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Effectiveness of *Bacillus subtilis* (Ehrenberg) Cohn. to Suppress the Intensity of Dry Spot Disease (*Alternaria solani* sor.) on Potato Plant (*Solanum tuberosum* L.)

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ABSTRACT

Background: Potato (*Solanum tuberosum* L.) is one of the priority horticultural crops to be developed in Indonesia. One of the factors affecting the decline in potato productivity is Plant Disturbing Organisms (PDO), namely *Alternaria solani* Sor. a pathogenic fungus that causes dry spot disease in potato plants. Infection by this disease can reach 100%, resulting in yield losses of up to 78%. One of the biological agents utilized is *Bacillus subtilis* (Ehrenberg) Cohn. the bacteria for controlling *Alternaria solani* Sor.

Aims: This study aims to determine the effectiveness and optimal concentration of *Bacillus subtilis* (Ehrenberg) Cohn in suppressing the intensity of *Alternaria solani* Sor—disease on potato plants.

Methods: The research was conducted using the Randomized Block Design (RBD) method, consisting of 6 treatments and 5 replications. The treatment applications used were: Bs-8 (*Bacillus subtilis* (Ehrenberg) Cohn. 8 ml/liter of water), Bs-10 (*Bacillus subtilis (Ehrenberg*) Cohn. 10 ml/liter of water), Bs-12 (*Bacillus subtilis* (Ehrenberg) Cohn. 12 ml/liter of water), Bs-14 (*Bacillus subtilis* (Ehrenberg) Cohn. 14 ml/liter of water), B1 (Positive control 80% mankozeb concentration 2 grams/liter of water), and B0 (Negative control without *Bacillus subtilis* (Ehrenberg) Cohn.).

Result: The results showed that *Bacillus subtilis* (Ehrenberg) Cohn. treatment suppressed the intensity of dry spot disease (*Alternaria solani* Sor.), and the concentration of *Bacillus subtilis* (Ehrenberg) Cohn. 14 ml/liter effectively suppresses the intensity of *Alternaria solani* Sor. disease by 88.89% in potato plants.

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1. Introduction

Potato (*Solanum tuberosum* L.) is one of the priority horticultural crops to be developed in Indonesia. Potatoes are prioritized in horticulture as an important source of carbohydrates for quality food diversification. In addition to being a high carbohydrate food alternative, potatoes also have potential as a raw material for the food industry (Gultom & Gea, 2020). Based on data from the Central Statistics Agency (BPS) in 2022, potato production in Indonesia has fluctuated with a significant increase in the last five years (2018-2022), reaching 1.5 million tons in 2022 with an increase of 10.5% from the previous year. Despite the increase in production, imports of fresh potatoes remained high, reaching around US$ 180 million in the same year. This indicates that domestic potato production has not been able to fulfil the overall domestic demand. In the long run, continued imports could negatively impact the country's export trade balance, exposing the imbalance between potato production and consumption in Indonesia. The following graph of potato production in Indonesia is shown in figure 1.

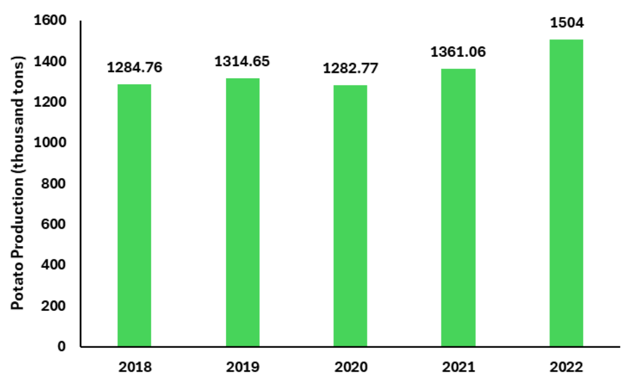

Figure 1. Chart of Potato Production in Indonesia in 2018-2022
Source: BPS, 2022 in Goodstats, 2023

One of the factors affecting the decline in potato productivity in Indonesia is the Plant Disturbing Organism (PEST), *Alternaria solani* Sor. a pathogenic fungus that causes dry spot disease in potato plants, in some years, the number of infected plants reaches 100%, and causes yield losses of up to 78% (Yuldashova *et al.,* 2023). The disease causes damage to potato plants characterised by spots with black spots that develop concentrically on the leaves, stems and tubers (Polyakova, 2023).

The damage caused by *A. solani* Sor. on potato plants can be overcome in several ways, one of which is spraying synthetic pesticides. The control that is usually carried out by farmers or breeders at the UPTD Potato Seed Centre of West Java Province uses synthetic pesticides, one of which is mankozeb. The application of synthetic pesticides, if carried out continuously, can cause several problems that can disturb the environmental balance (Prima, *et al.,* 2020). In addition, control using environmentally friendly biological agents is now an alternative in suppressing the development of diseases in potato plants in the UPTD Potato Seed Centre. One strategy that can be done to reduce the intensity of dry spot disease is control with the application of biological agents.

Control with the application of biological agents has been in the spotlight as one of the potential solutions in controlling diseases in plants. Based on research conducted by Irmawatie, *et al.* (2023) highlighted the important role of antagonistic microbes in plant disease control, offering environmentally friendly solutions that have the potential to increase agricultural productivity in a sustainable manner. One of the potential biological agents in controlling dry spot disease at the UPTD Potato Seed Centre is the microorganism *Bacillus subtilis* (Ehrenberg) Cohn.

B. subtilis (Ehrenberg) Cohn. is a bacterium that can inhibit pathogen development through the mechanisms of competition, antibiosis, and plant growth promotion (Wang *et al.,* 2018). The use of *B. subtilis* (Ehrenberg) Cohn. bacteria caused a decrease in the percentage of disease severity by 46.5% for diseases caused by *Rhizoctonia solani* and 55.2% for diseases caused by *Fusarium solani* (Madhi & Jumaah, 2020). Research by Cahya, *et al.* (2022) showed that the application of *Bacillus* sp.1 culture as much as 10 ml with a density of 1×108 cells/ml can suppress stem base rot disease by 53.38%. This shows the application of *B. subtilis* (Ehrenberg) Cohn. can be used as an alternative solution to suppress disease intensity effectively in potato plants. Therefore, exploration of the use of biological agents in controlling dry spot disease is relevant in an effort to increase the productivity and quality of potato yields at the UPTD Potato Seed Centre of West Java Province. One control that can be used and developed and has not been studied to reduce the intensity of *dry spot disease* disease is the use of biological agents *B. subtilis* (Ehrenberg) Cohn.

Based on preliminary identification in the field of the intensity of damage by *A. solani* Sor. pathogenic fungi that cause dry spot disease on potato clone Granola L, one of the screenhouses at the UPTD Potato Seed Centre of West Java Province has a percentage of intensity classified as 'Heavy' category, a significant increase from 75.9% at 79 DAP to 81.80% at 86 DAP. One of the controls carried out, namely using synthetic pesticides made from active mankozeb with a concentration of 2 grams per litre of water.

Pesticide control of the pathogenic fungus *A. solani* Sor. is not always effective, and there is currently a trend in plant science to reduce the use of synthetic agricultural chemicals by taking an ecological approach to crop cultivation, namely biological control (Yuldashova *et al.,* 2023). The use of the microbe *B. subtilis* (Ehrenberg) Cohn., either singly or in combination, has a significant impact in increasing plant resistance to blast disease in rice plants with inhibition ranging from 15.64% - 21.59% (Hersanti *et al.,* 2020). Based on research by Mutakin (2018), it shows that the biological agent *B. subtilis* (Ehrenberg) Cohn. with a concentration of 12 ml/litre can suppress the development of leaf spot disease caused by the pathogenic fungus *A. solani* Sor. and can increase yields in potato plants, the research was conducted in Lembang, West Java. The results of research by Istiqomah & Kusumawati (2018) showed that *B. subtilis* (Ehrenberg) Cohn. can significantly reduce the incidence of bacterial wilt disease by 50%. Thus, *B. subtilis* (Ehrenberg) Cohn. shows great potential as an effective biological agent to suppress plant disease intensity.

So far there has been no research related to information on the use of biological agents *B. subtilis* (Ehrenberg) Cohn. to suppress the intensity of dry spot disease on potato plants at the UPTD Potato Seed Centre West Java Province. Therefore, it is necessary to conduct research on the effectiveness of *B. subtilis* (Ehrenberg) Cohn. to suppress the intensity of *A. solani* Sor. disease on potato plants. This study aims to determine the extent of the effect of the use of *B. subtilis* (Ehrenberg) Cohn. on the intensity of dry spot disease on potato plants. and to know the concentration of *B. subtilis* (Ehrenberg) Cohn. which is effective to suppress the intensity of dry spot disease on potato plants.

1. Methods

This research was conducted at the UPTD Potato Seed Centre of West Java Province located at Baru Ibun, Sukamanah Village, Pangalengan District, Bandung Regency from 18 February 2024 to 30 May 2024. The location is located at an altitude of ±1500 metres above sea level, with an average daily temperature of between 17°C-26°C.

The research was conducted using the experimental method of Randomised Design Group (RAK), which consisted of 6 treatments and 5 replications, so that there were 30 research plots consisting of 12 potato plants per plot so as to obtain 360 plants. The 6 treatments of *B. subtilis* (Ehrenberg) Cohn. application used to be tested include:

Bs-8 = *Bacillus subtilis* (Ehrenberg) Cohn. 8 ml/litre
Bs-10 = *Bacillus subtilis* (Ehrenberg) Cohn. 10 ml/litre
Bs-12 = *Bacillus subtilis* (Ehrenberg) Cohn. 12 ml/litre
Bs-14 = *Bacillus subtilis* (Ehrenberg) Cohn. 14 ml/litre
B1 = Positive control mankozeb 80% concentration 2 g/l
B0 = Negative control without treatment Bacillus subtilis (Ehrenberg) Cohn.

Data analysis was carried out based on the linear model of Randomised Group Design according to Baihaki, et al. (1999) as follows:

$X_{ij} = \pi + t_i + r_j + e_{ij}$

Description:

X_{ij} : Observation of the i-th treatment in the j-th group
π : General mean
t_i : Effect of i-th treatment (i=1, 2, 3, ... j)
r_j : Effect of jth treatment (j=1, 2, 3, ... r)
e_{ij} : The effect of random factors on the i-th treatment in the j-th group

Research data were analysed using analysis of variance (ANOVA) with Duncan's multiple range test at the 5% real level to determine differences between treatments.

If the results of the F test show a significant difference, then to distinguish the averages of each treatment, further tests are carried out with the Duncan multiple range test method at a real level of 5% with the following formula:

LSR = SSR x $S\bar{x}$

Where:

LSR = Last Significant Rangers
SSR = Studentised Significant Rangers
$S\bar{x}$ = Standard Error of Mean

2. Results and Dicussion

3.1 Dry spot disease intensity (Alternaria solani Sor.)

Observations of disease intensity of *A. solani* Sor. on potato plants (*S. tuberosum* L.) were made twice, namely observations before application and after application. Observations before application were made to determine the initial disease intensity of dry spot disease. Subsequent observations were made 5 days after the application of *B. subtilis* biological agent concentration treatment carried out on 12 samples of potato plants in each research plot. The average results of observations of attack intensity from 6 weeks after planting to 12 weeks after planting are presented in Table 2.

Based on the results of Duncan's multiple range test analysis at a real level of 5%, the observation data of disease intensity of *A. solani* Sor. at the observation of 6 weeks after planting, 7 weeks after planting, and 8 weeks after planting, all *B. subtilis* treatments showed no significant difference with treatment B0 and comparison treatment B1 (positive control mankozeb 80% concentration of 2 grams/litre of water). This occurred because there was no treatment to control *A. solani* Sor. so there was no significant effect from each treatment plot. At 9 WAP observations were made after treatment application which showed that all treatments of *Bacillus subtilis* (Ehrenberg) Cohn. significantly different from the treatment of control B1 and control B0. Control B1 showed the lowest percentage of disease intensity of *A. solani* Sor. which was 11.10%. Based on the results, it indicates that *Bacillus subtilis* treatment has not been able to compete with the effectiveness of mankozeb 80% to control *A. solani* Sor. disease in potato plants. Although *Bacillus subtilis* can provide a significant difference compared to control B0 (no treatment) indicating a significant effect, its ability to suppress disease intensity is still limited. This is thought to be due to the slower mechanism of action of *Bacillus subtilis* in responding to infection compared to treatment B1 (positive control mankozeb 80% concentration of 2 grams/litre of water), which acts faster as a protective and curative fungicide (Sharma *et al.,* 2020). Mankozeb 80% was shown to be a highly effective contact fungicide in suppressing the development of *A. solani* Sor. which causes dry spot disease in potato plants. By inhibiting the formation of melanin necessary for pathogen cell wall strength, mankozeb interferes with the germination process and penetration of hyphae into plant tissues (Yan *et al.,* 2020).

Table 2. Results of analysis of average disease intensity of *Alternaria solani* Sor.

Treatment	Average Intensity Data of *Alternaria solani* Sor. in (%)						
	6 WAP	7 WAP	8 WAP	9 WAP	10 WAP	11 WAP	12 WAP
Bs-8	0.12ª	4.11a	12.62a	11.78b	9.95b	8.15b	5.81bc
Bs-10	0.14ª	4.09a	12.46a	11.69b	8.90c	7.10c	5.78bc
Bs-12	0.07ª	3.92a	12.42a	11.76b	9.06c	7.26c	5.72bc
Bs-14	0.7ª	4.11a	12.58a	11.73b	9.12c	7.21c	5.45c
B1	0.30ª	4.11a	12.54a	11.10c	8.96c	7.12c	6.41b
B0	0.43ª	4.02a	12.63a	25.03a	41.89a	43.69a	49.07a

Remarks:
- Numbers followed by the same letter in the same column indicate that they are not significantly different based on Duncan's multiple range test at the 5% real level.
- WAP = Week After Planting
- Treatment Bs-8= *B. subtilis* (Ehrenberg) Cohn. 8 grams/litre of water, Bs-10 = *B. subtilis* (Ehrenberg) Cohn. 10 grams/litre of water, Bs-12 = *B. subtilis* (Ehrenberg) Cohn. 12 grams/litre of water, Bs-14 = *B. subtilis* (Ehrenberg) Cohn. 14 grams/litre of water, B1 = Positive control mankozeb 80% concentration 2 grams/litre of water, B0 = Negative control *B. subtilis* (Ehrenberg) Cohn treatment.
- Data before processing was transformed by using square root transformation (x + 0.5).

Observations at 10 WAP and 11 WAP showed that all treatment applications of *B. subtilis* (Ehrenberg) Cohn. significantly different from the control treatment B0 (no treatment), but not significantly different from treatment B1 (Positive control mankozeb 80% concentration of 2 grams/litre of water) except treatment A. Based on the results of these observations indicate that all treatments have equivalent effectiveness with mankozeb 80% fungicide in suppressing the intensity of *A. solani* Sor. disease in potato plants. This finding supports previous research by Zhang *et al.* (2022) which explained that *Bacillus subtilis* microorganisms can significantly suppress the pathogenicity of *A. solani*, the control mechanism involves the production of anti-fungal compounds such as fengycins which can inhibit the germination of conidia and hyphal growth of *A. solani*.

Observations at 12 weeks after planting showed that all *B. subtilis* (Ehrenberg) Cohn. treatments were significantly different from the control treatment B0 (no treatment), but not significantly different from the control treatment B1 (Mankozeb 80%) except treatment Bs-14 which showed significant differences with the control treatment B0 (no treatment). Bs-14 which showed the lowest disease intensity of all treatments at 5.45%. Based on this observation, treatment Bs-14, which used a higher concentration than all treatments, showed that *B. subtilis* could reach optimum effectiveness faster, thus being able to effectively colonise the plants. This indicates the long-term effect of the biological agent *B. subtilis* in enhancing natural plant resistance to the pathogen *A. solani* through biocontrol mechanisms and induction of systemic resistance. This process does take time to show full effectiveness, in contrast to chemical fungicides such as mankozeb which have instant effects because they are contact based. Once adapted, *B. subtilis* can reduce pathogen populations on plants and significantly increase growth. Research by Zhang *et al.* (2022) stated that volatile organic compounds (VOCs) produced by *B. subtilis* were effective in reducing the colony size and mycelial penetration of *A. solani* and caused significant morphological changes in the pathogen. Thus, the use of biological agents *B. subtilis* (Ehrenberg) Cohn. not only provides a short-term solution to control diseases in plants, but also increases plant resistance in the long term.

B. subtilis (Ehrenberg) Cohn. is an antagonistic bacterium against several soil-borne and air-borne pathogens (Prihatiningsih *et al.,* 2019). *B. subtilis* (Ehrenberg) Cohn is one of the species of *Bacillus* sp. which is known to have potential as a biological agent for several plant pathogens due to the ability to form endospores, which allows these bacteria to survive in extreme environmental conditions (Slepecky & Henphill, 2019). *B. subtilis* (Ehrenberg) Cohn. is a bacterium that can inhibit pathogen development through the mechanisms of competition, antibiosis, and plant growth promotion (Wang *et al.,* 2018). *B. subtilis* bacteria can produce a variety of lipopeptides, namely fengycins, which are effective in inhibiting the growth of plant pathogens. Lipopeptides produced by *B. subtilis* bacteria have the ability to inhibit the growth of plant pathogens. The mechanism of action is to damage the cell membrane of the pathogen, create oxidative stress, and affect the immune system of the host plant (Sun *et al.,* 2023). Lipopeptides are produced by *B. subtilis* (Ehrenberg) Cohn. have great potential as an effective biological agent in suppressing the disease intensity of *A. solani* Sor. on potato (*S. tuberosum* L) plants.

3.2 Plant Height of Potato (Solanum tuberosum L.)

Observations of potato plant height were made after treatment application when the plants were 10 weeks old and 12 weeks old. The results of the observation analysis test are presented in Table 3 below:

Table 3. Analysed results of average potato plant height

Treatment	Data on Average Potato Plant Height (cm)	
	10 WAP	12 WAP
Bs-8	79.68ab	99.42a
Bs-10	98.11b	129.37b
Bs-12	74.90a	115.01ab
Bs-14	86.72ab	115.02ab
B1	86.37ab	103.04a
B0	81.16ab	101.33a

Remarks: Numbers followed by the same letter in the same column indicate that they are not significantly different based on Duncan's multiple range test at the 5% real level.

Based on Duncan's multiple range test at the 5% real level, the observation of 10 weeks after treatment application showed that all *B. subtilis* (Ehrenberg) Cohn. treatments were not significantly different from control B0 (no treatment) and positive control B1 (Mankozeb 80%). This is presumably because at this stage of growth, potato plants have been able to utilise nutrients and environmental conditions optimally, so the application of *B. subtilis* has not had a significant effect on increasing plant height. The potential of the applied *B. subtilis* may not be able to compensate for the influence of environmental factors and soil fertility that is homogeneous between plots (Munthali *et al.,* 2022).

At 12 weeks after planting, all *B.subtilis* treatments were not significantly different from the positive B1 and B0, except treatment Bs-10 (*B. subtilis* 10 grams/litre of water). Treatment Bs-10 is thought to be the optimal concentration to support plant growth, providing a balance between the number of bacteria and the ability of plants to absorb nutrients and water. Treatments Bs-8, Bs-12, and Bs-14 were comparable to controls B1 and B0, indicating that the plants did not respond optimally to the application of *B. subtilis* due to the already optimal availability of nutrients in the soil, so the addition of *B. subtilis* did not provide a significant increase (Munthali *et al.,* 2022). According to Kumar *et al.* (2022), *B. subtilis* can increase plant resistance to environmental stress, so that plants remain optimal even in the final phase. Thus, *B. subtilis* has the potential to increase height in potato plants.

3.3 Yield of Potato Tubers (Solanum tuberosum L.)

Observations on tubers can be made one week after pruning which is carried out at 96 Days After Planting (DAP) entering the harvest age, which is aged 103 Days After Planting (DAP) with the characteristic that the tuber skin has hardened which indicates that the tuber has reached the right maturity for harvesting. The following are the results of the observation analysis of the average weight of potato tubers presented in Table 4 below:

Table 4. Analysed results of average yield of potato crops

Treatment	Average Potato Tubers Weight (grams)/plot
Bs-8	457ab
Bs-10	569.40b
Bs-12	538.80b
Bs-14	569.80b
B1	465.40ab
B0	307.20a

Remarks: Numbers followed by the same letter in the same column indicate not significantly different based on Duncan's multiple range test at the 5% real level.

Based on Duncan's multiple range test at the 5% real level, the average weight of potato tubers showed that all *B. subtilis* (Ehrenberg) Cohn. treatments were not significantly different from the positive controls B1 (mankozeb 80% concentration of 2 grams/litre of water) and Bs-8, but significantly different from the negative control B0 (no treatment). This indicates that *B. subtilis* treatment can optimise the growth and development of potato plants, which is associated with reduced intensity of *A. solani* disease, so that photosynthesis takes place optimally. Kantar *et al.* (2020) explained that the application of *B. subtilis* increased growth parameters such as the number of leaves and stem diameter, which contributed to the increase in tuber weight stem diameter, which contributes to an increase in tuber weight. This is related to the intensity of *A. solani* disease which decreased due to the application of *B. subtilis*. In addition, Batool *et al.* (2020) stated that the application of *B.subtilis* resulted in higher tuber weight and yield compared to plants without Plant Growth-Promoting Rhizobacteria (PGPR). Root colonisation by *B. subtilis* increases tuber weight by improving nutrient availability and protecting roots from pathogen infection (Sun *et al.,* 2023). Thus, *B. subtilis* (Ehrenberg) Cohn. has the potential to be an environmentally friendly biological agent to increase tuber weight of potato plants.

3. Conclusions

Based on the research that has been done, the following conclusions can be obtained: 1) The application of biological agent *B. subtilis* (Ehrenberg) Cohn. has an effect to suppress the intensity of dry spot disease (*A. solani* Sor.) on potato (*S. tuberosum* L.) plants. 2) *B. subtilis* (Ehrenberg) Cohn. with a concentration of 14 ml/litre of water was effective to suppress the intensity of dry spot disease by 88.89% in potato plants. Based on the results and discussion of the research that has been conducted, to determine the effectiveness of the biological agent *B subtilis* (Ehrenberg) Cohn. thoroughly, it is recommended to conduct further research in open fields. In addition, it is necessary to evaluate the ability of *B. subtilis* to prevent or suppress *A. solani* Sor. disease in a preventive manner, to obtain a more comprehensive understanding of the benefits of its use in effective and sustainable potato crop management.

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