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Antagonistic Bacteria *Bacillus subtilis* Formulation as Biopesticide to Control Corn Downy Mildew caused by *Peronosclerospora philippinensis*

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Abstract— Downy mildew (DM) is a major disease of corn that can limit production. An alternative method of control that is currently being developed is the use of biopesticides which can inhibit the development of DM. Therefore, this study aims to test the effectiveness of *B. subtilis* antagonistic bacteria formulation in suppressing DM through seed treatment and foliar spraying. The study was carried out from June to October 2019 at Maros Experimental Farm of the Indonesian Cereals Research Institute. The first application was done by treating the seeds with the bacterial formulation with a dose of 8 g/kg of seeds. The second application was done at 16 DAP at a dose of 3 g/L. Spraying was done evenly throughout the leaves of the test plant. Observations made include incidents of downy mildew, presence of mycelium lignification, leaf chlorophyll content, stomatal density, plant height, and crop yield. Furthermore, the results showed that the application of the formula *B. subtilis* with seed treatment and spraying effectively suppressed the development of downy mildew and improved corn plants' growth. The test also showed that mycelium lignification occurred in the treatment using the formula *B. subtilis*. The treatment of B. *subtilis* tended to have higher leaf chlorophyll content in corn plants compared to control treatments. Considering the results, the Bima-15 and Perkasa variety have a relatively better response to *B. subtilis* biopesticides in inhibiting downy mildew infections, improving maize production.

***Keywords*— Resistant varieties; PGPR; biopesticide; seed treatment.**

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I. INTRODUCTION

Downy mildew (DM) is an important disease of corn plants that can reduce production. The typical symptom of the corn plant which DM attacks is pale yellow leaves with chlorotic stripes that extend parallel to the vein of the leaf with clear boundaries, while healthy leaves are normal green [1]. Biotic and abiotic environmental conditions are always changing to affect the development of disease in the field. The effort to control DM by cultural practices and synthetic pesticides has not shown satisfactory results. Therefore, it is necessary to find alternative methods to control the disease at every stage of the plant.

The negative impact of the massive use of synthetic pesticides on the environment and human health has stimulated the search for an environment-friendlier approach to control plant diseases. Bi biocontrol, which relies on using beneficial organisms or their products (bioactive molecules and hydrolytic enzymes), holds the greatest promise and is considered a pillar of integrated pest management [2]. Most Biological Control Agent (BCA) products can be applied through seed treatment and foliar spraying [3].

Over the years, the use of Bacillus genera, especially spore-producing *Bacillus subtilis* in crop protection industries, has increased due to their high survivability and wide range of antimicrobial compounds produced [4]. The antagonistic bacteria of *B. subtilis* TM4 used in this study can act as biological control of corn plant pathogens by producing various enzymes and secondary metabolites such as chitinase, protease, cellulase, antibiotics. They can increase plant growth with the ability to dissolve phosphate and potassium [5].

The initial response of plant resistance can be seen from the occurrence of mycelium lignification. Lignification will result in stunted growth and pathogen infections in plants [6]. Observation of chlorophyll content needs to be done to determine the effect of DM on chlorophyll due to chlorosis. The mechanism of stomata that opens and

closes automatically can enter organisms that play a role in the process of infection of plant pathogens. The greater the density of the stomata, the greater the chance of infection [7], [8].

The use of antagonistic bacteria as biopesticide can be one alternative in controlling the disease because it is still rare in Indonesia. This study aims to test the effectiveness of the formulation of antagonistic bacteria *B. subtilis* in inhibiting/suppressing the development of DM with seed treatment and spraying formulations on the field.

II. Materials and Method

This study was conducted at Maros Experimental Farm of ICERI from June to October 2019.

A. Experimental design

The treatments were arranged in factorial in randomized block design consisting of factor *A* (*B. subtilis* treatment) with three levels (A0=without *B. subtilis* treatment added, A1= with *B. subtilis* treatment, and A2=synthetic pesticides); and factor B (variety) with four levels (B1 = Anoman (control/susceptible variety), B2 = Bima 20 URI, B3 = Bima 15 Sayang, and B4= Perkasa (private-owned commercial variety). The *B. subtilis* formulation in powder form was made according to the formulation from the previous study [9].

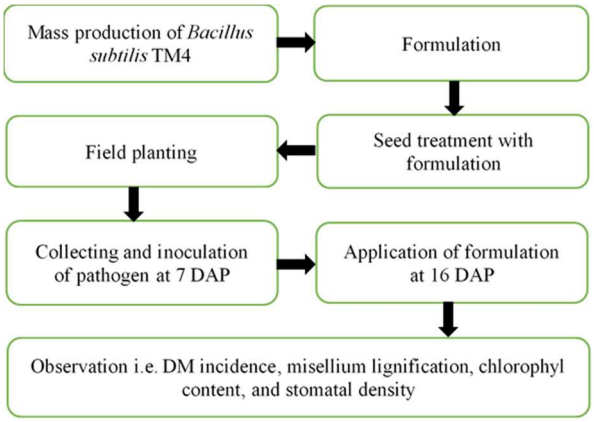

Fig. 1 Flowchart diagram of the procedures of biopesticide application.

B. Suppression Test of Downy Mildew by B. subtilis TM4Suppression Test of Downy Mildew by B. subtilis TM4

1) Collecting the Spores of Downy Mildew: The inoculum source was obtained from DM-infected plants in the Maros Experimental Farm of ICERI (Indonesian Cereals Research Institute). The collection of DM-infected leaves was carried out at 5 am by cutting the whole leaves and then put them in a plastic bag. Once collected, the leaves used as a source of inoculum were washed with running water to remove existing conidia and dirt on the leaf's surface. The base of the leaves was then soaked in sugar water with a concentration of 2% in a container in dark conditions to stimulate the formation of spores (sporulation). At 3:00 am., the spores were collected by rinsing the leaves that white-colored spores have covered in a container filled with water.

2) Planting and Inoculation: Seeds were planted in micro plots with an area of 5 m^2 with a spacing of 75 cm × 25 cm. After being planted, at the age of 7 days after planting (DAP), spore inoculation was carried out by spraying a conidia suspension of *P. philippinensis* (10^6 spores/ml) on the leaves of corn plants at around 03.00-04.00 am. Inoculation was repeated three days later to get optimal symptoms. Fertilizer was applied twice, at 10 DAP with the dose of 100 kg Urea/ha + 1 5 0 kg Phonska/ha and at 4 weeks after planting (WAP) by applying 100 kg Urea/ha. Watering was done every two days (conditioned according to soil conditions). Maintenance is carried out by removing weeds that grow and embroider plants that did not grow and making arrangements for irrigation not to lack water.

3) Application of B. subtilis Biopesticide Formulation: The *B. subtilis* TM4 formulation used in this study is formulated with talc as a carrier. The carrier is the major portion (by volume or weight) of the inoculant [10]. The formulation application was carried out twice. The first application was made just before planting by treating 8g/k g of corn seeds. The second application was made at 16 DAP at a dose of 3 g/L. Spraying was done evenly throughout the leaves of the test plant.

4) Observation: The parameters observed were the percentage of plants growth at 7 DAP, disease severity at 21, 28, 35, and 42 DAP, plant height at 28 and 42 DAP, and yields by counting weight and number of cobs harvest. The formula calculated the severity of the disease.

$$\text{Is} = \frac{A}{B} \times 100\%$$

Is = Percentage of DM incidence (%)
A = Number of infected plants
B = Total number of plants observed

C. Observation of Mycelium Lignification Observation of Mycelium Lignification

Observation of *P.philippinensis* mycelium lignification was carried out at 12 hours after inoculation. The observation was carried out by the Sass method [11] with modification. The plant leaves were cut approximately 1 cm, then soaked in phloroglucinol 1% solution in alcohol 96% during the first 5 minutes, then soaked in a solution of HCl 10% for 5 minutes and heated in a solution alcohol 50% for 30-60 seconds. Furthermore, the leaves were observed under the microscope. Purplish red tissue of mycelium indicates that mycelium was lignified.

D. Chlorophyll Content

Chlorophyll content was observed at 42 DAP using the SPAD-502 *Chlorophyll meter*. Leaf strands were sampled at the base, middle, and tip of the leaf

E. Stomata Density

Observation of the number of stomata was done once at 21 DAP. Each variety was represented by one sample plant. The leaf used was the third leaf from the top. The leaf's surface was cleaned from sand or soil and then smeared with 1 cm^2 nail polish on the underside of the leaf at the tip, middle, and base. After the paint dries, masking tape was applied to cover the paint layer. The tape was released slowly, so the nail

polish peels off. Masking tape clinging to the nail polish was affixed to the glass of the object. The surface of the leaves and stomata were recorded on a layer of nail polish like a mold. Stomata printouts were observed using optilab. Calculation of the number of stomata was carried out at an observation area of 876 × 656 μm^2.

F. Data Analysis

Data were analyzed with ANOVA and continued with the least significant difference test (LSD) at 5% level.

III. Results and Discussion

Mycelium lignification was observed in the *B. subtilis* treated samples. It is characterized by purplish-red mycelium. The control treatment did not show the mycelium lignification process (Figure 2). The occurrence of lignification 12 hours after inoculation in the biopesticide treated samples may indicate that *B. subtilis* triggers the response of plant resistance to pathogen infections.

The plant is known to have multi-layered recognition and resistance mechanisms. One layer of resistance involves the introduction of cell surfaces to microbial components, one of which is Microbe-Associated Molecular Patterns (MAMPs) which can trigger resistance (as an elicitor) [12]. MAMP can be a molecule derived from beneficial microbes or non-pathogens [13]. One of the epitopes (places of recognition) that acts as a MAMP elicitor is the flagella structure of bacteria [14], [15]. *B. subtilis* species is known to have a high number of flagella, so that there is a higher likelihood for contact recognition to trigger resistance [16].

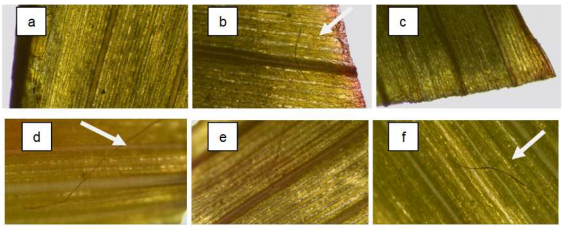

Fig. 2 Mycelium lignification of *P. philippinensis* on the leaves of corn plants 12 hours after inoculation; control + Anoman (a), formula *B. subtilis* + Anoman (b), formula *B. subtilis* + Bima-20 (c), formula *B. subtilis* + Bima-15 (d), formula *B. subtilis* + Perkasa (e), and synthetic pesticides + Bima-20 (f)

Lignification is one mechanism of plant resistance to a pathogen. Pathogens such as *Peronosclerospora* sp. can penetrate plants' tissues and grow internally between plant cells [17]. *Peronosclerospora* sp. was grouped into Oomycetes taxa [18]. In contrast to the fungi group, the Oomycetes cell wall comprises cellulose, glycan, and proteins rich in hydroxyproline [13]. The lignification process can inhibit the development of pathogens in the plant tissue as well as on the surface of the plant by strengthening the cell walls of plants through lignification process. The other mechanisms are to lignified pathogen cell containing components such as chitin, cellulose and protein-rich hydroxyproline, which can act as a matrix in the lignification process. As a result, pathogens will be lignified and become rigid and lose the plasticity required for development and penetration [19].

The effect varieties and the application of *B. subtilis* formulation to the plant growth and stomata density can be seen in Table 1. In general, the plant growth was not significantly different, except between the control treatments of Perkasa variety was significantly higher than the Bima-15 treated with synthetic pesticides with the plant growth 90% and 21% respectively. Meanwhile, the results of the analysis of variance on stomata density showed that the mean stomata density between varieties and between treatments was not significantly different. Anoman has the lowest density of stomata in synthetic pesticide treatment with 37.8/mm^2 and the highest was the *B.subtilis* treated Perkasa variety with the stomata density of 53.7/mm^2.

TABLE I
Effect of *B. Subtilis* Formulation on Plant Growth and Stomata Density on 4 Test Varieties. Maros 2019.

Seed Treatment	Plant growth (%) 10 DAP	Stomata Density (/mm^2) 21 DAP
Control + Anoman	85.0^{ab}	51.3
Control + Bima-20	78.7^{ab}	52.5
Control + Bima-15	57.9^{ab}	52.5
Control + Perkasa	90.4^{a}	53.7
B. subtilis + Anoman	85.4^{ab}	48.8
B. subtilis + Bima-20	69.6^{ab}	53.7
B. subtilis + Bima-15	65.0^{ab}	45.2
B. subtilis + Perkasa	80.0^{ab}	51.3
Synthetic + Anoman	67.9^{ab}	37.8
Synthetic + Bima-20	53.7^{ab}	51.3
Synthetic +Bima-15	21.2^{b}	43.9
Synthetic + Perkasa	97.9^{a}	47.6

Value in column followed by the same letter, are not significantly different according to Tukey test (HSD) at 5% level.

The effect of four corn varieties by *B. subtilis* formulation on DM incidence is presented in Table 2. Table 2 shows that the response of different corn varieties by the *B. subtilis* formulation can suppress *P. philippinensis*. The treatment of *B. subtilis* formulation in suppressing DM pathogen was significantly lower than in control. The statistical analysis showed that the incidence of DM on Anoman varieties was significantly higher compared to Bima-20, Bima-15, and Perkasa. There were no significant differences between the hybrid varieties.

The highest disease index was observed in the control (91%) at 42 days after treatment. The *B. subtilis* formulation treated group showed the lowest disease incidence, which was 6.5% and 24% in Bima-15and Bima-20, respectively (Table 2). This is presumably because Bima-15 has a better response to the treatment of *B. subtilis* formulation than Bima-20. Due to their genetic and metabolic diversity, *Bacillus* spp. are well-adapted to a wide range of environmental conditions. Such wide environmental adaptability with many beneficial traits makes *Bacillus* spp. a suitable candidate for their application as a biofertilizer or biocontrol agent [20].

In addition to inducing plant resistance, *B. Subtilis* is also known to directly inhibit the development of pathogens. *B. Subtilis* can produce several antimicrobial and volatile organic compounds that inhibit the development of pathogens [4], [21]. *B. Subtilis* has 4-5% of the entire genome to synthesize various antimicrobial compounds [22]. Among several antimicrobial compounds produced by *B. Subtilis*, compounds included in the cyclic lipopeptide group such as *surfactin, iturin*, and *fengicin* [23] are the best known for their potential because apart from being an antimicrobial

compound they are also included in the biosurfactant class. The ability of *B. subtilis* to inhibit the development of pathogens even in low concentrations in the treatment of seeds can indicate that *B. subtilis* is an environmentally friendly alternative disease control solution [24].

TABLE II
AVERAGE DM INCIDENCE ON TESTING FORMULATIONS OF B. SUBTILIS TO DOWNY MILDEW. MAROS 2019

Treatment	DM incidence (%) on			
	21 HST	28 HST	35 HST	42 HST
Control + Anoman	10.1 [b]	46.9 [a]	77.4 [a]	91.1 [a]
Control + Bima-20	1,4 [c]	3,3 [b]	20.1 [b]	28.7 [b]
Control + Bima-15	0.5 [c]	1.0 [b]	8.2 [b]	8.2 [b]
Control + Perkasa	0.0 [c]	2,8 [b]	7.3 [b]	13.2 [b]
B. subtilis + Anoman	17.7 [a]	41.6 [a]	71.7 [a]	75.2 [a]
B. subtilis + Bima-20	1.7 [c]	4,6 [b]	17.4 [b]	24.7 [b]
B. subtilis + Bima-15	1,2 [c]	2.2 [b]	5,4 [b]	6.5 [b]
B. subtilis + Perkasa	0.0 [c]	1.5 [b]	8.1 [b]	9.9 [b]
Synthetic + Anoman	3,9 [c]	37.8 [a]	59.4 [a]	81.9 [a]
Synthetic + Bima-20	0.0 [c]	1,2 [b]	12.6 [b]	19.4 [b]
Synthetic + Bima-15	0.0 [c]	9.3 [b]	6,4 [b]	6,4 [b]
Synthetic + Perkasa	0.4 [c]	4.0 [b]	5,3 [b]	9.2 [b]

Value in column followed by the same letter, are not significantly different according to Tukey test (HSD) at 5% level.

The treatment of *B. subtilis* biopesticide formulations tended to have higher leaf chlorophyll content in corn plants compared to control treatments. The result of observations showed that the highest chlorophyll level was found in the Bima-15 formulation treatment (43.7 g/ml), while the lowest with a chlorophyll content of 21.1 g/ml was found in Anoman for synthetic pesticide treatment. These findings can be explained by the fact that photosynthesis is mainly regulated by the stimulation of endogenous signals and the environment. The chlorophyll content also had greater in the *B. subtilis* formulation which may be explained by previously reported finding [25], i.e. that the *Bacillus subtilis* SL-13 may regulate signal transduction pathways that are related to photosynthesis, and biochar alters the soil environment, which results in plant growth stimulation. In downy mildew, plants undergo chlorosis which causes a decrease in chlorophyll and photosynthetic activity. Differences in the value of chlorophyll content between treatments can be caused due to the suppression effect of the treatment on disease progression so that the reduction rate of chlorophyll is lower in some treatments.

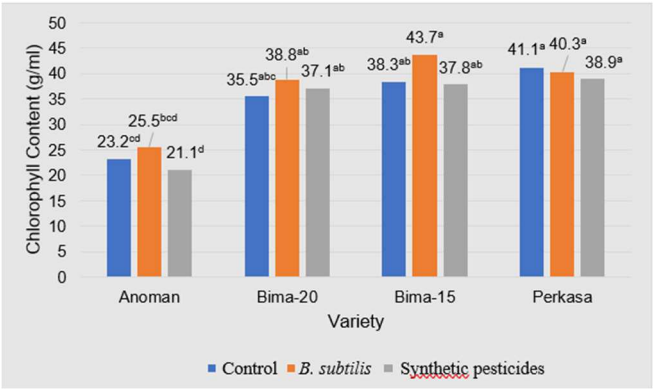

Fig. 3 Effect of application of *B. subtilis* formulation on four test varieties on leaf chlorophyll levels in the plant at 42 DAP. Maros 2019. The same letter indicating not significantly different according to the LSD test at the 5% level

Besides that *B. subtilis* can play a role as PGPR (Plant Growth Promoting Rhizobacteria) through photosynthetic pigment restoration activity [26]. The average chlorophyll content can be seen in Figure 3.

To determine the effect of the treatment of *B. subtilis* formulation on the growth and production of maize under conditions of downy mildew disease, several parameters were observed. Plant height at 28 DAP with the application of *B. subtilis* formulation showed significantly higher results compared to controls, and not significantly different from synthetic pesticides (Figure 4). The height of the corn plant in the treatment of *B. subtilis* formulation was 53.5 cm, and in the treatment of synthetic pesticides was 54.7 cm while in the control treatment, it was lower at 48.9 cm. *B. Subtilis* species have been widely reported as the PGPR that can trigger plant growth. Lobo *et al.* [27] reported that *B. subtilis* inoculation can cause an increase in plant height of 9-18%. Besides the ability of *B. Subtilis* to produce endospores like most other Bacillus genera, causing *B. Subtilis* can survive in less favorable environmental conditions.

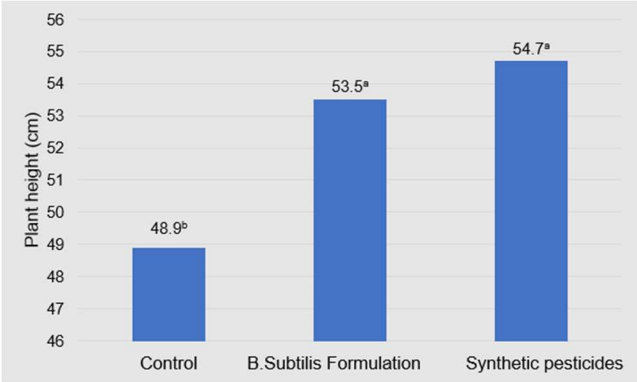

Fig. 4 Effect of *B. subtilis* formulation application on plant height at 28 DAP. Maros 2019. The same letter indicating not significantly different according to the LSD test at the 5% level

For observations on yields, parameters observed included the number of ears, 10 ears weight, and yields. The application of *B. subtilis* formulations showed the highest yields on Perkasa that were significantly different compared to Bima-15, Bima-20, and controls (Table 3). The yield on the Perkasa was 4.5 t/ha, followed by Bima 15 and Bima-20 was 1.8 and 1.7 t/ha respectively, and the lowest (Anoman) was 0.24 tons/ha.

TABLE III
COMPONENTS OF THE YIELD OF 4 VARIETIES IN THE TESTING OF *B. SUBTILIS* FORMULATIONS AGAINST DOWNY MILDEW. MAROS 2019.

Treatment	Yield components		
	Number of Ear	10 Ears Weight (kg)	Yield (t/ha)
Anoman	4 [c]	0.47 [c]	0, 24 [c]
Bima-20 URI	27 [b]	1.47 [b]	1.70 [b]
Bima-15 Sayang	25 [b]	1,24 [b]	1.80 [b]
Perkasa	57 [a]	2.0 2 [a]	4.51 [a]

The number in the column followed by the same letter is not significantly different according to the LSD test at the 5% level

IV. CONCLUSION

The present study showed the application of *B. subtilis* formulations by seed treatment and spraying on plants effectively inhibit the development of DM (*P. philippinensis*),

induce mycelium lignification, showed a higher leaf chlorophyll content and improve the growth of corn plants. Plant height of *B. subtilis* treated samples showed a significantly higher average (53.5cm) compared to controls. The combination of *B. subtilis* and Bima-15 showed the lowest disease incidence (6.5%) and highest chlorophyll content (43.7 g/ml) among the *B. subtilis* treated group. However, the Perkasa variety showed the highest yield (4.5 t/ha) compared to other DM stress conditions.

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REFERENCES

[1] C. Ginting *et al.*, "Identification of Maize Downy Mildew Pathogen in Lampung and the Effects of Varieties and Metalaxyl on Disease Incidence," *Annu. Res. Rev. Biol.*, vol. 35, no. May, pp. 23–35, 2020, doi: 10.9734/arrb/2020/v35i730244.

[2] F. Berini, C. Katz, N. Gruzdev, M. Casartelli, G. Tettamanti, and F. Marinelli, "Microbial and viral chitinases: Attractive biopesticides for integrated pest management," *Biotechnol. Adv.*, vol. 36, no. 3, pp. 818–838, May 2018, doi: 10.1016/j.biotechadv.2018.01.002.

[3] Y. Iftikhar, A. Sajid, Q. Shakeel, Z. Ahmad, and Z. Ul Haq, "Biological Antagonism: A Safe and Sustainable Way to Manage Plant Diseases," in *Plant Disease Management Strategies for Sustainable Agriculture through Traditional and Modern Approaches*, I. Ul Haq and S. Ijaz, Eds. Cham: Springer International Publishing, 2020, pp. 83–109.

[4] S. Caulier, C. Nannan, A. Gillis, F. Licciardi, C. Bragard, and J. Mahillon, "Overview of the antimicrobial compounds produced by members of the Bacillus subtilis group," *Front. Microbiol.*, vol. 10, no. FEB, pp. 1–19, 2019, doi: 10.3389/fmicb.2019.00302.

[5] N. Djaenuddin, Suwarti, S. Pakki, and A. Muis, "Characterization Of Physiological Properties Of Bacteria Isolates Tm4 And Bnt8 In Biopesticide Formulas," in *IOP Conference Series: Earth and Environmental Science (Accepted)*, 2020.

[6] E. Sabella *et al.*, "Xylella fastidiosa induces differential expression of lignification related-genes and lignin accumulation in tolerant olive trees cv. Leccino," *J. Plant Physiol.*, 2018, doi: 10.1016/j.jplph.2017.10.007.

[7] C. Tateda *et al.*, "The Host Stomatal Density Determines Resistance to Septoria gentianae in Japanese Gentian," *Mol. Plant-Microbe Interact.*, vol. 32, no. 4, pp. 428–436, 2019, doi: 10.1094/MPMI-05-18-0114-R.

[8] C. D. Muir, "A Stomatal Model of Anatomical Tradeoffs Between Gas Exchange and Pathogen Colonization," *Front. Plant Sci.*, vol. 11, no. October, pp. 1–12, 2020, doi: 10.3389/fpls.2020.518991.

[9] A. Muis, N. Djaenuddin, and N. Nonci, "Evaluasi Lima Jenis Inner Carrier Dan Formulasi Bacillus Subtilis Untuk Pengendalian Hawar Pelepah Jagung (Rhizoctonia Solani Kuhn)," *J. Hama Dan Penyakit Tumbuh. Trop.*, vol. 15, no. 2, p. 164, 2016, doi: 10.23960/j.hptt.215164-169.

[10] Y. Bashan, L. E. de-Bashan, S. R. Prabhu, and J. P. Hernandez, "Advances in plant growth-promoting bacterial inoculant technology: Formulations and practical perspectives (1998-2013)," *Plant and Soil*, vol. 378, no. 1–2. Kluwer Academic Publishers, pp. 1–33, May-2014, doi: 10.1007/s11104-013-1956-x.

[11] J. Sass, *Botanical microtechnique. 3rd Edition*. The Lowa State College Press, 1958.

[12] F. Agriculture *et al.*, "Nicotiana benthamiana LRR-RLP NbEIX2 mediates the perception of an EIX-like protein from Verticillium dahliae," 2020, doi: 10.1111/jipb.13031.

[13] O. M. Finkel, G. Castrillo, S. Herrera Paredes, I. Salas González, and J. L. Dangl, "Understanding and exploiting plant beneficial microbes," *Current Opinion in Plant Biology*. 2017, doi: 10.1016/j.pbi.2017.04.018.

[14] W. R. Chezem, A. Memon, F. S. Li, J. K. Weng, and N. K. Clay, "SG2-type R2R3-MYB transcription factor MYB15 controls defense-induced lignification and basal immunity in arabidopsis," *Plant Cell*, 2017, doi: 10.1105/tpc.16.00954.

[15] S. C. Bhatla, M. A. Lal, M. A. Lal, R. Kathpalia, R. Sisodia, and R. Shakya, "Biotic Stress," in *Plant Physiology, Development and Metabolism*, 2018.

[16] Z. Ahmad, J. Wu, L. Chen, and W. Dong, "Isolated Bacillus subtilis strain 330-2 and its antagonistic genes identified by the removing PCR," *Sci. Rep.*, 2017, doi: 10.1038/s41598-017-01940-9.

[17] C. J. R. Cumagun, "Host-Parasite Interaction During Development of Major Seed-Borne Fungal Diseases BT - Seed-Borne Diseases of Agricultural Crops: Detection, Diagnosis & Management," R. Kumar and A. Gupta, Eds. Singapore: Springer Singapore, 2020, pp. 233–244.

[18] R. Suharjo *et al.*, "Peronosclerospora australiensis is a synonym of P. maydis, which is widespread on Sumatra, and distinct from the most prevalent Java maize downy mildew pathogen," *Mycol. Prog.*, vol. 19, no. 11, pp. 1309–1315, 2020, doi: 10.1007/s11557-020-01628-x.

[19] E. Lamalakshmi Devi *et al.*, "Adaptation strategies and defence mechanisms of plants during environmental stress," in *Medicinal Plants and Environmental Challenges*, 2017.

[20] A. K. Saxena, M. Kumar, H. Chakdar, N. Anuroopa, and D. J. Bagyaraj, "Bacillus species in soil as a natural resource for plant health and nutrition," *Journal of Applied Microbiology*, vol. 128, no. 6. Blackwell Publishing Ltd, pp. 1583–1594, Jun-2020, doi: 10.1111/jam.14506.

[21] H. Gao, P. Li, X. Xu, Q. Zeng, and W. Guan, "Research on volatile organic compounds from Bacillus subtilis CF-3: Biocontrol effects on fruit fungal pathogens and dynamic changes during fermentation," *Front. Microbiol.*, vol. 9, no. MAR, Mar. 2018, doi: 10.3389/fmicb.2018.00456.

[22] T. Stein, "Bacillus subtilis antibiotics: Structures, syntheses and specific functions," *Molecular Microbiology*. 2005, doi: 10.1111/j.1365-2958.2005.04587.x.

[23] P. Mülner *et al.*, "Profiling for Bioactive Peptides and Volatiles of Plant Growth Promoting Strains of the Bacillus subtilis Complex of Industrial Relevance," *Front. Microbiol.*, vol. 11, no. June, 2020, doi: 10.3389/fmicb.2020.01432.

[24] N. Djaenuddin, Suriani, and A. Muis, "Effectiveness of Bacillus subtilis TM4 biopesticide formulation as biocontrol agent against maydis leaf blight disease on corn," *IOP Conf. Ser. Earth Environ. Sci.*, vol. 484, no. 1, 2020, doi: 10.1088/1755-1315/484/1/012096.

[25] S. Tao, Z. Wu, M. Wei, X. Liu, and Y. He, "Bacillus subtilis SL-13 biochar formulation promotes pepper plant 2 growth and soil improvement," *Can. J. Microbiol. Can. J. Microbiol*, 2019.

[26] N. Ghazy and S. El-Nahrawy, "Siderophore production by Bacillus subtilis MF497446 and Pseudomonas koreensis MG209738 and their efficacy in controlling Cephalosporium maydis in maize plant," *Arch. Microbiol.*, no. 0123456789, 2020, doi: 10.1007/s00203-020-02113-5.

[27] L. L. B. Lobo, R. M. dos Santos, and E. C. Rigobelo, "Promotion of maize growth using endophytic bacteria under greenhouse and field conditions," *Aust. J. Crop Sci.*, vol. 13, no. 12, pp. 2067–2074, 2019, doi: 10.21475/ajcs.19.13.12.p2077.