield.

Currently, local farmers' strategies to mitigate frost are predominantly limited to physical methods, such as covering plants with plastic or shade netting, which have not yet been demonstrated to be fully effective. Biological approaches utilizing plant growth-promoting rhizobacteria (PGPR) present a sustainable alternative for enhancing plant resilience against abiotic stresses. Bacteria of the genus *Serratia* sp. have been identified as potential PGPR with multifunctional capabilities, including phytohormone production, ACC deaminase activation, nitrogen fixation, phosphate solubilization, and increased antioxidant capacity (Kulkova *et al.*, 2024).

Bacteria of the genus *Serratia* sp. have been identified as potential PGPR capable of supporting potato cultivation. These bacteria can support potato cultivation by producing phytohormones and osmoprotectants that are crucial for maintaining cell turgor pressure under low environmental temperatures (Kour & Yadav, 2022). Furthermore, the role of *Serratia* sp. in nitrogen fixation and phosphate solubilization directly contributes to enhanced plant vigor and the development of a more extensive root system (Subramanian *et al.*, 2016). Robust root systems and adequate nutrient availability are essential for plants to sustain vegetative growth and survive under extreme environmental stresses, such as frost (El-Esawi *et al.*, 2020).

Although the potential of *Serratia* sp. as a biological agent to enhance plant resilience against abiotic stress has been widely reported, specific research regarding its effectiveness in mitigating the impact of frost on potatoes under field conditions is limited. Abdillah (2021) demonstrated that the application of *Serratia* sp. had a positive impact on soil parameters and plant growth; however, it did not specifically investigate resistance to low-temperature stress. However, most previous studies on *Serratia* sp. and other PGPR have been conducted under controlled or laboratory conditions, where environmental variability is minimal. The effectiveness of these biological agents under natural frost events in open field conditions remains poorly understood. In particular, the extent to which *Serratia* sp. can mitigate frost-induced damage and sustain potato productivity under real highland environments has not been sufficiently explored. Therefore, this study aimed to evaluate the effectiveness of *Serratia* sp. application in reducing frost damage and improving growth and yield of potato under natural field frost conditions in the Dieng Plateau.

2. MATERIALS AND METHODS

2.1. Time and Place of Research

The research was conducted on agricultural land in the Arjuna Temple Complex, Dieng Kulon Village, Batur District, Banjarnegara Regency, Dieng Plateau (altitude 2,000 masl; geographical coordinates 7°12'28"S 109°54'25"E; Figure 1) from May to August 2025. The propagation of *Serratia* sp. bacteria was carried out at the Microbiology Laboratory, Faculty of Agriculture and Business, Universitas Kristen Satya Wacana, Salatiga, in April 2025.

2.2. Materials and Tools

The research materials included *Serratia* sp. bacterial isolates with a density of 1×10^7 CFU (Colony Forming Unit)/ml, Tedjo MZ G1 variety potato seeds (Foundation Seed), manure, bacterial culture media (nutrient broth), sterile distilled water, and chemicals for analysis. The tools used included a digital thermometer, measuring tape, analytical balance (Sartorius BP 210 D), leaf area measurement tools (gravimetric method and Petiole Pro application), bacterial culture equipment (autoclave, incubator, laminar air flow cabinet), and standard potato cultivation equipment.

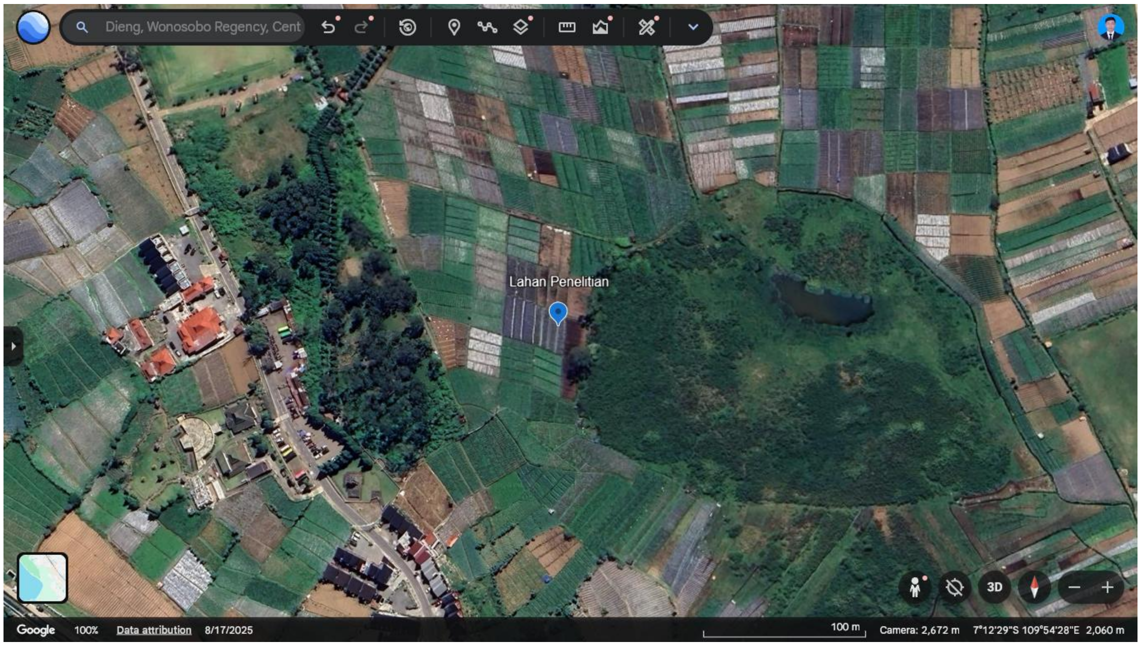

Figure 1. Location of the research site

2.3. Experimental Design

The study was arranged using a randomized complete block design (RCBD) with four treatments of *Serratia* sp. application rates and six replications. The treatments consisted of a control (0 ml/plant) and application volumes of 20 ml/plant, 40 ml/plant, and 60 ml/plant. Treatment allocation within each block was randomized to minimize the influence of field heterogeneity. Treatments consisted of different application volumes (0, 20, 40, and 60 ml/plant) of Serratia sp. suspension at a fixed concentration of 1×10^7 CFU/ml. Applications were performed on beds with a planting spacing of 50 cm × 70 cm. The experimental field was divided into 6 blocks (beds). Each block contained 4 experimental plots with exactly 1 plant per plot, accommodating the four treatment levels. Based on the planting spacing of 50 cm × 70 cm, the effective size of each single-plant plot was 0.35 m^2.

The land was cleared of weeds and previous crop residues, then tilled to a depth of 30 cm. Mature manure was applied at a rate of 20 ton/ha, equivalent to 300 g per planting hole, and mixed evenly with the soil one week prior to planting. MZ variety potato seed tubers, weighing 30–50 g per tuber, were planted at a depth of 10 cm.

Serratia sp. isolates were propagated in sterile nutrient broth medium and incubated at 28 °C for 24–48 hours with shaking at 150 rpm. Bacterial cell density was measured using a spectrophotometer at a wavelength of 600 nm and adjusted to a concentration of 1×10^7 CFU/ml prior to application. *Serratia* sp. application was performed via root zone drenching three times during the growth period. The first application was administered at 15 days after planting (DAP), the second at 28 DAP, and the third at 42 DAP. The bacterial suspension was applied according to the treatment volumes of 20 ml, 40 ml, and 60 ml per plant (at a fixed density of 10^7 CFU/ml).

2.4. Data Collection

Data collection included growth variables: (1) Plant height: measured using a ruler from the stem base at the soil surface to the highest growing point (apical shoot) of the main stem. Measurements were recorded at the maximum growth phase (104 DAP) to evaluate the vertical growth response to the treatments; (2) Leaf area: measured using the Petiole Pro application, validated by the gravimetric method, on the third fully expanded leaf from the main stem shoot. Leaf area was assessed at the maximum vegetative phase (55 DAP); (3) Incidence and intensity: Frost incidence and intensity were assessed visually on the entire plant canopy immediately after a frost event was detected in the morning, by calculating the percentage of leaf tissue damage based on a scoring scale; (4) Yield parameters: (tuber weight per plant, weight per tuber, number of tubers) were measured destructively at harvest (108 DAP); (5) Environmental temperature: monitored daily at 05:30 Western Indonesia Time (WIB) using a digital thermometer and sensors embedded near the experimental site.

2.5. Damage Level

Damage level due to frost case was evaluate using two parameters, namely damage incidence and damage intensity. Damage scores according to the damage levels and their symptoms for both parameters were explained in Table 1. The formula for damage incidence was adapted from the research of Wagiyanti *et al.* (2024):

$$IP = \left(\frac{\sum(n \times v)}{\sum N}\right) \times 100\% \qquad (1)$$

where *IP* is damage intensity (%), *n* is number of damaged leaves, *N* is total number of observed leaves, and *N* is the total number of observed leaves.

Meanwhile, the formula for frost damage intensity was adapted from the research of Sudika *et al.* (2010):

$$P = \frac{\sum(n_i \times v_i)}{(Z \times N)} \times 100\% \qquad (2)$$

where *P* is percentage of damage intensity, n_i is number of plants in each damage category, v_i is score of each damage category, *Z* is highest damage score value (= 4), and *N* is total number of observed plants.

Table 1. Scoring categories for frost damage incidence and damage intensity in potato plants

Damage Level	Score	Symptom for Damage Incidence	Symptom for Damage Intensity
Healthy	0	No damage observed	No necrotic symptoms on leaves.
Mild	1	Damage area 1–25%	Leaf tip necrosis and drying <25%.
Moderate	2	Damage area 25–50%	Yellowing leaves (chlorosis), necrosis 25–50%.
Severe	3	Damage area 50–75%	Wilting, leaves drying 50–75%.
Very Severe	4	Damage area 75–100% (Total Failure)	Total plant death, >75% drying.

Data Analysis

Observation data were analyzed using analysis of variance (ANOVA) and followed by the honestly significant difference (HSD) test at the 5% significance level if the treatment effect was significant. Prior to ANOVA, data were tested for normality and homogeneity of variance to meet statistical assumptions. Observation data were analyzed using analysis of variance (ANOVA) based on the Randomized Complete Block Design (RCBD) linear model:

$$Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}$$

where Y_{ij} is the observed response, μ is the overall mean, τ_i is the treatment effect, β_j is the block effect, and ε_{ij} is the random error. When the treatment effect was significant, the means were separated using Tukey's Honestly Significant Difference (HSD) test at the 5% significance level. All statistical analyses were performed using R Studio software.

3. RESULTS AND DISCUSSION

3.1. Environmental Conditions during the Study

As shown in Table 2, the environmental conditions observed during the study were characteristic of tropical highlands, with an average temperature of 8.25 °C (minimum 4.9 °C and maximum 11.6 °C), which is below the optimal temperature range for potato growth (15–20 °C). Frost events were documented on two occasions: on July 8 (0 °C) when plants were at 35 DAP, and the peak stress event on July 17 (-2 °C) at 44 DAP within the experimental site at the Arjuna Temple Complex.

The timing of these events was critical, occurring during the active vegetative growth phase (<60 DAP). Although the stress intensity was relatively mild (-2 °C) compared to potential extremes in Dieng (-4 °C to -6 °C), exposure during this sensitive provided sufficient selection pressure for testing purposes. High relative humidity, averaging 84.5% (range: 61–98%), fell within the optimal range for potato growth (80–90%) and supported the activity of soil microorganisms, including the applied strain *Serratia* sp. Meanwhile, very low rainfall averaging 6.7 mm (range: 0–11.2 mm) reflected dry season conditions, which was advantageous as it minimized pressure from diseases that typically thrive in wet conditions.

The sub-optimal temperatures encountered during the study constituted the primary limiting factor for plant growth. Yang *et al.* (2025) stated that low temperatures result in suboptimal agronomic growth and potato yields due to the disruption of basic physiological processes. The combination of low temperatures, high humidity, and low rainfall established ideal agroclimatic conditions for evaluating the effectiveness of *Serratia* sp. application in enhancing potato tolerance and productivity in the highlands, where plants require supplementary support to achieve optimal growth and productivity under abiotic stress conditions.

Table 2. Environmental conditions during the study

Date	**Plant Age (DAP)**	**Temperature (°C)**		**Rainfall (mm)**		**Humidity (%)**	
		Min	**Max**	**Min**	**Max**	**Min**	**Max**
20 June	17	6	15	0	6.8	78	95
26 June	23	6	12	0	5.1	80	97
2 July	29	9	12	0	4.7	73	95
8 July	35	0	10	0	6.0	62	92
14 July	41	9	12	0	4.2	72	94
17 July	44	-2	9	0	8.1	61	90
19 July	46	4	10	0	3.2	75	96
20 July	47	3	8	0	5.6	76	95
24 July	51	5	11	0	9.3	81	98
30 July	57	4	9	0	7.1	83	92
6 August	64	9	16	0	11.2	71	93
15 August	73	4	13	0	6.8	80	95
21 August	79	7	15	0	9.0	77	97
Average		**4.9**	**11.6**	**0**	**6.7**	**74.5**	**94.5**

Figure 2. Potato plant condition: (a) Healthy plant, (b) Frost-damaged plant

3.2. Effect of *Serratia* sp. on Plant Resistance

Figure 1 shows visual appearance of healthy and damaged potato plants due to frost. The resistance of potato plants toward frost attack is evaluate using damage intensity and damage incidence. Based on observation and calculation,

Table 3 presents the effect of application rate of *Seratia* sp. on the damage intensity and damage incidence of potato plants in the Dieng Plateau.

Table 3. Effect of *Serratia* sp. application rates on frost damage intensity and damage incidence in potato plants

Application rates *Serratia* sp. 10^7 CFU/ml	**Damage Intensity (%)**	**Damage Incidence (%)**
Control	0.49% ± 0.0272 a	1.99% ± 0.1180 a
20 ml/plant	0.41% ± 0.0360 ab	1.71% ± 0.1530 ab
40 ml/plant	0.28% ± 0.0226 c	1.18% ± 0.1310 b
60 ml/plant	0.19% ± 0.0318 d	0.44% ± 0.0625 c
Coefficient of Variation	9.78%	10.05%

3.2.1. Damage Intensity

The application of *Serratia* sp. had a significant effect on frost damage intensity. Treatment with 20 × 10^7/ml showed no significant difference compared to the control, with damage intensities of 0.41% and 0.49%, respectively. However, increasing the application rate to 40 ml/plant and 60 ml/plant resulted in a significant reduction in frost damage intensity. Specifically, the 40 ml/plant treatment resulted in an intensity of 0.28%, whereas the 60 ml/plant treatment yielded the lowest intensity at 0.19%.

Based on the damage scoring categories in Table 1, the damage intensity values ranged from 0.19% to 0.49%. These intensities were classified as very low (score < 1). This low level of damage is likely attributed to the recorded minimum frost temperature (-2 °C), which constitutes relatively mild stress compared to the potential extremes in the Dieng Plateau, which can reach -5 °C.

The reduction in damage intensity observed at higher application rates (40 ml/plant and 60 ml/plant) is likely closely associated with the role of *Serratia* sp. in modulating the plant's physiological responses. Although enzyme levels were not directly measured in this study, literature indicates that *Serratia* sp. application has the potential to activate endogenous antioxidant systems, such as superoxide dismutase (SOD) and peroxidase, which function to detoxify reactive oxygen species (ROS) generated by cold stress (Devi *et.al.*, 2016). Through this mechanism, cell membrane integrity can be better maintained, thereby minimizing tissue damage (necrosis) caused by the formation of ice crystals.

The effectiveness of *Serratia* sp. application was evident in the plants' ability to maintain high tuber weight and vigor. This is closely associated with the physiological adaptation of the bacteria (psychrotolerance), which ensures the continuity of nutrient uptake in the rhizosphere (Zhang *et al.*, 2009). The minimal physical damage observed on leaves further indicates the preservation of cell turgor pressure, which is a crucial factor for photosynthesis. This mechanism is suggested to be facilitated by the accumulation of osmoprotectants, which prevent tissue dehydration (Kang *et al.*, 2015). Additionally, the faster rate of vegetative growth recovery following stress exposure reflects a modulation of hormonal balance. This modulation re-stimulates cell division, thereby preventing growth stagnation (Saikia *et al.*, 2018).

3.2.2. Damage Incidence

Based on the damage incidence scoring categories in Table 1, both the highest incidence recorded in the control (1.99%) and the lowest incidence in the best treatment (0.44%) fell within the mild damage category (score 1, range >0 – 25%). The low percentage of affected plants is consistent with the mild frost stress conditions (-2 °C) observed in the field. Although damage appeared visually minimal across all plots, statistical analysis demonstrated that the application of *Serratia* sp. at a rate of 60 ml/plant was able to reduce the risk of damage incidence by 77.8% compared to the control. This indicates that the bacteria functioned as a preventive measure that may be associated with suppressing ice nucleation activity, resulting in fewer individual plants exhibiting initial damage symptoms.

Table 3 also indicates that the application of *Serratia* sp. had a significant effect on frost damage incidence. The application rate of 20 ml/plant showed no significant difference in frost damage incidence compared to the control, with incidence values of 1.71% and 1.99%, respectively. However, increasing the application rate from 40 ml/plant to

60 ml/plant significantly reducted frost damage incidence. Specifically, the 40 ml/plant treatment resulted in an incidence of 1.18% (a 40.5% reduction compared to the control), while the 60 ml/plant treatment yielded the lowest incidence at 0.44%.

Although the percentage of damage incidence was numerically low (<2%) across all treatments due to the relatively mild frost conditions (-2 °C), the significant differences observed between treatments carry important biological implications. In potato cultivation, preserving leaf canopy integrity, even to a minimal extent, is crucial for maintaining optimal photosynthetic rates. The reduction in damage risk observed in the 60 ml/plant treatment indicates that plants have a greater probability of surviving the critical phase, which ultimately contributes to improved tuber bulking efficiency.

3.3. Effect of *Serratia* sp. on Potato Plant Growth

Agronomic parameters to evaluate plant growth of potatoes during this research include plant height and plant leaf area. Table 4 provides effect of the application rate of *Serratia* sp. on plant height and leaf area of potato in the research location within Dieng Plateau.

Table 4. Effect of *Serratia* sp. application rates on the plant height and leaf area of potatoes

Application rates *Serratia* sp. 10^7 CFU/ml	Plant height (cm)	Leaf area (cm^2)
Control	58.33 ± 4.76a	25.70 ± 8.24c
20 ml/plant	55.17 ± 8.28a	27.50 ± 4.02bc
40 ml/plant	61.67 ± 2.66a	38.38 ± 6.90a
60 ml/plant	58.67 ±7.00a	32.98 ± 4.53ab
Coefficient of Variation	8.25%	16.04%

3.3.1. Plant Height

Based on the visualization in Table 4, an increasing trend in plant height was observed, corresponding to an increase in application rates. The 40 ml/plant treatment consistently resulted in taller plants. This increase in plant height indicates that *Serratia* sp. can facilitate vegetative growth recovery following exposure to frost stress.

It is noteworthy that plant height in this study was relatively consistent across treatments, likely due to the mild intensity of the frost stress (minimum temperature of -2 °C). Under more extreme frost conditions, the difference in plant height between the bacterial treatments and the control would likely be more pronounced, given the protective role of *Serratia* sp. in stabilizing the cell membranes and plant antioxidant systems (Kour & Yadav, 2022).

3.3.2. Leaf Area

The application of *Serratia* sp. demonstrated a significant effect on potato leaf area. As shown in Table 4, results from the HSD test at the 5% level indicate that the 20 ml/plant treatment (29.46 cm²) was not significantly different from the control (25.70 cm²). However, increasing the application rate to 40 ml/plant and 60 ml/plant resulted in a significant increase in leaf area.

The application of *Serratia* sp. had a notable impact on the leaf area of potatoes. As illustrated in Table 4, the results from the HSD test at the 5% significance level revealed that the 20 ml/plant treatment (29.46 cm²) did not differ significantly from the control (25.70 cm²). However, increasing the application rate to 40 ml/plant and 60 ml/plant led to a significant enhancement in leaf area.

The 40 ml/plant treatment achieved the largest leaf area (39.21 cm²), representing a 52.57% increase compared to the control, while the 60 ml/plant treatment resulted in a leaf area of 32.98 cm², or a 28.33% increase over the control. The 40 ml/plant and 60 ml/plant treatments were not significantly different from each other, suggesting that the 40 ml/plant rate was adequate for optimal leaf growth stimulation. The substantial increase in leaf area may be attributed to the environmental conditions during the study (average 8.25 °C), which remained within the tolerance limits that permit cell division and expansion processes to proceed. The recorded minimum temperatures (average 4.9 °C) did not reach the critical threshold that would entirely halt meristematic activity.

This significant increase in leaf area highlights the dual role of *Serratia* sp. Beyond its potential for stress mitigation, this bacterium primarily functions as a plant growth-promoting rhizobacterium (PGPR), stimulating vegetative growth in the early stages. Possessing a more extensive root system and larger leaf area prior to the mild frost event provided the plants with superior energy reserves and vigor, facilitating a more rapid recovery compared to control plants, which exhibited stunted growth. The increase in leaf area is crucial because it enhances the total photosynthetic capacity available for tuber development.

This observed expansion in leaf area was also consistent with the trends in plant height, suggesting a correlation between the two parameters, with the 40 ml/plant treatment exerting a significant influence on both. The application of higher *Serratia* sp. rates in this study demonstrated more optimal stimulation of potato leaf growth than that in untreated plants.

Devi *et al.* (2016) and Kour & Yadav (2022) showed that larger leaves possess superior antioxidant capacity and synthesize osmoprotectants. However, although the 60 ml/plant treatment resulted in a lower leaf area than the 40 ml/plant treatment, it yielded the highest tuber weight (1,363.00 g). This indicates a more efficient partitioning of photosynthates to tubers at this specific application rate.

3.4. Effect of *Serratia* sp. on Potato Yield

Table 5 summarizes the effect of application rate of the *Serratia* sp. on yield parameters of potato plants in term of tuber weight per plant, number of tubers, and unit tuber weight.

Table 5. Effect of *Serratia* sp. application rate on potato yield parameters

Application rates *Serratia* sp. 10^7 CFU/ml	Tuber weight (g/plant)	Number of tubers	Unit weight (g/tuber)
Control	755.83 ± 92.70 c	9.67 ± 1.86 (a)	81.32 ± 21.77 b
20 ml/plant	1106.67 ± 281.99 bc	9.83 ± 1.80 (a)	118.38 ± 48.16 ab
40 ml/plant	1310.83 ± 459.20 ab	7.83 ± 1.33 (b)	175.65 ± 88.21 a
60 ml/plant	1363.00 ± 367.77 a	8.75 ± 0.96 (ab)	164.87 ± 23.83 a
Coefficient of Variation	22.75%	16.38%	6.25%

3.4.1. Tuber Weight

Based on the results in Table 5, the application of *Serratia* sp. had a significant effect on the total tuber weight per plant. Treatment with 20 ml/plant yielded a tuber weight of 1,106.67 g per plant, which was not significantly different from the control (755.83 g), despite showing a numerical increase of 46.43%. This suggests that although lower application rates elicit a positive response, the effect is inconsistent, necessitating higher rates to achieve stable efficacy.

Increasing the application rate to 40 ml/plant resulted in a significant difference, with tuber weight reaching 1,310.83 g per plant, representing a 73.43% increase compared to the control. The 60 ml/plant treatment produced the best results, with the highest tuber weight (1,363.00 g per plant), an increase of 80.31% over the control. This result was significantly different from both the control and the 20 ml/plant treatment, but not significantly different from the 40 ml/plant treatment.

The increase in total tuber weight can be attributed to several factors. First, the significant expansion in leaf area observed in the 40 ml/plant (39.21 cm²) and 60 ml/plant (32.98 cm²) treatments compared to the control (25.70 cm²) enhanced the total photosynthetic capacity by 28–53%, thereby providing a greater supply of photosynthates for tuber starch accumulation. This process was further supported by the lower intensity (0.19–0.28%) and incidence (0.44–1.18%) of frost damage compared to the control (0.49% and 1.99%), which allowed the plants to sustain photosynthetic activity for a longer duration during the tuber bulking phase. Furthermore, the role of phytohormones, such as gibberellins produced by *Serratia* sp., has been suggested to contribute to the regulation of assimilate partitioning from leaves (source) to tubers (sink). Kang *et al.* (2015) explained that this microbial interaction can extend the duration of the active tuber bulking phase, enabling higher starch accumulation before the plants undergo senescence or are inhibited by low temperatures.

3.4.2. Number of Tubers

As shown in Table 5, the application of *Serratia* sp. had a significant effect on the number of tubers per plant, exhibiting an interesting pattern. The control and 20 ml/plant treatments yielded the highest tuber counts (9.67 and 9.83 tubers, respectively), with no significant difference observed between them. However, increasing the application rate to 40 ml/plant resulted in a significant decrease in tuber number to 7.83 tubers, representing a 19.03% reduction compared to the control. The 60 ml/plant treatment produced 8.75 tubers, which was not significantly different from either the control or the 40 ml/plant treatment, indicating an intermediate response.

The reduction in tuber number observed at higher application rates (40 and 60 ml/plant) reflects a physiological trade-off between tuber number and size. In control plants characterized by limited leaf area (25.70 cm²), the plants adopted a 'many small tubers' strategy as a survival mechanism. Conversely, in the *Serratia* sp. treatments with larger leaf areas (32.98–39.21 cm²), the plants shifted to a 'fewer, larger tubers' strategy, wherein energy was allocated to enlarging the existing tubers.

The hormonal balance modulated by gibberellins from *Serratia* sp. influences stolon initiation. Chen *et al.* (2024) elucidated that gibberellins interact with cytokinins to regulate the number of stolons formed. At higher application rates (40 – 60 ml/plant), elevated gibberellin concentrations suppress the initiation of new stolons but prolong the tuber bulking phase, thereby enhancing the allocation of photosynthates to existing tubers via increased activity of starch biosynthesis enzymes.

Low-temperature conditions (8.25°C) restricted the number of active stolons capable of optimal development into tubers. In the absence of *Serratia* sp. support, plants initiated numerous stolons; however, the majority resulted in small tubers because of limited photosynthetic capacity. The application of *Serratia* sp. facilitated optimal resource allocation by mitigating inter-sink competition through the restriction of the number of active stolons.

The enhanced plant resilience to frost observed in the *Serratia* sp. treatments (intensity 0.19–0.28% vs. control 0.49%) influenced the source-sink balance. The 60 ml/plant treatment achieved an optimal balance, yielding the highest total tuber weight (1,363.00 g) with an average individual tuber weight of 164.87 g.

3.4.3. Unit Weight of Potato Tuber

The application of *Serratia* sp. had a significant effect on the weight per tuber. The 20 ml/plant treatment yielded a weight per tuber of 118.38 g, which was significantly different from the control (81.32 g), representing an increase of 45.57%. Increasing the application rate to 40 ml/plant produced the best result, with weight per tuber reaching 175.65 g –an increase of 116% compared to the control– and was significantly different from all other treatments. The 60 ml/plant treatment resulted in a weight per tuber of 164.87 g (an increase 102.73% over the control); this was not significantly different from the 40 ml/plant treatment but was significantly higher than both the control and the 20 ml/plant treatment.

The achieved weight per tuber in the range of 164–175 g remains within the genetic potential limits of the Tedjo MZ variety and is commercially classified as Grade A or Super quality (>120 g) (Kusumiyati *et al.*, 2017). This indicates that the application of *Serratia* sp. can maintain optimal tuber bulking processes, ensuring that tuber sizes meet premium market standards despite the plants being exposure to low-temperature stress.

The HSD post-hoc test (Table 5) revealed that the 40 ml/plant and 60 ml/plant application rates consistently yielded the highest weight per tuber, differing significantly from the control. Conversely, the 20 ml/plant rate exhibited an intermediate response. This indicates that achieving a commercially significant increase in tuber size necessitates a minimum application rate of 40 ml/plant. Although the 40 ml/plant and 60 ml/plant rates were not statistically different, the 60 ml/plant treatment demonstrated superior size consistency compared to the 40 ml/plant rate.

Agronomically, an increase in tuber weight reflects the efficiency of photosynthate translocation, supported by healthier canopy conditions. Plants treated with *Serratia* sp. exhibited larger leaf area (Table 4), functioning as a more productive photosynthetic factory. Abundant supply of assimilates is supported by bacterial physiological mechanism capable of facilitating more efficient starch biosynthesis during the tuber bulking phase (Kang *et al.*, 2015). Therefore, despite the occurrence of low-temperature stress, dry matter accumulation in the tubers proceeded optimally.

Enhanced plant resilience to low-temperature stress enables the tuber bulking phase to proceed with greater efficiency. Stegner *et al.* (2022) noted that frost damage to potato leaves can reduce photosynthetic rates by up to 70% within 48 h following exposure to extreme temperatures. *Serratia* sp. treatment, by reducing damage intensity, enabled the plants to maintain a higher photosynthetic capacity throughout the tuber bulking period.

4. CONCLUSIONS AND RECOMMENDATIONS

The application of *Serratia* sp. significantly reduced frost damage and improved the growth and yield of potato under natural mild frost conditions (−2 °C) in the Dieng Plateau. Higher application volumes (40 and 60 ml/plant at 1×10^7 CFU/ml) were more effective in enhancing plant performance compared to the control. The 40 ml/plant treatment resulted in the highest vegetative growth, particularly in leaf area, while the 60 ml/plant treatment produced the highest tuber yield. This indicates a shift in assimilate allocation from vegetative growth to tuber development at higher application levels.

Overall, *Serratia* sp. shows potential as a biological approach to support potato production under field frost conditions. However, further studies under more severe frost events and across different environments are needed to validate its broader applicability.

AUTHOR CONTRIBUTION STATEMENT

Author	**C**	**M**	**So**	**Va**	**Fo**	**I**	**R**	**D**	**O**	**E**	**Vi**	**Su**	**P**	**Fu**
ISN	✓	✓		✓	✓	✓			✓					
YMZ	✓									✓		✓		
MMH	✓									✓	✓			

C: Conceptualization
M: Methodology
So: Software
Va: Validation
Fo: Formal Analysis
I: Investigation
D: Data Curation
R: Resources
O: Writing - Original Draft
E: Writing - Review & Editing
Vi: Visualization
Su: Supervision
Fu: Funding Acquisition
P: Project Administration

ACKNOWLEDGEMENT

The authors wish to express their gratitude to Mr. Sarmin for granting permission to use the experimental site in Dieng and for facilitating the provision of source seeds. The authors also extend their appreciation to the Dieng Weather Station IoT Team for providing real microclimate data obtained from sensors in the Arjuna Temple area, which served as supporting data for this study.

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