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Screening and Potential Testing of *Metarhizium* sp. Isolates for Controlling *Lepidiota stigma* (Fabricius) Larvae in Sugarcane (*Saccharum officinarum* L.)

Hanik Sulistyawati[1,✉], Wiwin Windriyanti[1], Yenny Wuryandari[1]

[1] Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" Jawa Timur, INDONESIA.

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Corresponding Author:
✉ hanilistyawati28@gmail.com
(Hanik Sulistyawati)

ABSTRACT

Lepidiota stigma is a major pest contributing to the decline in sugarcane quality in East Java, necessitating effective control measures that support sustainable agriculture. Metarhizium anisopliae is an entomopathogenic fungus that acts as a natural enemy of L. stigma and is expected to control its infestations. This study aims to evaluate the potential of isolated strains in killing the larvae and to identify the compounds produced by Metarhizium spp. The research involved exploration, morphological identification, virulence testing, and compound analysis. Isolate screening was conducted in two stages: the first stage involved screening 20 isolates down to 5 potential ones, and the second stage assessed the characteristics and effectiveness of these 5 isolates in controlling L. stigma larvae at spore concentrations of 10^6, 10^7, and 10^8 spores/ml in vitro. The parameters observed were larval mortality and metabolite content, analyzed using LC-HRMS (Liquid Chromatography High-Resolution Mass Spectrometry). Metarhizium spp. isolated from soil exploration showed an effect on L. stigma mortality. The fungus produced secondary metabolites, including destruxins A4, E, and Ed1, which contributed to the mortality of L. stigma larvae.

1. INTRODUCTION

Sugar cane is an important commodity for the Indonesian economy. National sugar demand increases along with the increase in Indonesia's population and the growth of the food and beverage industry. For 2013, sugar cane productivity reached 75.7 ton/ha, while in 2023 the productivity will reach 61.5 ton/ha. However, public consumption of sugar in 2023 will actually increase by 2.86% compared to 2013, namely 3.4 million tons. With a projected increase in population of around 1.25% and growth in the food and beverage industry of 6-7%/year, the projection for national sugar needs in 2030 will reach 9.8 million tons (Asia, 2024). East Java, as one of the sugarcane producing provinces in Indonesia, contributes 47.34% of the total national sugar production, namely 1.05 million tons out of every 2.35 million tons (Pusat Data dan Sistem Informasi Pertanian., 2022).

Like other cultivated plants, of course sugar cane experiences obstacles in its growth, both from climate change factors, conversion of agricultural land and also pests (Indrawan *et al*., 2024). One of the pests that attacks sugar cane plants is sugar cane ureth (*Lepidiota stigma*). This pest causes damage of up to 52% after 9 months (Sunarto Subiyakto, 2018) sporadically on sugar cane (Harrison & Wingfield, 2016) and spread to the districts of Sleman, Tulungagung, Purworejo, Kediri, Bondowoso, and Situbondo (Subiyakto & Sunarto, 2018b). In addition, the pest has polyphagous properties so it can attack other plants besides sugar cane such as corn, papaya, cassava, rubber, pineapple, coconut, coffee, nuts, soybeans, watermelon, pumpkin, canna and so on (Wiratmoko *et al*., 2021).

In efforts to control pests, farmers still rely on the use of pesticides, where the choice of control with pesticides takes into account the speed and effectiveness in controlling pests (Situmorang *et al*., 2021). Of course, this selection does not take into account the negative factors of controlling with pesticides, namely the impact on ecosystem balance,

environmental pollution, residues on agricultural products and pest resistance and resurgence. In Law No. 22 of 2019 concerning Sustainable Plant Cultivation Systems, it is stated that agricultural protection is carried out with an integrated pest management system and handling the impacts of climate change. Therefore, pest control efforts must minimize negative impacts by combining one component in integrated pest management.

The use of entomopathogenic microbes, including entomopathogenic fungi, is currently being developed as an alternative for biological control (Jati *et al*., 2021). Entomopathogenic fungi are capable of infecting insects and damaging the metabolic system in the insect's body structure (Widariyanto, 2016). *Metarhizium* sp., is the most prominent entomopathogenic fungus for controlling agricultural insect pests which is capable of infecting 200 species of insect pests (Gebremariam *et al*., 2021; Jitendra *et al*., 2012). The *Metarhizium* fungus is effective against insects that live in the soil and is reported to be effective in infecting the scarabaeidae group (Indrayani, 2017; Ravindran *et al*., 2016). *Metarhizium* sp. found in various agroecosystems, starting from desert areas, forests, agriculture, plantations (Kolczarek & Jankowski, 2014) and in the rhizosphere of sugar cane (Indrayani, 2017; Agastya, 2018). The fungus is saprophytic when in the soil, but is able to infect insects. This ability cannot be separated from secondary metabolite compounds including cyclopeptides, destruxins A, B, C, D, E and desmethyldestruxins B (Widiyanti & Muyadihardja, 2004). Destruxins will cause paralysis, inhibit the secretion process in insect cell DNA and RNA, inhibit secretion in Malpighian tubule fluid and suppress the insect's immune response (Golo *et al*., 2014).

Research on *Metarhizium* sp. To control sugarcane worms, the initial symptoms of insects infected with the fungus include a decrease in appetite, the body becomes weak, loses orientation, then does not move and finally dies. Insects experience changes in body color and black spots appear on the cuticle as traces of fungal penetration. With support from the environment, white mycelia will grow on the outer body of the insect. A reddish liquid will come out of the mouth of the infected insect larva. After death, the insect's body will become soft and within about five hours it will harden (undergo mummification). Within one day, the body will be covered by mycelia (Hasyim *et al*., 2016).

Currently, research on the compound *Metarhizium* sp. using Liquid Chromatography High Resolution Mass Spectometry (LC-HRMS) has not been widely done. Analysis in LC-HRMS combines physical separation of liquid chromatography (LC) with selective detection in high resolution mass spectrometry (HRMS) (Tuldjanah *et al*., 2024). The use of LC-HRMS provides more advantages compared to other analyses, namely accuracy in measuring the mass of the analyte, including non-target substances contained in the sample and does not require a reference standard for comparison with pre-existing references (Kumari *et al*., 2015).

Utilization of *Metarhizium* sp. as a biological control agent has been widely used. *Metarhizium* has specific characteristics according to its capacity as an entomopathogen, namely specific host and location (Gabarty *et al*., 2014). Meanwhile (Athifa *et al*., 2018) said that the isolate *Metarhizium* sp. Different geographical locations and host types will provide differences in aspects of physiology and pathogenicity. For this reason, this research will carry out exploration and isolation of *Metarhizium* sp. from the sugarcane rhizosphere which has the potential to control sugarcane rust in East Java, so it can be used as a recommendation as a means of biological control. This research aims to determine the potential of *Metarhizium* sp. which was discovered from the results of exploration in killing *L. stigma*, and to find out the insecticidal compound. This research provides benefits in providing information on *Metarhizium* sp. local isolate from East Java which has high virulence in killing *L. stigma* larvae.

2. RESEARCH MATERIALS AND METHODS

2.1. Research Location

The research was carried out at the Plant Health Laboratory, Faculty of Agriculture, UPN "Veteran" East Java and Research Hub Malang, geographically located at 7° 9' - 7° 21' South Latitude and 112° 36' - 112° 57' East Longitude. The research was conducted from August 2024 to March 2025.

2.2. Research Design

This research was arranged in a Factorial Randomized Block Design with spore density treatments of 10^6 spores/ml, 10^7 spores/ml, 10^8 spores/ml, which were repeated 3 times and 10 of 3rd instar *L. stigma* larvae in each treatment.

2.3. Tools and Materials

The tools needed for this research included a haemocytometer, spectrophotometry, ose needle, petridish, ose needle, Bunsen, Erlenmeyer, test tube, hand counter, beaker, sprayer, label paper, microscope, laminar air flow, autoclave, PCR machine, a set of electrophoresis equipment (tray, comb, chamber and electric current source), plastic jar measuring Ø 6 cm x 8 cm, saucepan, plastic bag, sieve, tissue and LC-HRMS instrument.

The materials used were ureth larvae, 70% alcohol, distilled water, 70% ethyl alcohol, PDA media, carrots, sterile soil, distilled water, TBE buffer, parafilm, agarose powder, 0.5x TAE buffer, ethidium bromide (EtBr) solution, 100 bp DNA ladder marker, forward primer ITS 1 (5'-TCCGTAGGTGAACCTGCGGA-3'), and reverse primer ITS 4 (5'TCC TCCGCTTATTGATATGC-3').

2.4. Research Implementation

2.4.1. PDA Media Preparation

100 grams of peeled potatoes were cut into cubes then put into an Erlenmeyer flask containing 200 ml of distilled water. Next, the potatoes were boiled for 5 minutes so that potato juice is produced, then filtered through gauze and measured until a volume of 200 ml is obtained. Potato juice was added to 300 ml of distilled water which has previously been mixed with 10 grams of glucose and stirred until dissolved. The mixture was then added with 15 grams of bacto agar and boiled until it boils. The media was then poured into petri dishes and sterilized using an autoclave at 1 atm pressure with a temperature of 121 °C within 1 hour.

2.4.2. Soil Sampling and Exploration of *Metarhizium* sp.

Exploration of *Metarhizium* sp. was carried out in the rhizosphere of healthy sugarcane plants, specifically among the plants showing symptoms of ureth attack. Soil sampling was carried out at two points and there were three points used for each sampling. The soil samples were taken from around the sugar cane roots. Soil was taken at a depth of 15-20 cm, amounting to approximately 0.5 kg using a spatula. The sample was then composited and placed in a plastic bag.

Isolation of rhizosphere fungi was performed using serial dilution method. The soil sample in each replication was weighed 10 grams, put into an Erlenmayer and 100 ml of distilled water was added until a suspension was formed. The suspension was shaken until homogeneous and left for approximately 10 minutes. Next, 1 ml of the resulting suspension was taken and then placed in 9 ml of sterile aqudes in a test tube. The solution was shaken again until homogeneous and the initial process is carried out to obtain the 2nd dilution or 10^{-2}. This process was carried out until the dilution was 10^{-7}. Exactly 0.1 ml of the suspension at dilutions 10^{-3} to 10^{-7} was taken and dropped using a pipette into a Petri dish containing PDA media. Next, the suspension was spread evenly with an L stick until it spread evenly in the PDA medium and incubated for 1-2 days at a temperature of 27-28 °C.

A fungal colony is the characteristic of a *Metarhizium* sp. fungal colony. Next, purification was carried out by looking at the morphology of the fungus in the petri dish in the form of the color and shape of the fungal colony found after isolation in the petri dish. Each fungal colony with the characteristics of a *Metarhizium* sp. fungal colony was taken and regrown in a petri dish containing PDA media and incubated for approximately 7 days. From the results of this exploration, it is hoped that 20 isolates of the *Metarhizium* sp fungus will be obtained.

2.4.3. Observation of the morphology of *Metarhizium* sp.

Morphological observations were carried out macroscopically and microscopically. Macroscopic observations include color, shape, texture and edge shape of the colony. Meanwhile, microscopic observations included conidia, conidiophores and hyphae.

2.4.4. Observation of the Morphology of *Metarhizium* sp.

Stock solution of *Metarhizium* sp. of each isolate was determined at a spore density of 1 x 10^{10} spores/ml. Meanwhile, to obtain other densities used in this research, dilution was carried out using the formula:

$$V1 \times N1 = V2 \times N2 \quad (1)$$

where V1 = volume of stock solution (ml), N1 = concentration of stock solution (spores/ml), V2 = is the expected volume of solution (ml), and N2 = expected solution concentration (spores/ml).

2.4.5. Provision of *Lepidiota stigma* Larvae

The research used *L. stigma* larvae at the 3rd instar stage, because this was the most damaging stage to sugarcane (Indrayani *et al*., 2018). To identify 3rd instar larvae, the head circumference of *L. Stigma* was measured. In 3rd instar larvae, the head circumference is 0.8–1 cm with a body length of 6–7 cm and a width of 1–1.5 cm (Alimin, 2022). The type of larvae was determined based on the raster pattern matching the characteristics of *L. stigma* (Achadian, 2022).

2.4.6. *Metarhizium* Virulence Test Exploration Results

The virulence test was carried out in 2 stages. The first stage of testing was using a spore density of 10^8 spores/ml and 20 isolates from exploration results, while the second stage was with a spore density of 10^6 spores/ml, 10^7 spores/ml, 10^8 spores/ml and the 5 best isolates in killing larvae from the first stage of testing. The virulence test was carried out by dipping the 3rd instar larvae into a fungal suspension in a petri dish for approximately 15 s, and placing them in a jar containing sterile soil for observation (Bintang *et al*., 2015).

2.4.7. Insect Mortality

L. stigma larvae that died due to parasitization will show symptoms of color change (melanization), stiffness (mummification), hyphae forming on the surface of the larva's body from white to greenish (Sari, *et al*., 2023). The mortality of the test insects during the first and second stage tests which was calculated using the formula:

$$M = \frac{n}{N} \times 100\% \quad (2)$$

where M = percentage of dead insects, n = is the number of dead insects, and N = is the number of test insects.

2.4.8. Compound Test

Test insecticidal compounds was analyzed using the LC-HRMS instrument. For the LC instrument, use Thermo Scientific™ Vanquish™ Hphenyl Hexyl Orizon UHPLC with Binary Pump (Germering Germany). The analytical column used Thermo Scientific™ AccucoreTM 100 mm length x 2.1 mm ID x 2.6 µm particle size (Lithuania). The mobile phase consisted of 2 solutions, namely solution A and solution B. Solution A was water and 0.1% formic acid, while solution B contained Acetonitrile + 0.1% formic acid. Specifications for instrument included flow: 0.30 mL/min, sample injection volume: 5 µL, running analysis time: 25 min, gradient 5% B and increased gradually to 90% in 16 min, maintained at 90% for 4 min and returned to 5% B up to 25 min.

For the HRMS instrument, the Themo Scientific™ Orbitrap™ Exploris HRMS machine (Bremen Germany) was used. Metabolomics sample preparation: 10 mg. Samples were diluted in 1000 µL of MS-grade methanol (Fisher Chemical) with a 2' vortex (Thermo Scienti-ic Digital Vortex Mixer) then ultrasonicated at 30' room temperature (PS-30A) with 1400 ×g centrifugation at 10' room temperature (Eppendorf Centrifuge 5430 R). The supernatant was filtered through a 0.2 um nylon filter (Charslton Technologies) into an HPLC bottle (Thermo Scienti-ic) and ready for injection. Compound Iiedentification with Thermo Scientific™ Compound Discoverer 3.3 Software (San Jose).

2.4.9. Data Analysis

Data analysis of virulence (mortality) test results was carried out using Rstudio software Analysis of Variety (ANOVA) based on R version 4.4.3. If a sig<alpha value (0.05), then continue testing the average differences between treatments using the DMRT test (Duncan Multiple Range Test) with a significance level of 5%.

3. RESULTS AND DISCUSSION

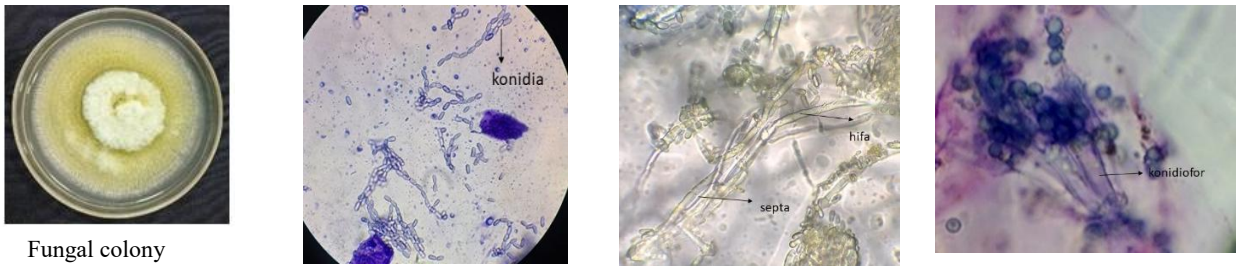
3.1. Morphological Screening of *Metarhizium* Isolates

The identification results on color, shape, texture, and colony edge shape of each isolate are presented in Table 1.

Table 1. Colony Morphological Characteristics of *Metarhizium* sp.

Isolate Code	Colony Color	Colony Form	Colony Texture	Colony Edge Shape
M1	Light green	Irregular	Like Cotton	Hairy
M2	Light green	Irregular	Like Cotton	Hairy
M3	Greenish White	Forming a Circular Pattern	Like Cotton	Hairy
M4	Greenish White	Irregular	Like Cotton	Hairy
M5	Greenish White	Forming a Circular Pattern	Like Cotton	Hairy
M6	Greenish White	Forming a Circular Pattern	Like Cotton	Hairy
M7	Greenish White	Forming a Circular Pattern	Like Cotton	Hairy
M8	Dark green	Forming a Circular Pattern	Like Cotton	Wavy
M9	Greenish White	Forming a Circular Pattern	Like Cotton	Hairy
M10	Dark green	Forming a Circle	Like Powder	Hairy
M11	Olive Green	Irregular	Like Powder	Wavy
M12	Olive Green	Forming a Circular Pattern	Like Cotton	Hairy
M13	Olive Green	Irregular	Like Powder	Wavy
M14	Olive Green	Irregular	Like Powder	Wavy
M15	Light green	Irregular	Like Powder	Wavy
M16	Light green	Irregular	Like Powder	Wavy
M17	Light green	Irregular	Like Powder	Wavy
M18	Young Hujau	Irregular	Like Cotton	Hairy
M19	Light green	Irregular	Like Powder	Wavy
M20	Greenish White	Forming a Circle	Like Cotton	Hairy

In this study, 20 isolates of *Metharizium* sp. discovered and identified macroscopically. Macroscopically, colonies of *Metarhizium* sp. The PDA media from exploration results has a white color that changes to greenish with varying colors, from light green, yellowish green to dark green to olive green, has irregular colony shapes and some form circular patterns, has a texture like cotton and some are thin like powder and have hairy and wavy colony edges (Table 1). Macroscopically, the colony shape of the fungus *Metarhizium* sp. shown in Figure 1a. Meanwhile, microscopically, *Metarhizium* sp. has round, cylindrical conidia that form chains and are hyaline (Figure 1b), have insulated and hyaline hyphae (Figure 1c) and conidiophores that are arranged upright and branched (Figure 1d). This is in accordance with research by Jati *et al*. (2021), that in general the conidia of *Metarhizium* sp. cylindrical and hyaline in shape and form chains. Meanwhile, research results (Akhsan *et al*., 2025) showed that the fungus colony *Metarhizium* sp. from plantation land isolated on Glucose Yeast Agar (GYA) media had olive green colonies with a prominent center and dark green colony edges, while at the edges of the media small and spread out colonies were formed. Apart from that, *Metarhizium* sp. have partitions on the hyphae, cylindrical conidia form chains and spread out.



Fungal colony

a b c d

Figure 1. Morphological characteristics of *Metarhizium* sp. macroscopically and microscopically

3.2. Identification of Mortality of *Lepidiota stigma* due to Metarizhium sp.

Symptoms of *Metarhizium* attacks on *L. stigma* include changes in the color and behavior of the insect. The larvae changed color from white to slightly blackened, shriveled, hard, odorless (Figure 2a) which occurred on the third day after application of *Metarhizium* sp., white hyphae appeared (Figure 2b) on 6 days after application (HSA) and then

green hyphae appeared (Figures 2e and 2f) on the 10th day after application. This raises the suspicion that the *Metarhizium* isolate used in the research has begun to make contact and penetrate into the urethral body. In line with the results of this research, (Sari *et al*., 2023) said that there were morphological changes in Leptocorisa acuta which was attacked by *Metarhizium anisopliae*. These changes include spots with a blackish brown color (melanization), then becoming stiff (mummification), the formation of white hyphae on the insect's body (mycosis) and then green fungal colonies appear.

a b c d e f

Figure 2. Symptoms of *Metarhizium* attacks on *Lepidiota stigma* : (a) healthy, (b) to (f): 3, 6, 7, 10, and 12 DAA

Changes in the behavior of L stigma are characterized by larvae that are not actively moving and have no appetite. This decrease in physiological activity is thought to be related to the activity of destruxins produced by *Metarhizium* sp., namely as an antifeedant and a cause of paralysis. This is in line with the results of research (Nababan *et al*., 2023) which states that infection with *Beauveria* sp. and *Metarhizium* sp. on *Spodoptera frugiperda* larvae showed physiological disturbances, namely a decrease in appetite and slow movement. (Amiri *et al*., 1999) said that destruxins A, B, and E, produced by the entomogenic fungus *Metarhizium anisopliae*, are insecticidal but relatively low doses have antifeedant properties, whereas (Samuels *et al*., 1988) said that injection of destruxins into lepidoptera larvae causes direct muscle paralysis followed by flaccidity caused by muscle depolarization with direct opening of Ca^{2+} channels in membrane. Muscles become soft, limp and lose their tone.

In the virulence test of 20 *Metarhizium* isolates against *L. stigma*, based on analysis, the density of 10^8 spores/ml, on the third day, had an effect on larval death (Table 2). This is in line with research from Anggraini & Wardati (2024) that *Metarhizium* sp. causes death of L stigma 48 hours after application. Likewise, Mora *et al*. (2018), said that fungi need between 24-48 hours to make contact with their hosts through insect integuments. Isolates M3, M5, M7, M9 and M12 gave the highest percentage of deaths among other isolates and will continue with the second phase of virulence testing.

Based on the results of the variance analysis in the second stage of the virulence test, the *Metarhizium* sp. showed an effect on the third day, especially on the isolate factor, while the spore density factor showed results on the eighth day after application. These results indicate that there was no interaction between isolate type and spore density on mortality. The isolate type factor partially had a significant effect on mortality on days 3 to 7. The spore density factor partially influenced mortality on days 3 to 6 and days 8 to 10. Isolate M3 in general is the best isolate with the highest mortality effect and causes mortality from day 2 and causes higher daily mortality compared to other treatments. Meanwhile, in the spore density factor, a density level of 10^8 spores/ml resulted in significantly higher mortality than other treatments. This is in accordance with the opinion of Jati *et al*. (2021) who said that the diversity of virulence of

Table 2. Mortality rate of *L. stigma* due to Metharizium at 2, 4, 6, and 8 DAA

Treatment	Mortalitas (%)			
	2	4	6	8
M1	0.00	0.00 e	36.67 cd	43.33 f
M2	6.67	16.67 cde	56.67 abcd	73.33 cde
M3	10.00	43.33 abc	73.33 ab	100.00 a
M4	0.00	16.67 cde	56.67 abcd	73.33 cde
M5	0.00	0.00 e	36.67 cd	83.33 abcd
M6	0.00	26.67 bcde	36.67 cd	66.67 cde
M7	0.00	16.67 cde	53.33 abcd	96.67 ab
M8	0.00	26.67 bcde	46.67 bcd	60.00 ef
M9	0.00	0.00 e	70.00 abc	100.00 a
M10	13.30	46.67 ab	63.33 abc	70.00 cde
M11	0.00	10.00 de	23.33 d	63.33 def
M12	6.67	63.33 a	86.67 a	100.00 a
M13	6.67	20.00 bcde	36.67 cd	56.67 ef
M14	3.33	23.33 bcde	50.00 bcd	70.00 cde
M15	10.00	26.67 bcde	50.00 bcd	70.00 cde
M16	0.00	16.67 cde	36.67 cd	70.00 cde
M17	6.67	23.33 bcde	46.67 bcd	70.00 cde
M18	3.33	36.67 bcd	66.67 abc	83.33 abcd
M19	6.67	36.67 bcd	63.33 abc	86.67 abc
M20	13.30	30.00 bcd	70.00 abc	76.67 bcde
DMRT 5%	tn	24.10	30.50	19.41

Note: numbers followed by the same letter in the same column indicate that they are not significantly different in the 5% DMRT test

Table 3. Mortality of *Lepidiota stigma* in the second stage until day 10

Treatment	Mortalitas (%)				
	2	4	6	8	10
M3	2.22	41.11 a	87.78 a	92.20	92.20
M5	0.00	8.89 b	51.11 b	87.80	90.00
M7	0.00	38.89 a	80.00 a	92.20	92.20
M9	0.00	27.78 a	68.89 ab	84.40	86.70
M12	0.00	27.78 a	67.78 ab	87.80	91.10
DMRT 5%	tn	16.68	20.65	tn	tn
^6	0.00	30.00 a	70.67 a	88.00 ab	88.00 b
^7	0.00	24.00 a	66.00 a	84.00 b	84.00 b
^8	1.33	32.67 a	76.67 a	94.67 a	99.33 a
DMRT 5%	tn	12.92	15.99	7.94	6.48

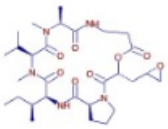
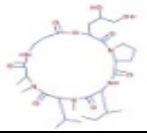
Note: numbers followed by the same letter in the same column indicate that they are not significantly different in the 5% DMRT test

entomopathogenic fungi against insects is influenced by the origin of the isolate because it is related to the type or strain of the entomopathogenic fungus. Besides that, (Suprayogi *et al*., 2015) stated that there is a relationship between conidia density and the effectiveness of the *M. anisopliae* fungus, where the higher the conidia density, the higher its effectiveness in killing insects.

3.3. Compound Test Results *Metarhizium* sp.

Results of testing for *Metarhizium* sp. metabolite compounds. carried out on the M3 isolate, which in analysis was the isolate that gave the highest mortality of the five test isolates. Tests using the LC-HRMS instrument found destruxins A4, E and Ed1 which were thought to have an influence on insect virulence (Table 4). Destructin causes paralysis and death in insects after 3-14 days (Poerwanto *et al*., 2022), which is possible due to disruption of vital physiological processes (Poprawski *et al*., 1994).

Table 4. Compound Test Results from Isolate M3 From LC-HRMS Results

Compound name	Formulas	Retension time (min)	Calc. MW	Chemical structure
Destruxins A4	$C_{30}H_{49}N_5O_7$	9.161	591.36123	
Destructin E	$C_{29}H_{47}N_5O_8$	7.114	593.34062	
Destructin Ed1	$C_{29}H_{49}N_5O_9$	5.973	611.35157	

Testing with LC-HRMS produces a whole chromatogram, which shows all the compounds produced during the testing process and shows the time used to produce a compound and its abundance. Besides that, the results of a single chromatogram for each compound are produced, which shows the x-axis as the ratio of mass to charge (m/z) and the y-axis as relative abundance. The single chromatogram of the destruxins produced by isolate M3 is shown in Figures 3-5. Destruxins, are compounds released by *Metarhizium anisopliae*. Chemically, destruxsins are hexadecipeptides formed from α-hydroxy acid and five amino acid residues. This compound has been reported to produce several bioactivities, including insecticidal, anti-tumor, antiviral and phytotoxic activities (Liu & Tzeng, 2012). In research, Destruxins E is thought to have the most crucial role in killing *L. stigma*. This is in accordance with several opinions, including Poprawski *et al*. (1994) stating that while destruxins E is reported to have the same toxicity as destruxins A when applied to insects, that Destruxins E is reported to cause rapid death in insects due to disruption of vital physiological processes. Destruxins E will disrupt the balance of antioxidants such as glutathione and ascorbate, resulting in increased levels of reactive oxygen species (ROS) which will cause widespread cell damage, resulting in loss of membrane integrity, cell swelling, vacuolization and cell leakage which ultimately results in larval death (Sowjanya Sree & Padmaja, 2008), affecting calcium flow and intracellular protein phosphorylation in insects which ultimately affects insect physiology and behavior such as muscle contraction, secretion, or immune defense which means that there will be dysfunction of important organs and systems, such as the nervous system. digestive, and

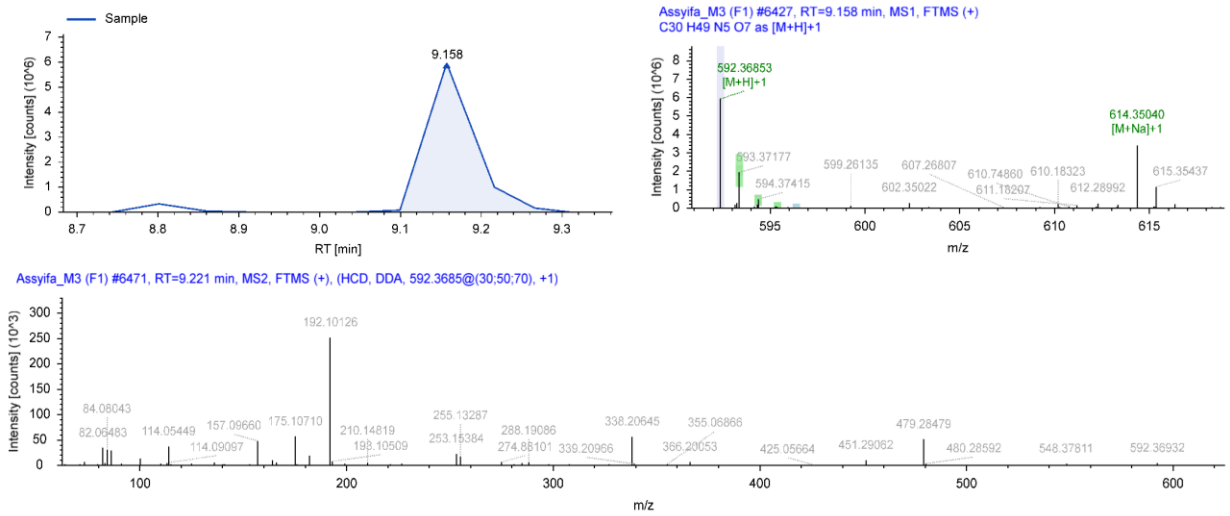


Figure 3. Single Chromatogram of Destruxins A4 Isolate M3 Compound

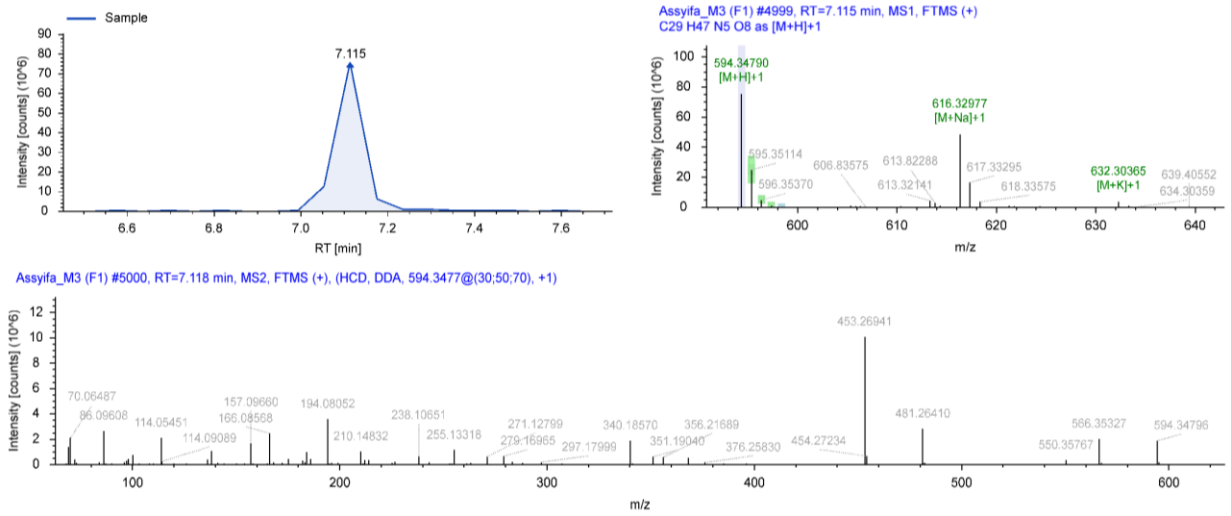


Figure 4. Single Chromatogram of Destruxins E Isolate M3 Compound

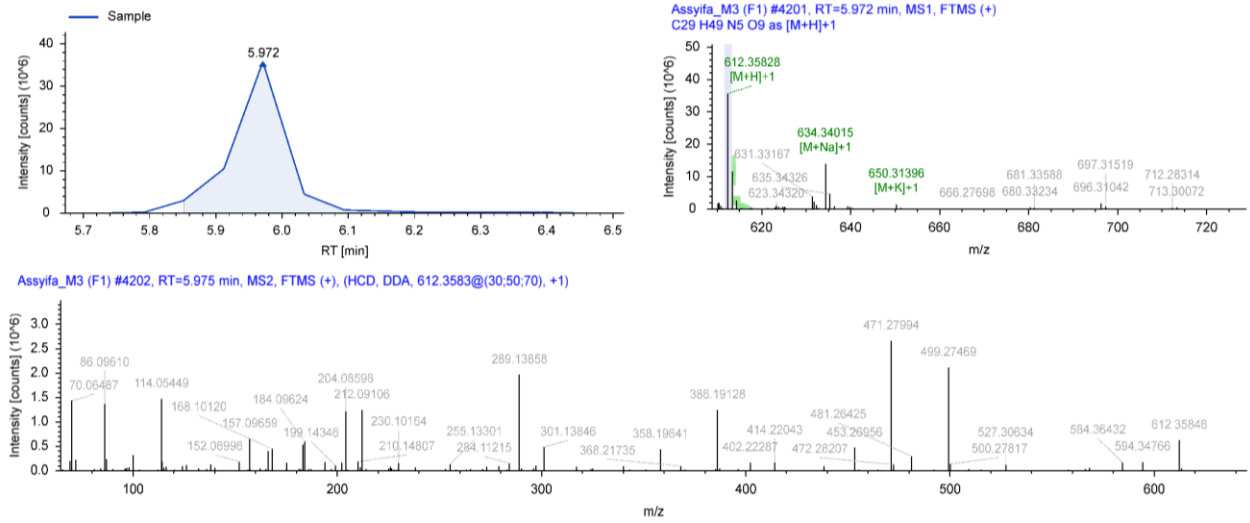


Figure 5. Single Chromatogram of Destruxins Ed1 Isolate M3 Compound

excretory systems, which can cause paralysis, tissue damage, and ultimately insect death (Dumas *et al*., 1994), inhibit the activity of H(+) type ATPase which will inhibit the degradation process of proteins, lipids, and other molecules and will affect insect nerve function which has the potential to cause paralysis or death (Yoshimoto & Imoto, 2002) and change the structure of insect hemocytes which causes cell stress and impaired immune function due to the influence of these toxins (Vey *et al*., 2002). 2011). Destruxins Ed1 is a cyclic hexadepsipeptide with a structure including pipecolic acid replacing proline in the second position. Research on the insecticidal activity of Destruxins Ed1 was reported by Jegorov *et al*. (1998) on Galeria melonella larvae, which proved that destruxins Ed1 has potential as a bioinsecticide. Meanwhile destruxins A4 is a derivative of destruxins A, but there has not been much research on destruxins A4 and its effects on insects. Destruxins A4 is reported to be able to induce the expression of the erythropoietin gene which is very important in regulating the production of red blood cells (erythrocytes), so that destruxins A4 is emphasized in its effects on human health.

4. CONCLUSION

The conclusion of this research is that *Metarhizium* sp. obtained from soil exploration results were able to influence the mortality of *L. stigma*. *Metarhizium* sp. produces secondary metabolites in the form of destruxins A4, E and Ed1 which cause mortality of *L. stigma*. Suggestions for future researchers to carry out in vivo testing on a field scale, to determine the effectiveness of *Metarhizium* sp. in more complex environmental conditions.

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