International Journal of Applied Science and Engineering Review

IJASER

ISSN: 2582-6271

Vol. 5, Issue.3, May-June 2024, page no. 58-75

To cite this article: Alfizar Alfizar, Amda Resdiar, Nana Ariska, Muhammad Sayuthi, Siti Shofiya Nasution, Novita Novita and Syaukani Syaukani (2024). EFFICACY ANTIMICROBIAL ENDOPHYTICS METABOLITES TO CONTROL SIGATOKA DISEASE (MYCOSPHAERELLA MUSICOLA) ON BANANAS IN NORTHERN SUMATERA, International Journal of Applied Science and Engineering Review (IJASER) 5 (3): 58-75 Article No. 199 Sub Id 299

EFFICACY ANTIMICROBIAL ENDOPHYTICS METABOLITES TO CONTROL SIGATOKA DISEASE (MYCOSPHAERELLA MUSICOLA) ON BANANAS IN NORTHERN SUMATERA

Alfizar Alfizar[1,2]*, Amda Resdiar[2], Nana Ariska[2], Muhammad Sayuthi[1], Siti Shofiya Nasution[1], Novita Novita[3], Syaukani Syaukani[4]

[1]Faculty of Agriculture, Department of Plant Protection, Universitas Syiah Kuala Banda Aceh, 23111, Indonesia
[2]Faculty of Agriculture, Department of Agrotechnology, Universitas Teuku Umar Meulaboh 23681, Indonesia
[3]Faculty of Teacher Training and Education, Universitas Syiah Kuala, Banda Aceh, 23111, Indonesia
[4]Faculty of Mathematics and Natural Sciences, Department of Biology, Universitas Syiah Kuala Banda Aceh, 23111, Indonesia

DOI: https://doi.org/10.52267/IJASER.2024.5309

ABSTRACT

The secondary metabolites show diverse biological activities, including phytotoxic, antibacterial, antifungal, and antitumor. This study aimed to obtain secondary metabolites that are more effective in suppressing the development of Sigatoka disease in bananas. A field trial was performed to screen the antifungal metabolitesactivity of three isolates against *Mycosphaerella musicola*. This research used a randomised block design with four treatments and six replications. The results showed that the fastest incubation period was obtained at 14 days in the control treatment, and with *Bacillus sp* occurred after 30 days, 25 days in the treatment of *Pseudomonas aeruginosa*, and 40 days in the treatment of *Trichoderma asperellum*. The percentage of disease incidence and spot area on the leaves was most significant in the control treatment, 62,5% and 66,45%, then followed by Pseudomonas aeruginosa respectively, 41,67% and 34,48%, and the lowest disease incidence and spot area in the treatment, *Trichoderma*sp were 25% and 21,78%, respectively. Further, the lowest disease severity was found in the secondary metabolite of *Trichoderma asperellum* at 23.36%, followed by *Pseudomonas aeruginosa* secondary metabolite at 32.57%. Meanwhile, the highest disease severity was found in the control treatment, with an average of 60.27%. Secondary metabolites of *Bacillus* sp, *Trichoderma asperellum* and *Pseudomonas aeruginosa* effectively suppress Sigatoka disease. The highest banana production was found in the secondary metabolite of *Trichoderma asperellum*, with a harvestweight of 13.98 kg, and the lowest harvest weight found in the control treatment was 11.90 kg.

International Journal of Applied Science and Engineering Review

IJASER

ISSN: 2582-6271

Vol. 5, Issue.3, May-June 2024, page no. 58-75

KEYWORDS: Banana, Sigatoka, endophytes, Bacillus, Pseudomonas, Trichoderma.

INTRODUCTION

Bananas (*Musa paradisiaca* L.) are Indonesia's leading horticultural commodities, which are very useful andhave high nutritional value. Nationally, this commodity is also the main contributor to 31% of superior fruit production. In Indonesia, bananas are in first place in terms of the wide distribution of plantations and the availability of suitable land for their growth very wide, from the lowlands to the highlands. Usually, different banana varieties are planted in small farming communities, and the farmers traditionally did not take cared seriously their plantation, so fungi and bacterial diseases often attack banana plants both in the dry season and more severely in the rainy season.

The development of banana plants in Indonesia is hampered by the emergence of various pathogens that attack banana plants, thereby disrupting banana plant production. Several diseases that attack banana plants include bacterial wilt, fusarium wilt, Cercospora leaf spot, and banana dwarf disease caused by viruses (Hermanto and Setyawati, 2002). Among these diseases that often attack banana plants are Sigatoka yellow spots. Sigatoka disease causes the leaves to scorch and dry, the fruit produced is small, can cause the ovaries to fall off, reduce the quality of the fruit, and ripen the fruit earlier, so that banana production decreases by up to 50% (Ploetz 2007). Sigatoka leaf spot caused yield losses in bananas by reducing the photosynthetic tissues through necrotic leaf lesions. If this disease is not controlled, it can cause major losses and even slowly kill banana plants.

The initial symptoms of yellow Sigatoka appear on the lower leaves in the form of small, pale-yellow spots. These small spots develop into pale yellow streaks, brown streaks and elliptic necrotic spots arranged parallel to the secondary veins. The spots then get bigger and longer over time, and the depressed grey centre is surrounded by a yellow halo, forming oval spots or elongated spots with a dry (Mulyanti, 2008). In advanced stages, these streaks enlarge to form necrotic lesions with a yellow halo and light grey centre (Stover, 1972). These necrotic lesions later coalesce, causing complete drying of the leaves and defoliation and leaf edges curled inward, leading to delayed flowering, reduction in the number of hands and fingers, premature ripening and peel splitting of the fruits Milsha et al, 2022).

The existence of the Sigatoka fungus can be suppressed by using biological agents, such as antagonistic endophyte microbes in the banana plant itself. Antagonistic microbes are organisms that can inhibit the growth of disease-causing microbes in plants, such as fungi and pathogenic bacteria. According to Marin et al. (2003), black Sigatoga disease in banana plantations can be controlled by applying fungicides, especially protectant fungicides such as mancozeb and chlorothalonil, and systemic fungicides such as

ISSN: 2582-6271

Vol. 5, Issue.3, May-June 2024, page no. 58-75

benomyl and benzimidazole used alternately. However, continuous application of protective and systemic fungicides causes pathogens to develop resistance to the active ingredients of fungicides.

Therefore, it is necessary to try other control methods that are environmentally friendly by using biological agents. Nowadays, the efforts to control Sigatoka disease are now starting to be non-chemical control. One alternative for controlling Sigatoka is by using antagonistic fungi and bacteria produced secondary metabolites. According to Demain and Fang (2000) Secondary metabolites serve as competitive weapons used against plant pathogen bacteria, fungi, amoebae, plants, insects, and large animals; as metal transporting agents; as agents of symbiosis between microbes and plants, nematodes, insects; as sexual hormones; and as differentiation effectors. The Bacillus genus is an antagonistic bacterium that is able to control several types of plant pathogens. Bacillus sp. is able to compete with pathogens and produce several secondary metabolites, such as antibiotics, siderophores, bacteriocins, and extracellular enzymes. These bacteria are also able to induce plant resistance compounds and can act as Plant Growth Promoting Rhizobacteria (PGPR) (Beneduzi et al., 2012) Bacillus sp. is able to produce fencing and bacillomycin compounds, which are known to be antifungal, and many other antibiotic peptide compounds produced by *Bacillus sp*. (Stein, 2005). Screening of microbial extracts reveals the large structural diversity of natural compounds with broad biological activities, such as antimicrobial, antiviral, immunosuppressive, and antitumor activities, that enable the bacterium to survive in its natural environment. These findings widen the potential industrial importance of *Bacillus spp*., particularly of B. thuringiensis, beyond insecticidal usage and may help explain the role of Bacillus spp. in the soil ecosystem (Sansinenea and Aurelio, 2011). Another type of bacteria, Pseudomonas sp, is used as a biological agent and is able to control plant diseases. This bacteria is capable of producing antibiotics (antifungal) compounds, siderophores, and additional secondary metabolites with characteristics that can prevent fungal growth (Coleman et al., 2011). Pseudomonas aeruginosa has been widely reported to be able to control soil-borne plant pathogens (Hass and Keel, 2003) and is known to be able to survive for a long time both in the rhizosphere and diphyllosphere. Several studies have shown that fluoride-emitting Pseudomonas bacteria are able to control disease, both in the roots and on the leaves (Gupta et al., 2001). Pseudomonas aeruginosa is a root- colonizing bacterium that produces salicylic acid and phytoalexin, which induce plant resistance to pathogens (Van Loon, 2006). P. fluorescens isolates 148, 35Q, 16Q, and 113 produce phenoloxidase (PO) and phenyl ammonia lyase (PAL), which can induce systemic resistance in cotton plants against bacterial blight (*Xanthomonas campestris* pv. malvacearum) (Fallahzadeh et al. 2009).

Apart from that, there are antagonistic fungi that also live in the soil, *Trichoderma* sp., a type of antagonistic fungus that has the potential to be a biological control agent for several plant diseases. Several previous studies showed that isolates of *Trichoderma* sp has the potential to produce secondary metabolites that are antibiotics, namely viridin and trichomidine (Nakkeeran et al., 2021). Viridin and

ISSN: 2582-6271

Vol. 5, Issue.3, May-June 2024, page no. 58-75

tricomidine can inhibit thegrowth of or even kill other fungi. This research is an initial study in the development of biopesticides with active ingredients from secondary metabolites of *Trichoderma* sp.

Based on this, it is necessary to conduct research on the potential of secondary metabolites from the biological agents *Bacillus sp*., *Trichoderma* sp., and *Pseudomonas aeruginosa* to control Sigatoga disease on bananas. It is hoped that secondary metabolites from this biological agent can suppress the pathogen that causes Sigatoka disease.

MATERIAL AND METHODS

Isolation of pathogenic fungus

The *Mycosphaerella musical with the* pathogenic fungus was isolated from a symptomatic banana leaf showing typical signs of Sigatoka yellow leaf streak infection. The banana leaf was obtained from the collection of banana plantations at the University Farm of the University of Teuku Umar in Indonesia. The leaf was carefully cleaned and washed to eliminate any airborne contaminants to ensure aseptic conditions. The symptomatic portion of the leaf was then cut into fragments measuring 1 cm x 1 cm. These fragments were treated with a 5% chlorine solution for 1 minute, followed by rinsing twice with sterile distilled water. Following that, the leaf fragments were placed on a Potato Dextrose Agar (PDA) medium supplementedwith Streptomycin at a concentration of 50 $mg.l^{-1}$ to inhibit bacterial growth. The cultures were incubated ina temperature-controlled incubator set at 27°C. As the fungal hyphae growth appeared, it was transferred toa fresh PDA medium to ensure the purity of the culture. This process was repeated until pure cultures of the *Mycosphaerella musicola* strain were obtained.

Morphological characterisation of pathogen

The characterisation of fungal isolates involved macroscopic and microscopic observations. This research was conducted in the plant pathology laboratory of the faculty of agriculture at Syiah Kuala University. The morphological characterisation of the fungal isolates was assessed by macroscopic examination, which involved observing the colony shape and colour, as well as microscopic examination to determine the morphology of hyphae, whether they were septate or non-septate, and the shape of conidiophores. All characterisation data obtained further are adjusted and identified using a fungus identification book, Illustrated Genera of Imperfect Fungi, by Barnett and Hunter, 1998.

Propagation of biological agents

Pseudomonas aeruginosa and Trichoderma asperellum were isolated by Alfizar et al. l, 2023 and Bacillus spwas a collection isolate from the Plant Pathology laboratory of the Agriculture Faculty, Universitas Syiah Kuala. Subsequently, the isolates were cultured at the Plant Disease Laboratory, Faculty of Agriculture, Syiah Kuala University. *T. asperellum* were cultured using *Potato Dextrose Agar* medium

ISSN: 2582-6271

Vol. 5, Issue.3, May-June 2024, page no. 58-75

(4g/L potato extract, 20g/L glucose, 15 g/L agar, Oxoid™) while bacterial isolates were cultured on King's B medium (20g/L protease peptone no. 3, 1.5 g/L K_2HPO_4, 1,5 g/L $MgSO_4.7H_2O$, 15 g/L agar, 10 ml glycerol, HiMedia®).

Production of secondary metabolites of bacteria and fungus

Pure cultures of *T. asperellum*, *Bacillus* sp, and *P. aeroginosa* were obtained and cultured in liquid media to promote the production of secondary metabolites. The production of secondary metabolites of *T. asperellum*,*Bacillus* sp, and *P. aeruginosa* used the method of Soesanto et al. l (2011). For the *T. asperellum* isolate, theliquid medium consisted of a combination of coconut water, rice water, and sugar. The propagation process involved meticulously blending 4 litres of rice washing water, 1 litre of coconut water, and 75 grams of sugar. The resulting mixture was thoroughly mixed, sterilised in an autoclave at 121°C for 30 minutes, cooled down, and transferred into a sterile jerry can. To initiate propagation, a pure culture of the *T. asperellum* isolate was diluted with 10 ml of sterile distilled water and added to the jerry can, which was sealed tightly. The jerry can then be placed on a shaker operating at 150 rpm for 10 days, providing optimal conditions for metabolite production. To obtain pure secondary metabolites, all secondary metabolites are precipitated for 12 hours and then filtered with a polycarbonate membrane (0,2 µm, 25 mm diameter, millipore, PC 110656N) to remove mycelium of fungus and bacteria cells.

Treatment of secondary metabolites and pathogen Mycosphaerella musicola

The design used in this study was a randomised block design with 4 treatments and 6 replications. Each treatment unit consisted of 4 banana seedlings, with 96 banana seedlings. The variety of banana (Musa acuminata) seedlings was 5 months old and taken from healthy banana clumps at the University Farm, Teuku Umar University. The method of applying secondary metabolites has been mixed with 2 ml.L-1 sticker to the leaves of banana, using an electric growing hand sprayer with one hole, a volume of 16 litres.

The applications to the foliar of banana by distilled water (T0), in a suspension of *Bacillus* sp secondary metabolites (T1), in a suspension of T. asperellum secondary metabolites (T2), and a suspension of P. *aeruginosa* secondary metabolites (T3). Two hours after the application of secondary metabolites, the pathogen Mycosphaerella musicola was suspended for 108 spores.ml-1, then were sprayed evenly onto the foliar of the banana. The parameter observed was the characterisation of the pathogen M. musicola

The incubation period (days). The incubation period was observed every day from the first day after treatment until the initial symptoms appeared - if the leaves showed yellowing symptoms, the initial symptoms could be counted.

The incidence of disease. The Incidence Parameters of the disease were calculated by the formula:

International Journal of Applied Science and Engineering Review

IJASER

ISSN: 2582-6271

Vol. 5, Issue.3, May-June 2024, page no. 58-75

$$\mathbf{IP} = \frac{\mathbf{n}}{\mathbf{N}} \mathbf{x\ 100\%}$$

where IP is the disease incidence, n is the number of infected leaves, and N is the number of leaves observed.

The disease severity. Parameters of disease severity were measured by looking at spots of leaves. The

$$\mathbf{KP} = \frac{\sum_{i=0}^{i}(n_i\ x\ v_i)}{N\ x\ V}\ \boldsymbol{x}\ \mathbf{100\%}$$

results of observations/scores were calculated using the formula:
with ni being the first score, the number of spots; vi being the value of the I score; N being the number of infected leaves observed; and V being the highest score contained in the scoring reference.

The disease severity (LSI = leaf symptom index) was carried out based on the symptoms of spot/streak on the leaves, and the severity of spot/streak on leaves (LDI = Leaves discolouration index) was carried out based on the percentage of a spot on the leaves. The scores and categories used in the LSI measurement, namely: score 0 with the percentage of leaf area symptomatic area (X) being 0 or no yellowing symptoms; score 1 with 1 X < 25 if the lower leaf edges begin to turn yellow spot; score 2 with 26 X < 50 if all the lower leaves turn yellow spot; score 3 with 51 X < 75 if all yellow spot banana leaves are found; and score 4 with 76 X < 100 if the plant dies. The categories used to measure the LDI are as follows: score 0 if the percentage of symptom area on the leaves (Y) is 0 or there are no symptoms of necrosis in the leaves tissue; score 1 with 1 Y < 25 if there is a little necrosis in the tissue on the leaves; score 2 with 26 Y < 50 if there is quite a lot of necrosis on the leaves tissue; score 3 with 51 Y < 75 if most of the necrotic leaves tissue is found; and score 4 with 76 X < 100 if all necrotic leaves tissue is found.

Data analysis

The data were analysed using the IBM SPSS Version 25 program. If the results of the analysis of variance showed significant differences, they were further tested using LSD 5%.

RESULT

Morphology characterisation

The morphological characterisation of the fungal isolate is as follows: This fungus has conidiophores that form tight, brown bundles that are pale, straight or slightly curved, not branched, not partitioned, does not have bends like a knee, narrows towards the tip, has no conidial bundle. Conidium is pale brown, tubular, or shaped like an inverted club, straight, curved, or bent, and the tip is blunt or rounded, 3-5 or more

International Journal of Applied Science and Engineering Review

IJASER

ISSN: 2582-6271

Vol. 5, Issue.3, May-June 2024, page no. 58-75

partitioned. These characterisations were similar to the characters of *Mycosphaerella musicola* obtained by (Goodw in et al., 2001; Crous, 2009).

Figure-1. Isolates M. Musicola, isolated from banana leaves with the symptom of Sigatoka

Figure-2. Hyphae of Mycosphaerella musicola (A), and conidiophores (B)

Effect of Secondary Metabolite Treatments

Incubation period

Based on the research results, it can be seen that, on average, the fastest incubation period occurred in the control treatment for 14 days (Fig. 1), and the longest incubation period occurred in treating T. asperellum metabolite for 40 days. Meanwhile, in the metabolite of *Bacillus* sp (T1), it occurred for 30 days and in the metabolite of *P. aeruginosa* (T3) it occurred for 25 days. Symptoms of the disease in the field are observed visually on the bananas, initially spot starting on lower old leaves, start on the 5^{th} and 6^{th} leaves of the shoot,which are bright yellow starting from the edge of the leaf, then continue to spread widely to the middle ofthe leaf with yellow-brown spots, then turn blackish brown. Small spots become larger over time, then merge with nearby other spots, causing tissue necrosis and eventually, the leaves scorch

International Journal of Applied Science and Engineering Review

IJASER

ISSN: 2582-6271

Vol. 5, Issue.3, May-June 2024, page no. 58-75

and dry, resulting from infection by the pathogen of *M. musicola*. Leaves that have dried up remain on the banana plant for 3 months. These infected dried leaves have the potential as a source of inoculum that can spread to healthy leaves through wind and rainfall. The average incubation period of yellow Sigatoka can be seen in figure-1 below.

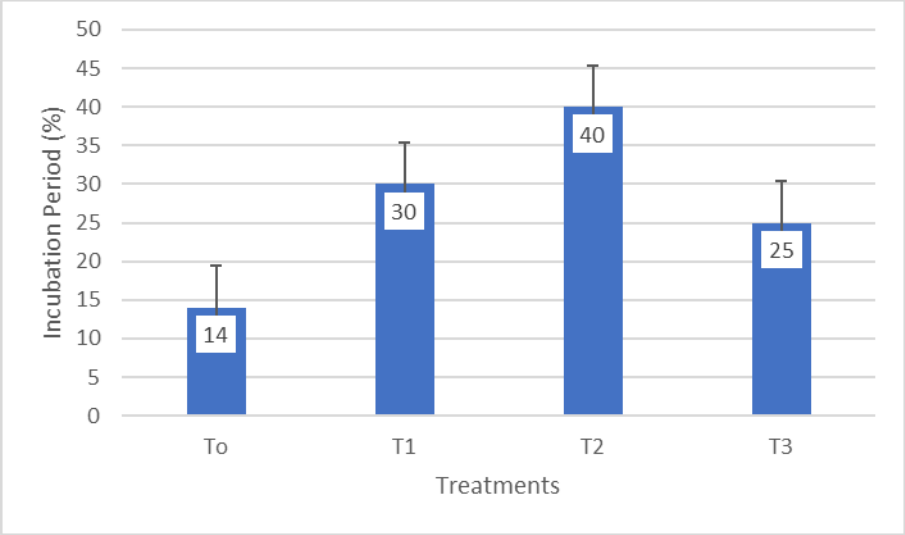

Figure-3. The incubation period of yellow Sigatoka on banana

According to Ajeng M K (2020), Sigatoka disease-affected leaves of bananas usually start on the fifth and sixth leaves of the shoot, with symptoms of elongated, pale yellow spots measuring 1-2 mm or more. Finally, the Sigatoka spots extend from the leaf veins to the edge of the leaf. Sigatoka disease does not kill banana plants but makes the leaves dry quickly. There are many factors Sigatoka disseminated from one banana to another. The disease is spread easily by seeds, wind, fertiliser, air, and rainfall, which carry diseases from one plant to another.

This study shows that secondary metabolites treatments extended the incubation period of Sigatoga disease compared to the control treatment. So, the secondary metabolites of *Bacillus* sp, *T. asperellum* and *P. aeruginosa* are proof of a valuable metabolite substance for controlling yellow Sigatoka disease caused by *M. musicola*. In the control treatment, yellow Sigatoka disease infection can reduce the area of photosynthesis, thereby decreasing banana production. The fastest incubation period occurred in the control treatment for 14 days, shown on $^{\text{the sixth}}$ leaves, and the most extended incubation period occurred in treating *T. asperellum* metabolite for 40 days. Janos Berdy (2005) Bioactive secondary microbial metabolites exhibitdiverse and versatile biological effects due to antimicrobial activities. Bioactive types of microbial metabolites were discovered to have the ability to act as antimicrobial, antifungal, antiviral,

ISSN: 2582-6271

Vol. 5, Issue.3, May-June 2024, page no. 58-75

antiprozoal, antibiotics, and antitumor (cytotoxic). Trichoderma-derived secondary metabolites comprise non-ribosomal peptides such as peptaibiotics, siderophores and diketopiperazines-like gliotoxin and gliotoxin, polyketides, terpenes, pyrones, and isocyanate metabolites (Susanne Zeilinger et al. l, 2016).

Disease incidence, area of spot, and disease severity (%)

Disease incidence (%)

The highest average percentage of disease incidence is found in the control treatment (T0), 62.50% (Fig. 3), while the lowest is in the *T. asperellum* secondary metabolite (T2), 25%. She was then followed by the secondary metabolite of *Bacillus* sp (T1) at 33.33% and the secondary metabolite *P. aeroginosa* at 41.67%. In the control treatment (T0), the first symptoms of Sigatoka were visible on the lower leaves of the shootsas elongated, pale-yellow spots with a length of 1-2 mm or more, directed parallel to the leaf veins. Some tiny spots become dark yellow-brown spots, eventually turning black and dry. Climatic factors, especially rainfall, dew and temperature, influence the spread of disease inoculum.

The secondary metabolite treatment of *T. asperellum* (T2) showed it took a long incubation period, and only then did the symptoms of Sigatoka disease appear on day 40. The content of secondary metabolites of *T. asperellum* in the form of enzymes could directly inhibit the germination of conidiospores from *M. musicola*on the leaf surface. The enzymes contained in the secondary metabolites of *Trichoderma* sp include protease, cellulase, cellobiase, chitinase, and 1,3-ß-glucanase (Soetanto, 2008; Dubey, Tripathi, Dureja, & Grover, 2011), which play an essential role in inhibit plant diseases. Secondary metabolites produced by *Trichoderma* sp can be elicitors in plant resistance to attack by plant pathogens. In addition, secondary metabolites contain complete compounds such as antibiotics, enzymes, hormones and toxins, which can reach the pathogen inoculum when applied to the surface of banana leaves. Mukherjee, Horwitz, & Kenerley(2012) and Vinale et al. (2014b) reported secondary metabolites from *Trichoderma* spp. Namely, 6-pentyl-a pyrone has been used to control plant diseases caused by fungi.

Further, *Trichoderma* sp. can potentially produce secondary metabolites containing antibiotics, namely viridin and trichomidine. Viridin and Tricomidine can inhibit growth or even kill pathogens. *Trichoderma* sp. has antagonistic properties against soil pathogens and several airborne pathogens. *Trichoderma* sp. can produce the hydrolytic enzymes β-1,3 gluconase, chitinase and cellulase (Soesanto, et.al., 2013). These enzymes actively degrade other fungal cells, which are mainly composed of β-1,3 glucan and chitin, so that they can penetrate the hyphae of other fungi. Other treatments on *Bacillus* sp. can produce secondary metabolite compounds, namely cyclolanostane, which can suppress mycelium growth and colonies ofpathogenic fungi. *Pseudomonas aeruginosa* (T3) can produce various secondary metabolites, such as pyocyanin and phenazine, which have antibiotic properties against fungal and bacterial pathogens (Nansathitet al., 2009).

International Journal of Applied Science and Engineering Review

IJASER

ISSN: 2582-6271

Vol. 5, Issue.3, May-June 2024, page no. 58-75

Leaf spot area (%)

The leaf spot area affected shows that the secondary metabolite of the biological agent can reduce Sigatoka pathogen infection. The secondary metabolite of *T. asperellum* is the best treatment and can inhibit infectionon the leaf surface so that the spot area on the leaves is only 21.78 per cent (Figure-2), then followed by the secondary metabolite of *P. aeruginosa* at 34.48 per cent, and finally the secondary metabolite of *Bacillus* sp.with a spot area of 46.12 per cent. However, all treatments were statistically not significantly different from the control treatment. In the control treatment, it was seen that the leaf spot area of the Sigatoka quickly expanded from the edge of the leaf and continued to the centre. Within 40 days, pathogen infection resulted in a spot area of 66.45 per cent, significantly different from other treatments. In general, it can be said that biological agent treatment helps inhibit the development of Sigatoka disease.

Symptoms of leaf spots caused by fungi are characterised by the appearance of small, brownish-yellow necrotic spots, which then widen and in the centre of the spots, it can see a grey colour that is circular or rhombic in shape on the upper surface of the leaves. The spots then spread to the edge of the leaf midrib and ultimately cause the leaves to dry out. According to Alves et al. (2018), *Trichoderma asperellum* can inhibit the pathogen *Mycosphaerella fijiensis* because *Trichoderma* produces chitinase and glucanase enzymes, which can damage the pathogen's cell walls. Apart from that, *Trichoderma* also produces secondary metabolite compounds with antimicrobial activity against *M. fijiensis*.

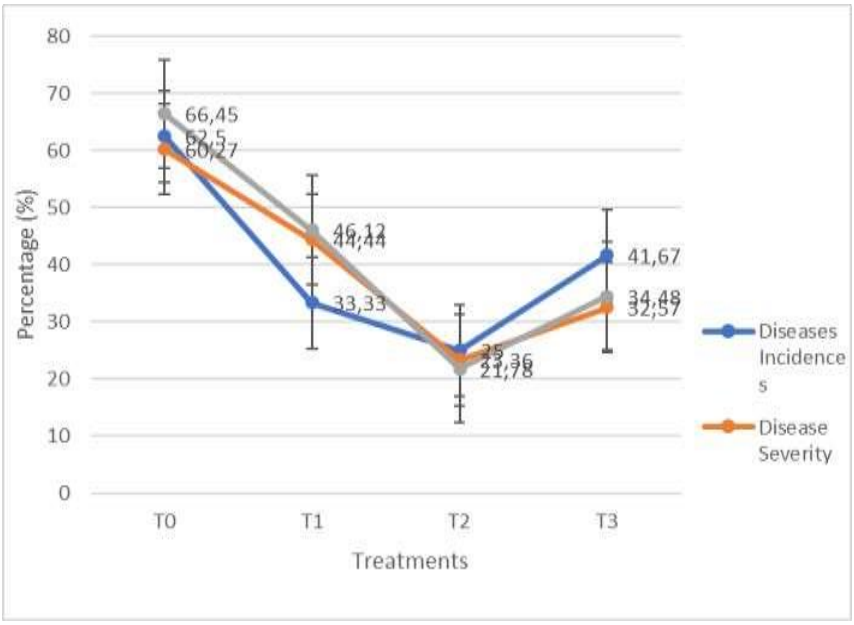

Figure-4. Disease incidence, area of spot, and disease severity (%)

International Journal of Applied Science and Engineering Review

IJASER

ISSN: 2582-6271

Vol. 5, Issue.3, May-June 2024, page no. 58-75

Disease Severity (%)

Figure 4 shows that the lowest disease severity was found in the secondary metabolite of *Trichoderma asperellum* at 23.36%), very significantly different from the control treatment and *Bacillus* sp treatment but not significantly different from the secondary metabolite of *Pseudomonas aeruginosa*. Then, it was followedby a secondary metabolite of *Pseudomonas aeruginosa* by 32.57%, significantly different from the control and Bacillus sp treatments. Meanwhile, the secondary metabolite of *Bacillus* sp could only reduce disease severity by 44.44% and was significantly different from the control treatment. The highest disease severity was found in the control treatment, with an average of 60.27%. This means that the pathogen Mycosphaerella musicola can colonise more than 50% of the leaf area within ten months. This is thought to be because *Trichoderma* can inhibit the growth of Sigatoka disease. According to research by Wahyuni and Yanti (2019), *Trichoderma* is antagonistic and can parasitise plant-pathogenic fungi. It can kill or inhibit the growth of other pathogenic fungi.

In addition, *Trichoderma* sp. can produce organic acids, such as glycine, citric or fumaric acid, which lower soil pH and solubilise phosphate, micronutrients and mineral cations such as iron, manganese and magnesium, which are beneficial for plant metabolism (Saba et al., 2012). The antagonistic bacteria *Pseudomonas aeruginosa* is reported to be able to produce secondary metabolites, including siderophores, pterins, pyrroles and phenazines. Siderophores can act as fungicides and bacteriostatics (Soesanto, 2008). According to Mehrotra (1980), the suppression mechanism by the genus *Bacillus* sp. is antibiosis, which produces the antibiotic bulbiformin, which is toxic to various plant pathogens. *B. subtilis* has an antibiotic called bulbiformin, which can inhibit the growth of Sigatoka *Mycosphaerella* sp. A type of *B. subtilis*, namely B. nato, produces the antibiotic bacitracin, which can inhibit the growth of several organisms.

Production

Harvesting occurs when the banana fruit has a physiological age (natural ripening) of approximately 9-10 months old. From flowering to harvest, it takes around 65-85 days. Results of harvesting observations in the research field show that one bunch of bananas produces 10-12 hands, weighing 11-14 kg per bunch. The observation at harvest showed that the hands of the bananas on the bottom weighed less than the hands onthe top of bunches, weighing between 0,8 Kg at the bottom and 1.5 kg at the top. One hand consists of 8-16 pieces of fruit, and one fruit weighs between 100-150 grams.

The highest banana production was found in the secondary metabolite treatment of *Trichoderma asperellum*,weighing 13.98 kg per bunch (figure-3). In this treatment, the banana fruit matures faster physiologically because the plants' leaves are limited; only the older 2-3 leaves are slightly affected by

International Journal of Applied Science and Engineering Review

IJASER

ISSN: 2582-6271

Vol. 5, Issue.3, May-June 2024, page no. 58-75

Sigatoka disease. Theremaining leaves above stay healthy, so the plant gets an ample supply of nutrients and minerals because all the existing nutrients are carried and processed in the leaves through photosynthesis with the help of leaf chlorophyll. This was followed by secondary metabolite treatment of *Bacillus* sp with a harvest weight of 13.44 kg and *Pseudomonas aeruginosa* treatment with a harvest weight of 12.21 kg. The harvest from the control treatment only weighed 11.90 Kg per bunch. This is due to a reduction, which disrupts the photosynthetic process of tissues, causing necrosis on the leaves. In the control treatment, yellow leaf streak Sigatoka generally attacks the lower to middle leaves. When the banana in the treatment control has five normal leaves at the harvest stage. Consequently, fruit from plants with a high level of infection displayed less weight volume than those from treatment plants with a low level of infection. It disrupted the photosynthesis process, limiting the assimilates produced to fruit filling and shoot formation.

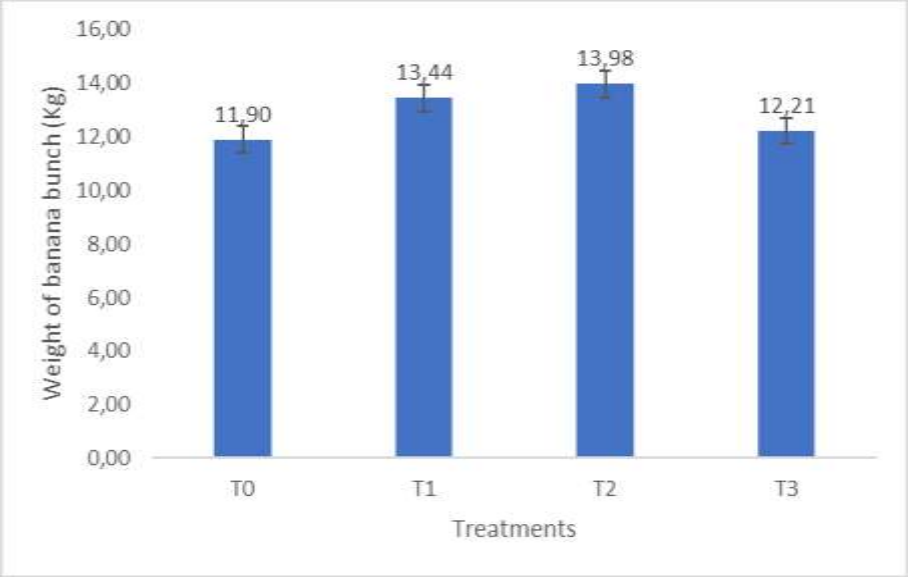

Figure-5. Production of banana (Kg) per bunch

Several studies have shown that *Trichoderma* treatment can produce positive effects on banana plant yields, especially through increasing root growth and increasing nutrient availability because *Trichoderma* producesPGRs that banana plants need; apart from that, *Trichoderma* sp also produces enzymes that can suppress the development of disease. The reduction in disease in banana plants has caused the growth and yield of bananaplants to increase. Research by Santos et al., (2019) proved that *Trichoderma harzianum* treatment can increase the growth and yield of banana plants. The results showed that *Trichoderma harzianum* treatment increased root length, plant weight and the number of fruit produced. According to Diaz et al., (2021), *Trichoderma asperellum* treatment significantly increased plant growth and reduced disease symptomsvisible on banana leaf leaves. However, fruit

ISSN: 2582-6271

Vol. 5, Issue.3, May-June 2024, page no. 58-75

from plants controlled with a high level of *Mycosphaerella musicola* infection weighed less than those from less infected plants from secondary metabolites treatments.

This result is similar to the result of F.P. Castelan et al. (2013). Further, using *Bacillus* sp. to control Sigatoka disease in plants affects plant growth and yield. This was shown in the increase in shoot dry weight, root dry weight, and fruit weight in the *Bacillus* sp. treatment. B64. These results relate to the abilityof *Bacillus* sp. to suppress the development of yellow Sigatoka disease. Low disease intensity allows plants to grow and develop better. In addition, *Bacillus* sp., known as a biological agent, is also reported to have PGPR properties (Radhakrishnan & Lee, 2016).

DISCUSSION

The secondary metabolites of *Bacillus* sp, *Trichoderma asperellum* and *Pseudomonas aeruginosa* were applied to the leaf of a banana and are proven a valuable metabolites substance for the control of yellow Sigatoka disease caused by *Mycosphaerella musicola*. This study showed that the incubation period of Sigatoga disease was extended by secondary metabolites treatment compared to the control. In the control treatment, yellow Sigatoka disease infection can cause a reduction in the area of photosynthesis, thereby causing banana production to decrease. The fastest incubation period occurred in the control treatment for 14days, and the longest occurred in treating *Trichoderma asperellum* metabolite for 40 days. Berdy (2005) Bioactive of secondary microbial metabolites exhibit diverse and versatile biological effects, first of all antimicrobial activities. The scientific literature describes hundreds of pathogenic and other microbes (Gram-positive, Gram-negative bacteria, fungi, yeasts, etc.) as test organisms in direct activity-based screenings. Bioactive types of microbial metabolites were discovered to have the ability to act as antimicrobial, antifungal, antiviral, antiprozoal, antibiotics, and antitumor (cytotoxic). Trichoderma-derived secondary metabolites comprise non-ribosomal peptides such as peptaibiotics, siderophores and diketopiperazines-like gliotoxin and gliotoxin, polyketides, terpenes, pyrones, and isocyanate metabolites. However, it has to be borne in mind that the production of these substances is species- and even strain-dependent, and not the whole repertoire will be biosynthesised by a given fungus under laboratory conditions as specific triggering stimuli may be required (Zeilinger et al. l, 2016).

Members of the *B. subtilis* group have long been known to produce a range of secondary metabolites, including polyketides (PKs), terpenes and siderophores, as well as ribosomally and non-ribosomally synthesised peptides (Colin et al., 2018). Sansinenea and Ortiz, 2011 show that *Bacillus* sp, *B. licheniformis*, *B. pumilus*, *B. subtillis*, *B. amyloliquefaciens* produce secondary metabolites asantibiotics such as Bacteriocins, Daitocidin Lichenysin, Pumilacidin, Mycosubtilin, Iturin, Fengycin, and Bacillomycin. Felix (2019) found bioactive secondary metabolites from *Bacillus subtilis* that have the potential as plant pathogen control agents, producing cyclic lipopeptides exhibiting strong surfactants

ISSN: 2582-6271

Vol. 5, Issue.3, May-June 2024, page no. 58-75

and antimicrobial activities, such as surfactants, returns, and fencing. Nina Neidig et al. (2011) Secondary metabolites form a key component of the defence response of soil pseudomonads against bacterivorous nematodes [4, 17] and likely contribute to improving bacterial fitness in soil. We showed that extracellular metabolites of P. fluorescens CHA0 drive complex interactions with nematode predators, affecting both nematode physiology and behaviour.

All studied secondary metabolites contributed to the bacteria's toxicity, with hydrogen cyanide efficiently repelling the nematodes and both hydrogen cyanide and 2,4-DAPG functioning as nematicides. The results suggest that bacterial secondary metabolites responsible for suppressing plant pathogens strongly inhibit bacterivorous nematodes and thus likely contribute to bacteria's resistance to predators in soil.

Rajesh R. et al. l (2021) shown the antifungal and bacterial activities of bacterial strains were evaluated against important plant pathogens in vitro; among them, PaRS was found to be most effective. The indole acetic acid production was recorded in all isolated *Pseudomonas* spp. Seed bacterisation with *P. aeruginosa* @ 6 g/kg seed proved better for germination and plant height. Further, field confirmations of the results are required for practical utility and feasibility. This can be suggested to the farmers for cost-effective and eco- friendly management of finger millet blast.

CONCLUSION

Succesful development of secondary metabolites of *Bacillus* sp, *Trichoderma asperellum* and *Pseudomonas aeruginosa* to control Sigatoka disease (*Mycosphaerella musicola*) proved that metabolites of endophytes tested are harmful weapons against pathogens of *Mycosphaerella musicola*. The metabolites' modes of action occur during the interaction between metabolites and pathogen spores on banana leaves. Hence, it proved that metabolites can reduce disease incidence, area of spot, disease incident and severity, and increase banana production. However, understanding the biology of *Mycosphaerella musicola* is the best way to get better metabolite application strategies, application timing, spraying number and interval of application of metabolites in the field.

ACKNOWLEDGMENTS

We thank the Universitas Teuku Umar for contributing funds (Leader: Alfizar) with financial Number 021/UN59.7/SPK-PPK/2022. The University farm of UTU team helped organize the land use and banana seedling requirements.

REFERENCES

Ajeng Mirayanti Kesuma, Rostamam, Marsandi K, 2020. Penyakit pada Tanaman pisang dan distribusinyadi wilayah Kecamatan Sumbang Kabupaten Banyumas. J. Agrowiralodra

ISSN: 2582-6271

Vol. 5, Issue.3, May-June 2024, page no. 58-75

3(1): 8-15.

Alfizar A, Amda R, Iwandikasyah P, Hendri B, Mohammad AB, Syaukani S. 2023. Efficacy of Trichoderma asperellum and Pseudomonas aeroginosametabolites against Fusarium wilt in Bananas. International Journal of Design & Nature and Ecodynamics, 18(6): 1525-1532.

Alves GC, Tomazetto P, Santos CMR, Mendes BV, Capoci IRG & Stephani R. 2018. Trichoderma asperellum T76B is a potential biocontrol agent against Mycosphaerella fijiensis. Biological Control, 116: 24-31.

Barnett & Hunter BB. 1998. Illustrated Genera of Imperfect Fungi By H. L. 4th Edition. Amer Phytopathological Society Press. 240p.

Beneduzi A, Ambrosini A, Passaglia LM. 20212. Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. Genet Mol Biol, 35(4 (suppl)):1044-51. doi: 10.1590/s1415-47572012000600020.

Coleman JJ, Ghosh S, Okoli I, Mylonakis E. 2011. Antifungal activity of microbial secondary metabolites. PLoS One. 2011;6(9):e25321. doi: 10.1371/journal.pone.0025321.

Colin RH, Jean-Marie M, Susanne P, Jose A. 2018. Secondary metabolite production and the safety of industrially important members of the Bacillus subtilis group. Microbiology Reviews, 42:721–738

Demain AL & Fang A. 2000. The natural functions of secondary metabolites. In: Scheper T (ed) Advances in biochemical engineering/biotechnology, 69: 1-39.

Diaz JF, Gutiérrez JF, D'Aquino L, Barrera V, León F. 2020. Trichoderma asperellum and fertilization effect on growth and disease control in the cultivation of banana (Musa AAA, Cavendish). Scientia Horticulturae, 265, 109243.

Díaz-Gutiérrez C, Arroyave C, Llugany M, Poschenrieder C, Martos S, Peláez C. 2021. Trichoderma asperellum as a preventive and curative agent to control Fusarium wilt in Stevia rebaudiana. Biological Control, 155, 104537.

Dubey SC, Aradhika T, Dureja P, Grover A. 2011. Characterization of secondary metabolites and enzymes produced by Trichoderma species and their efficacy against plant pathogenic fungi. Indian Journal of Agricultural Sciences, 81(5): 455-461.

Estibaliz S & Aurelio O. 2011. Secondary metabolites of soil Bacillus spp. Biotechnol lett 33:1523-1538.

Fallahzadeh-Mamaghani V, Ahmadzadeh M, Sharifi R. 2009. Screening systemic resistance-inducing fluorescent pseudomonads for control of bacterial blight of cotton caused by Xanthomonas campestris pv. malvacearum. Journal of Plant Pathology, 61: 663-670.

ISSN: 2582-6271

Vol. 5, Issue.3, May-June 2024, page no. 58-75

Estibaliz S & Aurelio O. 2011. Secondary metabolites of soil Bacillus spp. Biotechnol lett 33:1523-1538.

Fallahzadeh-Mamaghani V, Ahmadzadeh M, Sharifi R. 2009. Screening systemic resistance-inducing fluorescent pseudomonads for control of bacterial blight of cotton caused by Xanthomonas campestris pv. malvacearum. Journal of Plant Pathology, 61: 663-670.

Felix K, Peter N, Matthias G. 2019. Bioactive Secondary Metabolites from Bacillus subtilis: A Comprehensive Review. J. Nat. Prod., 82(7): 2038–2053

Fouré E. 1983. Les Cercosporioses du bananier et leurs traitments. Selection de molécules fungicides nouvelles. Activités Comparées de différentes molécules fungicides sur Mycosphaerella fijiensis Morelet, agent de la ‘‘maladie des raies noires’’ des bananiers et plantains de Gabon. Fruit, 38: 21–34.

Fastelan P, Abadie C, Hubert O, Chilin-Charles Y, de Lapeyre de Bellaire L, Chillet M. 2013. Severity of Sigatoka disease and banana quality characterized by pomological traits and fruit green life. Crop Protection, 50: 61-65

Gupta CP, Dubey RC, Kang SC, Maheshwari DK. 2001. Antibiosis-mediated necrotrophic effect of Pseudomonas GRC2 against two fungal plant pathogens. Current Science, 81(1): 91–94. http://www.jstor.org/stable/24105010

Haas D, Keel C. 2003. Regulation of antibiotic production in root-colonizing Peudomonas spp. and relevance for biological control of plant disease. Annu Rev Phytopathol, 41:117-53. doi:10.1146/annurev.phyto.41.052002.095656.

Hermanto C & Setyawati T. 2002. Pola sebaran dan perkembangan penyakit layu Fusarium pada pisang tanduk, rajasere, kepok, dan barangan. J. Hort., 12 (1): 64-70.

Janos B. 2005. Bioactive microbial metabolites a personal view. Journal of Antibiotics, 58(1): 1-26.

Jones DR & Stover RH. 2001. Fungal diseases of banana, fruit, and preharvest diseases. in Diseases of Banana, Abaca and Enset. D.R. Jones (ed.), pp. 173-190. CABI Publishing. Wallingford, UK.

Marin DH, Romero RA, Guzmán M, Sutton TB. 2003. Black Sigatoka: An increasing threat to banana cultivation, Plant Diseases, 87: 208 – 222.

Mehrotra RS. 1980. Plant Pathology. Tata McGraw-Hill, New Delhi, 771pp. Milsha GK, Anita C, Deepu M. 2022. Symptomatology of Sigatoka leaf spot disease in banana landraces and disease in banana landraces and identificationof its pathogen as Mycosphaerella eumusae. Journal of the Saudi Society of Agricultural Science, 21: 278-287

Mukherjee PK, Horwitz BA, Kenerley CM. 2012. Secondary metabolism in Trichoderma - a genomic p perspective. Microbiology, 158(1), 35–45. http://doi.org/10.1099/mic.0.053629-0

ISSN: 2582-6271

Vol. 5, Issue.3, May-June 2024, page no. 58-75

Mukherjee PK, Horwitz BA, Kenerley CM. 2012. Secondary metabolism in Trichoderma a genomic perspective. Microbiology, 158(1): 35-45.

Mulyanti N. 2008.Teknologi Budidaya Pisang. Badan Penelitian dan Pengembangan Pertanian. Hlm. 17-18.

Nakkeeran S, Rajamanickam S, Karthikeyan M, Mahendra K, Renukadevi P, Johnson I. 2021. Antimicrobial secondary metabolites from Trichoderma spp. as next generation fungicides,Editor(s): Sudisha Jogaiah, Biocontrol Agents and Secondary Metabolites, Woodhead Publishing, 257-282pp. https://doi.org/10.1016/B978-0-12-822919-4.00011-9.

Nansathit A, Apipattaraul S, Phaosiri C, Pongdontri P, Chantai S, Ruangviriyachai C. 2009. Synthesis, Isolation of Phenazine Derivatives and Their Antimicrobial activities. Walailak Journal Science and Technology, 6(1):79-91.

Nina N, Rudiger JP, Stefan S, Alexandre J. 2011. Secondary metabolites of Pseudomonas fluorescens CHAO drive complex non-Trophic interactions with bacterivorous nematodes. Microbiol Ecol., 61: 853-859.

Ploetz RC. 2007. Diseases of tropical perennial crops: challenging problems in diverse environments. Plant Disease, 91(6): 644-663.

Radhakrishnan R & In-Jung L. 2016. Gibberellins producing Bacillus methylotrophicus KE2 supports plantgrowth and enhances nutritional metabolites and food values of lettuce, Plant Physiology and Biochemistry, 09: 181-189 https://doi.org/10.1016/j.plaphy.2016.09.018

Rajesh RW & Ambalal NS. 2021. Impact of Pseudomonas spp on plant growth, lyctic enzymes and secondary metabolites production. Frontiers in Agronomy, 3, 752196.

Saba H, Vibhash D, Manisha M, Prashant KS, Farhan H, Tauseef A. 2012. Trichoderma - a promising plant growth stimulator and biocontrol agent. Mycosphere, 3(4): 524-531.

Sansinenea E & Ortiz A. 2011. Secondary metabolites of soil Bacillus spp. Biotechnol Lett. 33(8):1523- 38. doi: 10.1007/s10529-011-0617-5.

Santos JS, Silva RV, Alves LA & Silva GD. 2019. Effects of Trichoderma harzianum application on the growth and productivity of banana plants. Revista Brasileira deFruticultura, 41(1), e-332.

Soesanto LR & Prihatiningsih N. 2008. Penekanan beberapa mikroorganisme antagonis terhadap penyakit layu Fusarium gladiol. Agrivita, 30(1): 75–83.

ISSN: 2582-6271

Vol. 5, Issue.3, May-June 2024, page no. 58-75

Soesanto L, Mugiastuti E, Rahayuniati RF, Dewi RS. 2013. Uji Kesesuaian Empat Isolat Trichoderma spp. dan Daya Hambat In Vitro Terhadap Beberapa Patogen Tanaman. Jurnal HPT Tropika, 13(2): 117-123.

Soesanto L, Mugiastuti E, Rahayuniati RF. 2010. Kajian Mekanisme Antagonis Pseudomonas Fluorescens P60 terhadap Fusarium Oxysporum F. SP. Lycopersici pada Tanaman Tomat In Vivo. Jurnal Hama dan Penyakit Tumbuhan Tropika, 10 (2: 108–115. https://doi.org/10.23960/J.HPTT.210108-115

Soesanto L, Endang M, Ruth FR. 2011. Pemanfaatan beberapa kaldu hewan sebagai bahan formula cair Pseudomonas fluorescens P60 untuk mengendalikan Sclerotium rolfsii pada tanaman mentimun. Jurnal Perlindungan Tanaman Indonesia, 17(1): 7–17

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

Stover RH. 1972. Banana leaf spot disease caused by Mycosphaerella musicola: contrasting features of Sigatoka and black leaf streak control. Plant Disease Rep., 55: 437–439

Susanne Z, Sabine G, Ravindra B, Prasun KM. 2016. Secondary metabolism in Trichoderma – Chemistry meets genomics. Fungal Biology Review, 30: 74-90.

Van L, Rep LC, & Pieterse CM. 2006. Significance of inducible defense-related proteins in infected plants. Annu. Rev. Phytopathol., 44: 135-162.

Vinale F, Sivasithamparam K, Ghisalberti EL, Woo SL, Nigro M, Marra R, Lorito M. 2014. Trichoderma secondary metabolites active on plants and fungal pathogens. The Open Mycology Journal, 8(1): 27-38

Wahyuni SH & Nst DPY. 2019. Pengaruh kombinasi berbagai jenis pupuk organik yang didekomposisi dengan Trichoderma viride terhadap intensitas kerusakan bonggol tanaman pisang. Jurnal Pertanian Tropik, 6(3): 458-465.