



## **A Test for *Bacillus* Spp. Ability to Suppress *Pestalotiopsis* sp. Causing Rubber Leaf Drought Through In-Vitro**

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### **ABSTRACT**

Using synthetic chemical pesticides can cause environmental pollution, the death of biological agents, the emergence of residues, and is dangerous for humans. One environmentally friendly alternative for disease control is biological control using *Bacillus* spp. This study aimed to test the antagonistic ability of *Bacillus* spp., to obtain a morphological description of *Pestalotiopsis* sp. and get a type of *Bacillus* bacteria that can inhibit the fungus *Pestalotiopsis* sp. causes of rubber leaf fall disease in vitro. This research was carried out at the Plant Disease Laboratory, Faculty of Agriculture, Riau University and the Pekanbaru Class 1 Quarantine Laboratory. This research was carried out experimentally using a completely randomized design (CRD) consisting of 7 treatments and 4 replications. *Bacillus* spp that used namely (B0) Without *Bacillus* spp. (B1) *Bacillus amyloliquefaciens*, (B2) *Bacillus cereus*, (B3) *Bacillus pseudomycoides*, (B4) *Bacillus velezensis* 1, (B5) *Bacillus velezensis* 2, (B6) *Bacillus velezensis* 3. The data obtained from the research results were analyzed statistically using variance analysis and further tested using the DNMRT Test at the 5% level. All *Bacillus* spp. are able to inhibit the fungus *Pestalotiopsis* sp., but 2 *Bacillus*, namely B. *cereus* B. *pseudomycoides*, are not able to produce an inhibition zone. B. *amyloliquefaciens* is a type of *Bacillus* that is more capable of inhibiting the fungus *Pestalotiopsis* sp. with an inhibitory power of 69.85% and an inhibitory zone of 64.74% and can reduce the length and width of conidia.

Keywords: *Ability. Antagonistict, Bacillus* spp., *Pestalotiopsis* sp.

## 1. INTRODUCTION

The growth of rubber is closely linked to plant diseases. One such disease affecting rubber plants is leaf fall disease. Pestalotiopsis leaf fall disease is a relatively new issue. It was first reported to affect rubber plantations in North Sumatra in 2016 and South Sumatra in 2017. This disease has spread to various regions in Indonesia, covering an area of 22,804 hectares in 2018. By 2019, the disease had expanded, affecting a total area of 382,000 hectares in Indonesia. It has been identified in several areas, including North Sumatra, West Sumatra, Jambi, South Sumatra, Bangka Belitung, Bengkulu, Lampung, West Java, Central Java, South Kalimantan, West Kalimantan, Central Sulawesi, and Riau (PT Riset Nusantara, 2021).

The Pestalotiopsis leaf fall disease leads to sporadic leaf shedding of up to 75-90%, causing the canopy to become thin. This disease affects plants of all ages attacks all clones, and there are no resistant clones to this disease (Febbiyanti & Fairuzah, 2020).

The majority of farmers still rely on synthetic chemical pesticides to combat this disease. However, the use of these pesticides can lead to environmental pollution, the depletion of beneficial organisms, the presence of residues, and pose risks to human health. One eco-friendly alternative for disease control is through biological means.

Biological pest control can be achieved by using antagonistic bacteria like *Bacillus* spp. These bacteria have the potential to serve as effective biological agents due to their ability to adapt well, form endospores, and tolerate diverse environmental conditions. These traits give them an advantage in competing with pathogens through antibiosis, which involves toxin production. *Bacillus* bacteria associated with plants can also stimulate growth by producing metabolites that enhance root system sensitivity to nutrient absorption and promote the synthesis of growth-

regulating substances like auxin, gibberellin, and cytokinin. (Setiaji, Annisa, & Rahmandhias, 2023).

Hardiyanti *et al.* (2018) showed that *B. amyloliquefaciens* has the potential as a biological agent to control white root disease in rubber plants. The results of the study (Hidayah, N & Yulianti, 2014) showed that *B.cereus* was able to slow down the growth of *Rhizoctonia solani* on PDA media and could inhibit its growth by 68.9%, and these results indicate that *B.cereus* has the potential to be developed as a biological agent.

Elfina (2020) discovered 6 isolates of rhizobacteria, namely *B. amyloliquefaciens*, *B. cereus*, *B. pseudomycoides*, *B. velezensis* 1, *B. velezensis* 2, *B. velezensis* 3, that are capable of inhibiting the growth of *Fusarium oxysporum* f.sp. cepae, the causal agent of Fusarium wilt disease in shallots.

The study aimed to evaluate the antagonistic potential of newly isolated *Bacillus* spp. against various pathogens, such as *Pestalotiopsis* sp. responsible for rubber leaf fall disease. The research focused on identifying a specific strain of *Bacillus* capable of inhibiting the growth and altering the morphology of *Pestalotiopsis* sp. in laboratory conditions.

## 2. MATERIAL AND METHODS

The study was conducted at the Plant Disease Laboratory, Faculty of Agriculture, Riau University (0.480333,101.378639) and the Pekanbaru Class 1 Quarantine Laboratory (0.47070155677746484, 101.45598747597657). The experimental research utilized a completely randomized design (CRD) with 7 treatments and 4 replications, resulting in 28 experimental units, each comprising 2 Petri dishes. The treatments included an antagonistic test for *Bacillus* spp against the fungus *Pestalotiopsis* sp., specifically without *Bacillus* spp, *Bacillus amyloliquefaciens*, *Bacillus cereus*, *Bacillus pseudomycoides*, *Bacillus*

velezensis 1, *Bacillus velezensis* 2, and *Bacillus velezensis* 3. Statistical analysis of the data collected from the research was conducted using variance analysis. Furthermore, a post hoc test using Duncan's New Multiple Range Test (DNMRT) at a significance level of 5% was performed to compare the mean values among the treatments.

The parameters observed were the characteristics of the fungus *Pestalotiopsis sp.* before application of *Bacillus spp.*, the inhibitory power of *Bacillus spp.* against *Pestalotiopsis sp.* on PDA media, the bacterial inhibition zone of *Bacillus spp.* against *Pestalotiopsis sp.* on PDA media, macroscopic and microscopic characteristics of *Pestalotiopsis sp.* after application of *Bacillus spp.*, as well as the

length and width of the conidia of the *Pestalotiopsis sp.* after application of *Bacillus spp.*

### 3. RESULT AND DISCUSSION

#### 3.1 Characteristics of *Pestalotiopsis sp.* fungus before *Bacillus spp.* application

1. The attributes of the fungus responsible for rubber leaf fall disease prior to treatment consist of both macroscopic and microscopic features prior to the administration of *Bacillus spp.*, as detailed in Watanabe (2002) and Maharachchikumbura et al. (2012). The findings from the examinations are presented in Table 1, Figure 1, and Figure 2.

Table 1. Characteristics of fungi causing *Pestalotiopsis* rubber leaf fall disease on PDA media

| Morphological Characteristics | Research Result            | Watanabe (2002) and Maharachchikumbura (2012) |
|-------------------------------|----------------------------|---|
| Macroscopic:                  |                            |   |
| Colony color                  | White                      | White   |
| Direction of spread           | Lateral, concentric        | Lateral, concentric                           |
| Mycelium texture              | Fine                       | Fine  |
| Microscopic:                  |                            |   |
| Conidia form                  | Fusiform shape             | Fusiform                                      |
| Hyphae form                   | insulated Hyaline hyphae   | partitioned Hyphae                            |
| Conidia size                  | 20.82-22.15 × 7.39-7,48 µm |   |

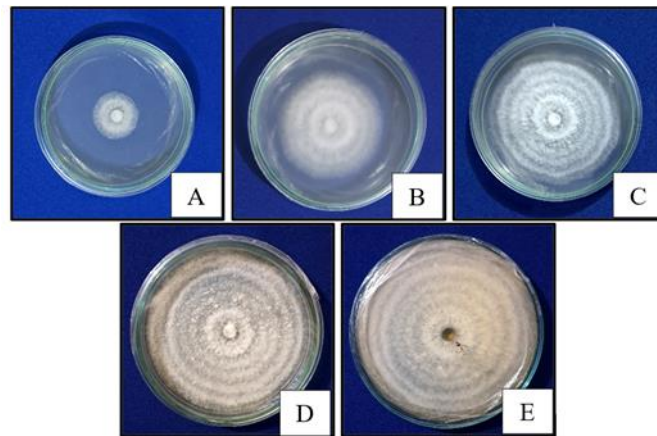
The fungus responsible for rubber leaf fall disease, known as *Pestalotiopsis sp.*, has been extensively studied through observations of both macroscopic and microscopic characteristics. These observations have been supported by the findings of Watanabe (2002) and Maharachchikumbura et al. (2012), as documented in Table 1. The macroscopic observations of the *Pestalotiopsis sp.* colony revealed that it appeared white, exhibited a flower-like pattern, and contained black conidiomata (Figure 1). These findings align with the research conducted by Kusdiana (2021), which also described the *Pestalotiopsis sp.* colony as white in color, smooth in

texture, resembling flowers or forming a circular pattern, and featuring irregularly growing black conidiomata that spread out from the center of the colony.

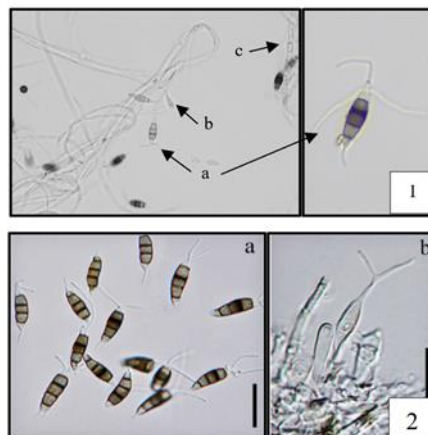
Microscopic examination reveals that *Pestalotiopsis sp.* fungus displays hyphae and fusiform conidia with insulation, measuring 20.82-22.15 × 7.39-7.48 µm. The conidia are composed of 5 cells and possess 4 dark-coloured septa with setula (Figure 20). These findings align with the findings of Febbiyanti and Fairuza (2019), who noted that *Pestalotiopsis sp.* fungus features insulated hyphae and lacks a nucleus. The conidia are fusiform and five-celled, with some being curved and others

straight. Additionally, Kusdiana et al. (2021) documented that the conidia of *Pestalotiopsis* sp. have hyaline, cone-shaped basal and apical cells. Each

conidium exhibits 2-4 setules at the apical part, while a tubular pedicel emerges at the end of the basal cell.



**Figure 1.** Macroscopic characteristics of the *Pestalotiopsis* sp. colony. (A) 3 hsi, (B) 5 hsi, (C) 7 hsi, (D) 11 HSI, (E) 11 HSI bottom view



**Figure 2.** Results of microscopic observations of *Pestalotiopsis* sp. before application of *Bacillus* spp. (1) results of observations of the fungus *Pestalotiopsis* sp. (2) microscopic *Pestalotiopsis* sp. according to Maharachchikumbura (2012) (a) Conidia, (b) conidiophores, (c) insulated hyphae

### 3.2 Inhibition of *Bacillus* spp. bacteria against *Pestalotiopsis* sp. on PDA media

The analysis of variance at the 5% level revealed that *Bacillus* spp. had a significant impact on inhibiting the growth of *Pestalotiopsis* sp. on PDA media. The results of the DNMRT test, as shown in Table 2, further support this finding.

Table 2 clearly demonstrates that *Bacillus* spp., specifically *B. amyloliquefaciens*, *B. velezensis* 3, *B. velezensis* 2, *B. velezensis* 1, *B. Pseudomycoides*, and *B. cereus*,

exhibited significantly different inhibitory effects compared to the treatment without *Bacillus* spp. This result indicates that *Bacillus* spp. possesses the ability to hinder the growth of *Pestalotiopsis* sp. This inhibition is attributed to the antagonistic mechanism of *Bacillus* spp., which involves the production of antibiotics that impede the growth of *Pestalotiopsis* sp. Among the treatments, *B. amyloliquefaciens* displayed the highest inhibitory power at 69.85%, although it was not significantly different from the treatment using *B. velezensis* 3,

which exhibited an inhibitory power of 68.19%. The *B. velezensis* 3 treatment, in turn, did not show a significant difference when compared to the *B. velezensis* 1

treatment, but it did differ significantly from the *B. velezensis* 2, *B. pseudomycoides*, and *B. cereus* treatments.

Table 2. Inhibition of *Bacillus* spp. bacteria against *Pestalotiopsis* sp.

| <i>Bacillus</i> spp.         | Inhibitory capacity A(%) |
|------------------------------|--------------------------|
| Without <i>Bacillus</i> spp. | 0.00 a                   |
| <i>B. cereus</i>             | 35.14 b                  |
| <i>B. pseudomycoides</i>     | 36.11 b                  |
| <i>B. velezensis</i> 2       | 61.76 c                  |
| <i>B. velezensis</i> 1       | 67.22 d                  |
| <i>B. velezensis</i> 3       | 68.19 de                 |
| <i>B. amyloliquefaciens</i>  | 69.85 e                  |

Note: Numbers followed by unequal lowercase letters are significantly different according to the DNMRT test results at the 5% level after being transformed by  $\text{arc sin}\sqrt{p}$

*B. amyloliquefaciens*, *B. velezensis* 3, *B. velezensis* 1, and *B. velezensis* 2 show greater potential as agents to control the *Pestalotiopsis* sp. fungus, as they exhibit inhibition rates above 50%. This aligns with the findings of Hardiyanti et al. (2018), suggesting that isolates with antagonistic power above 50% are suitable for further testing.

*Bacillus amyloliquefaciens* exhibits a stronger inhibitory effect compared to *B. velezensis* 3. This can also be attributed to the larger inhibition zones observed with *B. amyloliquefaciens*. The inhibition of pathogen growth is a result of the antagonistic mechanism of *Bacillus* spp. antibiosis against *Pestaliopsis* sp. According to the research findings by Jo et al. (2021), the strain N1 of *B. amyloliquefaciens* produces compounds such as bacillomycin D, surfactin, and fengcyn, which are lipopeptides with antifungal properties capable of inhibiting pathogen growth.

*B. velezensis* 3, *B. velezensis* 1, and *B. velezensis* 2 also exhibit stronger inhibitory effects compared to *B. pseudomycoides* and *B. cereus*. According to Gao et al. (2017), bacterial strains belonging to the *B. velezensis*-ZSY-1 species can produce various volatile compounds such as 2-tridecanone, pyrazine (2,5-dimethyl), benzothiazole, and phenol (4-chloro-3-

methyl) that function to suppress the formation of colonies of the fungi *Alternaria solani* and *Botrytis cinerea*.

The inhibitory effect varies for each treatment of *Bacillus* spp., suggesting that the differences may stem from the unique composition of secondary metabolite compounds in each bacterial isolate. These compounds differ not only between isolates but also in the quantity produced. This aligns with Pitasari and Ali's (2018) assertion that variations in inhibitory activity could result from differences in the types and amounts of compounds produced by each isolate for inhibition. Flori et al. (2020) further explain that the disparity in inhibitory diameters among bacteria is due to the diverse capability of bacterial isolates to produce inhibitory compounds. Additionally, Saputra et al. (2015) propose that physiological disparities in bacteria's utilization of nutrients in the media could also account for differences in inhibitory activity.

### 3.3 Zone of inhibition of *Bacillus* spp. Bacteria against *Pestalotiopsis* sp. on PDA media

The *Bacillus* spp. Bacteria significantly inhibit the growth of *Pestalotiopsis* sp. on PDA media after a variance analysis at the 5% level. The results of the LSD test at the 5% level are presented in Table 3.

Table 3 Zone of inhibition of *Bacillus* spp. bacteria against *Pestalotiopsis* sp.

| <i>Bacillus</i> spp.         | Zone of inhibition (%) |
|------------------------------|------------------------|
| Without <i>Bacillus</i> spp. | 0.00 a                 |
| <i>B. cereus</i>             | 0.00 a                 |
| <i>B. pseudomycooides</i>    | 0.00 a                 |
| <i>B. velezensis</i> 2       | 36.60 b                |
| <i>B. velezensis</i> 1       | 55.24 c                |
| <i>B. velezensis</i> 3       | 57.94 cd               |
| <i>B. amyloliquefaciens</i>  | 64.74 d                |

Note: Numbers followed by unequal lowercase letters are significantly different according to the DNMR test results at the 5% level after being transformed by  $\text{arc sin}\sqrt{p}$

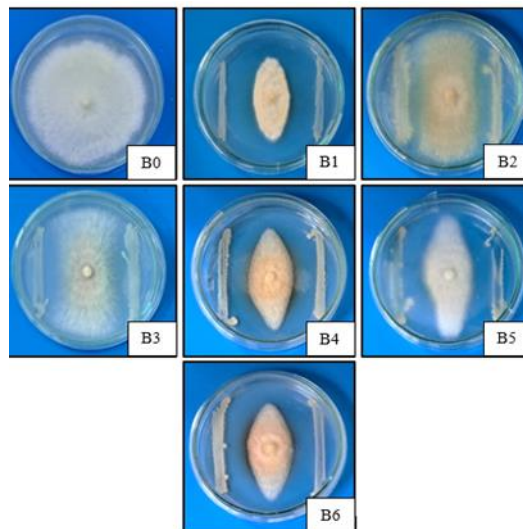
Table 3 illustrates that the absence of *Bacillus* spp leads to the absence of an inhibition zone in *B. cereus* and *B. pseudomycooides* treatments. This is in stark contrast to *B. velezensis* 2, *B. velezensis* 1, *B. velezensis* 3, and *B. amyloliquefaciens* treatments, which exhibit an inhibition zone. The variation in inhibition zone formation can be attributed to the different *Bacillus* species employed.

Among the treatments, the *B. amyloliquefaciens* treatment displays a larger zone of inhibition compared to the others. However, this difference is not statistically significant when compared to the *B. velezensis* treatment. On the other hand, the *B. velezensis* 3 treatment does not show a significant difference when compared to the *B. velezensis* 1 treatment, but it does exhibit a significant difference when compared to the *B. velezensis* 2, *B. pseudomycooides*, and *B. cereus* treatments.

*B. amyloliquefaciens* demonstrates a larger zone of inhibition in comparison to *B. velezensis* 3 treatment. These findings suggest that *B. amyloliquefaciens* possesses the capability to impede the growth of *Pestalotiopsis* sp., as evidenced by the presence of a distinct, clear zone. The formation of this clear zone is attributed to the capacity of *B. amyloliquefaciens* to generate secondary metabolite compounds, including antibiotics, which hinder the proliferation

of pathogens. This observation aligns with the study conducted by Delia et al. (2018), which highlights that the antibiosis mechanism of *Bacillus* Bn1 is characterized by the development of a clear zone serving as a growth inhibition area for the Stewart wilt pathogen. The size of the clear zone produced correlates with the efficacy of the bacteria in altering the substrate and suppressing the growth of pathogens.

*Bacillus* species can form an inhibition zone, which serves as evidence of an antibiosis mechanism (Saputra et al. 2015). This inhibition zone is a result of the production of antifungal compounds by the bacteria to outcompete plant pathogens and hinder their growth (Bawantari et al., 2020). The ability of *Bacillus* species to suppress the growth of *Rhizoctonia solani* on PDA media by generating an inhibition zone suggests their capacity to produce antibiotic substances (Margani et al., 2018). In order to combat *Colletotrichum gloeosporioides* infection, *B. velezensis* releases antimicrobial compounds by creating an inhibition zone in the growth medium (Wang and Zhu, 2023). The presence of an inhibition zone surrounding *B. velezensis* colonies indicates the bacteria's capability to produce siderophores (Li et al., 2023). The variations in the size of the inhibition zone among different treatments are illustrated in Figure 3.



**Figure 3.** Observation of inhibition and zone of inhibition of *Bacillus* spp. against *Pestalotiopsis* sp. (B0) Without *Bacillus* spp., (B1) *B. amyloliquefaciens* (B2) *B. cereus*, (B3) *B. pseudomycooides*, (B4) *B. velezensis* 1 (B5) *B. velezensis* 2, (B6) *B. velezensis* 3

### 3.4 Macroscopic and microscopic characteristics of *Pestalotiopsis* sp. after *Bacillus* spp. application.

The macroscopic and microscopic characteristics of *Pestalotiopsis* sp. following the application of *Bacillus* spp. can be observed through the color of the colony, the direction of distribution, the texture of the mycelium, and the shape of the conidia of the fungus. These observations are detailed in Table 4, Figure 3, and Figure 4. The utilization of *Bacillus* spp. can induce alterations in the macroscopic appearance of the fungus *Pestalotiopsis* sp. Table 4 and Figure 3 illustrate that treatment with *B. amyloliquefaciens*, *B. velezensis* 1, *B. velezensis* 2, and *B. velezensis* 3 resulted in the inhibition of hyphal growth, preventing the hyphae from filling the petri dish. Conversely, in the case of *B. cereus* and *B. pseudomycooides* treatments, the petri dish was almost filled, but the hyphae surrounding the bacteria appeared thinner. This phenomenon can be attributed to the production of inhibitory compounds by *Bacillus* that impede the growth of pathogenic fungal mycelium. According to Zuraidah et al. (2020), *Bacillus* is a

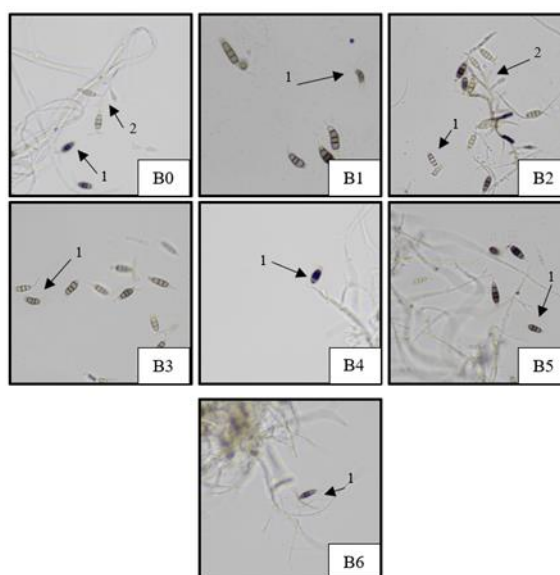
chitinolytic bacterium capable of producing a chitinase enzyme that degrades fungal cell walls, leading to lysis of the fungal cells and hindrance of their growth.

The antibiotic compounds synthesized by *Bacillus* can induce alterations in the color of fungal colonies. As illustrated in Table 4 and Figure 10, the absence of *Bacillus* spp. resulted in white fungal colonies, whereas treatments involving *B. amyloliquefaciens*, *B. cereus*, *B. pseudomycooides*, and *B. velezensis* 2 exhibited yellowish white colonies. Conversely, *B. velezensis* 1 and *B. velezensis* 3 treatments led to reddish white fungal colonies.

The application of *Bacillus* spp. Did not impact the conidia morphology of *Pestalotiopsis* sp. when compared to the control group without *Bacillus* spp. Despite the lack of changes in conidia shape during the antagonist test, *Bacillus* spp. demonstrated the ability to suppress the growth of *Pestalotiopsis* sp. through alternative antagonistic mechanisms. The microscopic features, specifically conidia, of *Pestalotiopsis* sp. post-application of select *Bacillus* spp. are depicted in Figure 4.

Table 4. Macroscopic and microscopic characteristics of *Pestalotiopsis sp.* after *Bacillus spp.* application.

| Treatment                    | Macroscopic and microscopic observations |                     |  |               |
|------------------------------|--|---------------------|--|---------------|
|                              | Colony color                             | Dispersal Direction | Mycelium Texture                             | Conidia Shape |
| Without <i>Bacillus</i> spp. | White                                    | Growing sideways    | Like cotton, thick                           | Fusiform      |
| <i>B. amyloliquefaciens</i>  | Yellowish                                | Growing sideways    | Like cotton, thick                           | Fusiform      |
| <i>B. cereus</i>             | white                                    | Growing sideways    | Like cotton, thick but thinner near bacteria | Fusiform      |
| <i>B. pseudomycooides</i>    | Yellowish                                | Growing sideways    | Like cotton, thick but thinner near bacteria | Fusiform      |
| <i>B. velezensis 1</i>       | White, reddish at the edges              | Growing sideways    | Like cotton, thick                           | Fusiform      |
| <i>B. velezensis 2</i>       | Yellowish                                | Growing sideways    | Like cotton, thick                           | Fusiform      |
| <i>B. velezensis 3</i>       | White, reddish at the edges              | Growing sideways    | Like cotton, thick                           | Fusiform      |



**Figure 4.** Microscopic characteristics of *Pestalotiopsis sp.* after application of *Bacillus* spp. (B0) No *Bacillus* spp., (B1) *B. amyloliquefaciens* (B2) *B. cereus*, (B3) *B. pseudomycooides*, (B4) *B. velezensis 1* (B5) *B. velezensis 2*, (B6) *B. velezensis 3*. Ket: 1. Conidia, 2. Conidiophores.

**3.5 Length and width of conidia of *Pestalotiopsis sp.* after application of *Bacillus* spp.**

*Bacillus* species significantly influence the length and width of *Pestalotiopsis sp.*

conidia after conducting a variance analysis (see Appendix 5). Further post hoc testing with a 5% significance level can be observed in Table 5 and Table 6.



Table 5. Conidia length of *Pestalotiopsis sp.* after *Bacillus spp.* application.

| <i>Bacillus spp.</i>         | Fungal conidia length <i>Pestalotiopsis sp</i> (µm) |
|------------------------------|---|
| Without <i>Bacillus spp.</i> | 21.31 a   |
| <i>B. cereus</i>             | 20.44 ab  |
| <i>B. velezensis 3</i>       | 19.86 abc   |
| <i>B. pseudomycooides</i>    | 19.01 bc  |
| <i>B. velezensis 1</i>       | 18.79 bc  |
| <i>B. velezensis 2</i>       | 17.97 cd  |
| <i>B. amyloliquefaciens</i>  | 16.39 d   |

Note: Numbers followed by unequal lowercase letters are significantly different according to the DNMRT test at the 5% level.

Table 6. Conidia width of *Pestalotiopsis sp.* after application of *Bacillus spp.*

| <i>Bacillus spp.</i>         | Fungal conidia length <i>Pestalotiopsis sp</i> (µm) |
|------------------------------|---|
| Without <i>Bacillus spp.</i> | 7.56 a  |
| <i>B. velezensis 1</i>       | 6.79 b  |
| <i>B. pseudomycooides</i>    | 6.72 b  |
| <i>B. velezensis 3</i>       | 6.67 b  |
| <i>B. cereus</i>             | 6.67 b  |
| <i>B. velezensis 2</i>       | 6.54 b  |
| <i>B. amyloliquefaciens</i>  | 6.40 b  |

Note: Numbers followed by unequal lowercase letters are significantly different according to the DNMRT test at the 5% level.

Table 5 reveals differences in conidia length between the fungus *Pestalotiopsis sp.* treated with *B. amyloliquefaciens* and *B. velezensis 2*, as well as other treatments. Additionally, Table 6 highlights variations in conidia width between the *B. amyloliquefaciens* treatment and treatments without *Bacillus spp.*, as well as other treatments.

The analysis of Tables 5 and 6 indicates that *Bacillus spp.* application impacts both the length and width of *Pestalotiopsis sp.* conidia. The treatment with *B. amyloliquefaciens* shows superior inhibition of conidia length and width compared to other *Bacillus* treatments. This efficacy is attributed to the production of inhibitory compounds by *B. amyloliquefaciens*, which impede conidia growth. Antagonistic bacteria-produced compounds can hinder conidia germination and the uptake of essential nutrients required for pathogen proliferation (Sriyanti *et al.*, 2015).

*Bacillus spp.* is known to produce two compounds, namely iturin and fengycin. These compounds possess the ability to

hinder the intracellular mechanism responsible for toxin production in pathogenic fungi. Additionally, they induce the breakdown of the mycelium and conidia by compromising the integrity of the fungal membrane (Hu *et al.*, 2019).

#### 4. CONCLUSION

*Bacillus* species have been found to possess the ability to inhibit the growth of the fungus *Pestalotiopsis sp.* However, it has been observed that two specific *Bacillus* strains, *B. cereus* and *B. pseudomycooides*, do not exhibit this inhibitory effect. On the other hand, *B. amyloliquefaciens* stands out as a *Bacillus* strain that demonstrates a higher capacity to inhibit the growth of *Pestalotiopsis sp.*, showing an inhibitory power of 69.85% and an inhibitory zone of 64.74%. Furthermore, *B. amyloliquefaciens* has been shown to reduce the length and width of conidia associated with *Pestalotiopsis sp.*

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