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Differential Responses of Antagonistic Microbes in Controlling Bacterial Wilt (*Ralstonia solanacearum)* in Potato Seed Production

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ABSTRACT

Ralstonia solanacearum is a soil-borne pathogen that can infect tubers, resulting in low-quality potato tubers and reduced yield. Antagonistic microbes may be used to control *Ralstonia solanacearum* in endemic areas. The aim of this research was to evaluate the efficacy of different antagonistic microbes in controlling *Ralstonia solanacearum* as a source of potato seeds. The research design was a factorial randomized block design with six replications. The first factor was the type of antagonistic microbes in four treatments, and the second factor was *Ralstonia solanacearum* inoculum concentration (two levels): low (10^4 CFU mL^{-1}) and high (10^8 CFU mL^{-1}). The *Ralstonia solanacearum* resistant microbial selection test was carried out in trays containing a substrate medium for eight weeks after cutting. The application of antagonistic microbes reduced bacterial wilt incidence by 55–76%. Based on Principal Component Analysis (PCA), in which the first two principal components explained 69.4% of the total variation, the microbes were grouped into: (1) *Bacillus subtilis* Garut and *Pseudomonas fluorescens*, and (2) *Bacillus subtilis* UNPAD and the microbial consortium.

1. INTRODUCTION

Indonesia's national potato production is currently unable to meet the demand for potatoes, which continues to increase in line with shifts in lifestyle consumption of foods other than rice [1, 2]. As demand increases, highland agricultural areas—where potatoes are predominantly grown in the tropics—are being cultivated more intensively [3, 4]. Intensive land use not only causes potato production to decrease in quantity, but also the quality of the tubers produced [5]. *Ralstonia solanacearum* is a soil-borne pathogen that can infect tubers, resulting in poor tuber quality and yield losses [6].

Ralstonia solanacearum is an aerobic, Gram-negative, non-spore-forming bacterium [7]. Moreover, *Ralstonia solanacearum* can survive in soil for long periods and spreads readily via water and soil; therefore, potato-growing areas where it is endemic may no longer produce high yields or high-quality tubers. Yuliar et al. [6] noted that soil-borne pathogens pose a greater constraint on crop production than seed-borne or airborne pathogens. In addition, the highland land suitable for potato cultivation in Indonesia is limited. Therefore, restoring soil health in fields affected by *Ralstonia solanacearum* is important for maintaining potato-growing areas and stabilizing national production [8, 9].

Efforts to control *Ralstonia solanacearum* using chemical pesticides on endemic land have not provided significant results on a widespread basis. Continuous use of pesticides can cause environmental and health problems [9, 10]. In addition, selection pressure resulting from repeated pesticide use can encourage the emergence of resistant *Ralstonia solanacearum* strains, resulting in reduced control effectiveness [11]. The use of microorganisms can be an option to control *Ralstonia solanacearum* in the long term in a sustainable manner [6, 10] and can be combined with other control methods [12, 13] and is very effective in endemic land that is difficult to control [8].

Antagonistic microbes are microorganisms that are able to inhibit the growth of plant pathogens through various mechanisms, such as competition for niche and nutrients, production of antibiosis compounds and volatile organic compounds (VOC), induction of plant resistance, and parasitism [10, 14, 15]. The effectiveness of antagonistic microbes, such as *Bacillus* spp. and *Pseudomonas* spp., has been widely reported to inhibit the growth of *Ralstonia solanacearum* in potato plants both in vitro [16, 17] and in vivo [16, 18-22], while *Pseudomonas* sp. has a high ability to inhibit *Ralstonia solanacearum* [8, 23, 24], is able to survive in various media, has a short generation time, and has high colonization mobility [22]. The study [2] reported that there were four best bacterial isolates that could inhibit the development of *Ralstonia solanacearum* in vitro, namely *Bacillus subtilis* UNPAD, *Bacillus subtilis* Garut, *Pseudomonas fluorescens*, and a consortium between *Bacillus subtilis* Garut + *Pseudomonas fluorescens* with an inhibition zone ranging from 0.65-1.95 cm. These four isolates have not been tested on substrate media to obtain healthy potato cuttings before being transplanted into the field as potato seed.

The study [23] stated that the successful identification of biocontrol strains in vitro does not always translate to successful biocontrol outcomes in vivo. Thus, field testing needs to be carried out because it is the easiest way to verify and confirm microbes' adaptation in actual conditions after obtaining selected microbes in vitro [24]. The study [5] reported that early application of *Bacillus subtilis* R31 could suppress wilt attacks on tomatoes. Thus, increasing the resistance of *Ralstonia solanacearum* when propagating seeds in substrate media with various types of antagonistic microbes is an effort to increase the production and quality of potato seed before they are transplanted for use as seed sources in the field. The aim of this research was to assess the inhibition rate and plant growth response of various types of microbes to control bacterial wilt of *Ralstonia solanacearum* in potato seeds.

2. MATERIALS AND METHODS

The research was carried out in the greenhouse of the West Java Province Potato Seed Center in Sukamanah Village, Pangalengan District, Bandung Regency, from January to April 2023. The microbial solution was made in the BPTPH Cianjur disease laboratory. The research design was a factorial randomized block design with six replications. The first factor was the kinds of antagonistic microbes in four treatments, and the second factor was the wilt concentration of *Ralstonia solanacearum* in two treatments (Table 1). The microbes used were the results of microbial exploration in research by Dianawati et al. [2]. The potato (*Solanum tuberosum* L.) cultivar used was 'Granola'. One experimental unit consisted of one tray. One tray consisted of 105 plants.

Table 1. Treatment of kinds of antagonism microbe and *Ralstonia solanacearum* concentration

Kinds of Antagonism Microbe Treatments
Bacillus subtilis UNPAD (Microbe A)
Bacillus subtilis Garut (Microbe B)
Pseudomonas fluorescens (Microbe C)
Bacillus subtilis Garut + *Pseudomonas fluorescens* (Microbe D)
***Ralstonia solanacearum* Concentration Treatments**
10^4 CFU mL^{-1} (low concentration)
10^8 CFU mL^{-1} (high concentration)

The four microbial types treated were microbes A and B identified as *Bacillus subtilis*, microbe C was *Pseudomonas fluorescens*, while D was a consortium of B and C. Microbe A was *Bacillus subtilis* strain H15, from a storage collection from Padjadjaran University with a host plant of potatoes, while microbes B and C were a collection of the National Research and Institute Agency (NRIA) obtained from farmers with a host plant of bamboo roots [2]. Microbes were propagated in nutrient broth medium, which was incubated in an orbital shaker for 42 hours. The density of the microbial suspension used was adjusted to a concentration of 10^8 CFU mL^{-1}. The *Ralstonia solanacearum* isolate came from the collection of the Disease Laboratory of the Ornamental Crops Research Institute, Indonesia, and its concentration depended on each treatment (10^4 and 10^8 CFU mL^{-1}).

The *Ralstonia solanacearum*-resistant microbial selection test was carried out in a tray with a substrate of burnt husks and compost that had been sterilized by steaming. Each tray that had been planted with one-week plantlet cuttings was soaked in a microbial suspension for one minute. Each tray that had been planted with one-week plantlet cuttings was soaked in a microbial suspension for one minute. Tray immersion was carried out in a plastic box containing a microbial suspension. One week later, each tray was inoculated with *Ralstonia solanacearum* at the concentration according to the treatment using a handsprayer to the seedlings at the ages of 2, 3, 4, and 5 weeks after cutting (WAC). Inoculation volume per tray by handsprayer was one liter for each concentration treatment of *Ralstonia solanacearum*. The *Ralstonia solanacearum* suspension was sprayed evenly over all parts of the plant at a spray distance of approximately 10-20 cm. Spraying was carried out until evenly wet but not excessively dripping. Spraying was carried out in the morning when the dew had disappeared around 7 a.m. with a temperature of around 20-22 °C and air humidity of 85-90%. Each tray was separated with plastic mulch to avoid contamination between treatments. The sample observed was 10 plants per experimental unit, randomly selected without involving borders. If the seeds were affected by wilting, they were removed and not counted again to prevent the spread of the wilt attack to the surrounding area. Plants were maintained for up to 8 WAC with watering to maintain media humidity. Pest and disease control was carried out if there were indications of an attack.

The variable observed in this study was the number of wilted plants every week until the age of 8 WAC. Criteria for determining wilt were wilted shoots, and then verified by immersing the tissue in water, and a white, mist-colored bacterial mass was formed [2]. The percentage of wilted plants was the percentage of the number of wilted plants compared to the total number of plants observed. The cumulative percentage of wilting was the sum of the percentage of wilted plants up to the last week of observation. Inhibition rate was the percentage of control wilted plants compared to the treatment and then divided by the percentage of control wilted plants [2]. Harvest observations were the fresh weight of stover, roots, and total plants.

$$\text{Inhibition rate} = \frac{\text{Pt} - \text{Pc}}{\text{Pc}} \times 100\%$$

Pt = percentage of wilted plants of the treatment.
Pc = percentage of wilted plants of the control.

Data were analyzed using variance analysis, and if there were significant differences, continued with the Duncan test and Orthogonal Contrast test with a confidence level of 95% ($p < 0.05$) and the SAS program version 9.4. Testing between observed variables used the Pearson correlation test and Principal Component Analysis (PCA) test with the R Studio program version 4.4.3. The calculation of the PCA value is the sum of dim1 (PCA1) and dim2 (PCA2) [2].

3. RESULTS

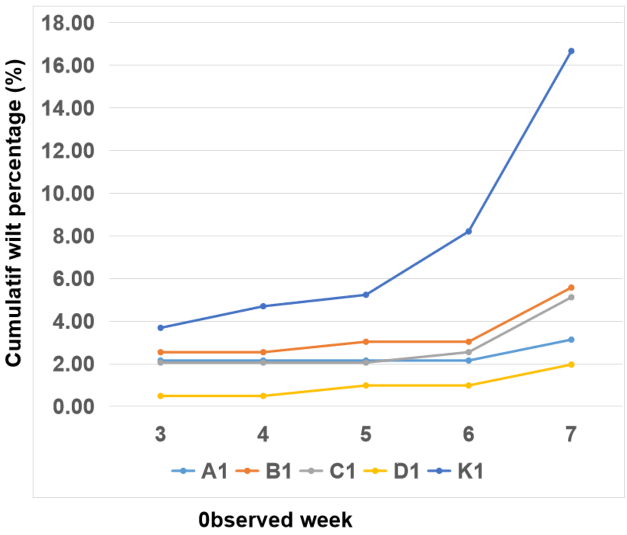
At a low *Ralstonia solanacearum* concentration, the cumulative percentage of wilting in the control treatment began to increase exponentially at 6 WAC (Figure 1(a)), whereas at a high *Ralstonia solanacearum* concentration, there was a linear and faster increase in the cumulative percentage starting at 3 WAC (Figure 1(b)), so that giving a high concentration of *Ralstonia solanacearum* accelerated the plants experiencing wilting. The highest cumulative percentage was found in the control treatment without microbes with high *Ralstonia solanacearum concentrations* (Figure 1(b)). At low *Ralstonia solanacearum* concentrations, various types of microbes could suppress *Ralstonia solanacearum* attack (Figure 1(a)), while at high *Ralstonia solanacearum* concentrations, application of microbes could inhibit *Ralstonia solanacearum* until the 6th week for microbes C and D and up to the 7th week for microbes A and B (Figure 1(b)).

There was an interaction between microbial type and *Ralstonia solanacearum* concentration on wilting percentage and number of leaves at 7 WAC (Table 2). The highest wilting percentage at 7 WAC was found in microbe B with a low *Ralstonia solanacearum* concentration and higher than microbes A, C, and D with various *Ralstonia solanacearum* concentrations and the control treatment with a high *Ralstonia solanacearum* concentration (Table 2). The number of leaves at 7 WAC in treatment D with high *Ralstonia solanacearum* concentrations was higher than that of microbes A and D at low *Ralstonia solanacearum* concentrations (Table 2).

Various types of microbes influenced the percentage of wilting at 2, 4, 5, 6 WAC, the cumulative percentage of wilting (3-7 WAC), plant height (4-7 WAC), and number of leaves (4-6 WAC), fresh weight of shoots and total plants, and dry weight of roots, shoots and total plants (Table 3). Treatment of various types of microbes compared to control could reduce the wilting percentage by 2, 4, 5, and 6 WAC and the cumulative wilting percentage in all observations (Table 3). The highest percentage of inhibition rate was found in microbe A at 76.86%, followed by microbes B, D, and C, respectively, at 72.31%, 54.87%, and 50.82% (Table 3). Plants with microbe D had higher plant height growth than microbe B, C, and controls, but the number of plant leaves was lower than that of microbe A. Meanwhile, microbe A, apart from having a large number of leaves, also had a high wet weight and dry weight, especially the dry weight of the plant compared to microbes B and C (Table 3).

Ralstonia solanacearum concentration affected wilting percentage 4 WAC, cumulative percentage 7 WAC, plant height 6 and 7 WAC, and number of leaves 4 WAC (Table 4). High *Ralstonia solanacearum* concentration could increase the wilting percentage by 4 WAC and the cumulative wilting percentage by 7 WAC (Table 4). High *Ralstonia solanacearum* concentration could increase wilt infection by 50.57%.

a) Low concentration of *Ralstonia solanacearum*



b) High concentration of *Ralstonia solanacearum*

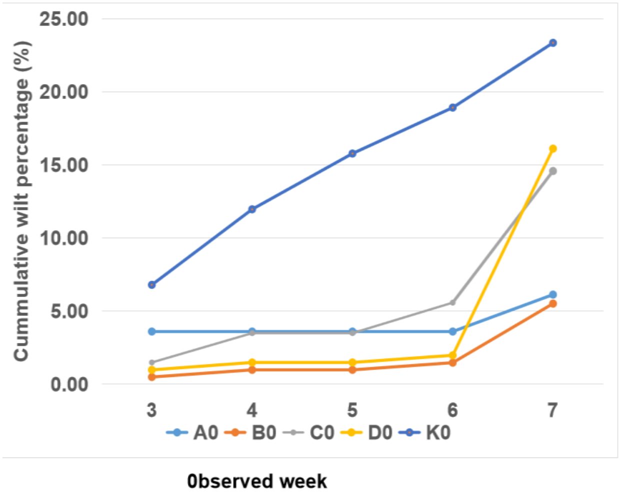


Figure 1. Wilt cumulative percentage on many observed weeks at low (a) and high (b) concentration of *Ralstonia solanaearum*

Notes: 1. A: *Bacillus subtilis* UNPAD, B: *Bacillus subtilis* Garut, C: *Pseudomonas fluorescens,* D: concorsium B + C, K: Control; 2. 0 or 1 = Low (0) or high (1) of *Ralstonia solanacearum* concentration

Table 2. Interaction of kinds of microbes at many concentrations of *Ralstonia solanacearum* on wilt percentage and number of leaves at 7 WAC

Concentration of *Ralstonia solanacearum*	Kinds of Microbes				
	Control	*Bacillus subtilis* UNPAD (A)	*Bacillus subtilis* Garut (B)	*Pseudomonas fluorescence* (C)	Consortium of B + C (D)
			Wilt percentage (%) at 7 WAC		
Low (10^4 CFU mL^{-1})	8.46 ab	4.04 b	14.13 a	0.98 b	2.57 b
High (10^8 CFU mL^{-1})	0.98 b	2.52 b	9.00 ab	4.43 b	2.54 b
CV (%) = 26.17*					
			Number of leaves at 7 WAC		
Low (10^4 CFU mL^{-1})	5.70 abc	4.80 c	5.23 abc	6.07 abc	5.10 bc
High (10^8 CFU mL^{-1})	6.03 abc	6.00 abc	5.70 abc	6.30 ab	6.57 a
CV (%) = 18.19					

Notes: 1. Data were transformed with * Log (x+4); 2. Numbers followed by different letters within a column were significantly different using Duncan's test ($p < 0.05$); 3. WAC: Weeks after cutting, CV: coefficient of variance.

Table 3. Effect of kinds of microbes on the observed variables

Observed Variables	Kinds of Microbes						
	Control	***Bacillus subtilis* UNPAD (A)**	***Bacillus subtilis* Garut (B)**	***Pseudomonas fluorescence* (C)**	**Consortium of B + C (D)**	**CV (%)**	**Control vs Microbes**
Wilt percentage (%)							
2 WAC	4.17 a	2.61 ab	1.26 b	0.74 b	0.74 b	25.45*	4.2 vs 1.3***
3 WAC	1.07	0.27	0.27	0.03	0.00	18.07*	1.1 vs 0.4
4 WAC	3.09 a	0.00 b	0.24 b	1.00 b	0.25 b	17.89*	3.1 vs 0.4***
5 WAC	2.18 a	0.00 b	0.24 b	0.00 b	0.24 b	15.89*	2.2 vs 0.1***
6 WAC	3.05 a	0.00 b	0.24 b	1.29 ab	0.45 b	19.54*	3.1 vs 0.4***
Cumulative wilt percentage (%)							
3 WAC	5.24 a	2.88 ab	1.52 b	1.77 ab	0.74 b	28.53*	5.2 vs 4.6***
4 WAC	8.33 a	2.88 b	1.76 b	2.77 b	0.99 b	28.50*	8.3 vs 1.7***
5 WAC	10.51 a	2.88 b	2.01 b	2.77 b	1.23 b	29.17*	10.5 vs 2.1***
6 WAC	13.56 a	2.88 b	2.25 b	4.06 b	1.48 b	28.75*	13.6 vs 2.2****
7 WAC	20.01 a	4.63 b	5.54 b	9.84 b	9.03 b	24.02*	20.0 vs 7.26***
Inhibition rate (%)							
		76.86	72.31	50.82	54.87		
Plant height							
4 WAC	1.39 c	1.95 b	1.38 c	1.49 c	2.52 a	22.26	1.4 vs 1.8***
5 WAC	1.94 b	2.46 a	1.78 b	1.74 b	2.86 a	25.62	1.9 vs 2.2
6 WAC	2.25 bc	2.53 ab	1.87 c	1.87 c	2.92 a	27.54	2.2 vs 2.3
7 WAC	2.50 bc	2.67 ab	2.03 cd	1.95 d	3.09 a	26.11	2.5 vs 2.4
Number of leaves							
4 WAC	4.97 b	6.03 a	5.38 b	5.20 b	5.35 b	13.62	4.9 vs 5.5***
5 WAC	5.68 b	6.42 a	5.83 ab	5.45 b	5.73 b	12.86	5.7 vs 5.9
6 WAC	5.87 ab	6.10 a	5.78 ab	5.45 ab	5.30 b	14.09	5.9 vs 5.7
Fresh weight (g/plant)							
Total plant	2.60 abc	3.17 a	2.42 bc	2.04 c	2.79 ab	28.92	2.6 vs 2.6
Root	1.24	1.58	1.26	1.32	1.42	23.58	1.2 vs 1.9
Stover	1.41 ab	1.58 a	1.16 bc	0.86 c	1.18 bc	24.42	1.4 vs 1.2
Dry weight (g/plant)							
Total plant	0.52 b	0.78 a	0.48 b	0.51 b	0.74 a	15.85**	0.5 vs 0.6
Root	0.35 b	0.66 a	0.41 b	0.45 b	0.65 a	16.48**	0.3 vs 0.5***
Stover	0.12 a	0.12 a	0.08 b	0.06 b	0.08 b	5.00**	0.1 vs 0.1***

Notes: 1. Data were transformed with * Log (x+4), **log (x+1); 2. Numbers followed by different letters in a column were significantly different using Duncan's test ($p < 0.05$); 3. Number followed by *** was significantly different using the contrast orthogonal test ($p < 0.05$); 4. WAC: Weeks after cutting, CV: coefficient of variance.

Table 4. Effect of *Ralstonia solanacearum* concentration on the observed variables

Observed Variables	Concentration of *Ralstonia solanacearum*		CV (%)
	Low (10^4 CFU mL^{-1})	**High (10^8 CFU mL^{-1})**	
Wilt percentage (%)			
2 WAC*	1.54	2.26	25.45
3 WAC*	0.64	0.41	18.07
4 WAC*	0.20 b	1.63 a	17.89
5 WAC*	0.3	0.76	15.89
6 WAC*	0.69	1.24	19.54
Cumulative wilt percentage (%)			
3 WAC*	2.18	2.67	28.53
4 WAC*	2.39	4.3	28.50
5 WAC*	2.69	5.07	29.17
6 WAC*	3.38	6.31	28.75
7 WAC*	6.49 b	13.13 a	24.02
Plant height (cm)			
4 WAC	1.71	1.78	22.26
5 WAC	2.07	2.24	25.62
6 WAC	2.11 b	2.46 a	27.54
7 WAC	2.23 b	2.64 a	26.11
Number of leaves			
4 WAC	5.68 a	5.09 b	13.62
5 WAC	5.87	5.77	12.86
6 WAC	5.87	5.53	14.09
Fresh weight (g/plant)			
Total plant	2.58	2.63	28.92

Root	1.34	1.39	23.58
Stover	1.24	1.24	24.42
	Dry weight (g/plant)		
Total plant**	0.58	0.63	15.85
Root**	0.47	0.53	16.48
Stover**	0.09	0.1	5.00

Notes: 1. Data were transformed with * Log (x+4), **log (x+1); 2. Numbers followed by different letters in a column were significantly different using Duncan's test ($p < 0.05$); 3. WAC: Weeks after cutting, CV: coefficient of variance.

Table 5. Correlation among observed variables

	L3	L4	L5	L6	L7	K3	K4	K5	K6	K7	TT4	JD4	TT5	JD5	TT6	JD6	TT7	JD7	BBB	BBA	BBT	BKB	BKA	BKT
L2	33	73*	83*	55	-9	96*	92*	91*	87*	60	-32	-32	-8	28	18	54	30	60	35	-5	62	-2	-19	79*
L3		32	34	52	-4	58	50	46	50	35	-65*	-26	-62	-36	-52	8	-47	1	-61	-31	-35	-49	-54	-21
L4			89*	81*	21	73*	92*	93*	94*	83*	-33	-80*	-18	-34	11	9	24	24	-4	-30	17	-35	-43	44
L5				63	-7	81*	91*	95*	92*	64*	-27	-61	-8	-13	16	31	30	44	9	-30	29	-31	-40	52
L6					41	63	77*	74*	84*	87*	-45	-67*	-38	-47	-15	-3	-7	7	-24	-47	7	-43	-61	26
L7						-9	5	2	11	67*	13	-55	12	-55	28	-71*	27	-37	-16	8	-19	10	6	-10
K3							94*	92*	90*	62	-46	-35	-25	13	0	49	13	52	13	-14	44	-16	-32	62
K4								99*	99*	77*	-43	-60	-23	-9	6	33	19	42	6	-23	34	-27	-40	58
K5									99*	75*	-39	-61	-19	-10	9	33	22	43	7	-25	33	-28	-40	57
K6										81*	-43	-66*	-25	-19	3	26	17	37	0	-31	28	-33	-47	53
K7											-24	-81*	-11	-47	19	-22	29	5	-9	-18	10	-19	-32	33
TT4												25	94*	24	82*	-31	75*	-6	55	45	17	81*	84*	17
JD4													20	69*	-7	42	-16	12	40	38	31	41	38	3
TT5														31	94*	-24	88*	-4	70*	60	36	83*	83*	39
JD5															22	69*	22	56	75*	33	74*	59	50	61
TT6																-24	99*	4	69*	57	41	77*	75*	53
JD6																	-17	81*	41	-13	64*	-8	-18	52
TT7																		14	69*	49	46	71*	67*	60
JD7																			42	-25	59	9	0	55
BBB																				53	90*	71*	59	82*
BBA																					29	64*	69*	24
BBT																						45	24	93*
BKB																							95*	45
BKA																								25

Notes: 1. * Significantly different from the Pearson correlation test ($p < 0.05$); 2. L3/4/5/6/7: wilt percentage at 3/4/5/6/7 weeks after cutting; K3/4/5/6/7 cumulative wilt percentage at 3/4/5/6/7 weeks after cutting; TT 4/5/6/7: high plant at 4/5/6/7 weeks after cutting; JD 4/5/6/7: number of leaves at 4/5/6/7 weeks after cutting; BBB/A/T = fresh weight of stover/root/total; BKB/A/T: dry weight of stover/root/total.

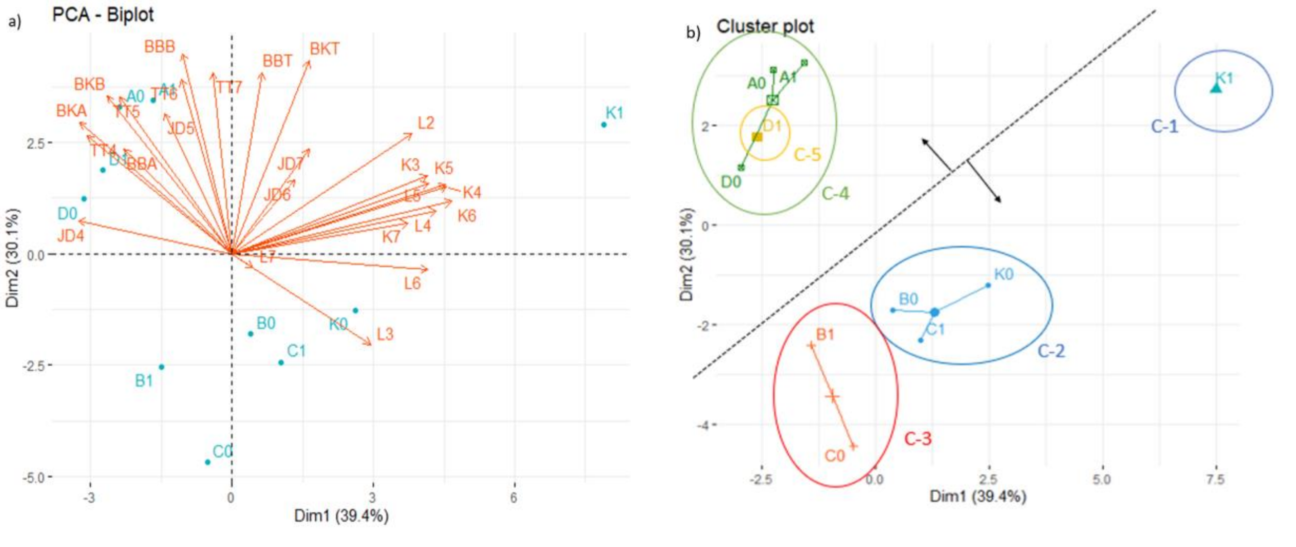


Figure 2. Principle component analysis (a) and clusters (b) on many treatment combinations and observed variables

Notes: 1. L2/3/4/5/6: wilt percentage at 2/3/4/5/6 weeks after cutting; K3/4/5/6/7 cumulative wilt percentage at 3/4/5/6/7 weeks after cutting; DH: inhibition power. TT 4/5/6/7: high plant at 4/5/6/7 weeks after cutting; JD 4/5/6/7: number of leaves at 4/5/6/7 weeks after cutting; BBB/A/T = fresh weight of stover/root/total; BKB/A/T: dry weight of stover/root/total; 2. A: *Bacillus subtilis* UNPAD, B: *Bacillus subtilis* Garut, C: *Pseudomonas fluorescence*, D: consortium of B + C, K: Control; 3. 0 or 1 = Low or high of *Ralstonia solanacearum* concentration.

Among all variables of wilting percentage and cumulative wilting, the wilting percentage in the 3rd and 7th weeks had a low correlation and was not significantly different, while the wilting percentage and the cumulative wilting percentage outside the observation time influenced each other with high correlation values and were significantly different (Table 5). The percentage of wilting and cumulative wilting did not have much influence on the growth of plant height and number of leaves, and plant weight, except for plant height of 4 WAC, number of leaves of 4 and 6 WAC, and total dry weight (Table 5).

Based on the PCA value of 69.5% (Figure 2(a) and (b)), the treatment combination can be divided into five clusters. The first three clusters had low growth in plant height, number of leaves, and plant weight, but had a high wilting percentage and cumulative wilting percentage, in contrast to the last two clusters (Figure 2(b)). The first cluster was a high *Ralstonia solanacearum* concentration control with a high percentage of wilting and cumulative wilting. The second cluster was control with low *Ralstonia solanacearum* concentration, microbe B with low *Ralstonia solanacearum*, and microbe C with high *Ralstonia solanacearum*. Meanwhile, the third cluster was microbe B with high *Ralstonia solanacearum* and microbe C with low *Ralstonia solanacearum*. The 4th cluster was microbe A with both high *Ralstonia solanacearum* and low *Ralstonia solanacearum,* and microbe D with low *Ralstonia solanacearum*, while the 5th cluster was microbe D with high *Ralstonia solanacearum*.

4. DISCUSSION

4.1 The control effect of antagonistic microorganisms on bacterial wilt

Preparing healthy seeds is very necessary so that they can grow well in the field and can withstand the threat of biotic and abiotic stress. Healthy seedlings can be conditioned by applying antagonistic microorganisms in the form of non-pathogenic bacteria from the start so that systemically induced resistance occurs, or what is known as induced systemic resistance (ISR) [25]. The study [10] stated that ISR causes plants to be able to survive in the presence of pathogens, whereas in the absence of microbes, the plants would die. Providing antagonistic microbes at the beginning of growth on sterile media will cause the microbes to grow quickly without competition with other unwanted microbes. However, to test the effectiveness of this microbial antagonism, it is necessary to assess it by giving the *Ralstonia solanacearum* pathogen at low (10^4 CFU mL^{-1}) and high (10^8 CFU mL^{-1}) concentrations to study its response to the growth of potato cuttings as a systemic acquired resistance (SAR) response. The study [25] stated that the combination of ISR and SAR could increase prevention against pathogen attacks and expand the spectrum of prevention against various pathogens.

All cuttings in this study experienced *Ralstonia solanacearum* attack at both low and high *Ralstonia solanacearum* concentrations (Figures 1 and 2). If *Ralstonia solanacearum* had entered the plant's environment through water, then *Ralstonia solanacearum* would easily infect plants because *Ralstonia solanacearum* could enter plants in various ways. The way in which *Ralstonia solanacearum* enters was through plant wounds, root movement, or the natural opening, and then it entered the xylem tissue and developed in the vessels of the plant stem [26, 27]. Extracellular polysaccharides (EPS) produced by *Ralstonia solanacearum* in the xylem would block the movement of water and nutrients in plant stems, causing the plant to wilt and ending with the plant dying as a whole [27].

The higher *Ralstonia solanacearum* concentration given would accelerate the occurrence of *Ralstonia solanacearum* attacks at 3 WAC compared to low *Ralstonia solanacearum* concentrations at 6 WAC (Figure 1). This was because high *Ralstonia solanacearum* concentration in this study could increase wilt attacks by 50.57%. Lowe-Power et al. [27] stated that with a low *Ralstonia solanacearum* inoculation of 10^4 CFU mL^{-1} on *Ralstonia solanacearum*-resistant cultivar plants, the plants could survive without showing symptoms of wilting. However, at a high concentration of *Ralstonia solanacearum* 10^8 CFU mL^{-1}, the plants would immediately show wilting symptoms. The rapid response of plants to *Ralstonia solanacearum* at high *Ralstonia solanacearum* concentration showed that the virulence of *Ralstonia solanacearum* in this study was relatively high due to the repeated application of *Ralstonia solanacearum* four times during growth. Kashyap et al. [28] reported that the *Ralstonia solanacearum* population increased rapidly after inoculation 48 hours later.

In environmental conditions polluted with *Ralstonia solanacearum*, the application of all types of microbes could help inhibit wilt attacks at both low and high *Ralstonia solanacearum* concentrations of 55-76% (Figure 1). The percentage of inhibition of wilting attacks was higher than in research conducted by Raza et al. [17], which ranged from 26-38%. The results of the orthogonal contrast test also showed that the wilting percentage and cumulative wilting percentage of the microbial treatment were higher than those of the control (Table 3). Even in this study, the cumulative percentage of wilting in various types of microbes at low *Ralstonia solanacearum* concentrations could be reduced below 6% (Figure 1(a)). Apart from that, the inhibition of *Ralstonia solanacearum* wilt attacks due to the application of microbes in this study was quite effective, with a delay of wilt attacks of around 2-3 weeks, namely up to 6 WAC for microbes C and D and 7 WAC for microbes A and B (Figure 1(b)). This delay was longer than in the study [28] research which was only delayed 10-14 days. Thus, this research showed that both *Bacillus subtilis* and *Pseudomonas fluorescens* microbes could inhibit *Ralstonia solanacearum* by various mechanisms, including niche and nutritional competition, antibiosis, resistance induction, parasitism, and VOC products. Sun et al. [5] reported that lipopeptides produced from *Bacillus subtilis* strain 31 played an important role in inhibiting *Ralstonia solanacearum*. Several studies report VOC that could inhibit *Ralstonia solanacearum* produced by *Bacillus subtilis*, such as pyrazine and benzothiazole [29], while by *Pseudomonas fluorescens* were 13-tetradecadien-1-ol, 2-butanone, and 2-methyl-n-1-tridecene [30]. These VOCs could damage the DNA of pathogens and induce changes in the expression of enzyme ontogenesis levels, which changed the target organisms to abnormal ones. Tahir et al. [7] reported abnormalities in this pathogen in the form of rupture and detachment of cell walls, movement of cytoplasm outward, and deformed cell shapes.

4.2 Differential response mechanisms of different microorganisms

The difference in inhibition rate against *Ralstonia* *solanacearum* attacks occurred between microbes, namely microbes A and B, respectively 76.86 and 72.31%, followed by microbes D and C at 54.87 and 50.82% (Table 3). The study [7] reported almost the same inhibition rate of *Pseudomonas fluorescens* and *Bacillus subtilis*, 49 and 47%, respectively, while Kashyap et al. [31] reported the respective inhibition rate of *Pseudomonas fluorescens* and *Bacillus subtilis* microbes of 72 and 69%. The inhibition rate of microbes A and B, namely *Bacillus subtilis*, in this study appeared to be higher than that of microbes C (*Pseudomonas fluorescens*) and D (*Bacillus subtilis* + *Pseudomonas fluorescens*), indicating that *Bacillus subtilis* was more effective at inhibiting *Ralstonia solanacearum* than *Pseudomonas fluorescens*. Initially, *Pseudomonas fluorescens* could inhibit *Ralstonia solanacearum* up to 6 WAC, but repeated application of *Ralstonia solanacearum* up to four times caused an increase in wilting attacks (Figure 1), so that *Pseudomonas fluorescens* might not be able to control *Ralstonia solanacearum*. In fact, *Pseudomonas fluorescens* was well known to have a high level of virulence [20], but it had a weakness, namely, it did not produce spores, so it was easily damaged [32]. The study [17] reported that the effectiveness of VOCs from *Pseudomonas fluorescens* in inhibiting *Ralstonia solanacearum* was determined by the ability of the bacteria to colonize roots. Apart from that, the high virulence of *Pseudomonas fluorescens* also needs to be considered to get the right concentration to avoid the reverse mechanism effect. The study [2] reported that soaking *Pseudomonas fluorescens* for 10 and 15 minutes could reduce the effectiveness of *Pseudomonas fluorescens* compared to soaking for 5 minutes because of its higher concentration. Meanwhile, *Bacillus* sp could produce endospores so that it was resistant to extreme conditions [7, 33] and could grow quickly in various media [34]. This made Bacillus easy to maintain, formulate, and store, and it can survive well in the soil without disturbing other bacterial populations [32]. This characteristic caused *Bacillus subtilis* in this study to appear superior to *Pseudomonas fluorescens*.

The difference response of microbe A and B of *Bacillus subtilis* with differences in strain may be attributed to strain-specific variation in the production of bioactive secondary metabolites, particularly lipopeptides and VOC. The study [35] demonstrated that different *Bacillus* strains produce distinct profiles and quantities of lipopeptides such as surfactin, iturin, and fengycin, which play key roles in antifungal and antibacterial activity. Likewise, variations in VOC composition can influence the degree of pathogen inhibition through direct antimicrobial effects or indirect mechanisms such as ISR activation [36]. Therefore, the higher inhibition rate observed in microbe A may indicate that *Bacillus subtilis* UNPAD produced a more effective combination or higher concentration of these bioactive compounds compared to *Bacillus subtilis* Garut. These findings suggest that differences in metabolite profiles among strains are likely a contributing factor to the observed variation in antimicrobial efficacy and should be further investigated through metabolomic or molecular analyses.

The low percentage of wilting and cumulative wilting due to the application of antagonistic microbes in this study did not always result in high plant growth (Tables 3 and 4). This was indicated by the low Pearson correlation value between the variables percentage wilting and cumulative wilting with the variables plant height, number of leaves, wet and dry weight of plants (Table 5), and the position of the variables plant growth and plant weight in different quadrants with the variables percentage wilting and cumulative wilting (Figure 2(a)). These conditions showed that each microbe tested had different characteristics in responding to wilting attacks and plant growth, so that two large groups of microbes or five clusters were obtained, as in Figure 2(b). Two large groups of microbes in response to wilting and plant growth, namely microbes with a high percentage of wilting and cumulative wilting with low plant growth, namely controls, microbes B and C, while microbes A and D had a low percentage of wilting and cumulative wilting with high plant growth. Identifying the characteristics of the four microbes used by PCA value of 69.4% was a novelty from this research. High inhibition rate on microbe B did not necessarily produce high plant growth, while low inhibition rate on microbe D still could increase plant growth. Meanwhile, microbes A and C showed the same positive and negative tendencies between inhibition rate and plant growth (Figure 2).

Microbes A and B were *Bacillus subtilis,* which could overcome wilting at both low and high *Ralstonia solanacearum* concentrations (Figure 1). However, the influence of these two microbes on plant growth was very different. The study [37] stated that different *Bacillus subtilis* strains would produce different hydrolytic enzymes, including cellulase, protease, and β-glucanases, so that they would produce different plant growth responses. The high ability to inhibit wilting by microbial A was in line with the high plant height at 5 WAC, the number of leaves at 4, 5, and 6 WAC, the fresh weight of shoot and total plant, dry weight of shoot, root, and total plant (Table 3). This showed that *Bacillus subtilis* in treatment A could not only inhibit wilting, but could also increase plant growth (Table 3). Apart from producing compounds that could kill pathogens, *Bacillus subtilis* could also produce plant growth-promoting rhizobacteria (PGPR), which could help plant growth [28]. The study [5] reported that *Bacillus subtilis* strain R31 could indirectly inhibit the growth of *Ralstonia solanacearum* and the bacterial community within it, thereby restoring the abundance of other normal bacterial communities that contributed to metabolic functions that influence plant growth.

Meanwhile, microbe B could only inhibit wilt attacks, but could not increase plant growth because plant growth was the same as the control (Table 3). The *Bacillus subtilis* Garut strain of microbe B may not produce enough PGPR to increase plant growth, so it was only able to inhibit *Ralstonia solanacearum*. This was the same as research by Istifadah et al. [38] in chilies, where *Bacillus subtilis* could suppress *Ralstonia solanacearum* wilt, but did not increase plant growth. Soesanto et al. [18] stated that although microbes could inhibit pathogen attacks, this was not always followed by increased plant growth because the production of PGPR, which played a role in growth, was not optimal. This was because microbes to produce PGPR take time [16] and need to be applied early and repeatedly [5, 7, 39].

Microbe C had a lower percentage of inhibition rate than microbes A and B (Table 3). Thus, C microbes at both high and low *Ralstonia solanacearum* concentrations had low growth (Figure 2). This showed that *Pseudomonas fluorescens* in this study was no better than the control for increasing plant growth. *Pseudomonas fluorescens'* inability to overcome *Ralstonia solanacearum* in the 7th week was thought to have disrupted plant growth.

Microbe D had a lower percentage of inhibition rate than microbes A and B, but plant height growth and plant dry weight were higher than controls and microbes B and C, and were not different from microbe A (Table 3). This could be explained because the plant seeds affected by wilt in this study were observed and then removed and not counted again, so that the remaining plants were plants that could survive the wilt attack. Yuliar et al. [6] stated that isolates taken from the rhizospheres of diseased plants could suppress disease more than those from the rhizospheres of healthy plants. Plants that could survive wilt attacks would induce SAR by producing certain hormones to fight *Ralstonia solanacearum* attacks so that plant growth was better [40]. Success in inducing this resistance could occur due to the combination of compatible microbes such as *Bacillus subtilis* and *Pseudomonas fluorescens* in the microbes D. The study [41] stated that although each microbe in a microbial consortium has a different mode of action, combining various compatible microbes in a consortium would broaden the spectrum of activity against diverse pathogens. In addition, consortia of microbes could contribute to PGPR and/or increase the activity of other microorganisms [42]. Thus, microbial consortia were not only related to virulence stability, but also to the application spectrum [14]. Several studies also reported the success of the *Bacillus subtilis* and *Pseudomonas fluorescens* consortium microbes in various plants such as chilies [43], potatoes [9], tomatoes [44], and garlic [45].

4.3 Implications for potato seed production

The results of this research showed that the response of plant seeds to various types of microbes was very diverse. *Bacillus subtilis* UNPAD and consortia *Bacillus subtilis* + *Pseudomonas fluorescens* were the microbes with the best plant growth. However, *Pseudomonas fluorescen*s alone had a low inhibition rate and plant growth. Even though the type of microbe was the same, namely *Bacillus subtilis*, its effect on the growth of cuttings could be different depending on the strain. *Bacillus subtilis,* which could inhibit wilt attacks, may not necessarily increase plant growth as happens with microbe B, and this phenomenon was common in the world of microbiology [46]. Meanwhile, the microbial consortium D, which was initially able to inhibit *Ralstonia solanacearum* but then *Ralstonia solanacearum* increased, showed better growth because it induced a SAR by utilizing the excess properties of *Bacillus subtilis* and *Pseudomonas fluorescens*. Thus, *Bacillus subtilis* UNPAD and the microbial consortium are recommended as effective antagonistic agents for potato seed production.

5. CONCLUSIONS

The application of all microbes could inhibit wilt attacks of *Ralstonia solanacearum* at 55-76%. There were two groups of microbes in response to wilting and plant growth based on partial component analysis value of 69.5%, namely 1) *Bacillus subtilis* Garut and *Pseudomonas fluoresence* and 2) *Bacillus subtilis* UNPAD and the microbial consortium. *Bacillus subtilis* UNPAD and the microbial consortium are recommended as effective antagonistic agents for potato seed production.

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REFERENCES

[1] Dianawati, M., Yulyatin, A. (2021). Alternative potential of various processed potato varieties in the highland. E3S Web of Conferences, 316: 03003. https://doi.org/10.1051/e3sconf/202131603003

[2] Dianawati, M., Hanudin, Hamdani, K.K., Nuryani, W., Sulastrini, I., Haryati, Y., Cartika, I., Rahardjo, I.B., Muharam, A., Rahayuniati, R.F. (2024). Microbial antagonism of *Pseudomonas fluorescens* and *Bacillus subtilis* to overcome *Ralstonia solanacearum* in potato seed production with an aeroponic system in Indonesia. Chilean Journal of Agricultural Research, 84(4): 500-512. https://doi.org/10.4067/S0718-58392024000400500

[3] Maharijaya, A., Syukur, M., Salma, L.N., Sanubary, U.L. (2021). Diversity and performance of eight new promising potato (*Solanum tuberosum*) genotypes in Garut District, West Java, Indonesia. Biodiversitas, 22(5): 2848-2858. https://doi.org/10.13057/biodiv/d220549

[4] Dianawati, M., Cahyaningrum, H., Suwitono, B. (2023). The determination of nitrogen concentration in the G2 potato seed production on fertigation system. E3S Web of Conferences, 425: 01005. https://doi.org/10.1051/e3sconf/202342501005

[5] Sun, Y., Su, Y., Meng, Z., Zhang, J., Zheng, L., Miao, S., Qin, D., Ruan, Y., Wu, Y., Xiong, L., Yan, X., Dong, Z., Cheng, P., Shao, M., Yu, G. (2023). Biocontrol of bacterial wilt disease in tomato using *Bacillus subtilis* strain R31. Frontiers in Microbiology, 14: 1281381. https://doi.org/10.3389/fmicb.2023.1281381

[6] Yuliar, Asi Nion, Y., Toyota, K. (2015). Recent trends in control methods for bacterial wilt diseases caused by *Ralstonia solanacearum*. Microbes and Environments, 30(1): 1-11. https://doi.org/10.1264/jsme2.ME14144

[7] Tahir, H.A.S., Gu, Q., Wu, H., Raza, W., Safdar, A., Huang, Z., Rajer, F.U., Gao, X. (2017). Effect of volatile compounds produced by *Ralstonia solanacearum* on plant growth promoting and systemic resistance inducing potential of Bacillus volatiles. BMC Plant Biology, 17(1): 1-16. https://doi.org/10.1186/s12870-017-1083-6

[8] Elsayed, T.R., Jacquiod, S., Nour, E.H., Sørensen, S.J., Smalla, K. (2020). Biocontrol of bacterial wilt disease through complex Interaction between tomato plant, antagonists, the indigenous rhizosphere microbiota, and *Ralstonia solanacearum*. Frontiers in Microbiology, 10: 2835. https://doi.org/10.3389/fmicb.2019.02835

[9] Dianawati, D., Purnamasari, N.R., Priani, R.A. (2024). Land preparation by Trichoderma and mulch application to increase potato yield. AIP Conference Proceedings, 2957: 040020. https://doi.org/10.1063/5.0184454

[10] Lahlali, R., Ezrari, S., Radouane, N., Kenfaoui, J., Esmaeel, Q., El Hamss, H., Belabess, Z., Barka, E.A. (2022). Biological control of plant pathogens: A global perspective. Microorganisms, 10(3): 1-33. https://doi.org/10.3390/microorganisms10030596

[11] Wang, Z., Luo, W., Cheng, S., Zhang, H., Zong, J.,

Zhang, Z. (2023). *Ralstonia solanacearum* – A soil borne hidden enemy of plants: Research development in management strategies, their action mechanism and challenges. Frontiers in Plant Science, 14: 1-12. https://doi.org/10.3389/fpls.2023.1141902

[12] Dianawati, M., Budiarti, S.W. (2024). Response of processed potato cultivars to biofertilizer application on yield. AIP Conference Proceedings, 2957: 040030. https://doi.org/10.1063/5.0184453

[13] Choudaker, K., Singh, V.K., Kashyap, A.S., Patel, A.V., Sameriya, K.K., Yadav, D., Manzar, N., Kamil, D., Prasad, L., Saharan, M.S. (2024). Evaluating the efficacy of microbial antagonists in inducing resistance, promoting growth, and providing biological control against powdery mildew in wheat. Frontiers in Microbiology, 15: 1419547. https://doi.org/10.3389/fmicb.2024.1419547

[14] Maciag, T., Kozieł, E., Rusin, P., Otulak-Kozieł, K., Jafra, S., Czajkowski, R. (2023). Microbial consortia for plant protection against diseases: More than the sum of its parts. International Journal of Molecular Sciences, 24(15): 1-20. https://doi.org/10.3390/ijms241512227

[15] Mahawer, A.., Dubey, A.K., Awasthi, O.P., Singh, D., Dahuja, A., Sevanthi, A.M., Kumar, A., Goswami, A.K., Sharma, N., Yadav, J., Kesharwani, A.K., Kashyap, A.S., Kulshreshtha, A., Singh, R.P., Morade, A., Sharma, R.M. (2023). Elucidation of physio-biochemical changes in *Citrus* spp. incited by *Xanthomonas citri* pv. citri. Horticulturae, 9(3): 324. https://doi.org/10.3390/horticulturae9030324

[16] Soesanto, l., Mugiastuti, E., Manan, A., Wachjadi, M. (2013). Ability test of several antagonists to control potato bacterial wilt in the field. Agrivita, 35(1): 30-35. https://doi.org/10.17503/agrivita-2013-35-1-p030-35

[17] Raza, W., Ling, N., Yang, L., Huang, Q., Shen, Q. (2016). Response of tomato wilt pathogen *Ralstonia solanacearum* to the volatile organic compounds produced by a biocontrol strain Bacillus amyloliquefaciens SQR-9. Scientific Reports, 6: 24856. https://doi.org/10.1038/srep24856

[18] Soesanto, L., Mugiastuti, E., Khoeruriza. (2019). Granular formulation test of *Pseudomonas fluorescens* P60 for controling bacterial wilt (*Ralstonia solanacearum*) of tomato in planta. Agrivita, 41(3): 513-523. https://doi.org/10.17503/agrivita.v41i3.2318

[19] Miljaković, D., Marinković, J., Balešević-Tubić, S. (2020). The significance of *Bacillus* spp. In disease suppression and growth promotion of field and vegetable crops. Microorganisms, 8(7): 1-19. https://doi.org/10.3390/microorganisms8071037

[20] Kyaw, H.W.W., Iiyama, K., Yonehara, S., Naing, T.A.A. (2022). Occurrence of bacterial wilt disease of ginger caused by *Ralstonia solanacearum* species complex in Myanmar. Journal of the Faculty of Agriculture, Kyushu University, 67(2): 129-132. https://doi.org/10.5109/4797818

[21] Lyng, M., Kovács, A.T. (2023). Frenemies of the soil: Bacillus and Pseudomonas interspecies interactions Trends in Microbiology, 31(8): 845-857. https://doi.org/10.1016/j.tim.2023.02.003

[22] Nasir, S. (2016). Review on major potato disease and their management in Ethiopia. International Journal of Horticulture and Floriculture, 4(5): 239-246.

[23] Clough, S.E., Jousset, A., Elphinstone, J.G., Friman, V.P. (2022). Combining in vitro and in vivo screening to identify efficient Pseudomonas biocontrol strains against the phytopathogenic bacterium *Ralstonia solanacearum*. Microbiologyopen, 11(2): e1283. https://doi.org/10.1002/mbo3.1283

[24] Nguyen, H.T., Pham, T.T., Nguyen, P.T., Dinh, N.C.G., Le, M.T., Nguyen, T.D., Nguyen, T.T., Nguyen, V.B. (2025). Microbial biocontrol in agriculture: From mechanistic understanding to field application. Discover Plants, 2(1): 334. https://doi.org/10.1007/s44372-025-00421-y

[25] Salwan, R., Sharma, M., Sharma, A., Sharma, V. (2023). Insights into plant beneficial microorganism-triggered induced systemic resistance. Plant Stress, 7(7): 100140. https://doi.org/10.1016/j.stress.2023.100140

[26] Rostand, R.C., Joel, M., Viviene, M., Steven, R. (2018). Isolation and characterization of *Ralstonia solanacearum* strains causing bacterial wilt of potato in Nakuru County of Kenya. African Journal of Biotechnology, 17(52): 1455-1465. https://doi.org/10.5897/ajb2018.16659

[27] Lowe-Power, T.M., Khokhani, D., Allen, C. (2018). How *Ralstonia solanacearum* exploits and thrives in the flowing plant xylem environment. Trends in Microbiology, 26(11): 929-942. https://doi.org/10.1016/j.tim.2018.06.002

[28] Kashyap, A.S., Manzar, N., Nebapure, S.M., Rajawat, M.V.S., Deo, M.M., Singh, J.P., Kesharwani, A.K., Singh, R.P., Dubey, S.C., Singh, D. (2022). Unraveling microbial volatile elicitors using a transparent methodology for induction of systemic resistance and regulation of antioxidant genes at expression levels in chili against bacterial wilt disease. Antioxidants, 11(2): 404. https://doi.org/10.3390/antiox11020404

[29] Gao, Z., Zhang, B., Liu, H., Han, J., Zhang, Y. (2017). Identification of endophytic *Bacillus velezensis* ZSY-1 strain and antifungal activity of its volatile compounds against *Alternaria solani* and *Botrytis cinerea*. Biological Control, 105: 27-39. https://doi.org/10.1016/j.biocontrol.2016.11.007

[30] Park, Y.S., Dutta, S., Ann, M., Raaijmakers, J.M., Park, K. (2015). Promotion of plant growth by *Pseudomonas fluorescens* strain SS101 via novel volatile organic compounds. Biochemical and Biophysical Research Communications, 461(2): 361-365. https://doi.org/10.1016/j.bbrc.2015.04.039

[31] Kashyap, A.S., Manzar, N., Rajawat, M.V.S., Kesharwani, A.K., et al. (2021). Screening and biocontrol potential of rhizobacteria native to Gangetic plains and hilly regions to induce systemic resistance and promote plant growth in chilli against bacterial wilt disease. Plants, 10(10): 2125. https://doi.org/10.3390/plants10102125

[32] Radhakrishnan, R., Hashem, A., Abd Allah, E.F. (2017). Bacillus: A biological tool for crop improvement through bio-molecular changes in adverse environments. Frontiers in Physiology. 8: 1-14. https://doi.org/10.3389/fphys.2017.00667

[33] Yadav, D.K., Devappa, V., Kashyap, A.S., Kumar, N., Rana, V.S., Sunita, K., Singh, D. (2023). Boosting the biocontrol efficacy of *Bacillus amyloliquefaciens* DSBA-11 through physical and chemical mutagens to control bacterial wilt disease of tomato caused by *Ralstonia solanacearum*. Microorganisms, 11(7): 1-15. https://doi.org/10.3390/microorganisms11071790

[34] Wu, L., Wu, H.J., Qiao, J., Gao, X., Borriss, R. (2015). Novel routes for improving biocontrol activity of Bacillus based bioinoculants. Frontiers in Microbiology, 6: 1-13. https://doi.org/10.3389/fmicb.2015.01395

[35] Saiyam, D., Dubey, A., Malla, M.A., Kumar, A. (2024). Lipopeptides from Bacillus: Unveiling biotechnological prospects—sources, properties, and diverse applications. Brazilian Journal of Microbiology, 55(1): 281-295. https://doi.org/10.1007/s42770-023-01228-3

[36] Khan, A.R., Ali, Q., Wu, X., Chen, S., Ayaz, M., Tariq, H., Bilal, M.S., Li, L., Mundra, S., Gu, Q., Wu, H., Li, X., Gao, X. (2025). Bacillus species compatibility enhances VOC-mediated systemic resistance against *Botrytis cinerea*. World Journal of Microbiology and Biotechnology, 41(11): 421. https://doi.org/10.1007/s11274-025-04634-x.

[37] Hashem, A., Tabassum, B., Abd_Allah, E.F. (2019). Bacillus subtilis: A plant-growth promoting rhizobacterium that also impacts biotic stress. Saudi Journal of Biological Sciences. 26(6): 1291-1297. https://doi.org/10.1016/j.sjbs.2019.05.004

[38] Istifadah, N., Sapta, D., Krestini, H., Natalie, B., Suryatmana, P., Nurbaity, A., Hidersah, R. (2018). The effect of dosages of microbial consortia formulation and synthetic fertilizer on the growth and yield of field-grown chili. IOP Conference Series: Earth and Environmental Science, 142: 012012. https://doi.org/10.1088/1755-1315/142/1/012012

[39] Soesanto, L., Dwijayanto, H., Mugiastuti, E., Manan, A. (2021). Application of secondary metabolites of two *Pseudomonas fluorescens* isolates to control bacterial wilt of potato. KnE Life Sciences, 7(3): 278-293. https://doi.org/10.18502/kls.v7i3.11130

[40] Lal, M., Kumar, A., Chaudhary, S., Singh, R.K., Sharma, S., Kumar, M. (2022). Antagonistic and growth enhancement activities of native *Pseudomonas* spp. against soil and tuber-borne diseases of potato (*Solanum tuberosum* L.). Egyptian Journal of Biological Pest Control, 32(22): 1-14. https://doi.org/10.1186/s41938-022-00522-w

[41] Kozieł, E., Otulak-Kozieł, K., Rusin, P. (2024). Glutathione-the ‘master’ antioxidant in the regulation of resistant and susceptible host-plant virus-interaction. Frontiers in Plant Science. 15: 1373801. https://doi.org/10.3389/fpls.2024.1373801

[42] Liu, Q., Cheng, L., Nian, H., Jin, J., Lian, T. (2023). Linking plant functional genes to rhizosphere microbes: A review. Plant Biotechnology Journal, 21(5): 902-917. https://doi.org/10.1111/pbi.13950

[43] Resti, Z., Sulyanti, E., Reflin. (2018). Endophytic bacterial consortium as biological control to *Ralstonia solanacearum* and growth promoter for chili plant. Proceedings of the National Seminar on Biodiversity Society Indonesia, 4(2). https://doi.org/10.13057/psnmbi/m040219

[44] Istiqomah, I., Kusumawati, D.E. (2018). Utilization of *Bacillus subtilis* and *Pseudomonas fluorescens* in biological control of *Ralstonia solanacearum* the cause of bacterial wilt disease in tomato. Journal of Agro, 5(1): 1-12. https://doi.org/10.15575/2305

[45] Dianawati, M., Haryati, Y., Ruswandi, A., Sutrisna, N., Susanto, B., Amisnaipa, Saidah, Rahayu, M., Tandi, O.G., Nurawan, A., Surdianto, Y., Ishaq, I., Handoko, S., Rosliani, R. (2022). Increasing production of various garlic local cultivars in off season by gliocompost, Periodicals of Engineering and Natural Sciences, 10(1): 656-665. https://doi.org/10.21533/PEN.V10I1.2744

[46] Yan, P., Ahmad, S., Xu, Z., Jia, H., Zhang, R., Song, J., Manzar, N., Kashyap, A.S., Zhang, W. (2024). Isolation and characterization of *Bacillus* sp. HSY32 and its toxin gene for potential biological control of plant parasitic nematode. Chemical and Biological Technologies in Agriculture, 11(1): 1-12. https://doi.org/10.1186/s40538-024-00720-8