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Potential of The Biological Agent *Bacillus spp* in Inhibiting Fusarium Wild Disease and its Effects on The Growth and Production of Cayenne Papper (*Capsicum frutescens* L.)

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Abstract

Cayenne pepper (Capsicum frutescens L.) is a horticultural crop in Indonesia that often has many obstacles. The main obstacle that is often faced is wilt disease caused by the fungus Fusarium oxysporum. Bacillus spp. bacteria have the potential to control the fungus F. oxysporum. The purpose of this study was to determine the ability of Bacillus spp. bacteria in controlling fusarium wilt disease. This research was conducted from August to November 2023 at the Plant Health Laboratory and screen house of the Faculty of Agriculture, National Development University "Veteran" East Java. The study used a Factorial Complete Randomized Block Design with three replications and nine treatments so that there were 27 experimental units. The observation variables included incubation period, disease intensity, plant height, number of leaves, first flower appearance and fruit wet weight. The research data were analyzed using ANOVA analysis of variance procedure to determine whether there is an effect of each treatment. If it is known that there is a significantly different effect from the treatment, it will be continued with the DMRT test at the 5% level. The results showed that the combination of Bacillus spp bacteria and dosage had an effect on the incubation period and disease intensity. The results showed that the combination of Bacillus spp bacteria and doses had an effect on vegetative phase parameters, namely plant height and number of leaves. However, the combination of Bacillus spp and dosage showed that the generative phase parameters did not significantly affect the first flower appearance and fruit wet weight

Keywords: *Bacillus spp, Cayenne pepper, Dosage, Fusarium wild, Growth and production*

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INTRODUCTION

Cayenne pepper (*Capsicum frutescens* L.) is one of the horticultural plants of the vegetable type that is often used in various seasonings. According to (BPS, 2019) cayenne pepper consumption by households increased from 2017 to 2019, namely 390.28 tons, 486.38 tons, and 533.35 tons. This is not proportional to its productivity which according to (BPS, 2019) the productivity of cayenne pepper plants per hectare is still low, namely 4 - 5 tons ha-1 compared to its production potential, which is> 20 tons ha-1. The main obstacle that is often faced in the cultivation of cayenne pepper plants is the disease wilt caused by the fungus *Fusarium oxysporum*. According to (Phabiola *et al.,* 2019) *F. oxysporum* is one type of deadly soil-borne pathogen. In addition (Djajakirana *et al.,* 2022) also stated that the fungus *F. oxysporum* attacks chili plants and results in a decrease in chili production, losses and crop failure of up to 50%. Symptoms that appear due to fusarium wilt disease are characterized by plant leaves that begin to turn pale, especially on the upper leaves then followed by wilting and rolling of the lower leaves, petioles also begin to duck and then become completely wilted (Rusman *et al.*, 2018). In addition, according to Endah *et al.,* (2014) The initial symptom of fusarium wilt disease is the pallor of the leaf bones, especially the upper leaves. Then followed by rolling up the older leaves (epinasty) due to the ducking of the petiole and finally the plant becomes completely wilted.

The utilization of *Bacillus spp*. biological agents as control of fusarium wilt disease has been widely conducted. According to research (Florianus *et al.,* 2020). *Bacillus sp*. isolates from the rhizosphere of pepper plants were able to inhibit the growth of Fusarium sp. with the highest inhibition of 13.92 mm. In addition, (Prihatiningsih *et al.,* 2017) stated that *B. subtilis* B315 at a dose of 1000 ml/8 potato tubers was able to delay the incubation period by 7 days and control bacterial wilt disease with an effectiveness of 64.9%. *B. subtilis* B315 was also able to increase plant growth in leaf area. Research results (Djaenuddin & Muis, 2015) showed that the application of *Bacillus* sp. as a bicontrol agent to control fusarium wilt disease in chili plants carried out by watering around the plant root area as much as 10 ml / plant can increase the best growth with plant height reaching 57.39 cm and can control fusarium wilt disease which is 12.96% and 11.11%.

Bacillus spp. bacteria used consist of Bcz 14, Bcz 16, and Bcz 20 which from previous studies have been conducted research on its potential with the results of the largest inhibition zone in inhibiting bacterial wilt in vitro (Zinidin *et al.,* 2022). Therefore, it is necessary to conduct further research related to the potential of *Bacillus* sp. in inhibiting fusarium wilt *in vivo*. Based on this background, a study was conducted entitled "The Potential of *Bacillus spp*. Biological Agents in Inhibiting Fusarium Wilt Disease and its Effect on the Growth and Production of Cayenne Pepper (*Capsicum frutescens* L.)".

RESEARCH METHODS

This research was conducted from August to November 2023 at the Plant Health Laboratory and screen house of the Faculty of Agriculture, National Development University "Veteran" East Java. The materials used included *Bacillus spp* bacterial isolates with isolate codes Bcz 14, Bcz 16, and Bcz 20 from the collection of Dr. Ir. Yenny Wuryandari, MP., then the pathogenic fungus *Fusarium* sp. came from the collection of the Agrotechnology Plant Health Laboratory, UPN "Veteran" East Java.

Trial Design

This study used a factorial experiment with two factors, the first factor was the dose of *Bacillus spp*. 20 ml/per plant (D1), 25 ml/per plant (D2) and 30 ml/per plant (D3). The second factor is the type of isolate of *Bacillus spp*. Bcz 14 (M1), Bcz 16 (M2), and Bcz 20 (M3). There were 9 treatments and repeated 3 times, the control used was negative control by only giving the pathogenic fungus *Fusarium* sp. then a total of 27 experimental units and arranged using a Randomized Complete Factorial Design (RALF). The research data were analyzed using the ANOVA analysis of variance procedure to determine whether there was an effect of each treatment. If it is known that there is a significantly different effect from the treatment, it will be continued with the DMRT test at the 5% level.

Research Implementation

The population of *Bacillus spp*. bacteria used is 10^9 cfu/ml. the density of *Fusarium* sp. fungi used is 10^6 spores/ml. *In vivo* testing requires 30-day-old cayenne pepper plant seeds or there are already approximately 4-6 leaves. The planting media used for in vivo testing on cayenne pepper plants are soil and compost. The ratio between soil and compost used is 1 : 1.

Treatment and Maintenance

Inoculation of *F. oxysporum* with a density of 10^6 spores/ml as much as 25 ml per plant, and the application of *Bacillus spp*. bacteria were carried out simultaneously at the time of transplanting from the nursery and chili plants aged 30 HSS. Before inoculating the fusarium fungus, the plants were first wounded at the roots. *Bacillus spp*. application was done by sprinkling *Bacillus spp*. suspension at the base of the stem according to the dose treatment. The dose treatment used consisted of doses of 20 ml/plant, 25 ml/plant and 30 ml/plant with a bacterial density of 10^9 cfu/ml.

Observation Variable

Inhibition of *Bacillus spp* against fusarium wilt was done by calculating the incubation period and intensity of fusarium wilt disease. The incubation period of wilt disease caused by *Fusarium oxysporum* was observed from inoculation until the appearance of the first symptoms of Fusarium wilt on chili plants. Observation of disease intensity was done by observing the development of wilt symptoms on the leaves every 1 week until the 30th day after inoculation. Disease severity was assessed using the following scale:

0 = no symptoms
1 = 1-10% of leaves show wilting
2 = 11-30% of leaves show wilting
3 = 31-60% of leaves show wilting
4 = 61-99% of leaves show wilting
5 = 100% of leaves show wilting

The disease index is calculated by the formula (Arwiyanto, 1999).

$$I = \frac{\sum_{i=0}^{k} k.nk}{Z \times N} \times 100\%$$

Description,
I = disease index
k = scale
nk = number of leaves showing symptoms from each attack category
N = total number of plants
Z = highest attack category

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Observation parameters included plant height, number of leaves, first flower appearance and fruit wet weight. Plant height observations were made a week after planting with a one-week interval until the plants entered the generative phase or the first flowers appeared. Plant height was measured from the base of the lowest stem to the highest plant growth point. Observation of the number of leaves was done once a week along with the observation of plant height. The first flower appearance is done every day when the plant has started to appear flowers. The criteria for the first flower appearance is the appearance of cayenne pepper flower buds. Observation of the weight of the fruit per plant is done after harvesting, harvesting begins when the cayenne pepper plants enter the age of 65 HST with the harvest criteria being reddish orange colored fruit.

RESULTS AND DISCUSSION

Incubation Period Based on the results of the analysis of variance, the treatment of a combination of biological agents *Bacillus spp*. and dosing had a significant effect on the incubation period of fusarium wilt disease. The treatment of *Bacillus spp*. bacteria and the dose that can best delay the incubation period is the treatment of *Bacillus spp*. isolate Bcz 20 and a dose of 20 ml/plant. The application of *Bacillus spp*. isolate Bcz 20 and a dose of 20 ml/plant caused a delay in the incubation period of 7 days from the control treatment and was the highest treatment in delaying the incubation period. The treatment of giving *Bacillus spp*. isolate Bcz 20 at a dose of 20 ml/plant was the highest treatment in delaying the incubation period. It is suspected that isolate Bcz 20 can inhibit pathogenic compounds of *Fusarium* sp. more than isolates Bcz 14 and Bcz 16. Zinidin *et al.,* (2022) stated that the results of measuring the diameter of the inhibition zone of *Bacillus spp*. bacteria against the pathogen *Ralstonia solanacearum* showed that isolate Bcz 20 had an inhibition zone of 34.2 mm.

Table 1. Incubation period of fusarium wild disease

Treatment	Average Incubation Period (HST)
Control	6,50 a
M1D1	10,44 bc
M1D2	10,00 b
M1D3	11,28 bc
M2D1	10,67 bc
M2D2	12,17 bc
M2D3	12,00 bc
M3D1	13,56 c
M3D2	12,33 c
M3D3	11,94 bc

Description: M0D0= Without *Bacillus spp* administration, M1D1= *Bacillus spp* Bcz 14 + 20 ml dose, M1D2= *Bacillus spp* Bcz 14 + 25 ml dose, M1D3= *Bacillus spp* Bcz 14 + 30 ml dose, M2D1= *Bacillus spp* Bcz 16 + 20 ml dose, M2D2= *Bacillus spp* Bcz 16 + dose of 25 ml, M2D3= *Bacillus spp* Bcz 16 + dose of 30 ml, M3D1= *Bacillus spp* Bcz 20 + dose of 20 ml, M3D2= *Bacillus spp* Bcz 16 + dose of 25 ml, M3D3= *Bacillus spp* Bcz 20 + dose of 30 ml. The same letter in the same column indicates not significantly different in the 5% DMRT test.

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Increasing the dose in the treatment had no effect on delaying the incubation period for the *Fusarium* sp pathogen. This was proven by the addition of a higher dose to the Bcz 20 isolate treatment with a dose of 30 ml/plant which was no better in delaying the incubation period than the Bcz 20 isolate treatment with a dose of 20 ml/plant. The lowest treatment was shown by the treatment of *Bacillus spp*. isolate Bcz 14 and a dose of 25 ml/plant with a delay in the incubation period of 4 days. The treatment of isolate Bcz 14 at a dose of 25 ml/plant showed the lowest delay in the incubation period, probably because isolate Bcz 14 produced fewer inhibitory compounds than the treatment of isolates Bcz 16 and Bcz 20. . Zinidin *et al.,* (2022) that the results of measuring the diameter of the inhibition zone of Bacillus spp. bacteria against the pathogen *Ralstonia solanacearum* showed that isolate Bcz 14 was 30.33mm. All treatment combinations of *Bacillus spp* bacteria and doses gave a significant effect on the control (without *Bacillus spp* bacteria), see in Table 1.

Based on the results of observations, the delay in the incubation period for fusarium wilt disease in the combination treatment of *Bacillus spp* bacteria and dose is thought to be because *Bacillus spp* bacteria have a secondary metabolite compound, namely the enzyme chitinase, which can slow the development of the disease. This is supported by the statement (Florianus *et al.,* 2020) the ability of *Bacillus* sp. bacteria as antagonistic agents cannot escape the secondary metabolite compounds produced by *Bacillus* sp. in inhibiting the growth of pathogenic fungi, including bacillin, bacitracin, bacillomycin, difficidin, oxidificidin, lecithinase, subtilisin, and fengycin. In addition, the treatment of *Bacillus spp* bacteria, namely isolates Bcz 14, Bcz 16 and Bcz 20, can delay the incubation period due to the ability of the antibiosis mechanism possessed by *Bacillus spp* bacteria. Zinidin *et al.,* (2022) that the antibiotic mechanism of *Bacillus spp*. against *R. solanacearum* with isolate codes Bcz 14 and Bcz 20 has moderate inhibitory effectiveness, where the pathogen *R. solanacearum* is able to grow on day 4 while isolate code Bcz 16 has the least effectiveness with the growth of *R. solanacearum* on day 2. Wildan *et al.,* (2021) state that the length of the incubation period is determined by the pathogen, host plant, and environment.

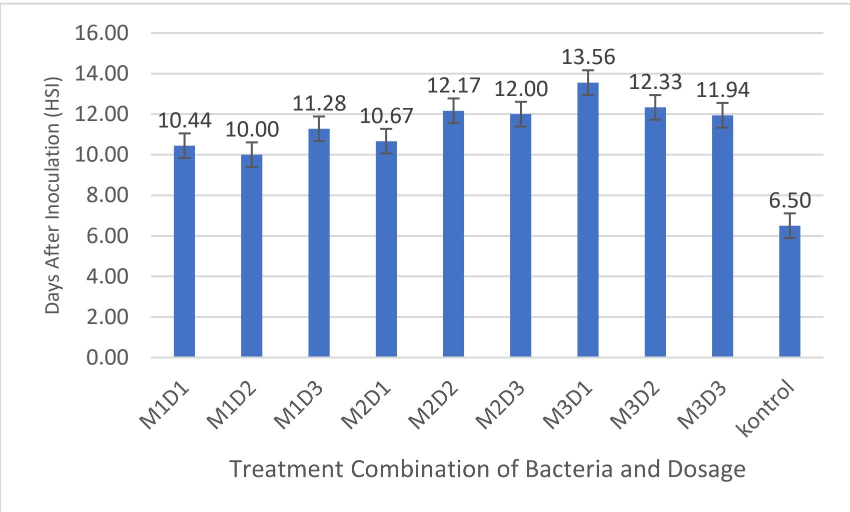

Figure 1. Graph of the Average Incubation Period in Cayenne Pepper Plants

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The graph of the incubation period of *Bacillus spp* bacteria (Figure 1) shows that the control treatment has the lowest incubation period compared to the combination treatment of *Bacillus spp* bacteria and dosage. The control treatment did not apply biological agents so that there was no inhibition of the appearance of symptoms of fusarium wilt disease. Ekowati *et al.*, (2023) revealed that disease development is closely related to the incubation period, pathogen virulence, environmental conditions, and susceptible host plants. In the life cycle of *Fusarium* sp., nutrient requirements are necessary to maintain a spore germination rate of 20-30%. Germination can decrease if there is competition for nutrients with other microorganisms (Ruman *et al.,* 2018).

Disease Intensity

The results of the analysis of variance showed that the treatment combination between *Bacillus spp* bacteria and dosage had a significant effect on the intensity of Fusarium wilt disease in cayenne pepper plants. Observation of disease intensity was carried out for 30 days with 4 observations from week 1 to week 4 (see in Table 2). The disease intensity at week 1 showed that the results were not significantly different. The disease intensity in week 1 in the control treatment was 28.87%, in all treatments the combination of *Bacillus spp* bacteria and doses in week 1 had not yet appeared symptoms so that the disease intensity was not visible. The intensity of the disease at week 2 showed significantly different results. The control treatment at week 2 experienced an increase in disease intensity from 28.87% to 49.97%. The combination treatment of *Bacillus spp* bacteria and dosage in week 2 showed the highest disease intensity in the treatment of *Bacillus spp* bacteria isolate Bcz 14 at a dose of 30 ml/plant at 16.67%. While the treatment with the smallest intensity was shown by the treatment of *Bacillus spp* isolate Bcz 20 and a dose of 20 ml/plant which amounted to 7.77%.

Table 2. Intensity of Fusarium Wilt Disease in Cayenne Pepper Plants

Treatment	Disease Intensity (%)			
	Week 1	Week 2	Week 3	Week 4
M0D0	28,87 b	49,97 b	66,67 b	66,67 c
M1D1	0,00 a	0,00 a	16,67 a	16,67 ab
M1D2	0,00 a	8,87 a	21,07 a	21,07 ab
M1D3	0,00 a	16,67 a	24,43 a	24,43 b
M2D1	0,00 a	13,33 a	24,43 a	24,43 b
M2D2	0,00 a	0,00 a	6,67 a	13,33 ab
M2D3	0,00 a	0,00 a	20,00 a	20,00 ab
M3D1	0,00 a	7,77 a	8,87 a	12,20 ab
M3D2	0,00 a	0,00 a	15,53 a	24,40 ab
M3D3	0,00 a	0,00 a	7,73 a	7,73 a

Description: M0D0= Without *Bacillus spp* administration, M1D1= *Bacillus spp* Bcz 14 + 20 ml dose, M1D2= *Bacillus spp* Bcz 14 + 25 ml dose, M1D3= *Bacillus spp* Bcz 14 + 30 ml dose, M2D1= *Bacillus spp* Bcz 16 + 20 ml dose, M2D2= *Bacillus spp* Bcz 16 + dose of 25 ml, M2D3= *Bacillus spp* Bcz 16 + dose of 30 ml, M3D1= *Bacillus spp* Bcz 20 + dose of 20 ml, M3D2= *Bacillus spp* Bcz 16 + dose of 25 ml, M3D3= *Bacillus spp* Bcz 20 + dose of 30 ml. The same letter in the same column indicates not significantly different in the 5% DMRT test.

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The results of the analysis of variance at week 3 and week 4 showed significantly different results, with the control treatment. Disease intensity in the control treatment in week 3 continued to increase, namely 66.67%. At week 3 and week 4, all treatments of a combination of *Bacillus spp* bacteria and doses were significantly different from the control treatment. The treatment with the smallest disease intensity at week 3 and week 4 was shown by the treatment of *Bacillus spp* isolate Bcz 20 and a dose of 30 ml/plant, which amounted to 7.73%.

The treatment with the application of *Bacillus spp* isolate Bcz 20 and a dose of 30 ml/plant was able to suppress the intensity of Fusarium wilt disease with the highest suppression compared to other treatments, this is thought to be isolate Bcz 20 has the greatest inhibitory power in inhibiting the pathogen *Fusarium* sp. This is in accordance with research Zinidin *et al.*, (2022) that the results of measuring the diameter of the inhibition zone of *Bacillus spp*. bacteria against the pathogen *Ralstonia solanacearum* with isolate Bcz 20 is 34.2 mm. In addition, the treatment of isolate Bcz 20 and a dose of 30 ml/plant has the highest intensity suppression compared to other treatments because the higher the dose given, the ability of *Bacillus spp* bacteria to inhibit pathogens is getting better. Puspita *et al.*, (2018) stated that the higher the number of *Bacillus* sp. colonies, the faster it will colonize plant roots and help absorb nutrients.

Bacillus sp. isolates are known to produce several types of antimicrobial compounds such as bacitracin, bacillin, bacillomycin B, dificidin, oxidificidin, lestinase and subtilisin, which play a role in inhibiting plant pathogens (Purnawati, 2022). It can be seen that the highest disease intensity was shown by the control treatment, while the smallest disease intensity was shown bythe treatment of *Bacillus spp*. isolate Bcz 20 and a dose of 30 ml/plant. The low disease intensity in the treatment of isolate Bcz 20 in a dose of 30 ml/plant is thought to be isolate Bcz 20 can produce a compound that is higher in inhibiting the pathogen *Fusarium* sp. This is in accordance with research Zinidin *et al.*, (2022) that isolate Bcz 20 is bactericidal because it does not grow R. solanacearum isolates until the 10th day of the incubation period.

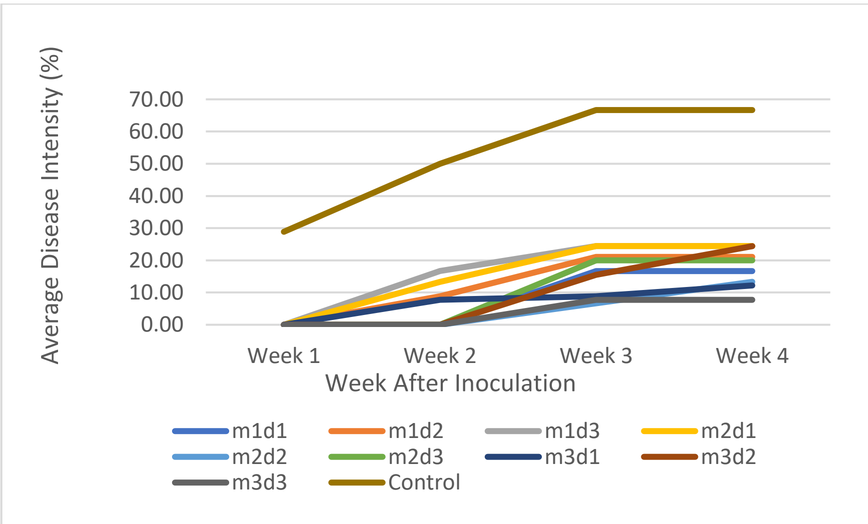

Figure 2. Graph of disease intensity

Plant Height

The results of variance analysis showed that the treatment of *Bacillus spp*. and dosing had a significant effect on plant height parameters. It is known that the treatment of *Bacillus spp*. and dosing were able to increase plant height parameters from week 1 to week 6 compared to the control treatment. The three isolates namely Bcz 14, Bcz 16 and Bcz 20 and all doses tested were able to increase the height of cayenne pepper plants (Table 3.).

Table 3. Plant Height of Cayenne Pepper

Treatment	Plant Height (cm)					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
M0D0	6,76 a	7,97 a	8,59 a	10,42 a	11,55 a	12,09 a
M1D1	11,22 b	13,03 b	16,17 bc	20,67 b	24,89 bc	26,89 b
M1D2	10,97 b	13,39 bc	15,94 b	21,78 bc	24,78 b	28,00 bc
M1D3	11,08 b	13,58 bc	17,86 bc	23,06 bc	26,33 bc	31,44 bc
M2D1	11,19 b	14,08 bc	19,19 d	26,36 c	31,28 c	35,67 bc
M2D2	10,97 b	13,39 bc	16,72 bc	23,00 bc	26,72 bc	31,17 bc
M2D3	11,33 b	14,42 bc	17,92 bc	25,81 bc	30,06 bc	34,39 bc
M3D1	12,31 b	15,58 c	19,94 d	27,39 d	31,11 bc	36,56 c
M3D2	11,69 b	13,86 bc	18,11 bc	24,44 bc	27,94 bc	32,89 bc
M3D3	11,92 b	14,94 c	19,06 cd	27,11 d	32,33 d	38,44 d

Description: M0D0= Without *Bacillus spp* administration, M1D1= *Bacillus spp* Bcz 14 + 20 ml dose, M1D2= *Bacillus spp* Bcz 14 + 25 ml dose, M1D3= *Bacillus spp* Bcz 14 + 30 ml dose, M2D1= *Bacillus spp* Bcz 16 + 20 ml dose, M2D2= *Bacillus spp* Bcz 16 + dose of 25 ml, M2D3= *Bacillus spp* Bcz 16 + dose of 30 ml, M3D1= *Bacillus spp* Bcz 20 + dose of 20 ml, M3D2= *Bacillus spp* Bcz 16 + dose of 25 ml, M3D3= *Bacillus spp* Bcz 20 + dose of 30 ml. The same letter in the same column indicates not significantly different in the 5% DMRT test.

The results showed that all treatments of a combination of *Bacillus spp* bacteria and doses increased plant growth in the vegetative phase, especially in plant height parameters compared to the control treatment (without *Bacillus spp* bacteria and doses). According to Purnawati, (2022) *Bacillus* sp. is known as a biological agent, also reported to be PGPR (*Plant Growth Promoting Rhizobacteria*). In the last observation, the highest treatment was shown by the treatment of isolate Bcz 20 at a dose of 30 ml/plant. The treatment gave the highest results because isolate Bcz 20 in the in vitro test against the pathogen *Ralstonia solanacearum* is bactericidal. It is suspected that Bcz 20 bacteria are bactericidal so that they can suppress the development of the pathogen *Fusarium* sp. and spur plant growth. This is in accordance with research Zinidin *et al.,* (2022) that isolate Bcz 20 is bactericidal because it does not grow *R. solanacearum* isolates until the 10th day of the incubation period.

Figure 3. Plant Height (A) Bacillus spp treatment and dosage, (B) Control treatment.

The combined treatment of *Bacillus spp* bacteria and dosage showed a better increase in plant height than the control treatment. The control treatment does not use the addition of *Bacillus spp* bacteria which are PGPR bacteria so that the growth of plant height is lower than the combination treatment of *Bacillus spp* bacteria and dosage (Figure 3.). Jatnika *et al.,* (2013) stated that PGPR such as *Bacillus* sp. and *Pseudomonas* sp. are able to provide a direct effect that can trigger plant growth (biostimulants), while the indirect effect is that bacteria can inhibit the growth of harmful microbes such as disease-causing (plant pathogens).

Number of Leaves

The results of variance analysis showed that the treatment of *Bacillus spp*. and the addition of doses had a significant effect in increasing the number of leaves of cayenne pepper plants. All treatments had a significant effect with the control treatment. The treatment of Bcz 20 at a dose of 30 ml/plant and Bcz 16 at a dose of 30 ml/plant showed the highest number of leaves, namely 20.89 strands and 20.94 strands. The treatment of Bcz 16 dose 25/plant showed no significant effect with the control treatment which was 14.83 strands in increasing the number of leaves of cayenne pepper plants (Table 4.).

Table 4. Average number of leaves of cayenne pepper plants

Treatment	Number of Leaves (Sheet)					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
M0D0	6,17 a	7,50 ab	8,17 a	8,33 a	9,67 a	11,83 a
M1D1	6,00 a	7,06 a	9,67 ab	14,06 b	15,50 bc	17,61 bc
M1D2	5,94 a	7,28 ab	9,72 ab	14,28 b	15,89 bc	17,61 bc
M1D3	6,50 a	8,06 ab	11,39 b	15,89 b	19,78 c	21,39 c
M2D1	6,22 a	8,17 ab	10,56 b	14,17 b	17,06 bc	21,39 c
M2D2	6,00 a	7,67 ab	9,28 ab	11,78 ab	13,00 ab	14,83 ab
M2D3	6,61 a	8,33 ab	10,94 b	14,50 b	17,83 bc	20,94 c
M3D1	6,11 a	8,89 b	11,33 b	16,67 c	18,50 c	21,33 c
M3D2	6,67 a	7,89 ab	9,72 ab	14,61 b	15,89 bc	18,44 bc
M3D3	6,33 a	8,11 ab	11,50 b	16,22 b	19,11 c	20,89 c

Description: M0D0= Without *Bacillus spp* administration, M1D1= *Bacillus spp* Bcz 14 + 20 ml dose, M1D2= *Bacillus spp* Bcz 14 + 25 ml dose, M1D3= *Bacillus spp* Bcz 14 + 30 ml dose, M2D1= *Bacillus spp* Bcz 16 + 20 ml dose, M2D2= *Bacillus spp* Bcz 16 + dose of 25 ml, M2D3= *Bacillus spp* Bcz 16 + dose of 30 ml, M3D1= *Bacillus spp* Bcz 20 + dose of 20 ml, M3D2= *Bacillus spp* Bcz 16 + dose of 25 ml, M3D3= *Bacillus spp* Bcz 20 + dose of 30 ml.

The same letter in the same column indicates not significantly different in the 5% DMRT test.

Based on the results of the study, the increase in the number of leaves of cayenne pepper plants every week has increased both the combination treatment of *Bacillus spp*. bacteria and doses and the control treatment. The highest treatment of the combination of *Bacillus spp*. bacteria and the dose in the parameter of the number of leaves was shown by the treatment of *Bacillus spp*. bacteria isolate Bcz 16 dose of 20 ml/plant and Bacillus spp. bacteria isolate Bcz 20 dose of 30 ml/plant. The treatment of *Bacillus spp*. isolate Bcz 16 and Bcz 20 showed the highest value in the parameter of the number of leaves presumably because *Bacillus spp*. isolate Bcz 20 has the ability to inhibit the pathogen *Fusarium* sp. as well as the highest disease suppression and better plant growth. In addition, according to Zinidin *et al.*, (2022) isolates Bcz 16 and Bcz 20 in the *in vitro* test against the pathogen *Ralstonia solanacearum* have a large inhibition zone and are bacteriostatic and bactericidal. So as to suppress the development of plant pathogens and maximize plant growth. In addition, the increase in the number of leaves shows that the addition of more doses is one of the factors in increasing the number of leaves of cayenne pepper plants. According to Puspita *et al.*, (2018) the large number of *Bacillus* sp. colonies will colonize the roots and stimulate the growth of lateral roots of cocoa seedlings by producing plant growth-promoting hormones so that nutrient absorption is more optimal.

First Flower Appearance

The results of variance analysis showed that the combination treatment of *Bacillus spp* bacteria and the addition of doses did not significantly affect the time of first flower appearance. The flower emergence time in all treatments showed a faster flower emergence time compared to the control treatment (Table 5).

Table 5. Time to first flower

Treatment	Time to First Flower (Days)
M0D0	67,00 b
M1D1	35,33 a
M1D2	54,17 a
M1D3	55,33 a
M2D1	54,89 a
M2D2	53,50 a
M2D3	52,55 a
M3D1	38,11 a
M3D2	34,33 a
M3D3	55,33 a

Description: M0D0= Without *Bacillus spp* administration, M1D1= *Bacillus spp* Bcz 14 + 20 ml dose, M1D2= *Bacillus spp* Bcz 14 + 25 ml dose, M1D3= *Bacillus spp* Bcz 14 + 30 ml dose, M2D1= *Bacillus spp* Bcz 16 + 20 ml dose, M2D2= *Bacillus spp* Bcz 16 + dose of 25 ml, M2D3= *Bacillus spp* Bcz 16 + dose of 30 ml, M3D1= *Bacillus spp* Bcz 20 + dose of 20 ml, M3D2= *Bacillus spp* Bcz 16 + dose of 25 ml, M3D3= *Bacillus spp* Bcz 20 + dose of 30 ml. The same letter in the same column indicates not significantly different in the 5% DMRT test.

The treatment of *Bacillus spp* isolate Bcz 20 at a dose of 25 ml/plant showed the fastest flower emergence time of 34.33 days or at week 5. The average flower emergence time of the combination treatment of *Bacillus spp* bacteria and dose was at week 6 or 55 days. The longest treatment that shows the time of flower appearance is the control treatment where flowers appear at 67.00 days. At the end of the observation, many flowers did not bloom completely, fell and did not succeed in becoming chili fruit candidates, but there were still some flowers that succeeded in becoming chili fruit candidates.

It is known that the treatment of *Bacillus spp*. bacteria in this study, namely isolates Bcz 14 and Bcz 16, is bacteriostatic while isolate Bcz 20 is bactericidal. Bacillus bacteria that are bacteriostatic cannot control plant pathogens and can only inhibit disease development. This is supported by research Zinidin *et al.,* (2022) Bacteria isolates that are bacteriostatic are isolates Bcz 14 and Bcz 16, where isolate growth occurs during the 10-day incubation period. While isolate Bcz 20 is bactericidal because it does not grow *R. solanacearum* isolates until the 10th day of incubation. Therefore, isolate Bcz 20 is the best treatment in the parameter of the first flower appearance of cayenne pepper plants because in this treatment the pathogen *Fusarium* sp. can be controlled and can spur the formation of flowers quickly.

It is known that all treatment combinations of *Bacillus spp* bacteria and doses can appear the first flower because *Bacillus spp* bacteria produce hormones that promote flower growth in plants. One of the hormones possessed by *Bacillus spp* is the IAA hormone. According to Agustiansyah *et al.,* (2013), that biological agents produce IAA which functions to spur growth. In addition, Purnawati (2022) also states that the hormone IAA has an important role in various aspects, one of which is flowering.

Fruit Wet Weight

The results of variance showed that the treatment of a combination of *Bacillus spp*. bacteria and the addition of doses did not significantly affect the total wet weight of cayenne pepper fruit. The treatment of *Bacillus spp*. isolate Bcz 20 at a dose of 30 ml/plant still gave the best results of the wet weight parameter of cayenne pepper fruit, which was 5.40 g. The treatment of *Bacillus spp*. isolate Bcz 16 at a dose of 20 ml/plant showed the smallest result with a fruit weight of 2.19 g (Table 6).

The treatment of giving *Bacillus spp*. isolate Bcz 20 into the highest treatment is thought to be isolate Bcz 20 has a greater inhibitory compound than isolate Bcz 14 and isolate Bcz 16 in inhibiting the pathogen *Fusarium* sp. so that plant growth in the generative phase becomes more optimal. This is in accordance with the statement Zinidin *et al.,* (2022) that isolates Bcz 16 and Bcz 20 in the in vitro test against the pathogen *Ralstonia solanacearum* have a large inhibition zone and are bacteriostatic and bactericidal. So as to suppress the development of plant pathogens and maximize plant growth.

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Table 6. Total wet weight of cayenne pepper fruit

Treatment	Fruit Wet Weight (g)
	Total Fruit Wet Weight
M0D0	1,86 a
M1D1	2,65 a
M1D2	2,33 a
M1D3	2,69 a
M2D1	2,19 a
M2D2	3,70 a
M2D3	3,80 a
M3D1	3,90 a
M3D2	3,25 a
M3D3	5,40 b

Description: M0D0= Without *Bacillus spp* administration, M1D1= *Bacillus spp* Bcz 14 + 20 ml dose, M1D2= *Bacillus spp* Bcz 14 + 25 ml dose, M1D3= *Bacillus spp* Bcz 14 + 30 ml dose, M2D1= *Bacillus spp* Bcz 16 + 20 ml dose, M2D2= *Bacillus spp* Bcz 16 + dose of 25 ml, M2D3= *Bacillus spp* Bcz 16 + dose of 30 ml, M3D1= *Bacillus spp* Bcz 20 + dose of 20 ml, M3D2= *Bacillus spp* Bcz 16 + dose of 25 ml, M3D3= *Bacillus spp* Bcz 20 + dose of 30 ml. The same letter in the same column indicates not significantly different in the 5% DMRT test.

In addition, the addition of the highest dose amount is still the best treatment compared to the lower dose treatment. The results of this study are in line with research (Nugraheni *et al.*, 2022) that the A4 treatment with 25 ml *B. megaterium* application which is the highest dose treatment is able to provide the best results for the variable number of leaves, fresh weight and wet weight of plants. The low wet weight of cayenne pepper fruit is thought to be because *Bacillus spp* bacteria have not been able to control the *Fusarium* sp. fungus perfectly so that it has an impact on the quality of the wet weight of cayenne pepper fruit. *Bacillus spp* bacteria in this study are bacteriostatic so they have not been able to control Fusarium wilt perfectly. Wildan *et al.,* (2021) stated that the symptoms of fusarium wilt disease are that the affected leaves experience fatigue starting from the bottom, yellowing and radiating upwards to young twigs. If the infection progresses the plant wilts. The color of root and stem tissue becomes brown. The infection site is covered with white cotton-like hyphae. If the attack occurs at the time of maximum plant growth, the plant can still produce fruit. However, if the infestation has reached the stem, the small fruits will fall. In addition, environmental factors also affect the wet weight of cayenne pepper fruit, at the time of the study the conditions around the planting area were very humid and high rainfall so that chili flowers could not become fruit and fruit that succeeded decreased in quality. According to (Rusman *et al.,* 2018) low air temperature will cause low soil temperature, and vice versa. In addition to affecting plant growth, temperature also affects the development of the disease.

CONCLUSIONS

The treatment of *Bacillus spp*. isolate Bcz 20 dose of 20 ml/plant was the greatest in delaying the incubation period of 7 days. The treatment of *Bacillus spp*. isolate Bcz 20 dose of 30 ml/plant showed suppression of pathogen intensity of *Fusarium* sp. with the highest suppression of 58.94%. The application of *Bacillus spp*. isolate Bcz 20 at a dose of 30 ml/plant gave the best results on plant height and also gave the best results on the number of leaves, and gave the highest results on fruit wet weight.

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