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In Vitro Evaluation of *Trichoderma* spp. against Sugarcane Eye Spot Disease (*Bipolaris* sp.)

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ABSTRACT

Sugar cane (*Saccharum officinarum* L.) are the main ingredient in sugar production. Sugarcane is widely cultivated in warm and tropical areas and used as a sweetener because it contains a lot of fructose and glucose. National sugar production shows a decline, caused mainly by pathogens, especially the fungus *Bipolaris* sp., which can produce up to 85% damage per ha if no control is applied. Biological control using *Trichoderma* spp. can control this pathogen and promote sugarcane growth. This study aimed to determine the effectiveness of *Trichoderma* spp. in controlling *Bipolaris* sp. in vitro. The method used is an antagonistic and volatile bioassay test. Testing was conducted in PDA medium and observed every day for 7 days. The results showed that isolates TD1, TD2, and TD3 could inhibit *Bipolaris* sp. antagonistically and volatilely; the antagonist test showed a result of 86% (TD1), while the volatile test was 65% (TD2). In conclusion, Trichoderma spp. can inhibit the growth of *Bipolaris* sp. on an *in vitro* scale.

Keywords: BCA, *Bipolaris* sp. *Trichoderma* spp.

ABSTRAK

Tebu (*Saccharum officinarum* L.) adalah bahan utama dalam produksi gula. Tebu banyak dibudidayakan di daerah hangat dan tropis dan banyak digunakan sebagai pemanis karena kandungan fruktosa dan glukosanya. Produksi gula nasional menurun, yang sebagian besar disebabkan oleh patogen, terutama jamur *Bipolaris* sp. yang mampu menimbulkan kerusakan hingga 85% per hektare jika tidak dikendalikan. Pengendalian hayati menggunakan *Trichoderma* spp. dapat mengendalikan patogen ini dan mampu mendorong pertumbuhan tebu. Tujuan penelitian ini adalah untuk mengevaluasi efektivitas *Trichoderma* spp. dalam mengendalikan *Bipolaris* sp. secara *in vitro*. Metode yang digunakan adalah uji bioasay antagonis dan volatil; diuji dalam medium PDA dan diamati setiap hari selama 7 hari. Hasil penelitian menunjukkan isolat TD1, TD2, Tdan D3 mampu menghambat *Bipolaris* sp. Secara antagonis dan volatil, uji antagonis menunjukkan hasil 86% (TD1) sedangkan uji volatil mencapai 65% (TD2). Disimpulkan bahwa *Trichoderma* spp. dapat menghambat pertumbuhan *Bipolaris* sp. pada skala *in vitro*.

Kata kunci: APH, *Bipolaris* sp. *Trichoderma* spp.

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is the primary material in the sugar industry. It is a warm and tropical place to cultivate and has a long bamboo shape; sugarcane is often used as a sweetener because it contains high fructose and glucose. Locals use it as a base for their molasses production. National sugarcane production from 2015 to 2016 showed a decline of 0.2 million tons, according to the Indonesia Ministry of Agriculture (2016). According to Wulansari (2021), the East Java sugar cane plantation sector from 2011 to 2020 showed a yearly decline of 8.680 tons, whereas forecasting production results from 2021–2025 also showed a decline. The main cause of the decline is pathogenic fungi that attack the sugarcane throughout the year.

Bipolaris sp. is a pathogenic ascomycete fungus from the genus Bipolaris that can cause eye spot lesions on the sugarcane leaf. According to Yandoc (2004), if left untreated, *B. Sacchari* can cause damage up to 85% per hectare. *Bipolaris* sp. infects the leaf through air-borne spores. According to Dallagnol *et al.* (2011), the fungi infect the host leaf when the warm season comes; it can cause irregularly shaped lesions to appear on the surface of the leaf blade. Sugarcane needs its leaf to produce the carbohydrates, which are then stored in its stem and grow large; without the leaf, there's no photosynthesis, and the plant will die. Most of the control efforts were done using chemical pesticides, and because it is an air-borne fungus, many pesticide residuals can be found in the leaf and the soil.

Trichoderma sp. is a soil fungus that has many anti-fungal properties. Vinale (2008) reported that *Trichoderma* sp. produces numerous biologically active compounds, including cell degradation enzymes and secondary metabolites. Also, it is widely available in all types of soil; because of this, many researchers often use *Trichoderma* sp. as a main BCA (biocontrol agent). Mukhopadhyay (2020) stated that 20 species of the genus *Trichoderma* sp. that act as bioagents. They attack the pathogen by mycoparasitism and antibiosis. *Fusarium* sp. is the most widely used soil pathogen to

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measure *Trichoderma* sp. effectiveness; Dwiastuti (2015) reported that there was a 49.7% growth inhibition between *Trichoderma* sp. And *Fusarium* sp. in strawberries (*Fragaria x ananassa Dutch*).

Trichoderma sp. also emits a volatile compound known as VOC (*Volatile Organic Compound*) that can act as a PGPR (*Plant Growth Promoting Rhizobium*) or as a BCA (*Bio Control Agent*). Lee *et al.* (2016) reported that nine strains of VOCs can produce plant growth by increasing their biomass (37.1%–41.6%) and chlorophyll content (82.5–89.3%) in Thale cress (*Arabidopsis*). Ruangwong (2021) reported that producing VOCs is an effective antibiosis mechanism of *Trichoderma* species because it is associated with antimicrobial ability, induces plant defense response, and promotes plant growth. Guo (2019) experiment reported that *Trichoderma* sp. Volatile can inhibit the growth of an air-borne fungi *Colletotrichum capsici* up to 37.16%, while Contreras (2014) reported that *Trichoderma* sp. VOCs can induce plant protection and immunity against *Botrytis cinerea* in grapes; therefore, Trichoderma sp. can inhibit the growth rate of air-borne fungi. This study aims to determine *Trichoderm* spp. effectiveness via its antibiosis and VOCs properties against *Bipolaris* sp.

MATERIALS AND METHODS

Isolation and Identification of *Trichoderma* spp.

The sample was obtained from sugarcane soil. Soil dilution will be used as the isolation method for *Trichoderma* spp. using a dilution of 10^{-3} based on Rahman's (2011) experiment. *Trichoderma* spp. identification based on macroscopic and microscopic observation. 3 types of *Trichoderma* spp. (TD1, TD2, TD3) found in the isolation process will be randomly selected.

Isolation and Identification of *Bipolaris* sp.

The sample was collected from the Sukodono sugarcane farm in Sidoarjo. It has all the characteristic symptoms caused by *Bipolaris sacchari*. Manamgoda (2014) stated that *Bipolaris* sp. symptoms vary based on the host plant, but most of it showed a ring-like smut with a reddish edge on the leaf. The infected part of the leaf was cut and cleaned using sterilized water. PDA (potato dextrose agar) was used as a growth medium and incubated for 7 days.

Koch's postulate will be conducted to determine the symptoms of *Bipolaris* sp. Healthy sugarcane leaves were used as the substrate and placed on a petri dish with wet tissue. Bipolaris sp. spore density was accounted for. According to Yandoc (2004) *B. Sacchari* spore density of 10^5 spores mL^{-1} can cause a severe foliar blight in cogon grass (*Imperata cylindrica*).

In vitro Test between *Trichoderma* spp. and *Bipolaris* sp.

*In vitro t*est was conducted to determine the effectiveness of *Trichoderma* spp. Agains *Bipolaris* sp. growth in a controlled environment using a dual cultured method between *Trichoderma* spp. And *Bipolaris* sp. placed against each other in a 9 cm petri diameter with a 3 cm gap from each other. Colony diameters of plant pathogens were measured and converted into the percentage of inhibition through the following formula (Muksin *et al.,* 2013).

$$R = \frac{R1 - R2}{R1} X\ 100\ \%$$

Description:
R1 = The mycelial growth of the plant on the control plate
R2 = The mycelial growth of the plant pathogen on the tested plate

Volatile Antifungal Bioassay

The Trichoderma sp. volatile test will be conducted using the sealed plate method by Dennis (1971) but with some modifications based on Intana *et al.* (2021). *Trichoderma* sp. was grown on PDA for three days, and then an agar plug (0.5 cm) was cut from the culture plate, inserted centrally, and the petri dish lid was removed. The bottom part of the dish containing PDA with *Bipolaris* sp. agar plug (0.5 cm) was then sealed with *Trichoderma* sp. plate using plastic wrap; after that, it was incubated for 3 days. A control test using a similar method but without *Trichoderma* sp. Colony diameters of plant pathogens were measured and converted into the percentage of inhibition through the following formula (Ruangwong *et al.* 2021).

$$\text{Percent inhibition (\%)} = ((Dc - Dt)/Dc) \times 100\%$$

Description:
Dc = The mycelial growth of the plant pathogen on the control plate
D*t* = The mycelial growth of the plant pathogen on the tested plate

RESULT AND DISCUSSIONS

Isolation and Identification

Bipolaris sp. growth rate is considered slow within its first 5 days after purification. The colony size only reaches up to 3–4 cm in diameter. Almaguer *et al.* (2013) stated that *Bipolaris* sp. required a temperature range from 28–30°C to sprout more quickly. The result showed that macroscopic-wise, the colony has a white cottony surface (Figure 1). In contrast, the microscopic observation showed that the spores have an ellipsoidal shape with a septum (Figure 2). The conidiophores are curved with slightly tapering ends (Figure 3). This is

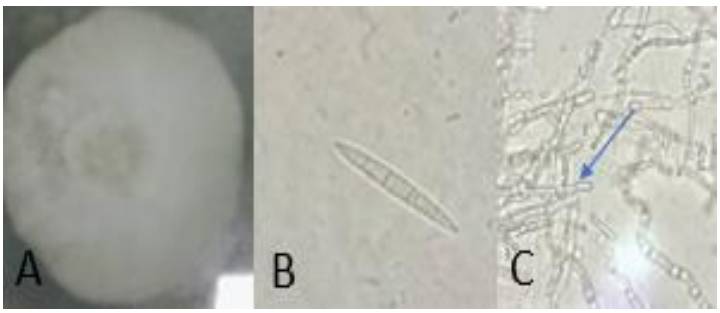

Figure 1 (A) *Bipolaris* sp. colony (7-Days-old); (B) *Bipolaris* sp. conidia; (C) *Bipolaris* sp. conidiophores.

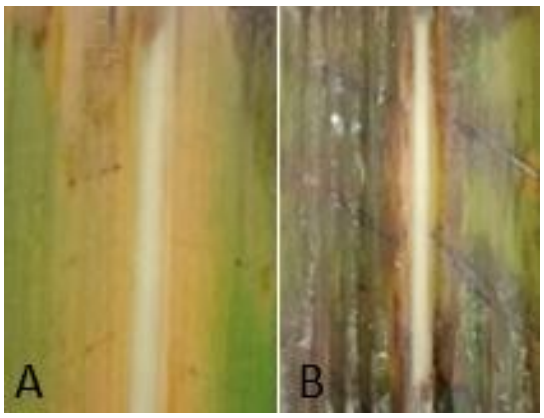

Figure 2 (A) Healthy sugarcane leaf; (B) *Bipolaris* sp. infection on sugarcane leaf.

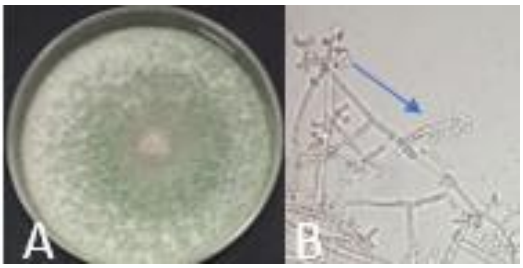

Figure 3 (A) Morphological of *Trichoderma* spp.; (B) *Trichoderma* spp. Conidiospore.

aligned with Manamgoda (2004) research about *Bipolaris* genus.

Bipolaris sp. Koch's postulate began by preparing healthy sugarcane leaf samples for pathogen growth. The sample was then cleaned using 70% alcohol with cotton and cut according to petri dish size. Wet tissue was placed beneath the sample to ensure it was moisturized. *Bipolaris* sp. inoculation began by pouring a suspension containing spore density of 4,3 X 10^{-5} and 10 ml of sterilized water. According to Yandoc (2004), a suspension of 10^{-5} can cause severe foliar blight. Incubation time for *Bipolaris* sp. lasted around 1 week (7 days) to ensure the symptom was correct, and it was then isolated to confirm macroscopically and microscopically. The infected leaf will be isolated to reaffirm that the cause of the symptom is done by *Bipolaris* sp.

Trichoderma spp. isolation began by diluting 1 g of soil to perform a dilution series. According to Rahman (2011), *Trichoderma* sp. can be obtained from a dilution of 10-3, then it was inoculated in PDA and incubated for 3 days. The *Trichoderma* spp. obtained showed a greenish cottony texture, while microscopic observation showed a green coccus spore and a pinecone-like conidiophore. Rahman (2011) stated that *Trichoderma* sp. morphology has a white cotton color at first, then gradually changes into a greenish color, and Taribuka (2017) stated that *Trichoderma* sp. conidiophore has a triangle shape like a branch for the spore to develop and sporulate.

Antagonistic Test Between *Trichoderma* spp. and *Bipolaris* sp.

- ***In vitro* antagonistic test**

The antagonistic test used a week old *Bipolaris* sp. and *Trichoderma* spp. Isolate. Thambugala (2020) stated that using an isolate that reaches maturity can increase the effectiveness of that fungi, while *Trichoderma* sp. has a faster growth rate than *Bipolaris* sp. by using a 1 week old isolate it can even the playfield between the BCA and the pathogen.

PDA was used as the primary growth medium using PDA instant by MERCK containing (potato extract 4g/L, dextrose 20 g/L, and agar 15 g/L), and placed in a 9 cm petri dish. The test was observed for around 1 week or until the *Trichoderma* spp. took over *Bipolaris* sp. The inhibition growth zone and microscopic observation determined the effectiveness of *Trichoderma* sp. against *Bipolaris* sp.

Antagonistic tests showed that in 4 days, *Trichoderma* spp. all showed a result of dominancy against *Bipolaris* sp. (Figure 4) based on the diameter of the *Trichoderma* spp. and *Bipolaris* sp. According to Howell (2003), *Trichoderma* sp. has the highest ability to control any pathogenic fungi because of its ability to mycoparasitism and produce antibiotics. *Trichoderma* sp. affects its host by coiling around the pathogen hyphae, penetrating it, and subsequently dissolving the host cytoplasm, thus making it abnormal.

Data appeared that *Trichoderma* spp. can inhibit the growth of *Bipolaris* sp. TD1 showed the best result at 86% inhibition, followed by TD2 at 81%, then TD3 at 76% (Figure 5). *Trichoderma* spp. Inhibits *Bipolaris* sp. hyphae by coiling and mycoparasite (Figure 4 B and C).

Volatile Test between *Trichoderma* sp. and *Bipolaris* sp.

The sealed plate method was conducted to evaluate the effects of the VOCs emitted by *Trichoderma* sp. on the fungal growth of *Bipolaris* sp. The diameter colony of *Bipolaris* sp. will be measured after the 7 days/1 week incubation period along with *Trichoderma* spp. colony. The result showed that before the 1-week mark, all the *Trichoderma* spp. reach the diameter of the petri dish (9 cm) (Figure 6) in just about 4 days, while the control showed a growth of around 3-4 cm when it was one week old.

The VOC's test result showed that the *Trichoderma* spp. emitted a compound with antifungal abilities for inhibiting the fungal pathogen growth (Fig. 6). Fungal growth in the tested plate showed a smaller diameter than the control plate, and in one the tested plate showed a non-existent fungal growth. TD2 has the highest inhibition rate (65%) among the three isolates (Figure 7), according to Ruangwong *et al.* (2021) *Trichoderma* sp. VOC is the most effective way to control fungal growth of airborne, soilborne, and postharvest pathogenic fungi. Therefore, it can be concluded that *Trichoderma* spp. can inhibit *Bipolaris* sp. growth rate.

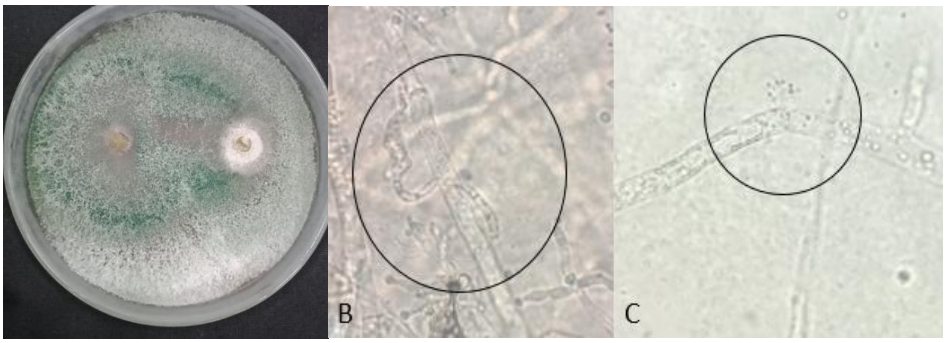

Figure 4 (A) Antagonistic test between TD1 and *Bipolaris* sp.; (B) *Bipolaris* sp. hyphae being coiled by *Trichoderma* sp. hyphae. (C) *Bipolaris* sp. hyphae infected with *Trichoderma* sp.

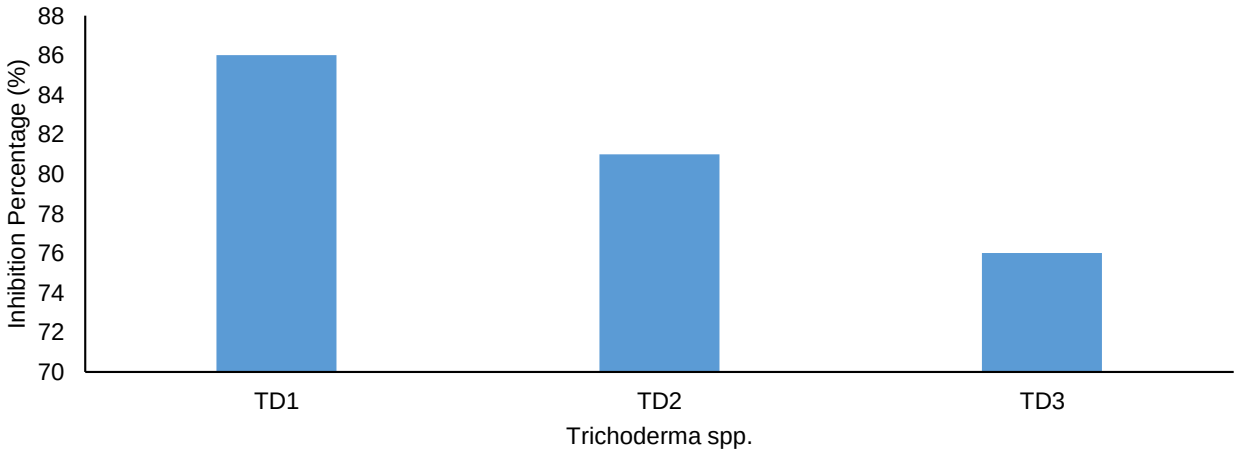

Figure 5 Percentage inhibition antagonistic test *Trichoderma* spp. against *Bipolaris* sp. The same letters indicate non-significant differences among treatments ($p < 0,05$).

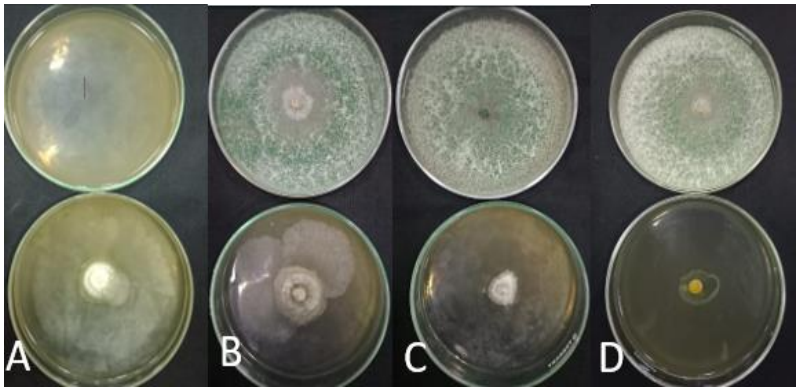

Figure 6 (A) Volatile control test; (B) Volatile test TD1; (C) Volatile test TD2; (D) Volatile test TD3.

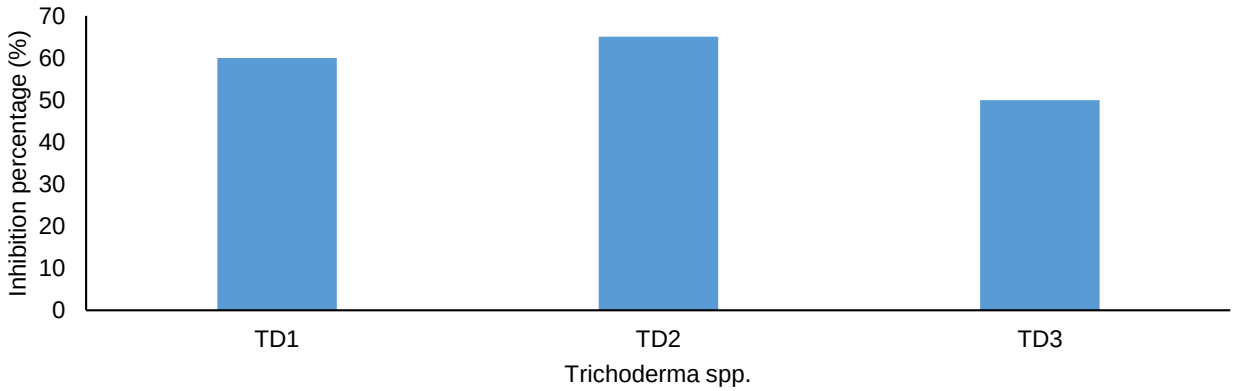

Figure 7 Percentage inhibition of VOCs emitted from *Trichoderma* spp. against fungal pathogen *Bipolaris* sp. using the sealed plate method. The same letters indicate non-significant differences among treatments ($p < 0,05$).

CONCLUSION

Bipolaris sp. is an air-borne fungus that infects sugarcane leaves, causing a lesion; most of the time, farmers often control it by cutting the infected leaves and spraying them with fungicide. The study aims to show an alternative way by using *Trichoderma* spp. based on its antagonistic and volatile ability i*n vitro.* The result showed that all the isolates can inhibit *Bipolaris* sp. growth. The antagonistic test is 86% by TD1, while the volatile test is 65% by TD2.

Furthermore, the application of *Trichoderma* spp. against *Bipolaris* sp. or airborne fungi needs to be tested on the field to know how well *Trichoderma* spp. will react outside of their soil habitat, and applying them on sugarcane leaves also promotes the growth of *Bipolaris* sp., which can be done by using an incubator at a specific temperature.

REFERENCES

Almaguer M, Rojas TI, Dobal V, Batista A, Aira MJ. 2013. Effect of temperature on growth and germination of conidia in Curvularia and Bipolaris species isolated from the air. *Aerobiologia*. 29: 13–20. https://doi.org/10.1007/s10453-012-9257-z

Contreras-Cornejo HA, Macías-Rodríguez L, Herrera-Estrella A, López-Bucio J. 2014. The 4-phosphopantetheinyl transferase of *Trichoderma virens* plays a role in plant protection against *Botrytis cinerea* through volatile organic compound emission. *Plant and Soil*. 379: 261–274. https://doi.org/10.1007/s11104-014-2069-x

Dallagnol LJ, Rodrigues FA, Martins SC, Cavatte PC, DaMatta FM. 2011. Alterations on rice leaf physiology during infection by *Bipolaris oryzae*. *Australasian Plant Pathology*. 40(4): 360–365. https://doi.org/10.1007/s13313-011-0048-8

Dwiastuti, Mutia E. 2015. Potensi Trichoderma Spp. Sebagai Agens Pengendali Fusarium Spp. Penyebab Penyakit Layu Pada Tanaman Stroberi. *Jurnal Hortikultura*. 25(4): 331–339, https://doi.org/10.21082/jhort.v25n4.2015.p331-339

Dennis C, Webster J. 1971. Antagonistic properties of species-groups of *Trichoderma*, II. Production of volatile antibiotic. *Trans. Br. Mycol. Soc. 57*: 41–48. https://doi.org/10.1016/S0007-1536(71)80078-5

Guo Y, Ghirardo A, Weber B, Schnitzler JP, Benz JP, Rosenkranz M. 2019. Trichoderma species differ in their volatile profiles and in antagonism toward ectomycorrhiza *Laccaria bicolor*. *Frontiers in Microbiology*. 10: 244–259. https://doi.org/10.3389/fmicb.2019.00891

Howell CR. 2003. Mechanisms employed by Trichoderma species in the biological control of plant diseases: the history and evolution of current concepts. *Plant Disease*. 87(1): 4–10. https://doi.org/10.1094/PDIS.2003.87.1.4

Lee S, Yap M, Behringer G, Hung R, Bennett JW. 2016. Volatile organic compounds emitted by Trichoderma species mediate plant growth. *Fungal Biology and Biotechnology*. 3(1): 1–14. https://doi.org/10.1186/s40694-016-0025-7

Manamgoda DS, Rossman AY, Castlebury LA, Crous PW, Madrid H, Chukeatirote E, Hyde KD. 2014. The genus Bipolaris. *Studies in Mycology*. 79(1): 221–288. https://doi.org/10.1016/j.simyco.2014.10.002

Mukhopadhyay R, Kumar D. 2020. Trichoderma: a beneficial antifungal agent and insights into its mechanism of biocontrol potential. *Egyptian Journal of Biological Pest Control*. 30(1): 1–8. https://doi.org/10.1186/s41938-020-00333-x

Muksin R, Rosmini I, Panggeso J. 2013. *Uji Antagonisme Trichoderma sp. terhadap Jamur Patogen* Alternaria porri *Penyebab Penyakit Bercak Ungu pada Bawang Merah secara in-Vitro* (Doctoral dissertation, Tadulako University).

Rahman A, Begum MF, Rahman M, Bari MA, Illias GNM, Alam MF. 2011. Isolation and identification of Trichoderma species from different habitats and their use for bioconversion of solid waste. *Turkish Journal of Biology*. 35(2): 183–194. https://doi.org/10.3906/biy-0905-8

Ruangwong OU, Wonglom P, Suwannarach N, Kumla J, Thaochan N, Chomnunti P, Sunpapao A. 2021. Volatile organic compound from *Trichoderma asperelloides* TSU1: Impact on plant pathogenic fungi. *Journal of Fungi*. *7*(3): 187–199. https://doi.org/10.3390/jof7030187

Intana W, Kheawleng S, Sunpapao A. 2021. *Trichoderma asperellum* T76-14 released volatile organic compounds against postharvest fruit rot in muskmelons (*Cucumis melo*) caused by *Fusarium incarnatum*. *Journal of Fungi*. 7(1): 46–59. https://doi.org/10.3390/jof7010046

Kementerian Pertanian RI DP. 2016. *Tebu*. Retrieved from http://ditjenbun.pertanian.go.id/tinymcpuk/gambar/file/statistik/2017/Tebu-2015-2017.pdf

Taribuka J, Sumardiyono C, Widyastuti SM, Wibowo A. 2017. Eksplorasi dan identifikasi Trichoderma endofitik pada pisang. *Jurnal Nasional*. 16(2): 115–124. https://doi.org/10.23960/j.hptt.216115-123

Thambugala KM, Daranagama DA, Phillips AJ, Kannangara SD, Promputtha I. 2020. Fungi vs. fungi in biocontrol: An overview of fungal antagonists applied against fungal plant pathogens. *Frontiers in Cellular and Infection Microbiology*. 10: 604923. https://doi.org/10.3389/fcimb.2020.604923

Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Woo SL, Lorito M. 2008. Trichoderma–plant–pathogen interactions. *Soil Biology and Biochemistry*. 40(1): 1–10. https://doi.org/10.1016/j.soilbio.2007.07.002

Yandoc CB, Charudattan R, Shilling DG. 2004. Suppression of cogongrass (*Imperata cylindrica*) by a bioherbicidal fungus and plant competition. *Weed Science*. 52(4): 649–653. https://doi.org/10.1614/WS-03-044R2

Wulansari E. 2021. *Analisis Trend Produksi Tebu Jawa Timur Tahun 2011-2020*. (Doctoral dissertation, Universitas Muhammadiyah Malang).