Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
https://pjp.pakps.com

RESEARCH ARTICLE

Storage Duration of *Bacillus* spp . Consortium Formula for Controlling Bacterial Wilt Disease and Increasing Growth and Production of Chile Pepper

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Article History:
Submitted: May 03, 2025; Revised: August 07, 2025; Accepted for Publication: November 25, 2025.

A B S T R A C T

Bacterial wilt disease caused by *Ralstonia syzigii* subsp*. indonesiensis* is an important disease of chili plants that can reduce yields up to 90%. Utilization of *Bacillus* spp*.* consortium in the form of a formula is an alternative control that is cheap and environmentally friendly. The study aimed to obtain a better storage period of *Bacillus* spp*.* consortium solid waste formula for the control of *R. syzygii subsp*. *indonesiensis* and increase the growth and yield of chili plants. The study was structured in to two distinct phases, namely: 1). Formulation of solid waste-based carrier for *Bacillus* spp. consortium. 2). The CFU count and biological control efficacy of the *Bacillus* spp. consortium in the solid waste carrier were assessed at different storage intervals against *R. syzigii* subsp. *indonesiensis*. Phase 2 of the study was conducted using CRD (completely randomized design) consisting of 19 treatments and 4 replications. The formulated solid waste based *Bacillus* spp. consortium was applied to chili seeds and seedlings at the time of sowing and transplanting respectively. Inoculation with *R. syzygii* subsp. *indonesiensis* was performed after 35 days of planting. The observed variables include the viability of solid waste formula of *Bacillus* spp*.* consortium, disease development, seedling and plant growth and yield of chili plants. The results showed that 9 solid formulas of *Bacillus* spp*.* consortium that has the potential to control *Ralstonia syzigii subsp. indonesiensis* disease are Bran + Sugarcane Dregs (4 weeks' storage), Bran + Rice Straw (4 weeks' storage), Bran + Rice Straw (6 weeks storage), Bran + Sugarcane Dregs + Rice Straw (4 weeks storage). Additionally, some formulations have potential the potential to increase the growth and yield of chili plants are Bran + Sugarcane Dregs (stored 4 weeks), Bran + Sugarcane Dregs (stored 6 weeks), Bran + Rice Straw stored (4 weeks), Bran + Rice Straw (stored 6 weeks), Bran + Rice Straw (stored 8 weeks), Sugarcane Dregs + Rice Straw (stored 4 weeks), Sugarcane Dregs + Rice Straw (stored 8 weeks).

Keywords: *Bacillus* spp . , chili, formulation, *R. syzigii* subsp. *Indonesia* , solid waste, viability.

INTRODUCTION

Chili pepper (*Capsicum annuum* L.) is a major vegetable and spice crop cultivated worldwide and widely consumed in fresh, dried, and processed forms. It contributes substantially to dietary nutrition and farm income, and is considered a high-value horticultural commodity in tropical and subtropical regions (Azizy *et al* ., 2020; Tang *et al.,* 2023; Usman *et al.,* 2025). Global production of chilies and peppers was about 36–42 million tons from approximately 3.7 million ha in 2020, with Asia contributing more than 60% of total output (Poudyal *et al.,* 2023; Tahir *et al.,* 2023). Indonesia is consistently ranked among the top four producing countries for fresh red chili in the world, and chili is grown on more than 130,000 ha nationally, making it one of the largest vegetable crops in terms of area and a key driver of food price stability (Sudardono *et al.,* 2021). The major diseases affecting chili are caused by diverse fungal, viral, and bacterial pathogens remain key constraints to sustainable production. Important fungal diseases include anthracnose caused by *Colletotrichum* spp., which leads to severe fruit losses, and Cercospora leaf spot caused by *Cercospora capsici*, which reduces leaf area and plant vigor (Sharma *et al.,* 2021; Sutomo *et al.,* 2022; Leite *et al.,* 2025). Phytophthora blight and leaf spot caused by *Phytophthora capsici* are also major problems in chili cultivation, particularly under high soil moisture conditions (Muro-Culebras *et al.,* 2024; Ali *et al.,* 2025). In addition, viral diseases such as yellow leaf curl disease, caused by begomoviruses including *Chilli leaf curl virus* and *Pepper yellow leaf curl virus*, frequently result in severe growth suppression and yield losses in chili-growing regions (Shingote *et al.,* 2022; Pandey *et al.,* 2024.) one of which is bacterial wilt by *Ralstonia syzygii* subsp. *indonesiensis* is an important disease in chili plants and causes considerable losses (Begum *et al.,* 2012; Yanti *et al.,* 2018). The commonly recommended approaches to manage bacterial wilt include crop rotation with non-host crops, the use of pathogen-free or disinfected soil and planting material, and the deployment of resistant or tolerant cultivars (Abu Bakar *et al.,* 2025; Matloob *et al.,* 2025)., the use of resistant varieties (Hassan *et al.,* 2010), technical culture measures through sanitation echnical cultural measures such as field sanitation and removal of infected plants, as well as the use of pathogen-free soil and non-host crop rotation, are commonly recommended to reduce bacterial wilt incidence (Ajayasree *et al.,* 2022). Chemical control with bactericides offers only limited effectiveness because the pathogen persists in soil and water (Wang *et al.,* 2023). Therefore, biological control using beneficial microorganisms has been promoted as an alternative and more sustainable management strategy (Azeem *et al.,* 2020; Abu Bakar *et al.,* 2025). One of the biological agents currently developed in controlling plant pathogens is from the Plant Growth Promoting Rhizobacteria (PGPR) group (Yanti *et al.,* 2019), one of which is the *Bacillus* spp . group which functions as a biofertilizer and biopesticide. The application of *Bacillus* spp . singly causes the ability of *Bacillus* spp . to not last long and is not optimal either so it needs to be consorted and formulated so that microbes can maintain their vitality and increase their ability to control pathogens. The purpose of the study was to obtain the length of storage of solid waste formula of *Bacillus* spp . consortium that has the potential to control Ralstonia syzygii subsp. indonesiensis and increase the growth and yield of chili plants.

MATERIALS AND METHODS

Rejuvenation of *Bacillus* spp. : The bacteria were obtained from the collection of Dr. Yulmira Yanti, SSi., MP., produced at the Microbiology Laboratory, Faculty of Agriculture, Department of Plant Protection, Universitas Andalas, namely *B. cereus* strain SLBE3.1AP and *B. toyonensis* strain AGBE 1.2.TL from microtubes were rejuvenated by the scratch method on TSA medium, then incubated for 2 x 24 hours.

Gram test: One colony of *Bacillus* spp*.* from overnight grown culture was taken using a chair and then placed on a glass object and mixed with one drop of 3% KOH solution (Schaad *et al.,* 2001). The results of the Gram test of *Bacillus* spp*.* is Gram positive is characterized by a mixture of bacteria that does not thicken.

Hypersensitive Reaction Test (HR): Test was conducted by following the method of Klement *et al.* (1990), *Bacillus* spp*.* isolates were suspended (10 ^{8} CFU/ml) and infiltrated into the lower tissue of Mirabilis jalapa leaves using a syringe. Furthermore, the infiltrated leaves were covered with plastic for 2 x 24 hours in conditions of incubation (Yanti, 2017). *Bacillus* spp*.* showed a negative hypersensitivity reaction (HR) characterized by the absence of necrosis symptoms on the leaves.

Compatibility Test between Different spp. of *Bacillus*: *Bacillus* spp . compatibility test using the cross streak method. Two *Bacillus* isolates (*B. cereus* strain SLBE3.1AP and *B. toyonensis* strain AGBE1.2.TL) were scratched by crossing (vertically and horizontally) on a Petri dish containing TSA media, then incubated for 2x24 hours at room temperature. Furthermore, the inhibition zone that appeared between isolates was observed (Nurbailis *et al.,* 2023 Figure 1.

Fiqure 1. Compatibility test *of B. cereus* strain SLBE3.1AP and *B. toyonensis* strain AGBE1.2.TL (2x24 hours) which showed no growth inhibition (Clear Zone).

Preparation of *Bacillus* spp. Consortium: *Bacillus* spp. consortium was prepared through a two stage formulation process. In the first stage of pre-culture, a single colony of each *Bacillus* spp. isolates was inoculated into 10mL Nutrient Broth and incubated on a rotary shaker at 150 rpm for 24 hours at 28 ±2°C. In the second stage, main-culture of *Bacillus* spp . consortium was made by combining two compatible *Bacillus* species. Furthermore, 1 ml of liquid culture of each *Bacillus* species (pre-culture results) was transferred into 23 ml of sterile coconut water in a culture bottle (50 ml) and incubated for 2x24 hours on a rotary shaker at 150 rpm at temperature (Yanti *et al.,* 2020). To determine the density of the bacterial population, the turbidity of the bacterial suspension was compared with McFarland scale 8 solution with a population density of 10^{8} CFU/ml. (Yanti *et al.,* 2018).

***Bacillus* spp. Consortium Solid Waste Formula: Preparation of Carrier Materials:** Solid waste carrier materials are bagasse, bran, and rice straw, which are pulverized using a blender. Each carrier material was put into a 100 ml Schott bottle as much as 9.5g. Then 0.5 grams of sucrose (5% of the total weight of the media) was added to each formula and sterilized by autoclaving at 121°C and 1 atm pressure for 15 minutes. After that, the formula material was cooled and 5 ml of main-culture suspension of 10^{8} CFU/ml was added.

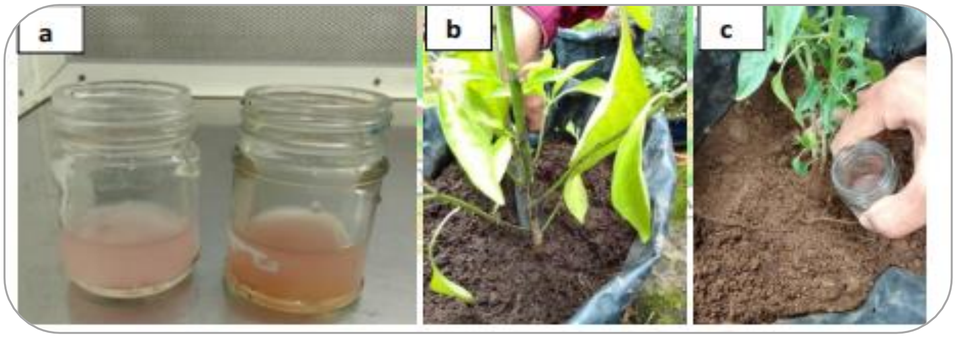
Viability Test of *B. cereus* strain SLBE3.1AP and *B. toyonensis* strain AGBE1.2.TL. One gram of each solid formulation was suspended in 9mL of sterile distilled water and subjected to serial ten-fold dilutions (10^{-1} to 10^{-8}). From dilutions 10-7 and 10-8, 1 ml was transferred, poured into liquid TSA medium in a test tube and then homogenized with a vortex. After that, pour on petridish and incubate for 2 x 24 hours. The number of colonies that appeared was counted with a colony counter (Yanti *et al.,* 2017).

The results of the viability test of each formula of *B. cereus* strain SLBE3.1AP and toyonensis strain AGBE1.2.TL based on carrier materials stored for different times (without storage, 4, 6, and 8 weeks) were used as a reference formula to be used during planting. The basis for using the formula is seen from the highest number of bacterial colonies that can survive with a minimum population of 10^{8} CFU/ml on each medium that has been stored for different times (without storage, 4, 6, and 8 weeks).

Preparation of Planting Media: The planting medium used was a mixture of soil and manure (2:1 v/v). The planting medium was put in a plastic bag and then sterilized in a boiler for 1 hour at 100 °C. The planting medium was put 5 g into a pot tray for nursery and 10 kg into polybags for planting (Yanti *et al.,* 2018).

Introduction of *Bacillus* spp . consortium formula: *Bacillus* spp . consortium formula was introduced twice to chili seeds and seedlings. Chili seeds were surface sterilized using 1% NaOCl for 1 minute, then dried. Next, the seeds were soaked with each formulation (Figure 7a) for 15 minutes, positive control and negative control were soaked with sterile distilled water and soaked with active ingredient Streptomycin sulfate 20%. Chili seeds were sown as many as 2 seeds per pot tray hole. Maintenance was carried out for 21 days. Chili seedlings aged 21 (DAP) were removed from the pot tray then the soil attached to the roots was washed with running water and soaked in 100 ml of *Bacillus* spp . consortium formula for positive control and negative control soaked with sterile distilled water. The next treatment was given an antibiotic made from the active ingredient Streptomycin at a concentration of 0.2% (Ashmawy, *et al.* 2021).

Isolation and Inoculation of *R. syzygii* subsp. indonesiensis: Diseased chili plants exhibiting bacterial wilt symptoms were collected from naturally infected field sites. Isolation of *R. syzygii* subsp. *indonesiensis* was carried out by following method of Lu *et al.* (2021)). Bacterial ooze was extracted from vascular tissues by transversely cutting the base of wilted stem and suspending the cut end in sterile distilled water for 10 minutes to allow bacterial streaming. The bacterial mass was then cultured purely streaked on TZC medium, to determine the virulent colonies of *R. syzygii* subsp. *indonesiensis*, then concentrated for 2x24 hours. Pure isolates of bacteria were observed for their characteristics, like milky white in color and pink to red in the middle. *R. syzygii* subsp. *indonesiensis* was inoculated on chili plants aged 35 HST, by cutting the roots on two sides of the plant 5 cm away from the stem (Figure 2b). After that, 30 mL of suspension of *R. syzygii* subsp. indonesiensis with a population of 10^{7} CFU/ml (Figure 2a) was poured onto the plants (Figure 2c) (Perera *et al.,* 1992).



Fiqure 2. Inoculation of *R. syzygii subsp. Indonesiensis* (a) 30 mL of bacterial suspension *of R. syzygii* subsp. *Indonesiensis* with a population density of 10 ^{7} CFU/ml (b) the roots of chili plants were cut on two sides with a distance of 5 cm from the stem (c) bacterial suspension *of R. syzygii* subsp. *Indonesiensis* with a population density of 10 ^{7} CFU/ml was poured onto the plants

Maintenance and Harvesting: Plant maintenance includes watering, staking, weeding, and fertilizing. Plants are watered twice a day in the morning and evening. Stakes were installed when the plants were 3 weeks old. Weed management was performed weekly by manual removal of weeds. Chili fruits were harvested starting at 15 weeks after planting, when 75% of fruit attained physiological maturity (red coloration), with subsequent harvest at 5-7 weeks' intervals (Yuliana *et al.,* 2023). Chili fruit is harvested along with the stalk manually (by hand). The post- harvest chili fruits were classified into damaged (diseased) and intact ones (healthy) groups.

Viability of *Bacillus* spp. Consortium Solid Waste Formula: Observations of bacterial population density in Solid formula stored for no storage, 4, 6, and weeks were calculated using the formula (Klement *et al.,* 1990).

Disease Development: Incubation Period: The incubation period was observed every day after inoculation of *R. syzigii* subsp. *indonesiensis* until the plants showed the first symptoms of bacterial wilt in chili. The effectiveness of suppressing the incubation period was calculated using the formula of Yanti *et al.,* (2018).

Disease incidence: Disease incidence is the proportion of pathogen-affected plants in a plant population. Disease incidence was observed after inoculation at 1 week intervals. Disease incidence was observed until one of the controls died. Disease incidence was observed every day after inoculation until the appearance of visual symptoms of attack. Disease incidence was calculated using the following Formula 1:

$$\mathrm{DI} = \frac{\mathrm{A}}{\mathrm{B}}\, x\; 100\ (\%)$$

Where :

DI : disease incidence (%),

A : number of plants infected with stalk rot, and

B : number of plants observed in each line

Disease Severity: Disease severity was determined by observing plants with wilt symptoms. Observations were counted on the same day as disease incidence. Disease severity was calculated until one of the controls died. The percentage of disease severity can be calculated using the Formula 2:

$$\mathrm{DS} = \frac{\sum(\mathrm{n\ x\ v})}{\mathrm{Z\ x\ N}}\, x\; 100\ (\%)$$

where

DS : disease severity

N : number of infected plants in each category;

V : scale value on each affected plant;

Z : highest scale value;

N : number of plants observed in each treatment.

The resistance categories of the test genotypes to s

Growth of Chili Seedlings: Seed Germination Power of Chili: Seed germination test was conducted using the Bhat, *et al.,* 2024. This test was conducted in the laboratory using stencil paper that had been sterilized. Seeds were soaked in a suspension of endophytic bacterial consortium for 10 minutes at a density of 10 ^{8} CFU/ml. Chili seeds were arranged in 1 stencil paper that had been moistened first as many as 50 seeds with an arrangement of 10 x 5, then the seeds were covered again using moist stencil paper.

Seedling Height (cm): Seedling height was measured when the seedlings emerged into the field and were 7 days old. Seedling measurements start from the base of the stem to the highest point of the body. Seedling height was observed for 3 weeks in 1 week intervals. The effectiveness of each treatment was calculated using Formula 3.

$$\mathrm{E} = \frac{\mathrm{P} - \mathrm{K}}{\mathrm{K}}\, x\; 100\ (\%)$$

Where :

E = Effectiveness (%)

P = Treatment

K = Positive control

Number of Leaves (strands): Observed when the first leaf appears every 1 week until the first flower appears. Leaves that had been included previously were added to the newly counted leaves and each growing leaf was marked. Field emergence of chili seedlings observed every day until 21 days after planting (DAP) to evaluate the seed vigor and establishment under field conditions. The effectiveness of treatments in improving seedling emergence was determined according to Sivan and Chet (1986) as modified by Yanti *et al.,* (2018) using Formula 3.

$$E = \frac{P - K}{K}\, x\, 100\ (\%)$$

Where :

E = Effectiveness (%)

P = Treatment

K = Positive control

Root Length (cm): Three week after seedling emergence, the seedlings were removed from the pot tray and cleaned from the planting medium. The number of seedlings observed was 4 seedlings for each treatment. Measurements were taken from the base of the root to the growing point of the longest root. The effectiveness of each treatment was calculated using Formula 3.

Fresh Weight and Dry Weight (g): Seedlings were cleaned from the planting medium and weighed to obtain fresh weight. To measure dry weight, the seedlings were wrapped in stencil paper and dried in an oven at 30 °C for 5 hours and weighed (until the weight was constant). The effectiveness is calculated using Formula 3.

Plant Growth: Vegetative Phase: Plant Height (cm): Measured from the base of the stem to the highest growing point. Plant height was observed once every 1 week starting after the plants were 14 DAP until the plants flowered. The effectiveness of each was calculated using Formula 3.

Number of Leaves (strands): The number of leaves was observed when the first leaf appeared, counted once every 1 week for 4 weeks. Previously counted leaves were added to the newly counted leaves and each growing leaf was marked. The effectiveness of each was calculated using Formula 3.

Generative phase: First flower appearance (strands): Observations of the first flower appearance were made on the first day after the appearance of flowers on each plant until 4 weeks of observation. Treatment effectiveness was calculated using Formula 3.

Number of fruits: The number of fruits was observed by counting the fruits in each treatment after harvest. The effectiveness of each treatment was calculated using Formula 3.

Fruit Weight (g): The harvested chili fruits were weighed and summed up after each harvest. The effectiveness of each treatment was calculated using Formula 3.

DATA ANALYSIS

Data were analyzed by variance analysis, using the SPSS if significantly different followed by DNMRT at the 5% level.

RESULTS AND DISCUSSION

Viability of *Bacillus* spp. consortium solid waste formula: Viability of *Bacillus* spp. consortium in combination with Solid Formula with different storage lengths, showed a relatively stable population every week with density of 10^9 CFU/ml from no storage to 8 weeks of storage. The initial population of *Bacillus* spp. consortium amounted to 2.01×10^9 CFU/ml (Figure 3).

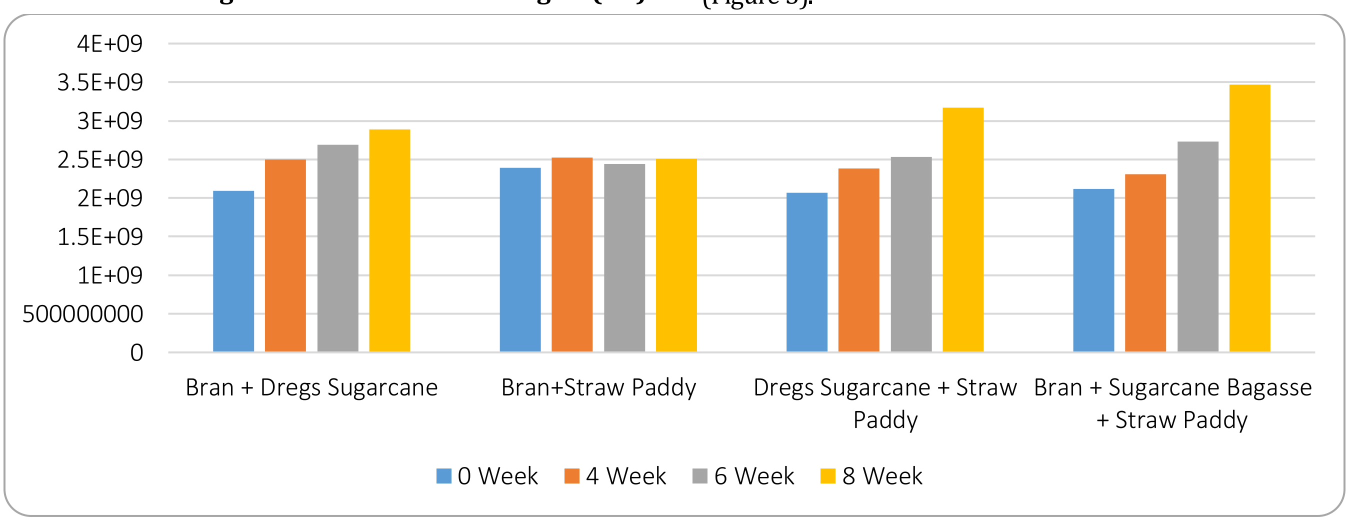


Figure 3. Population density of solid waste formula of *Bacillus* spp. consortium with different storage lengths

The ability of solid waste formula of *Bacillus* spp. consortium to control bacterial wilt disease in chili plants

Disease progress: Incubation period (days after inoculation): Chili plants introduced by bacterial formulas *B. cereus* strain SLBE3.1AP and *B. toyonensis* strain AGBE1.2.TL with different storage lengths showed significantly different results to the variance analysis. After the DNMRT test at the 5% significance level, the results are presented in Table 1. *Bacillus* spp. consortium formulations significantly prolonged the incubation period of bacterial wilt compared to the negative control. The incubation period ranged from 36.75 to 42.00 hours across treatments whereas the negative control exhibited the shortest incubation period of 12.25 hours.

Table 1. Incubation period of bacterial wilt disease in chili plants introduced with the solid waste formula of *Bacillus* spp. consortium with different storage lengths.

Treatment	Strored (weeks)	Incubation period (dpi)	Disease incidence (%)	Disease severity (%)	Criteria
Streptomycin	-	38.50 a	100.00 a	100.00 a	Heavy
control -	-	12.25 b	100.00 a	19.50 b	Light
Bran + Bagasse + Rice Straw	0	40.25 a	50.00 ab	19.14 b	Light
Basasse + Rice Straw	0	38.50 a	50.00 ab	18.55 b	Light
Bran + Rice Straw	0	38.50 a	50.00 ab	11.92 b	Light
Bran + Bagasse	0	36.75 a	25.00 ab	5.50 b	Light
Bran + Bagasse + Rice Straw	4	42.00 a*-	25.00 ab	0.98 b	Light
Basasse + Rice Straw	4	42.00 a*-	0.00 b	0.00 b	Light
Bran + Rice Straw	4	42.00 a*-	0.00 b	0.00 b	Healthy
Bran + Bagasse	4	42.00 a*-	0.00 b	0.00 b	Healthy
Basasse + Rice Straw	6	42.00 a*-	50.00 ab	9.98 b	Healthy
Bran + Rice Straw	6	42.00 a*-	50.00 ab	5.43 b	Light
Bran + Bagasse	6	40.25 a	25.00 ab	1.09 b	Light
Basasse + Rice Straw	6	36.75 a	0.00 b	0.00 b	Light
Bran + Rice Straw	8	38.50 a	75.00 ab	13.16 b	Healthy
Bran + Bagasse	8	38.50 a	50.00 ab	9.58 b	Light
Bran + Bagasse + Rice Straw	8	36.75 a	50.00 ab	6.56 b	Light
Bran + Bagasse + Rice Straw	8	36.75 a	25.00 ab	5.07 b	Light

1. (*-) Indicates that the plant did not show symptoms until the last day of observation (42 dpi).
2. Numbers followed by the same lowercase letter in the same column are not significantly different according to DNMRT at the 5% level.

Disease Occurrence: Chili plants introduced with *Bacillus* spp. consortium formula with different storage lengths showed significant differences according to variance analysis. After the DNMRT test at the 5% level, the results can be seen in Table 1. *Bacillus* spp. consortium formula was able to suppress the incidence of bacterial wilt disease and significantly different from the negative control. Notably four formulations achieved complete suppression (0.00% disease incidence), Bran + Rice Straw (4 and 6 weeks storage), Bran + Sugarcane Dregs + Rice Straw (4 weeks storage), and Bran + Sugarcane Dregs (4 weeks storage). These treatments were significantly superior to both the negative control and streptomycin (both 100% incidence).

Disease Severity: The results of the ANOVA, further confirmed by the 5% DNMRT test, indicated that storage duration of the *Bacillus* spp. consortium formulations significantly influenced chili seedling growth (Table 1). *Bacillus* spp. consortium formulas significantly suppressed bacterial wilt disease severity compared to the positive control (streptomycin) . All tested formulas—Bran + Rice Straw, Bran + Sugarcane Dregs, Sugarcane Dregs + Rice Straw, and Bran + Sugarcane Dregs + Rice Straw—across all storage periods (0, 4, 6, and 8 weeks) showed disease severity values of 0.00–19.14%, while the streptomycin exhibited 100% severity (Table 1)

Growth of Chili Seedlings Sprouting Power of Chili Seeds: The solid waste formula of *Bacillus* spp. consortium was able to increase the germination of chili seeds. Sugarcane Dreg + Rice Straw formula with 0, 4, and 8 weeks storage, Bran + Sugarcane Dreg + Rice Straw without storage, Bran + Sugarcane Dreg with 4 and 6 weeks storage, and Bran + Rice Straw with 4, 6 and 8 weeks storage have the potential to increase seed germination with 96.00% germination rate (Figure 4).

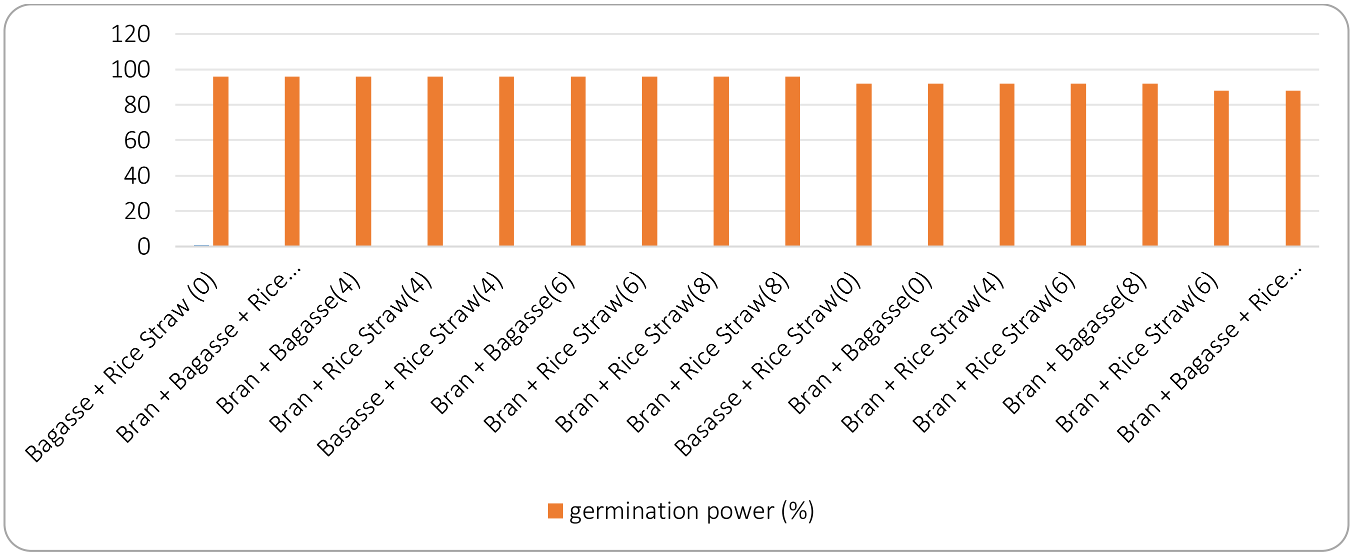


Figure 4. Growth of Chili Seedlings Sprouting Power of Chili Seeds.

Field Emergence: Solid waste formula of *Bacillus* spp. consortium was able to increase the field emergence of seeds. The formulation of Bran + Sugarcane Dregs with 4 and 6 weeks storage, Sugarcane Dregs + Rice Straw with 4 weeks and 8 weeks storage, and Bran + Rice Straw with 4, 6, and 8 weeks storage have the potential to increase the field emergence of seeds with an effectiveness of 25.00% (Table 2).

Table 2. Field emergence and seedling height of chili seeds introduced with solid waste formula of *Bacillus* spp . consortium with different storage duration.

Treatment	Storage period (weeks)	Field emergent power (%)	Height (cm)	Effectiveness (%)
Bran + Dregs Sugarcane	4	100.00	11.55 a	25.00
Dregs Sugarcane + Rice Straw	4	100.00	11.55 a	25.00
Bran + Straw Paddy	4	100.00	11.50 a	25.00
Bran + Dregs Sugarcane	6	100.00	11.48 a	25.00
Bran + Straw Paddy	6	100.00	11.45 a	25.00
Bran + Straw Paddy	8	100.00	11.28 a	25.00
Dregs Sugarcane + Rice Straw	8	100.00	11.23 ab	25.00
Bran + Straw Paddy	0	96.00	10.88 abc	20.00
Bran + Dregs Sugarcane + Straw Paddy	4	96.00	10.88 abc	20.00
Bran + Dregs Sugarcane + Straw Paddy	6	96.00	10.88 abc	20.00
Bran + Dregs Sugarcane	8	96.00	11.20 ab	20.00
Bran + Dregs Sugarcane	0	92.00	10.70 abc	15.00
Dregs Sugarcane + Rice Straw	0	92.00	10.63 abc	15.00
Bran + Dregs Sugarcane + Straw Paddy	0	92.00	10.33 bc	15.00
Dregs Sugarcane + Rice Straw	6	92.00	10.88 abc	15.00
Bran + Dregs Sugarcane + Straw Paddy	8	88.00	10.33 bc	10.00
Streptomycin	-	83.00	10.05 cd	3.75
Control +	-	80.00	9.33 d	0.00

Numbers followed by the same lowercase letter in the same column are not significantly different according to DNMRT at the 5% level.

Seedling Height (cm): The *Bacillus* spp. consortium solid waste formulas significantly increased chili plant height compared to the positive control. Eight formulas—including combinations of Bran, Sugarcane Dregs, and Rice Straw at various storage periods (0, 4, 6, and 8 weeks)—produced seedling heights of 11.20–11.55 cm, while the positive control showed the lowest height at 9.33 cm (Table 2).

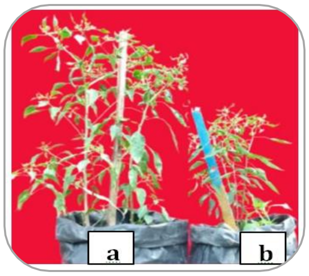


Figure 5. Comparison of the height of chili plants introduced by solid waste formula of *Bacillus* spp . consortium (35 hst) (a) Bran + Sugarcane Tooth 4 weeks storage, (b) Positive control Solid waste formula of *Bacillus* spp .

Number of Seedling Leaves (strands): Chili seeds introduced with the waste formula in the *Bacillus* spp . Consortium with different storage lengths showed significantly different based on variance analysis CFU (Figure 5). The waste formula in the *Bacillus* spp . consortium is able to increase the number of leaves of chili seedlings and is significantly different from the positive control. the formula of Bran + Rice Straw for 4 weeks storage, Bran + Sugarcane Dregs + Rice Straw without storage, 4 weeks, and 8 weeks has the potential to increase the number of leaves of seedlings and is significantly different from the positive control and streptomycin.

Seedling Root Length (cm): Chili seeds introduced with solid waste formula of *Bacillus* spp. consortium with different storage lengths showed 6.25-7.00 leaves lowest number of leaves of chili plant seedlings is found in the positive control, which is 5.25 leaves. After the DNMRT test at the 5% level, the results can be seen in Table 3.

The solid-waste *Bacillus* spp. consortium formulations significantly enhanced chili seedling root number and length, with all effective combinations (Sugarcane Dregs + Rice Straw; Bran + Sugarcane Dregs + Rice Straw; Bran + Rice Straw; and Bran + Sugarcane Dregs at 4–8 weeks storage) producing root lengths of 3.45–4.75 cm, compared with only 2.18 cm in the positive control +.

Fresh Weight and Dry Weight (g) : Chili seeds that are introduced to the solid waste formula of *Bacillus* spp . consortium with different storage the results can be seen in Table 3.

Table 3. Root length and Fresh weight of chili seedlings introduced with solid waste formula of *Bacillus* spp . consortium with different storage lengths.

Treatment	Storage (weeks)	Root length (cm)	Fresh whey (g)
Dregs Sugarcane + Straw Paddy	4	4.75 a	0.54 a
Bran + Dregs Sugarcane + Straw Paddy	4	4.50 ab	0.53 ab
Dregs Sugarcane + Straw Paddy	4	4.50 ab	0.52 abc
Bran + Straw Paddy	6	4.50 ab	0.51 abc
Bran + Dregs Sugarcane + Straw Paddy	6	4.50 ab	0.48 abc
Bran + Dregs Sugarcane	8	4.33 abc	0.42 abcd
Bran + Dregs Sugarcane	8	4.25 abcd	0.42 abcd
Bran + Straw Paddy	8	3.55 abcde	0.41 cde
Bran + Dregs Sugarcane	4	3.45 bcde	0.41 cde
Bran + Dregs Sugarcane + Straw Paddy	6	3.33 bcdef	0.40 cde
Dregs Sugarcane + Straw Paddy	0	3.25 bcdef	0.40 cde
Bran + Dregs Sugarcane	6	3.25 bcdef	0.35 de
Bran + Straw Paddy	0	3.20 cdef	0.32 de
Bran + Straw Paddy	0	3.15 cdef	0.32 de
Dregs Sugarcane + Straw Paddy	0	3.03 def	0.32 de
Bran + Dregs Sugarcane + Straw Paddy	8	3.00 def	0.31 de
Streptomycin	-	2.35 ef	0.30 de
Control +	-	2.18 f	0.29 e

Numbers followed by the same lowercase letter in the same column are not significantly different according to DNMRT at the 5% level.

The *Bacillus* spp. consortium solid waste formulas significantly increased seedling fresh weight compared to the positive control and Streptomycin. Formulas combining Bran, Sugarcane Dregs, and Rice Straw at 4, 6, and 8 weeks storage achieved fresh weights of 0.42–0.54 g, while the positive control showed the lowest value at 0.29 g.

Plant Growth: Vegetative Phase: Plant Height (cm): Chili plants that were introduced to the solid waste formula of *Bacillus* spp . consortia with different storage lengths showed significantly different according to the analysis of variance Table 4.

Table 4. Dry weight and height of chili seedlings introduced with solid waste formula of *Bacillus* spp . consortium with different storage duration.

Treatment	Storage (weeks)	Dry weight (g)	Height (cm)
Bran + Dregs Sugarcane	4	0.34 a	51.25 a
Bran + Straw Paddy	0	0.21 abcd	47.45 ab
Bran + Dregs Sugarcane + Straw Paddy	4	0.34 a	45.75 abc
Dregs Sugarcane + Straw Paddy	8	0.18 bcd	45.63 abc
Dregs Sugarcane + Straw Paddy	6	0.28 ab	43.65 abc
Bran + Dregs Sugarcane + Straw Paddy	0	0.19 bcd	43.05 abc
Dregs Sugarcane + Straw Paddy	0	0.18 bcd	42.78 abc
Dregs Sugarcane + Straw Paddy	4	0.27 ab	42.75 abc
Bran + Dregs Sugarcane	8	0.18 bcd	42.50 abc
Bran + Dregs Sugarcane	0	0.17 bcd	41.70 bc
Bran + Dregs Sugarcane	6	0.18 bcd	40.50 bc
Bran + Dregs Sugarcane + Straw Paddy	6	0.18 bcd	40.50 bc
Bran + Straw Paddy	6	0.16 bcd	39.50 bc
Bran + Dregs Sugarcane + Straw Paddy	8	0.16 bcd	39.25 bc
Bran + Straw Paddy	8	0.17 bcd	39.25 bc
Bran + Straw Paddy	4	0.26 abcd	36.75 c
Streptomycin	-	0.16 bcd	29.75 c
Control +	-	0.11 cd	26.18 d

Numbers followed by the same lowercase letter in the same column are not significantly different according to DNMRT at the 5% level.

Bacillus spp. consortium in solid waste carriers significantly enhanced seedling dry weight compared to the positive control. Five formulations, Bran + Sugarcane Dregs for (4 weeks storage), Bran + Sugarcane Dregs + Rice Straw (for 4 weeks storage), Sugarcane Dregs + Rice Straw (for 4 weeks and 6 weeks storage) recorded dry weight between 0.27-0.34g. The lowest dry weight is obtained from the positive control which is 0.10g.

Solid waste formula of *Bacillus* spp . consortium is able to increase the number of leaves of chili plants and is significantly different from the positive control. formula of Bran + Sugarcane Dregs 4 weeks storage, Bran + Sugarcane Dregs + Rice Straw without storage, Sugarcane Dregs + Rice Straw 4 weeks storage has the potential to increase the number of leaves of chili plants compared to the positive control and streptomycin with a range of 69.00-74.00 leaves. The lowest number of leaves of chili plants is found in the negative control with 55.00 leaves.

Generative Phase: First Flower Appearance (day): Chili plants introduced with solid waste formula of *Bacillus* spp . Consortium with different storage lengths showed no significant difference based on variance analysis. The solid waste formula of *Bacillus* spp . consortium was able to accelerate the first flower appearance of chili plants and was not significantly different from the positive control. all formulas have the potential to accelerate the first flower appearance compared to the positive control and streptomycin, the first flower appearance range is 42.00-50.75 days with an effectiveness of 3.33% - 20%. The lowest first flower appearance was obtained from the positive control which was 52.50 days with an effectiveness of 0.00%. In (Figure 2), it can be seen that the comparison of the first flower appearance of chili plants introduced by the solid waste formula of *Bacillus* spp . consortium is better than positive control.

Number of Fruits: Chili plants introduced with solid waste formula of *Bacillus* spp . consortium with different storage duration showed The solid waste formula of *Bacillus* spp . consortium is able to increase the number of chili fruits and is significantly different from the positive control. formula of Sugarcane Dreg + Rice Straw without storage, Bran + Rice Straw 4 weeks storage, Sugarcane Dreg + Rice Straw 4 weeks storage is a formula that has the potential to increase the number of chili fruits compared to the positive control and streptomycin with a range of 41.50-61.50 chili fruits. The lowest number of chili fruit was found in the positive control which was 23.50 fruit. In (Figure 4), it can be seen that the comparison of the number of chili fruits introduced by the solid waste formula of *Bacillus* spp . consortium is better than the positive control Figure 6.

Figure 6. Comparison of the number of chili fruits intoduced by *Bacillus* spp . concortium solid waste formula (a). Positive control, (b). Sugarcane dregs + rice straw treatment 4 weeks storage.

Chile plants introduced with solid waste formula of *Bacillus* spp . consortium with different storage duration in Table 5

Table 5. Weight of chili fruit introduced with solid waste formula of *Bacillus* spp . consortium with different storage lengths.

Treatment	Storage (weeks)	Fruit weight (g/plants)	Fruit weight (t/ha)
Sugarcane Bagasse + Rice Straw	4	143.41 a	9.58
Bran + Sugarcane Bagasse + Rice Straw	4	142.20 a	9.5
Bran + Rice Straw	4	134.56 ab	8.99
Sugarcane Bagasse + Rice Straw	8	134.45 ab	8.98
Bran + Sugarcane Bagasse + Rice Straw	0	134.22 ab	8.97
Bran + Rice Straw	6	131.58 ab	8.79
Bran + Sugarcane Bagasse + Rice Straw	6	128.98 abc	8.62
Bran + Rice Straw	8	128.02 abcd	8.55
Bran + Sugarcane Bagasse + Rice Straw	8	125.28 abcd	8.37
Sugarcane Bagasse + Rice Straw	0	123.85 abcd	8.27
Bran + Sugarcane Bagasse	4	120.42 abcd	8.04
Sugarcane Bagasse + Rice Straw	6	116.40 bcde	7.78
Bran + Sugarcane Bagasse	6	114.65 bcde	7.66
Bran + Rice Straw	0	105.81 cde	7.07
Bran + Sugarcane Bagasse	0	104.57 cde	6.99
Bran + Sugarcane Bagasse	8	103.08 de	6.89
Streptomycin	-	102.50 de	6.85
Control +	-	93.00 e	6.21

Solid waste formula of *Bacillus* spp . concortium was able to increase the weight of chili fruit and significantly different from the positive control. Eleven formulas showed a significantly different effect from the positive control and Streptomycin, namely Sugarcane Dreg + Rice Straw storage 4 weeks and 8 weeks, Bran + Sugarcane Dreg + Rice Straw without storage, 4 weeks, and 6

DISCUSSION

Viability of *Bacillus* spp . consortium formula at different storage shows a relatively stable formulation every week at a density of 10 ^{9} CFU/ml (Table 2). It is due to the addition of glucose at various storage, apparently *Bacillus* spp. able to utilize the glucose added in the formulation media which contains nutrients and contains simple elements. According to Bashan *et al.* (2014), the addition of glucose nutrients has a function as an additional food source for bacteria during shelf life. Talk formula with the addition of glucose can maintain the viability of Pseudomonas GanoEB3 until 12 months storage and increase the growth of palm oil. Furthermore, Yanti *et al.* (2017), stated that the viability of RBI isolates formulated with carrier agents of tapioca flour, peat and tofu solid waste showed stability after being stored for up to 8 weeks, besides that it was caused by the difference in the content of nutrients available in the 3 different combinations of carrier materials which influenced the formation of microorganism CFU during the shelf life. Rice bran contains high levels of carbohydrates, proteins, lipids, and minerals, making it a suitable organic substrate for supporting microbial activity and enhancing plant growth (Patil & Khan, 2021; Thakur *et al.,* 2022). Sugarcane bagasse also provides substantial structural carbohydrates mainly cellulose (45%), hemicellulose (28%), lignin (20%), sugar (5%), minerals (1%) and ash (2% which serve as important carbon sources during microbial decomposition (Verma *et al.,* 2020; Singh *et al.,* 2023). Fiber consists weeks, Bran + Rice Straw storage 4 weeks and 6 weeks are formulas that have the potential to increase the weight of chili fruit compared to positive control and streptomycin with a range of fruit weight 128. 98 -143.41 g and 8.62-9.58 t/ha . The lowest chili fruit weight is found in the positive control which is 93.00 g/plant and 6.21 t/ha . Rice straw contains 34–47% cellulose, 21–28% hemicellulose, and 5–20% lignin, making it an effective amendment for improving soil organic matter and nutrient cycling (Zhou *et al.,* 2021; Hassan *et al.,* 2023)

The solid waste formula of *Bacillus* spp . consortium with different storage lengths on chili plants was able to extend the incubation period, reduce the incidence and severity of bacterial wilt disease compared to the negative control (inoculated with *Ralstonia syzigii* subsp. *indonesiensis*) and Streptomycin. six Formulas that have the potential to control *Ralstonia syzigii* subsp. *Indonesiaensis* until the end of observation (42 days)namely the Formula of Bran + Sugarcane Dregs stored 4 weeks, Bran + Rice Straw stored 4 weeks and 6 weeks, Sugarcane Dregs + Rice Straw stored 4 weeks and 6 weeks, Bran + Sugarcane Dregs + Rice Straw stored 4 weeks based on the recapitulation of observation results. This is thought to be due to the presence of nutritional content of the carrier material that supports bacteria in maintaining its population during storage where the completeness of Nutrient availability is essential for the effectiveness of *Bacillus* spp. bioformulations in suppressing plant pathogens . also produces antibiotic compounds and lytic enzymes so that it can inhibit the growth of *Ralstonia syzigii* subsp. *indonesiensis*. According to Jinal *et al.,* (2022) that the use of organic materials (peat flour, rice flour) and inorganic (talc and bentonite) as carriers compounds increases the stability and effectiveness of bioformulations. according to (Caulier *et al.,* 2019; Rabbee *et al.,* 2024), reported that four *Bacillus* spp . formulations were able to inhibit the growth of *X. oryzae* pv *oryzae* which showed that the antibiosis mechanism occurs where *Bacillus* spp . in its metabolic process produced antibiotic compounds which were secreted when the bacteria formed a stationary phase and produced enzymes such as chitinase enzymes, mycobacillin, bacitrasin and others. Furthermore, Yanti *et al.* (2021), stated that the formula of tofu pulp and tofu pulp + corn cob is the best formula in reduce stem base rot disease (*Sclerotium rolfsii*) in tomato plants.

The introduction of solid waste formula of *Bacillus* spp . consortium with different storage lengths on chili seeds can increase seed germination, seed field emergence, seedling height, seedling leaf number, seedling root length, fresh weight and dry weight of chili seedlings compared to the positive control. The eight formulation that have the potential to increase the growth of chili seedlings are, the formula of Bran + Sugarcane Dregs stored 4 weeks and 6 weeks, Bran + Rice Straw stored 4 weeks, 6 weeks and 8 weeks, Sugarcane Dregs + Rice Straw stored 4 weeks and 8 weeks, Bran + Sugarcane Dregs + Rice Straw stored 4 weeks based on the recapitulation of observation results. This is because the *Bacillus* spp bacteria produce growth hormones, increase the availability of nutrients, and the formula has a role in increasing plant growth. Méndez-Gómez, et al 2023, *Bacillus* spp . are also reported as bacteria that induce plant resistance and as Plant Growth Promoting Rhizobacteria (PGPR) that can increase plant growth. According to Ullah *et al.,* 2022 Increased plant growth by treatment with *Bacillus* spp . allegedly because *Bacillus* spp can increase nitrogen fixation, photosynthetic activity, and production of indole acetic acid (IAA) Endophytic bacteria *Bacillus* spp produce growth hormones, such as IAA is thought to be the cause of increasing the speed of root growth of chili plants in the soil. *Bacillus* spp. can stimulate root development by promoting the formation of lateral roots and increasing root density, thereby enhancing the plant's nutrient absorption capacity (Bhattacharyya & Lee, 2022;). Recent studies also show that seed treatment with *Bacillus* spp. can significantly enhance root development, increasing both root length and root biomass during the early seedling (Ansari *et al.,* 2023). In addition to stimulating root growth, *Bacillus* spp. promote overall seedling vigor through improved nutrient uptake and hormonal modulation. In addition to increasing plant growth in the seedling phase, the *Bacillus* spp . consortium formula introduced to chili seedlings is also able to increase chili plant growth in the vegetative phase. The solid waste formula of *Bacillus* spp . consortium that has the potential to increase the growth of chili plants in plant height and number of leaves is the Bran + Sugarcane Dregs formula which is stored for 4 weeks based on the recapitulation of observations. This is because the solid waste formula of *Bacillus* spp . consortium used contains nutrients and produces hormones to increase plant growth in the vegetative phase of chili plants. growth hormones that can stimulate plant growth produced from *Bacillus* spp . such as indoleacetic acid, gibberellic acid, cytokinin, and ethylene. According to Bacon and Hinton (2006), *Bacillus* spp . can increase plant growth by increasing the availability of plant nutrients such as nitrogen, phosphate and other minerals, and stimulate plant growth by producing growth hormones. In accordance with the results of research by Yanti *et al.* (2021), the ability of *B. cereus* strain TLE1.1 to produce growth hormones so that the solid formula of *B. cereus* strain TLE1.1 can increase the growth of tomato plants in the vegetative phase.

The introduction of solid waste formula of *Bacillus* spp . consortium with different storage lengths can increase the growth of chili plants in the generative phase compared to the control. formulas that have the potential to accelerate the appearance of the first flower and increase the number of fruits and fruit weight are the formula of Bran + Sugarcane Dregs stored for 4 weeks, Sugarcane Dregs + Rice Straw stored for 4 weeks based on the reaccumulation of observation results This is because the formula carrier material has nutrients and the role of *Bacillus* spp . consortium as PGPR can produce gibberalin hormone which functions to stimulate flower and fruit formation, and also has the ability as a phosphate solvent. Phosphate elements are more available to be absorbed by plants so that plant growth is optimal and will increase the number and weight of chili plant fruits. Sarker & Kim, 2021; Méndez-Gómez *et al.,* 2023 stating that *Bacillus* spp bacteria also produce other hormones such as auxins, cytokinins, and gibberellins that support plant growth and yield. furthermore Yanti *et al.*(2016), which states that chili plants introduced with PGPR can accelerate the appearance of the first flower and increase the fruit weight of chili plants.

CONCLUSION

Based on the results of the above research, it can be stated that the research results of the solid waste formula of the *Bacillus* spp . consortium have the potential to control *Ralstonia syzygium* subsp. *indonesian* and can increase the growth and yield of chili peppers, namely Bran + Sugarcane Dregs 4 weeks storage, Bran + Sugarcane Dregs 6 weeks storage, Bran + Rice Straw 4 weeks storage, Bran + Rice Straw 6 weeks storage, Bran + Rice Straw 8 weeks storage, Sugarcane Dregs + Rice Straw 4 weeks storage, Sugarcane Dregs + Rice Straw 6 weeks storage, Sugarcane Dregs + Rice Straw 8 weeks storage, Bran + Sugarcane Dregs + Rice Straw 4 weeks storage.

ACKNOWLEDGEMENT

The authors would like to thank the Directorate General of Higher Education through LPPM UNAND following the Agreement Letter for the Implementation of Basic Research Publication Assignments with SK Number 561/UN16.R/XII/KPT/2023 and Contract Number T/2/UN.16.17/PP.Pangan - which has funded this research.

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