

MORPHOLOGY VARIATION OF *METARHIZIUM ANISOPLIAE* FROM SOIL IN NORTH SUMATRA AREAS

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Abstract

Metarhizium anisopliae is an important entomopathogenic fungus that mainly used for biological control. Isolates of *M. anisopliae* extracted from soil from 15 oil palm plantations located in several districts on the Province of North Sumatra, Indonesia have been investigated to identify their morphological diversity. The morphology of assessed fungi was constructed in the form of conidia, conidia size, color and shape colony and growth diameter of the 15 isolates. All of conidia forms showed cylindrical shape and string together. The length/width (μm) ratio of conidia size was minimum 2,16 and maximum 3,54. The highest growth rate isolates were found in M3 (Bandar Selamat) and M12 (Mancang) treatment and the lowest rate was M11 (Teluk Parit Kaca) treatment. The color of colonies were varied among the isolates. They were greenish yellow, dark green, yellowish and showing zonation/no zonation.

Keywords : *Metarhizium anisopliae*, biological character, morphological diversity

1. INTRODUCTION

Currently, the development of several biological controls as one of the benefits of integrated pest management is increasing. This is done to reduce or eliminate the use of chemical pesticides, both on agricultural land and forestry, so that the impact is less to human health and the environment (Sapna Bai *et al.*, 2015). The entomopathogenic fungi, *M. anisopliae*, was discovered by Sorokin in 1883, has a wide range of hosts. This fungus is widely used as a biological agent throughout the world, mainly as an inundation control (Hoe *et al.*, 2009; Zimmermann, 2007).

Microbial control is an aspect of biological insect control and consists of the rational use of pathogens to maintain pest balances in agricultural environments, with increases in the numbers of other natural enemies often being observed in fields where microbial control has been used (Tiago *et al.*, 2014). *Metarhizium* is wide spread in nature and is found in soil, at the rhizosphere of plants or arthropod cadavers as saprophyte and parasitizes a broad range of insect. *Metarhizium* grows vegetatively as hyphae producing mycelia and conidia that are the infective propagules on arthropods host and culture media. Although the overall host range of *M. anisopliae* is broad, individual strains can target only particular hosts (Schrank *et al.*, 2010). The aim of this study was to establish the level of diversity among several isolates of *M. anisopliae*, isolated from various locations of oil palm plantations.

2. MATERIALS AND METHODS.

2.1. Isolation of *M. Anisopliae* from Soil Samples of Several Districts

M. anisopliae was obtained from the isolation of soil samples obtained from the palm oil plantation from 5 (five) districts of North Sumatra province where samples from each district were taken from 3 (three) subdistricts and each district was taken 1 (one) village. Soil samples were taken around the roots of the plant by using a small shovel at a depth of 10-15. Then put in a plastic bag and labeled according to the sampling location and time. The soil sample is taken to the laboratory and stirred until it is flat (composited). Isolation of *M. anisopliae* was performed by modifying the larvae *T. molitor* trap method proposed by Trizelia *et al.* (2011) and Inglis *et al.* (2012). The trap method by inserting 300 g into a plastic container and putting 10 larvae of *T. molitor*. Then incubated at room temperature (28°C) for 10-14 days. Dead larvae that have symptoms of *M. anisopliae* disease were taken and sterilized the surfaces with 1% sodium hypochlorite and rinsed twice with sterile water and dried. Isolation of larvae by cutting larvae of larvae and taking part in the larvae body and inoculated onto sterile SDAY medium (Inglis *et al.*, 2012) in petri dishes. *M. anisopliae* cultures were taken with a 5 mm cork drill, then implanted on SDAY medium and incubated for 16 days at 28°C.

2.2. Experimental design

The method used in this research is Completely Randomized Design (RAL) non factorial with 15 treatment of three replications. The treatment was conducted in locations as follow:

M1 = village Kampung Baru, sub district of Bilah Barat in Labuhan Batu district

- M2 = village Bandar Rejo, sub district of Rantau Selatan in Labuhan Batu district
M3 = village Bandar Selamat, sub district of Rantau Utara in Labuhan Batu district
M4 = village Sipenggeng, sub district of Batang Toru in Tapanuli Selatan district
M5 = village Perdamean, sub district of Muara Batang Toru in Tapanuli Selatan district
M6 = village Bandar Tarutung, sub district of Angkola Sangkunur in Tapanuli Selatan district .
M7 = village Lanbau, sub district of Bandar in Simalungun district
M8 = village Bandar Tonga, sub district of Bandar Huluan in Simalungun district
M9 = village Lias Baru, sub district of Bandar Masilam in Simalungun district
M10 = village Perdamaian, sub district of Binjai in Langkat district
M11 = village Teluk Parit Kaca, sub district of Secanggang in Langkat district
M12 = village Mancang, sub district of Selesai in Langkat district
M13 = village Gunung Rintih, sub district of STM Hilir in Deli Serdang district
M14 = village Namotualang, sub district of Sibiru-biru in Deli Serdang district
M15 = village Kutambelin, sub district of Pancur in Deli Serdang district

3. RESULT AND DISCUSSION

Identification of *M. anisoplae* was observed by seeing the colony morphology, size, shape and conidia. Morphological of conidia in SDAY under bright field microscope are cylindrical shape with round end and commonly light green in color and string together (Fig. 1). The fifteen isolates of this study, were identified as *M. anisoplae*. The conidial size with minimum and maximal width is respectively 1.81 μm and 2.76 μm , and the minimum and maximum length is respectively 5,96 μm and 6,54 μm . This is consistent with research conducted by Ghayedi and Abdollahi (2013) that conidia *M. anisoplae* is cylindrical to oval and measuring $<9 \mu\text{m}$. Ratio length/width of conidia size was minimum 2,16 and maximum 3,54. This similar to Tangthirasunun *et al.* (2010) who reported conidia dimension of *M. anisoplae*. They grouped the length/width ratio into 3 groups. Low ratio length/width group of conidia is ≤ 2.18 , medium ratio length/width group of conidia is between 2.33 and 2.87 and high ratio length/width group of conidia is ≥ 2.91 .



Fig. 1. The morphology of conidia under bright field light microscope (100x) with lactophenol blue staining.

The colony color differ the growth of colony was observed on SDAY at 28°C for 16 days. Fungal micelium are initially white or cream. Becoming shades of yellow, shades of green/yellow to shade of dark during sporulation. Most isolates are greenish yellow and yellowish, except M13 is dark green (Fig. 1). For the results of observation color colony aged 16 days, it can be seen in general colonies are white to greenish yellow to dark green. This is the same as the research conducted by Ghayedi and Abdollahi (2013) that the colony of *M. anisoplae* is yellowish green or olive green. *M. anisoplae* has been described as having yellowish green, olivaceous, dark-herbage gree, pink or vinaceous buff (Bridge *et al.*, 1993). Sapna Bai *et al.*, (2015) also reported the morphology of colony color *M. anisoplae* are pale green and dark green.

Table 1. Size of conidia and colony color of isolate *M. anisoplae*

Isolate	Host	Origin	Average size of Konidia (μm)		Colony color
			Width	Length	
1	Soil	Kampung Baru, Labuhan Batu	2.31	6.23	Greenish yellow
2	Soil	Bandar Rejo, Labuhan Batu	2.10	6.06	Greenish yellow
3	Soil	Bandar Selamat, Labuhan Batu	1.87	5.98	Greenish yellow
4	Soil	Sipenggeng Tapanuli Selatan	1.93	6.26	Greenish yellow, with zonation
5	Soil	Perdamean Tapanuli Selatan	2.21	6.02	Greenish yellow

6	Soil	Bandar Tarutung Tapanuli Selatan	2.22	6.25	Greenish yellow, with zonation
7	Soil	Lanbau Simalungun	2.33	6.48	Greenish yellow, with zonation
8	Soil	Bandar Tonga Simalungun	2.43	6.41	Greenish yellow
9	Soil	Lias Baru Simalungun	2.01	6.54	Greenish yellow
10	Soil	Perdamaian Langkat	1.94	6.16	Greenish yellow, with zonation
11	Soil	Teluk Parit KacaLangkat	1.95	6.22	Greenish yellow
12	Soil	Mancang Langkat	1.81	6.40	Greenish yellow
13	Soil	Gunung Rintih Deli Serdang	2.01	6.38	Dark green, with zonation
14	Soil	Namotualang Deli Serdang	2.76	5.96	Yellowish
15	Soil	Kutambelin Deli Serdang	2.31	6.47	Greenish yellow

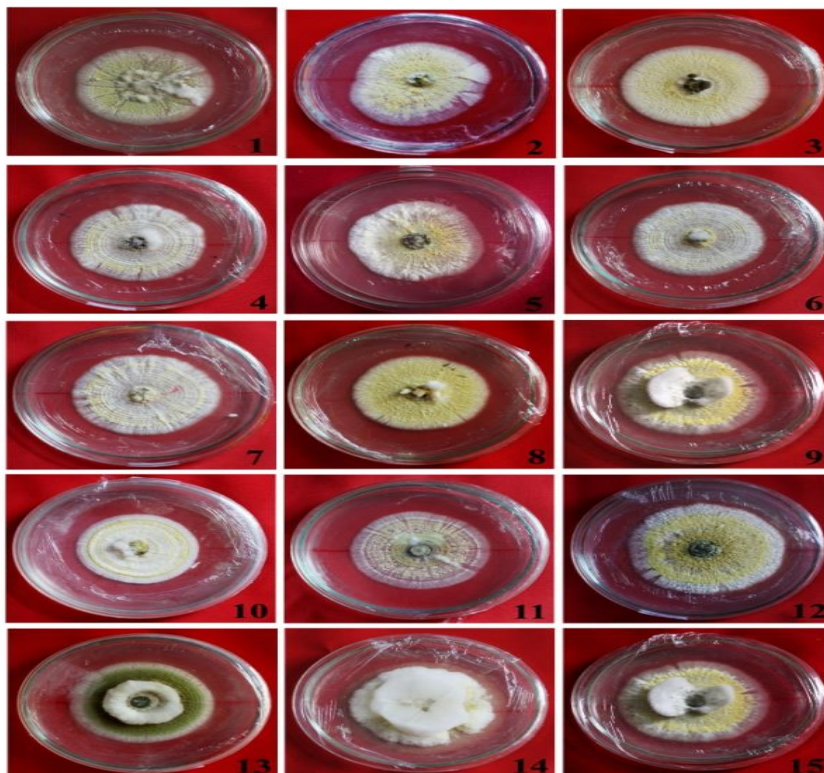


Fig. 2. *Metarhizium anisopliae* originated: 1. Kampung Baru (Labuhan Batu) 2. Bandar Rejo (Labuhan Batu), 3. Bandar Selamat (Labuhan Batu), 4 Sipenggeng (Tapanuli Selatan), 5. Perdamean (Tapanuli Selatan), 6. Bandar Tarutung (Tapanuli Selatan), 7. Lanbau (Simalungun), 8. Bandar Tonga (Simalungun), 9. Lias Baru (Simalungun), 10. Perdamaian (Langkat), 11. Teluk Parit Kaca (Langkat), 12. Mancang (Langkat), 13. Gunung Rintih (Deli Serdang), 14. Namotualang (Deli Serdang), Kutambelin (Deli Serdang).

Tabel 2. Growth diameter *M. anisopliae* at 1st until 16th day

Treatment	Growth diameter <i>M. anisopliae</i> at 1st until 16th day															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
M1	7,33	7,83	9,67	12,17	15,00	17,33	20,33	22,83	25,33	27,83	30,00	33,33	36,67	39,33	41,50	44,67
M2	7,00	9,33	11,00	13,00	17,33	19,67	23,17	25,33	30,50	31,17	34,33	37,33	40,33	47,17	49,17	52,5
M3	9,00	11,67	15,67	19,33	23,67	26,33	33,33	38,33	37,00	40,50	51,50	54,17	57,67	65,33	66,00	69,33
M4	7,17	8,50	10,67	14,50	16,67	21,33	23,00	26,33	29,17	32,67	35,17	38,33	41,83	44,67	47,50	51,33
M5	7,67	8,67	10,00	12,50	16,50	19,50	21,50	25,17	27,50	31,17	34,67	36,17	38,50	43,83	46,67	50,33
																bcd

M6	7,00	9,50	11,00	15,00	17,67	21,00	23,33	26,67	29,50	31,00	32,67	35,67	42,33	46,00	48,50	51,83	bed
M7	7,00	9,50	10,50	15,00	17,17	21,33	24,33	27,17	31,50	35,17	37,33	41,33	44,17	46,83	50,17	53,33	b
M8	7,83	9,33	11,50	15,17	16,50	18,67	21,83	24,17	27,00	29,17	33,00	38,00	41,67	43,83	46,67	48,83	bcd
M9	7,67	9,83	10,83	13,00	15,17	17,17	21,00	24,17	26,83	29,33	31,33	34,00	37,33	40,00	42,67	46	bcde
M10	7,00	9,17	11,33	14,67	17,17	21,50	23,00	26,50	30,17	33,17	35,50	37,33	39,00	43,00	46,00	48,17	bcde
M11	7,67	8,00	9,50	11,83	14,50	16,17	18,83	21,17	24,00	25,50	27,50	30,83	34,33	36,33	38,50	40,5	e
M12	8,83	9,67	13,50	17,00	21,00	24,67	27,17	31,00	34,83	40,17	45,17	47,17	51,50	54,17	58,83	63 a	47,33
M13	8,17	10,33	12,67	13,67	16,17	18,00	23,00	25,00	28,33	30,17	34,17	36,50	38,17	40,00	44,00	45,33	bcde
M14	8,17	9,67	10,33	13,17	15,50	17,50	20,00	22,33	25,00	28,17	31,33	34,50	36,83	40,67	43,50	50,5	cde
M15	8,33	9,50	10,67	14,67	18,17	21,33	24,17	26,00	30,00	32,50	35,67	38,00	41,17	43,33	47,67	51,83	bcd

Note. The numbers in the columns followed by the same letter do not differ significantly according to the Duncan test at the 5% level.

In the Tabel 2. showed M3 (Bandar Selamat) and M12 (Mancang) are faster growth than other isolates. Then among M6, M4, M15, M5, M8, M10, M13 and M9 is significantly different from M14, M1 and M11. In nature isolates of *M. anisopliae* contain diverse assemblage of genotypes and probably comprise species complexes. Therefore, it is not surprising that within the isolates, individual isolate can show variation biological characters (Rachappa *et al.*, 2009). Milner *et al.* (2002) founded ariation in colony characters of *M. anisopliae* collected from geographical locations and host insects.

4. CONCLUSION

M. anisopliae has a morphological diversity from the shape and color of colonies, conidia size and diameter growth. But for the level of virulence to the insects, it is necessary to obtain a virulent isolate that can be used for biological control. It is also recommended that molecular testing be conducted to see the genetic diversity.

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