Bitki Koruma Bülteni / Plant Protection Bulletin

http://dergipark.gov.tr/bitkorb

Orijinal araştırma (Original article)

Potential of a *Bacillus* spp. consortium as a biological control agent of purple spot (*Alternaria porri* (Ellis) Cif.) and enhancement of shallot growth and production

Bacillus spp. konsorsiyumunun mor leke (*Alternaria porri* (Ellis) Cif.) hastalığının biyolojik kontrol ajanı olarak potansiyeli ve arpacık soğanı büyümesi ve üretiminin artırılması

Yulmira Yanti[a*], Hasmiandy Hamid[a], Nurbailis Nurbailis[a], Ilham Wibowo[a]

https://orcid.org/0000-0002-2853-3226, https://orcid.org/0000-0003-2532-9411,
https://orcid.org/0000-0002-1082-7816, https://orcid.org/0009-0005-8208-1581

[a]*Universitas Andalas, Agriculture Faculty, Department of Plant Protection, Limau Manis, Padang, Indonesia, 251632*

ARTICLE INFO

Article history:
DOI: 10.16955/bitkorb.1472420
Received : 25-04-2024
Accepted : 17-05-2025

Keywords:
Alternaria porri, shallot, purple spot, consortium

* Corresponding author: Yulmira YANTİ
mira23@agr.unand.ac.id

ABSTRACT

Purple spot disease caused by *Alternaria porri* poses a significant threat to shallot plants, potentially leading to yield reductions of up to 40%. Addressing this issue is crucial for maintaining agricultural productivity and ensuring food security. One promising approach to controlling purple spot disease is the utilization of *Bacillus* spp. consortium, which offers a cost-effective and environmentally friendly solution. In a recent study, researchers aimed to identify the most effective *Bacillus* spp. consortium for controlling *A. porri* while also enhancing the growth and yield of shallot plants. The study was employed a completely randomized design (CRD) with seven treatments and five replicates. These treatments included various combinations of *Bacillus* strains along with positive and negative controls and a fungicide containing mancozeb 80%. The results of the study demonstrated that the consortium treatment consisting of *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1 + *B. cereus* SNE 2.2 was the most effective in reducing purple spot disease development. This treatment exhibited a disease incidence of 17.00% and disease severity of 13.33%. Moreover, the consortium treatment significantly promoted the growth and production of shallot plants. Specifically, plants treated with this consortium exhibited a notable increase in plant height, leaf number, and both fresh and dry bulb weights. The enhanced growth parameters included a plant height of 49.83 cm, a number of leaves of 53.33 strands, and fresh and dry bulb weights of 127.08 g and 96.65 g, respectively.

INTRODUCTION

Shallots (*Allium ascalonicum* L.) hold significant importance as a horticultural commodity within Indonesia, being utilized diversely in various sectors such as health (Aryanta 2019), the food industry (Ibrahim and Elihami 2020), and export markets. Over recent years, Indonesia has witnessed a notable increase in shallot productivity, with yields reaching 13.16 tons/ha in 2019, 15.12 tons/ha in 2020, and 18.15 tons/ha in 2021. Similarly, in West Sumatra, productivity surged from 12.02 tons/ha in 2019 to 19.07 tons/ha in 2021 (Anonymous 2021). Despite these advancements, shallot

productivity continues to fall short of the optimal level of 20 tons/ha (Susanti et al. 2018).

The relatively low productivity of shallots can be attributed, in part, to various plant pathogens (Rachmatunnisa et al. 2017). Among the pathogens affecting shallot plants are *Peronospora destructor*, causing feather dew disease; *Colletotrichum gleosporoides*, responsible for anthracnose disease; *Fusarium oxysporum* f. sp. *cepae*, leading to Fusarium wilt disease (Supyani et al. 2021, Yağmur et al. 2024); *Erwinia caratovora* pv. *caratovora*, causing tuber rot disease; *Pantoea ananatis*, inducing bacterial leaf blight disease (Yanti et al. 2021); *Cercospora duddiae*, resulting in leaf spot disease; the Onion yellow dwarf virus, causing onion mosaic disease; and *Alternaria porri*, responsible for purple spot disease (Aldo and Putra 2020).

Purple spot disease poses a significant threat to onion plants, often reducing bulb production (Kim et al. 2022). Uncontrolled infections of *A. porri* in shallots can lead to yield losses of up to 40% (Sutariati et al. 2020). Typical symptoms of an *A. porri* infestation include the development of white spots that evolve into purplish lesions, expanding with a surrounding yellow halo (Hersanti et al. 2019). Control measures against the *A. porri* pathogen have been predominantly cultural and technical, involving environmental management through appropriate cultivation practices (Agastya et al. 2017), mechanical methods such as the removal of infected plant parts (Sumartini 2012), as well as the utilization of resistant cultivars and synthetic fungicides containing propineb (Ruswandari et al. 2020). However, the excessive and continuous use of synthetic fungicides poses environmental and health risks (Bansal 2020), necessitating the exploration of alternative, cost-effective, and eco-friendly control methods such as biological agents (Kim et al. 2022). *Bacillus* spp. have been investigated as potential biological agents for controlling plant pathogens, as they can suppress pathogens and enhance plant growth quality directly or indirectly (Miljaković et al. 2020).

The utilization of Bacillus spp. for controlling plant diseases is typically conducted individually (Miljaković et al. 2020). However, to enhance its effectiveness, Bacillus can be applied in combination by utilizing two or more isolates, a practice known as a consortium (Aiman et al. 2017). A consortium refers to a blend of two or more species of microorganisms that collaborate synergistically to provide various more efficient control mechanisms (Yanti et al. 2021). The objective of the study was to identify the most effective *Bacillus* spp. consortium for the control of *Alternaria porri* while simultaneously promoting shallot growth and increasing production.

MATERIALS AND METHODS

The study was conducted using a completely randomized design (CRD) consisting of seven treatments and five replicates. The bacterial isolates used in this study were obtained from previous exploration research conducted by Yanti et al. (2019). These are individual bacteria that were isolated and characterized in that study, not commercial products. The combinations of Bacillus species used in this study were based on previous research by Yanti et al. (2021). According to Yanti et al. (2021), the combinations were determined through compatibility testing between the bacterial isolates. The compatibility tests enabled the identification of bacterial combinations that could effectively work together without inhibiting each other's growth or antagonistic effects. The specific combinations that passed these compatibility tests are shown in Table 1.

The research was conducted in the microbiology laboratory of the Department of Andalas University and the experimental garden of Andalas University from July 2023 to January 2024.

Table 1. Treatment and consorsium *Bacillus* spp.

Treatment	Accepted
A	*Bacillus pseudomycoides* strain EPL 1.1.4 + *Bacillus cereus* strain TLE 2.3
B	*Bacillus pseudomycoides* strain EPL 1.1.4 + *Bacillus cereus* strain TLE 2.3 + *Bacillus cereus* strain SNE 2.2
C	*Bacillus pseudomycoides* strain EPL 1.1.4 + *Bacillus cereus* strain TLE 2.3 + *Bacillus cereus* strain TLE 1.1
D	*Bacillus pseudomycoides* strain EPL 1.1.4 + *Bacillus cereus* strain TLE 2.3 + *Bacillus cereus* strain TLE 1.1 + *Bacillus cereus* strain SNE 2.2
E	Positive control: without *Alternaria porri* inoculation and without *Bacillus* spp. consortium treatment.
F	Negative control: inoculated with *Alternaria porri* and without *Bacillus* spp. consortium treatment.
G	Fungicide: active ingredient mancozeb 80%

Preparation of Bacillus spp.

Bacillus spp. samples were gathered in microtubes, revitalized using the scratch technique on Tryptic Soy Agar (TSA) medium, and cultivated for two consecutive periods of 24 hours each. Following this incubation, they were examined for morphological traits of colony growth. To ensure purity, *Bacillus* spp. isolates underwent verification through Gram staining and hypersensitivity reaction testing.

Isolation and identification of Alternaria porri

The fungal inoculum of *A. porri* was obtained from symptomatic shallot leaves through isolation using the moist chamber method. When symptomatic plants were discovered in the field, they were promptly transported to the laboratory. Subsequently, surface sterilization was performed by immersing the symptomatic plant parts, cut into 1x1 cm^2 pieces, in sterile distilled water and alcohol for 1 minute. These pieces were then placed onto Petri dishes containing sterile Potato Dextrose Agar (PDA) media and incubated for seven days (Wiyatiningsih 2009). Identification of the pathogenic fungi was conducted through both macroscopic and microscopic observations. Macroscopic observation of *A. porri* morphology focused on colony growth, shape, color, and texture, while microscopic examination involved assessing the shape of hyphae and conidia, as well as the color and shape of conidiophores, using a binocular microscope with a magnification of 40x10 (Rachmatunnisa et al. 2017).

Pathogenicity test of Alternaria porri

A Petri dish containing a pure culture of A. porri fungus was filled with 10 ml of sterile distilled water. Subsequently, the fungus was gently crushed using a small sterile brush to release the hyphae and conidia. The resulting suspension, which primarily contained conidia, was transferred to a test tube and homogenized using a vortex. Serial dilutions were performed up to 10^{-3}. The fungal suspension was examined using a Haemocytometer Neubauer Improved, and the conidia density was calculated under a microscope. The required conidia density for the test was 10^7 conidia/ml (Hersanti et al. 2019). The homogenized conidial suspension was then transferred into a sprayer. The pathogenicity test of *A. porri* was conducted on Birma variety shallot plants two weeks after planting. The fungus was inoculated by spraying 5 ml per plant onto shallot leaves that had been wounded with a sterile needle. Observations were subsequently made to determine whether the leaves exhibited symptoms of purple spot disease.

Bacillus spp. complex propagation

Bacillus spp. that were revitalized on TSA media underwent a multiplication process in liquid culture, which comprised two stages—in the initial stage, the pre-culture phase, a single colony of *Bacillus* spp. pure culture was transferred into 25 ml of TSB medium within a culture bottle. Subsequently, this mixture was incubated on a rotary shaker at 150 rpm for 24 hours. Following this, the second stage, referred to as the main culture phase, involved the creation of the Bacillus consortium by combining two compatible Bacillus species. For this stage, 1 ml of liquid culture from each Bacillus species obtained from the pre-culture phase was transferred into 198 ml of sterile coconut water within a culture bottle. The mixture was then incubated for 48 hours on a rotary shaker at 150 rpm, maintaining room temperature. After the incubation period, the concentration of the bacterial suspension used in the experiment was determined by comparing the turbidity of the suspension with a 0.5 McFarland standard, which corresponds to approximately 1.5×10^8 CFU/ml.

Preparation of planting media

The shallot planting substrate consisted of soil, which was thoroughly cleaned and sifted to remove any impurities, combined with manure sourced from the Animal Husbandry UPT of Universitas Andalas at a ratio of 2 parts soil to 1 part manure (Yanti et al. 2019). This mixture was then filled into transparent, heat-resistant plastic containers with a capacity of 5 kg. The planting medium underwent sterilization in a pot for one hour, after which the soil was allowed to cool for a day. Subsequently, the planting substrate was transferred into polybags.

Bacillus spp. consortium introduction and planting

The introduction of the consortium involved cutting the top one-third portion of the shallot bulb. Subsequently, the cut portion was immersed in the *Bacillus* spp. consortium solution corresponding to the designated treatment for 15 minutes. Following this, two treated bulbs were planted by submerging them into a single polybag with a capacity of 10 kg, filled with soil mixed with manure (Hersanti et al. 2019).

Inoculation of Alternaria porri

The inoculation of the *A. porri* fungus occurs when the shallot plants reach the age of two weeks. The fungus is inoculated by spraying 5 ml of *A. porri* conidia suspension (10^7 conidia/ml) onto the shallot leaves that have been previously wounded with a sterile needle. Subsequently, the wounded leaves are covered with clear plastic for three days (Hersanti et al. 2019). The variables under scrutiny encompassed disease development, which involved assessing the incubation period, disease incidence, and disease severity.

Incubation period (days after planting)

The incubation period was observed after inoculation with *A. porri* on the shallot plants, noting the first appearance of purple spot disease symptoms on the plants.

Disease incidence (%)

Disease incidence was monitored weekly after inoculation until the plants were 8 weeks old. The disease incidence was calculated using the following formula:

$$DI\% = \frac{n}{N} X100\% \quad \text{..... Formula 1}$$

where:

DI%: Percentage of disease incidence

n: Number of leaves infected by A. porri

N: Total number of leaves

Disease severity (%)

Disease severity was assessed weekly after inoculation until the plants were 8 weeks old. The severity of the disease was calculated using the following formula:

$$S = \frac{\Sigma(ni \times vi)}{N \times V} X100\% \quad \text{..... Formula 2}$$

where:

S: Disease severity

ni: Number of plants with the same disease score

vi: Disease score for each plant

N: Total number of sample plants

V: Maximum value of the damage category

Table 2. Scale and degree of infection by *Alternaria porri* (modified from Hersanti et al. 2019)

Scale	Symptoms
0	No symptoms
1	Leaves infected 0 < X < 12%
2	Leaves infected 13% < X < 25%
3	Leaves infected 26% < X < 50%
4	Leaves infected 51% < X < 75%
5	Leaves infected 76% < X < 100%

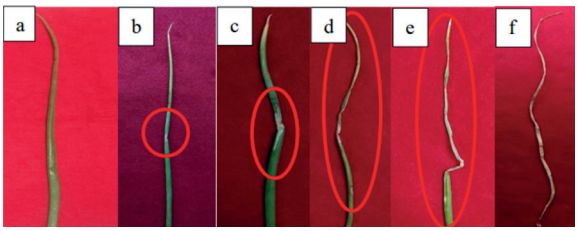

Figure 1. Scale of purple spot disease symptoms on shallots: (a) scale 0, (b) scale 1, (c) scale 2, (d) scale 3, (e) scale 4, (f) scale 5

Shallot plant growth

Number of leaves (count): Counting was performed once a week from 1 week after planting until 6 weeks after planting.

Plant height (cm): Measurement was done once a week from 1 week after planting until 6 weeks after planting. Observations were made from the soil surface to the tip of the highest leaf on the sample plants.

Fresh bulb weight (g): The weight was measured by weighing the shallot bulbs per cluster in each treatment within the polybag. The bulbs were cleaned of any remaining soil, then the leaves at the top of the bulbs were trimmed before weighing.

Dry bulb weight (g): The dry weight was measured after the shallot bulbs from each cluster were air-dried for 14 days, then the bulbs were weighed.

Data analysis

Data were analyzed using analysis of variance (ANOVA). If significant differences were found, further analysis was conducted using Duncan's New Multiple Range Test (DNMRT) at a 5% significance level.

RESULTS

Disease progression

Incubation period (hsi)

Shallot plants treated with a *Bacillus* spp. consortium showed a notable difference in the incubation period of purple spot disease compared to the negative control. Specifically, Consortium D demonstrated a significant difference when compared to the treatment with Consortium B. In contrast, while the treatments with Consortiums C and A did not show significant differences from each other, they were significantly different from both the fungicide (active ingredient: mancozeb 80%) and the negative control (Table 3).

Disease incidence (%)

Disease incidence was monitored weekly after inoculation until the plants were 8 weeks old. The percentage of disease incidence was calculated using the following Formula 1. Shallot plants treated with the *Bacillus* spp. consortium exhibited a notably distinct impact on the incidence of purple spot disease compared to the control group. The negative control exhibited a notable variation between Consortia C and A, while no significant differences was found between the two consortia themselves. Conversely, Consortium B displayed significant distinctions from both the fungicide treatment and Consortium D. Notably, no significant difference was observed between the fungicide treatment of 80% mancozeb and Consortium D (Table 4).

Table 3. The incubation period of purple spot disease in shallot plants introduced with the *Bacillus* spp. consortium

Treatment	Incubation period (the day after inoculation) ± SD
(D) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1 + *B. cereus* SNE 2.2	9.67 ± 0.57 a
(B) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* SNE 2.2	9.00 ± 0.00 b
(C) *B. pseudomycoides* EPL 1.1.4 + B. cereus TLE 2.3 + *B. cereus* TLE 1.1	8.67 ± 0.57 bc
(A) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3	8.33 ± 0.57 bc
(G) Fungicide: active ingredient mancozeb 80%	8.00 ± 0.00 cd
(F) Negative control	5.00 ± 0.00 d

Numbers followed by identical lowercase letters within the same column do not display significant differences, as determined by Duncan's New Multiple Range Test (DNMRT) at the 5% significance level.

Table 4. The incidence of purple spot disease in shallot plants following their exposure to the *Bacillus* spp. consortium

Treatment	Disease incidence (%) ± SD
(F) Negative control	54.73 ± 4.33 a
(C) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1	39.77 ± 1.16 b
(A) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3	36.90 ± 1.15 b
(B) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* SNE 2.2	28.17 ± 2.28 c
(G) Fungicide: active ingredient mancozeb 80%	20.23 ± 0.63 d
(D) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1 + *B. cereus* SNE 2.2	17.00 ± 2.64 d

Numbers followed by identical lowercase letters within the same column do not display significant differences, as determined by Duncan's New Multiple Range Test (DNMRT) at the 5% significance level

Disease severity (%)

Disease severity was assessed weekly after inoculation until the plants were 8 weeks old. The severity of the disease was calculated using the following Formula 2. Shallot plants treated with the *Bacillus* spp. consortium exhibited a significantly varied impact on the severity of purple spot disease compared to the control group. Notably, the negative control group exhibited a significant difference when compared to the treatments with Consortiums C and A; however, no significant difference was found between the two consortia. Consortium B showed significant differences when compared to the fungicide treatment and Consortium D. Interestingly, there was no significant difference observed between the fungicide treatment and Consortium D (Table 5).

A comparison of the severity of purple spot disease is shown in Figure 2. The initial symptoms caused by the *A. porri* fungus appear as minor white spots on the leaf surface. Subsequently, with continued infection, the color transitions to a purplish hue, and shallot leaves exhibit curling, accompanied by yellow circles forming along the leaf edges. Severe infections may lead to complete leaf necrosis, thereby delaying bulb maturation.

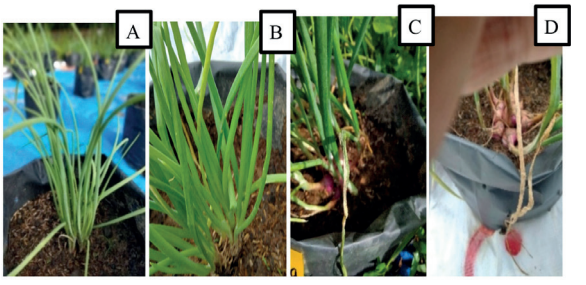

Figure 2. Severity of purple spot disease: (a) shallot leaves without symptoms of purple spot disease, (b) early symptoms of purple spot disease, (c) purple spot disease begin to spread, (d) purple spot disease begins to spread on all parts of the affected leaves, and leaves die

Table 5. The severity of purple spot disease in shallot plants following their exposure to the *Bacillus* spp. consortium

Treatment	Disease Severity (%) ± SD
(F) Negative control	53.20 ± 3.55 a
(C) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1	35.93 ± 2.06 b
(A) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3	33.10 ± 1.22 b
(B) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* SNE 2.2	26.70 ± 3.11 c
(G) Fungicide: active ingredient mancozeb 80%	17.57 ± 2.41 d
(D) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1 + *B. cereus* SNE 2.2	13.33 ± 0.90 d

Numbers followed by identical lowercase letters within the same column do not display significant differences, as determined by Duncan's New Multiple Range Test (DNMRT) at the 5% significance level

Onion plant growth

Number of leaves (blade)

Onion plants that were introduced to a consortium of *Bacillus* spp. demonstrated a significantly different effect on the number of leaves compared to the control group. Specifically, Consortia D and B showed significant differences when compared to Consortia A and C. However, there were no significant differences among the treatments within the same consortium. Additionally, both Consortia A and C exhibited significant differences when compared to the fungicide and the control group, but again, there were no significant differences among the treatments within the consortia (see Table 6).

Conversely, no significant difference was observed between the fungicide treatment and the control across the treatments (Table 7).

Fresh weight of tuber (g)

Shallot plants introduced with the Bacillus spp. consortium exhibited a notably distinct impact on bulb fresh weight compared to the control group. Notably, Consortium D demonstrated a significantly different effect compared to Consortium A treatment. Furthermore, Consortium A treatment displayed significant differences from the treatments of both Consortium B and C. However, no significant difference was observed between Consortium B and C treatments, although they were significantly different

Table 6. Number of leaves of shallot plants inoculated with *Bacillus* spp. complex

Treatment	Number of leaves (Leaves) ± SD (%)
D) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1 + *B. cereus* SNE 2.2	55.33 ± 3.01 a
(B) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* SNE 2.2	54.66 ± 3.07 a
(A) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3	51.33 ± 1.36 b
(C) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1	51.33 ± 1.36 b
(G) Fungicide: active ingredient mancozeb 80%	35.00 ± 2.09 c
(E) Positive control	33.16 ± 2.31 c

Numbers followed by identical lowercase letters within the same column do not display significant differences, as determined by Duncan's New Multiple Range Test (DNMRT) at the 5% significance level.

Plant height (cm)

The consortium exhibited a notably distinct impact on plant height compared to the control group. Interestingly, all *Bacillus* spp. consortia did not demonstrate significant differences amongst themselves. However, they significantly differed from the fungicide treatment and the control. from both the fungicide treatment and the positive control (Table 8).

Tuber dry weight (g)

Shallot plants treated with the Bacillus spp. consortium exhibited a notably distinct impact on the dry weight

Table 7. The plant height measurements of shallots following their exposure to the *Bacillus* spp. consortium

Treatment	Plant Height (cm) ± SD
D) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1 + *B. cereus* SNE 2.2	49.83 ± 4.87 a
(B) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* SNE 2.2	48.83 ± 2.73 a
(A) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3	48.33 ± 4.95 a
(C) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1	47.33 ± 3.98 a
(G) Fungicide: active ingredient mancozeb 80%	38.50 ± 1.37 b
(E) Positive control	35.50 ± 1.87 b

Numbers followed by identical lowercase letters within the same column do not display significant differences, as determined by Duncan's New Multiple Range Test (DNMRT) at the 5% significance level.

Table 8. The weight for shallot bulbs following their exposure to the *Bacillus* spp. consortium

Treatment	Fresh Weight (g) ± SD
D) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1 + *B. cereus* SNE 2.2	127.08 ± 5.91 a
(A) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3	87.52 ± 2.03 b
(B) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* SNE 2.2	67.95 ± 1.59 c
(C) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1	64.90 ± 1.58 c
(G) Fungicide: active ingredient mancozeb 80%	51.33 ± 1.54 d
(E) Positive control	49.43 ± 1.45 d

Numbers followed by identical lowercase letters within the same column do not display significant differences, as determined by Duncan's New Multiple Range Test (DNMRT) at the 5% significance level.

Table 9. The dry weight measurements for shallot bulbs following their exposure to the *Bacillus* spp. consortium

Treatment	Dry Weight (g) ± SD
D) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1 + *B. cereus* SNE 2.2	96.65 ± 0.80 a
(A) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3	81.27 ± 1.37 b
(B) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* SNE 2.2	47.07 ± 1.44 c
(C) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1	46.43 ± 0.83 c
(G) Fungicide: active ingredient mancozeb 80%	38.43 ± 1.27 d
(E) Positive control	35.45 ± 1.98 e

Numbers followed by identical lowercase letters within the same column do not display significant differences, as determined by Duncan's New Multiple Range Test (DNMRT) at the 5% significance level.

of the bulbs compared to the control group. Notably, Consortium D demonstrated a significantly different effect compared to Consortium A treatment. Furthermore, the Consortium A treatment displayed significant differences from the treatments of both Consortium B and C. However, Consortium B and C treatments were significantly different from the fungicide treatment (Table 9).

DISCUSSION

The introduction of a *Bacillus* spp. consortium in shallot plants has been found to extend the incubation period, reduce disease incidence, and mitigate disease severity compared to both the negative control and fungicide treatments. Among the various treatments, the consortium consisting of *B. pseudomycoides* EPL 1.1.4, *B. cereus* TLE 2.3,

B. cereus TLE 1.1, and *B. cereus* SNE 2.2 proved to be the most effective. This can be attributed to the utilization of multiple isolates in the consortium, which yields superior outcomes in suppressing the development of purple spot disease. The efficacy of *Bacillus* spp. consortium in acting as a biocontrol agent against the *A. porri* pathogen in shallot plants is underscored by its collaborative action in inhibiting pathogen growth. This notion is supported by Hadi et al. (2021), who suggest that bacterial consortia exhibit enhanced efficacy in plant protection due to their interactive mechanisms that control pathogen intrusion and exert physiological influences.

Utilizing the consortium consisting of *B. pseudomycoides* EPL 1.1.4, *B. cereus* TLE 2.3, *B. cereus* TLE 1.1, and *B. cereus* SNE 2.2 confers an advantage in suppressing plant diseases compared to alternative treatments. This Bacillus consortium is known to produce salicylic acid, which plays a pivotal role in inducing plant resistance (Lugtenberg and Kamilova 2009). Each Bacillus strain harbors unique advantages when incorporated into the consortium, as it facilitates various mechanisms and synergistic effects that effectively suppress plant diseases. Including *B. cereus* and *B. pseudomycoides* in the consortium significantly enhances the production of chitinase enzymes, which help decompose chitin substances and hinder the growth of plant pathogens. This finding is supported by research conducted by Win et al. (2021), demonstrating that a combination of *B. subtilis*, *B. velezensia*, and *Penicillium* sp. resulted in a substantial reduction (60-63%) in disease severity caused by *Fusarium* sp. and *Alternaria* sp. on banana plants. Additionally, Krestini et al. (2020) reported promising outcomes in reducing the intensity of Fusarium wilt disease in garlic plants by employing a consortium comprising *B. subtilis*, *T. harzianum*, *A. chroococcum*, and *P. cepacian*.

Bacillus spp. possess the capability to produce antibiotic compounds and enzymes that serve as signaling molecules, prompting the attacked plant to activate its self-defense mechanisms. Among these enzymes, chitinase, lipoxygenase, and glucanase are notable examples found in plants as part of their self-defense mechanisms against pathogens. This statement is supported by research conducted by Butarbutar et al. (2018), which highlighted that *Bacillus* sp. has the ability to produce chitinase enzymes, fix nitrogen, and solubilize phosphate. These capabilities allow *Bacillus* sp. to outcompete white root fungi for nutrients in plants, thereby suppressing the growth of the fungi. Additionally, Mageshwaran et al. (2022) reported the antagonistic activity of *B. subtilis* against wilt disease in chickpea plants caused by various soil-borne pathogens. Furthermore, studies by El-Kareem et al. (2021) demonstrated that *B. pumilus* effectively mitigated the severity of black rot disease in strawberry plants induced by pathogens such as *F. solani*, *R. solani*, and *Pythium* sp. by 65.3% to 67.3%. Moreover, Djaenuddin et al. (2017) found that *B. subtilis* significantly suppressed the development of the soil-borne pathogenic fungus *R. solani* in corn plants by 63.4%. Additionally, Saputri et al. (2020) reported that *Bacillus* spp. they inhibited dauqan midrib rot disease caused by the pathogen *R. solani* by 56.93% and 51.52% in corn plants.

The introduction of a *Bacillus* spp. consortium to shallot plants has been shown to enhance plant growth and productivity. Among the Bacillus consortia tested, the combination of *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1 + *B. cereus* SNE 2.2 emerged as the most effective in promoting plant height, leaf count, and bulb weight, with plant height reaching 49.83 cm, leaf count at 55.33 leaflets, fresh bulb weight of 127.08 g, and dry bulb weight of 96.65 g. This enhancement in growth is attributed to the production of Indole Acetic Acid (IAA) by Bacillus, known as a plant growth promoter. Additionally, *Bacillus* spp. contribute to plant growth through the synthesis of phytohormones and siderophores. Rabbe et al. (2019) highlighted that Bacillus spp. can produce siderophores, bacteriocins, and other volatile compounds that stimulate plant growth. This finding is corroborated by Gau et al. (2021), who reported that *B. subtilis* application in shallots increases plant height, leaf count, and fresh bulb weight. Furthermore, Ernita et al. (2016) demonstrated through their research that *B. pumilis* can augment plant height, leaf count, and shallot yield, reaching 15.2 tons/ha.

The application of the consortium comprising *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1 + *B. cereus* SNE 2.2 exhibited superior efficacy in mitigating the incidence and severity of purple spot disease, resulting in a disease incidence of 17.00% and disease severity of 13.33%. Additionally, this treatment demonstrated notable enhancements in shallot plant growth and production, evidenced by a plant height of 49.83 cm, a total of 53.33 leaves, a fresh bulb weight of 127.08 g, and a dry bulb weight of 96.65 g. In our study, the antagonistic potential of the isolate appeared to be suppressed in consortium D compared to consortium C. This could be attributed to several factors. The increased complexity of consortium D, which includes *Bacillus pseudomycoides* EPL 1.1.4, *Bacillus cereus* TLE 2.3, *Bacillus cereus* TLE 1.1, and *Bacillus cereus* SNE 2.2, might lead to competitive interactions among the strains for resources such as nutrients and space. This competition could reduce the overall antagonistic activity of each strain. Additionally, certain strains may produce antimicrobial compounds or engage in quorum sensing that affects the

antagonistic capabilities of other strains. In consortium D, the inclusion of *Bacillus cereus* SNE 2.2 might have altered the production or effectiveness of these compounds, leading to a diminished antagonistic effect. Furthermore, while consortium D effectively reduced disease incidence and severity, the interactions among the four strains may not have been synergistic, impacting the overall effectiveness. These findings suggest that optimizing the composition of microbial consortia to balance strain interactions could enhance their disease-suppressing capabilities. The use of the consortium *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1 + *B. cereus* SNE 2.2 has advantages in suppressing the development of plant diseases compared to other treatments. This is because the Bacillus consortium produces salicylic acid which functions to induce plant resistance (Lugtenberg and Kamilova 2009). Each of the Bacillus used has advantages when introduced as a consortium because the consortium has various mechanisms and synergistic effects that can suppress plant diseases. The use of *B. cereus* and *B. pseudomycoides* produces the chitinase enzyme where the enzyme can decompose chitin, so that the chitin produced by Bacillus is able to inhibit the growth of plant pathogens. This is supported by research by Win et al. (2021) reported that the combination of *B. subtilis*, *B. velezensis* and *Penicillium* sp. was able to reduce the severity of disease from various types of pathogenic fungi in banana plants caused by *Fusarium* sp. and *Alternaria* sp. pathogens by up to 60-63%.

ACKNOWLEDGEMENTS

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.

Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The author declared no conflict of interest.

ÖZET

Alternaria porri'nin neden olduğu mor leke hastalığı, arpacık soğanı bitkileri için önemli bir tehdit oluşturmakta ve potansiyel olarak %40'a varan verim kayıplarına yol açmaktadır. Bu sorunun ele alınması, tarımsal verimliliğin sürdürülmesi ve gıda güvenliğinin sağlanması açısından hayati önem taşımaktadır. Mor leke hastalığının kontrolüne yönelik umut vadeden bir yaklaşım, uygun maliyetli ve çevre dostu bir çözüm sunan *Bacillus* spp. konsorsiyumunun kullanılmasıdır. Yakın zamanda yapılan bir çalışmada, araştırmacılar *A. porri*'yi kontrol altına alırken aynı zamanda arpacık soğanı bitkilerinin büyümesini ve verimini de artıran en etkili *Bacillus* spp. konsorsiyumunu belirlemeyi amaçlamıştır. Çalışmada, yedi uygulama ve beş tekerrürden oluşan tamamen tesadüfi tasarım (CRD) kullanılmıştır. Bu uygulamalar, pozitif ve negatif kontrollerin yanı sıra çeşitli *Bacillus* strainlerinin kombinasyonlarını ve %80 oranında mancozeb içeren bir fungisiti içermektedir. Çalışmanın sonuçları, *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1 + *B. cereus* SNE 2.2'den oluşan konsorsiyum uygulamasının mor nokta hastalığının gelişimini azaltmada en etkili olduğunu göstermiştir. Bu uygulama %17.00 hastalık insidansı ve %13.33 hastalık şiddeti göstermiştir. Dahası, konsorsiyum uygulaması arpacık soğanı bitkilerinin büyümesini ve üretimini önemli ölçüde teşvik etmiştir. Özellikle, bu konsorsiyum ile tedavi edilen bitkiler bitki boyu, yaprak sayısı ve hem taze hem de kuru soğan ağırlıklarında dikkate değer bir artış göstermiştir. Geliştirilmiş büyüme parametreleri arasında 49.83 cm bitki boyu, 53.33 şerit yaprak sayısı ve sırasıyla 127.08 g ve 96.65 g taze ve kuru soğan ağırlığı yer almıştır.

Anahtar kelimeler: *Alternaria porri*, arpacık soğanı, mor leke, konsorsiyum

REFERENCES

Agastya I.M.I., Julianto R.P.D., Amir H., 2017. Teknik pengendalian penyakit antraknosa (patek) di sentra tanaman cabai (*Capsicum annuum* L.) menggunakan pendekatan PHT. Jurnal Akses Pengabdian Indonesia, 1 (2), 28-31.

Aiman U., Tantriati, Sriwijaya B., 2017. Pemberian macam konsorsium bakteri hasil isolasi tumbuhan pantai pada kangkung (*Ipomoea reptans* Poirs.). (abstract in English). Planta Tropika: Jurnal Agrosains (Journal of Agro Science), 5 (1), 1-6. https://doi.org/10.18196/pt.2017.065.1-6

Aldo D., Putra S.E., 2020. Expert system for diagnosis of pests and diseases of shallots using the dempster shafer method. Komputika: Journal of Computer Systems, 9 (2), 85-93. https://doi.org/10.34010/komputika.v9i2.2884

Anonymous, 2021. Agricultural data and information center. Ministry of Agriculture, Ministry of Agriculture of the Republic of Indonesia, Central Bureau of Agricultural Statistics, Jakarta Central Java Agriculture.

Aryanta I.W.R., 2019. Bawang merah dan manfaatnya bagi kesehatan (abstract in English). Jurnal Widya Kesehatan, 1 (1), 29-35. https://doi.org/10.32795/widyakesehatan.v1i1.280

Butarbutar R., Marwan H., Mulyati S., 2018. Eksplorasi *Bacillus* spp. dari rizosfer tanaman karet (*Hevea brasilliensis*)

dan potensinya sebagai agens hayati jamur akar putih (*Rigidoporus* sp.). Jurnal Agroecotania, 1 (2), 31-41.

Djaenuddin N., Nonci N., Muis A., 2017. Efektivitas formula *Bacillus subtilis* TM4 untuk pengendalian penyakit pada tanaman jagung. (abstract in English). Jurnal Fitopatologi Indonesia, 13 (4), 113-118. https://doi.org/10.14692/jfi.13.4.113

Bansal O.P., 2020; Impact of pesticides on human and environment: A review. Egyptian Scientific Journal of Pesticides, 6 (1), 1–7.

El-Kareem F.A, Elshahawy I.E., Abd-elgawad M.M.M., 2021 Application of *Bacillus pumilus* isolates for management of black rot disease in strawberry. Egyptian Journal of Biological Pest Control, 31, 25. https://doi.org/10.1186/s41938-021-00371-z

Ernita M., Zahanis, Jamilah., 2016. Aplikasi rizobakteri dalam meningkatkan pertumbuhan, hasil dan ketahanan pada tanaman bawang merah. (abstract in English). Jurnal Pengabdian Kepada Masyarakat, 22 (3),131-134.

Gau A.D.T., Syam'um E., Ulfa F., 2021. Application of *Bacillus subtilis* on red onion (*Allium ascalonicum*). IOP Conference Series: Earth and Environmental Science, 921: 012078. doi: 10.1088/1755-1315/921/1/012078

Hadi A.E., Khalisha A., Pambudi A., Effendi Y., 2021. Potential of bacteria consortium as growth controller of pathogenic fungi *Fusarium oxysporum* f.sp. *cubense* (Foc). IOP Conference Series: Earth and Environmental Science, 637 (1), 1-11.

Hersanti H., Sudarjat S., Damayanti A., 2019. Kemampuan *Bacillus subtilis* dan *Lysinibacillus* sp. dalam silika nano dan serat karbon untuk menginduksi ketahanan bawang merah terhadappenyakit bercak ungu (*Alternaria porri* (Ell.) Cif). (abstract in English). Jurnal Agrikultura, 30 (1), 8-16. https://doi.org/10.24198/agrikultura.v30i1.22698

Ibrahim I., Elihami E., 2020. Pembuatan bawang goreng raja di kabupaten enrekang. (abstract in English). Maspul Journal of Community Empowerment, 2 (2), 6-17. e-ISSN: 2716-4225. https://ummaspul.e-journal.id/pengabdian/article/view/766

Krestini E.H., Rusmawati U., Susilawati A., 2020. Effectiveness of microbial consortium on growth, yield, and intensity of withered disease (F*usarium oxysporum* Schelecht) on garlic plants. ICWEB 2019, BIO Web of Conferences, 20, 4 p. https://doi.org/10.1051/bioconf/20202003009

Lugtenberg B., Kamilova F., 2009. Plant growth promoting rhizobacteria: bacteria that cause indirect plant growth promotion or biological control. Annual Review of Microbiology, 63, 541-56. doi: 10.1146/annurev.micro.62.081307.162918

Mageshwaran V., Gupta R., Singh S., Sahu P.K., Singh U.B., Chakdar H., 2022. Endophytic *Bacillus subtilis* antagonizes soil-borne fungal pathogens and suppresses wilt complex disease in chickpea plants (*Cicer arietinum* L.). Frontiers in Microbiology, 13:994847. doi: 10.3389/fmicb.2022.994847

Miljaković D., Marinković J., Balešević-Tubić S., 2020. The significance of *Bacillus* spp. in disease suppression and growth promotion of field and vegetable crops. Microorganisms, 8 (7), 1037. https://doi.org/10.3390/microorganisms8071037

Kim M.Y., Han J.W., Dang Q.L., Kim J-C., Kim H., Choi G.J., 2022. Characterization of *Alternaria porri* causing onion purple blotch and its antifungal compound magnolol identified from *Caryodaphnopsis baviensis*. PLoS ONE, 17 (1): e0262836. https://doi.org/10.1371/journal.pone.0262836

Rabbe M.F., Ali M.D.S., Choi J., Hwang B.S., Jeong S.C., Baek K.H., 2019. *Bacillus velezensis*: a valuable member of bioactive molecules within plant microbiomes. Molecules, 24 (6), 1046 https://doi.org/10.3390/molecules24061046.

Rachmatunnisa R., Rukmi I., Pujiyanto S., 2017. Aktivitas antagonistik kapang endofit duwet (*Syzygium cumini* (L.) skeels) terhadap *Alternaria porri* penyebab bercak ungu pada bawang merah (*Allium ascalonicum* L.) secara *in-vitro*. (abstract in English). Jurnal Akademika Biologi, 6 (1), 71-78.

Ruswandari V.R., Syauqi A., Rahayu T., 2020. Uji antagonis jamur *Trichoderma viride* dalam menghambat pertumbuhan jamur patogen *Alternaria porri* penyebab penyakit bercak ungu pada tanaman bawang merah (*Allium ascalonicum* L.): Fungi antagonism test of *Trichoderma viride* in inhibiting growth pathogenic fungi of *Alternaria porri* that causes of the purple spot on shallots (*Allium ascalonicum* L.). Jurnal Ilmiah Biosaintropis (Bioscience-Tropic), 5 (2), 84–90. https://doi.org/10.33474/e-jbst.v5i2.255

Saputri A., Soesanto L.E., Mugiastuti A., Umayah, Sarjito A., 2020. Eksplorasi dan uji virulensi bakteri *Bacillus* sp. endofit jagung terhadap penyakit busuk pelepah jagung. (abstract in English). Jurnal Ilmu-Ilmu Pertanian Indonesia, 22 (2), 70-78. https://doi.org/10.31186/jipi.22.2.70-78

Sumartini. (2012). Penyakit tular tanah (*Sclerotium rolfsii* dan *Rhizoctonia solani*) pada tanaman kacang-kacangan dan umbi-umbian serta cara pengendaliannya. (abstract in English). Jurnal Litbang Pertanian, 31 (1), 27-34.

Supyani S.H.P., Supriyadi F.I.P., Putri D.H., Putri D.T., Hadiwiyono, 2021. Disease intensity of moler and yield

losses of shallot cv. Bima caused by *Fusarium oxysporum* f.sp. cepae in Brebes Central Java. IOP Conference Series: Earth and Environmental Science, 905 (1), 11-16. https://doi.org/10.1088/1755-1315/905/1/012049

Susanti H., Budiraharjo K., Handayani M., 2018. Analisis pengaruh faktor-faktor produksi terhadap produksi usahatani bawang merah di kecamatan Wanasari kabupaten Brebes. (abstract in English). Agrisocionomics: Jurnal Sosial Ekonomi dan Kebijakan Pertenian, 2 (1), 23-30. https://doi.org/10.14710/agrisocionomics.v2i1.2673

Sutariati G.A.K., Khaeruni A., Madiki A., 2020. Bakteri asal Wakatobi menghambat pertumbuhan koloni *Alternaria porri* dan *Fusarium oxyporum* penyebab penyakit pada bawang merah secara *in vitro*. (abstract in English). Jurnal Fitopatologi Indonesia, 16 (3), 105-111). https://doi.org/10.14692/jfi.16.3.105-111

Win T.T., Bo B., Malec P., Fu P., 2021. The effect of a consortium of *Penicillium* sp. and *Bacillus* spp. in suppressing banana fungal diseases caused by *Fusarium* sp. and *Alternaria* sp. Journal of Applied Microbiology, 131 (4), 1890-1908. doi: 10.1111/jam.15067

Wiyatiningsih S., 2009. Etiologi penyakit moler pada bawang merah. UPN University Press. Surabaya.

Yağmur A., Demir S., Canpolat S., Danesh Y.R., Farda B., Djebaili R., Pace L., Pellegrini M., 2024. Onion Fusarium basal rot disease control by arbuscular mycorhizal fungi and *Trichoderma harzianum*. Plants, 13, 386. https://doi.org/10.3390/plants1303038.

Yanti Y., Arneti N.M., 2019. Characterization of biocontrol ability of indigenous endophytic bacteria for the control of *Ralstonia syzygii* subsp. *indonesiensis* in chili. National Seminar in the framework of the 43rd Anniversary of UNS 2019, 3 (1).

Yanti Y., Hamid H.R., 2021. Development of the PGPR and cyanobacteria consortium for growth promotion and control of *Ralstonia syzigii* subsp. *indonesiensis* of tomato. IOP Conference Series: Earth and Environmental Science, 709, 1-11. doi:10.1088/1755-1315/709/1/012085

Yanti Y., Hamid H., Yaherwandi R., 2021. Biological control of *Sclerotium rolfsii* on tomato seedlings using *Bacillus* spp. consortium. Earth and Environmental Science, 741 (1), 1-5. doi: 10.1088/1755-1315/741/1/012063

Yanti Y., Hamid H., Yaherwandi R., Warnita, Habazar T., 2020. The ability of indigenous *Bacillus* spp. consortia to control the anthracnose disease (*Colletrotricum capsici*) and increase the growth of chili plants. Biodiversity, 21 (1), 179-186. doi: 10.13057/biodiv/d210123

Cite this article: Yanti, Y., Hamid, H., Nurbailis, N., Wibowo, I. (2025). Potential of a *Bacillus* spp. consortium as a biological control agent of purple spot (*Alternaria porri* (Ellis) Cif.) and enhancement of shallot growth and production. Plant Protection Bulletin, 65-3. DOI: 10.16955/bitkorb.1472420

Atıf için: Yanti, Y., Hamid, H., Nurbailis, N., Wibowo, I. (2025). *Bacillus* spp. konsorsiyumunun mor leke (*Alternaria porri* (Ellis) Cif.) hastalığının biyolojik kontrol ajanı olarak potansiyeli ve arpacık soğanı büyümesi ve üretiminin artırılması. Bitki Koruma Bülteni, 65-3. DOI: 10.16955/bitkorb.1472420