BIODIVERSITAS
Volume 21, Number 7, July 2020
Pages: 2935-2945

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d210709

Evaluation of in vitro antagonistic activity of fungi from peatlands against *Ganoderma* species under acidic condition

SUPRIYANTO[1,3,♥], PURWANTO[2], S.H. POROMARTO[2], SUPYANI[2]

[1]Department of Agricultural Science, Graduate School, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia. Tel./fax.: +62-271-637457, ♥email: supriyantountan2013@gmail.com

[2]Faculty of Agriculture, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia.

[3]Department of Agriculture Cultivation, Faculty of Agriculture, Universitas Tanjungpura. Jl. Prof. Dr. H. Hadari Nawawi, Pontianak 78124, West Kalimantan, Indonesia

Manuscript received: 26 March 2020. Revision accepted: 8 June 2020.

Abstract. *Supriyanto, Purwanto, Poromarto SH, Supyani. 2020. Evaluation of in vitro antagonistic activity of fungi from peatlands against* Ganoderma *species under acidic conditions. Biodiversitas 21: 2935-2945.* The use of peatlands is a significant contributor to the world's palm oil production. A serious problem of oil palm plantations in peatlands is the high incidence of basal stem rot (BSR) disease caused by *Ganoderma*, which has a higher attack rate than on mineral soils. There is no effective way to control *Ganoderma* in peatlands. At present, the effort for the same focuses on environment-friendly biological methods; however, this is constrained by the unavailability of appropriate biological agents for peatlands. The development of biological control agents for peatlands is hampered by limited data on biological control of *Ganoderma* in peatlands. This research was conducted to evaluate the in vitro antagonistic activity of fungi isolated from a peatland in acidic pH conditions. Twenty-seven *Ganoderma*-antagonistic fungi from peatland were evaluated for their activity and their ability to antagonism in vitro within a pH range of 2-7. The results show that most antagonistic fungi from peatland, based on biomass weight, the sporulation ability, and germination of conidium, were able to grow optimally at pH 3.0-4.0, indicating that most of the *Ganoderma*-antagonistic fungi from peatland can be used as biological control agents for BSR on oil palms in peatlands.

Keywords: Acidic pH, biological control, *Ganoderma,* oil palm, peatland

INTRODUCTION

The utilization of peatlands is important to global palm oil production. In 2015, the area of oil palm plantation in peatlands in the world was 3.1 million ha. Indonesia, the largest palm oil-producing country in the world, has a large area of oil palm plantations in peatlands, which is 2.046 million ha, equivalent to 14.58% of the total area of Indonesian palm oil, which until 2017 was 14.03 million ha (Miettinen et al. 2016; Dirjenbun 2017). Most oil palm plantations in peatlands in Indonesia are spread on the islands of Sumatera and Kalimantan. In Kalimantan island, almost half (42.33%) of oil palm plantation in peatlands is in the province of West Kalimantan, which covers 309.32 thousand ha (Miettinen et al. 2016).

A serious problem of oil palm plantations in peatlands is the high incidence of basal stem rot (BSR) disease caused by *Ganoderma* where the attack is higher and appears earlier than in the mineral soils (Azahar et al. 2011; Susanto et al. 2011; Rakib et al. 2017). Economically, *Ganoderma* attacks are detrimental because they could reduce the yields of crops when the oil palms suffer from BSR diseases by an average of 43.32% in 6 months (Assis et al. 2016). *Ganoderma* attacks usually cause palm death, thus shortening the production period. The intensity of BSR disease also always increases with age and oil palm plantation regeneration (Chong et al. 2017). *Ganoderma* species are saprophytic fungi indigenous tropical rainforest; however, in certain situations it becomes pathogenic (Pilotti 2005; Cooper et al. 2011). In addition, *Ganoderma* is transmitted through air, besides through root contact, with basidiospores (Rakib et al. 2014), has many host plants (Muniroh et al. 2019), and forms a retaining structure like sclerotium (pseudosclerotia) (Darmono 2000).

There are no effective methods to control the spread of BSR disease in peatlands. Some methods that were developed such as making a mounding at the stem base, "stem surgery" (removing diseased tissue), making isolation trenches, sanitation, land ploughing during oil palm plantation replanting, using fungicides, using cover crop legumes, or oil palm fertilizing improvements. The results of these methods vary greatly (Fee 2011; Wong et al. 2012; Priwiratama et al. 2014; Muniroh et al. 2019). Biological control is an alternative control method that is the focus of current development (Muniroh et al. 2019). However, there are no reports on the biological agents that can be effectively used in peatlands. Its development is constrained by the lack of data on biological control in peatlands, which have different characteristics compared to mineral soils. The characteristic of tropical peatlands which is considered a serious obstacle in biological control is its extremely low pH level. Tropical peatlands in the lowlands of Kalimantan have an average pH of 3.3 for the deepest peatland, whereas shallow peatlands have an average pH of 4.3 (Andriesse 1988).

Various *Trichoderma* species are known to be the main antagonists of *Ganoderma* (Naher et al. 2015; Alexander et al. 2017; Musa et al. 2018). The pH value of the growing medium was reported as the most important parameter that influences the growth and sporulation of *Trichoderma* because it determines the availability of minerals and influences the metabolic rate and enzymatic activity (Zehra et al. 2017). Acidic soil conditions usually increase spore germination, conidiophore production, mycelial growth, antibiotic production, and reduce the fungus conidium fungistatic (Bakker et al. 2012). However, evaluation of the activity and dynamics of the antagonistic capabilities of various *Ganoderma* antagonists in low pH environments has not been widely reported. This research aims to determine the fungal biomass growth, sporulation, and germination of the conidia of *Ganoderma*-antagonistic fungi from peatlands and their antagonism ability in the low pH range. This information is important in the development of strategies for controlling the BSR disease of oil palm in peatlands caused by the *Ganoderma* species.

MATERIALS AND METHODS

The origin of *Ganoderma* isolates and their antagonists

Ganoderma isolates were isolated from *Ganoderma* fruit bodies taken from diseased oil palms at PT. Bumi Pratama Khatulistiwa (PT. BPK) in Kubu Raya District, West Kalimantan Province, Indonesia (0° 2'44.11"N, 109°26'6.20"E). The *Ganoderma*-antagonistic fungal isolates were collected from peat soils of 10 sample blocks in PT. BPK (Figure 1). Three samples were taken from each block at depths of 0-15 cm and 50-60 cm. The samples from each block were made into a composite based on a depth of 0-15 cm as a layer, which was routinely disturbed by land maintenance activities, and a depth of 50-60 cm as a layer that is considered to be minimally disturbed during land maintenance. The pH value of each peat sample was measured by mixing 2.5 mL of fresh peat with 4 mL of 0.01 M $CaCl_2$ solution and allowed to stand for at least an hour and the pH was measured (Notohadiprawiro 1985).

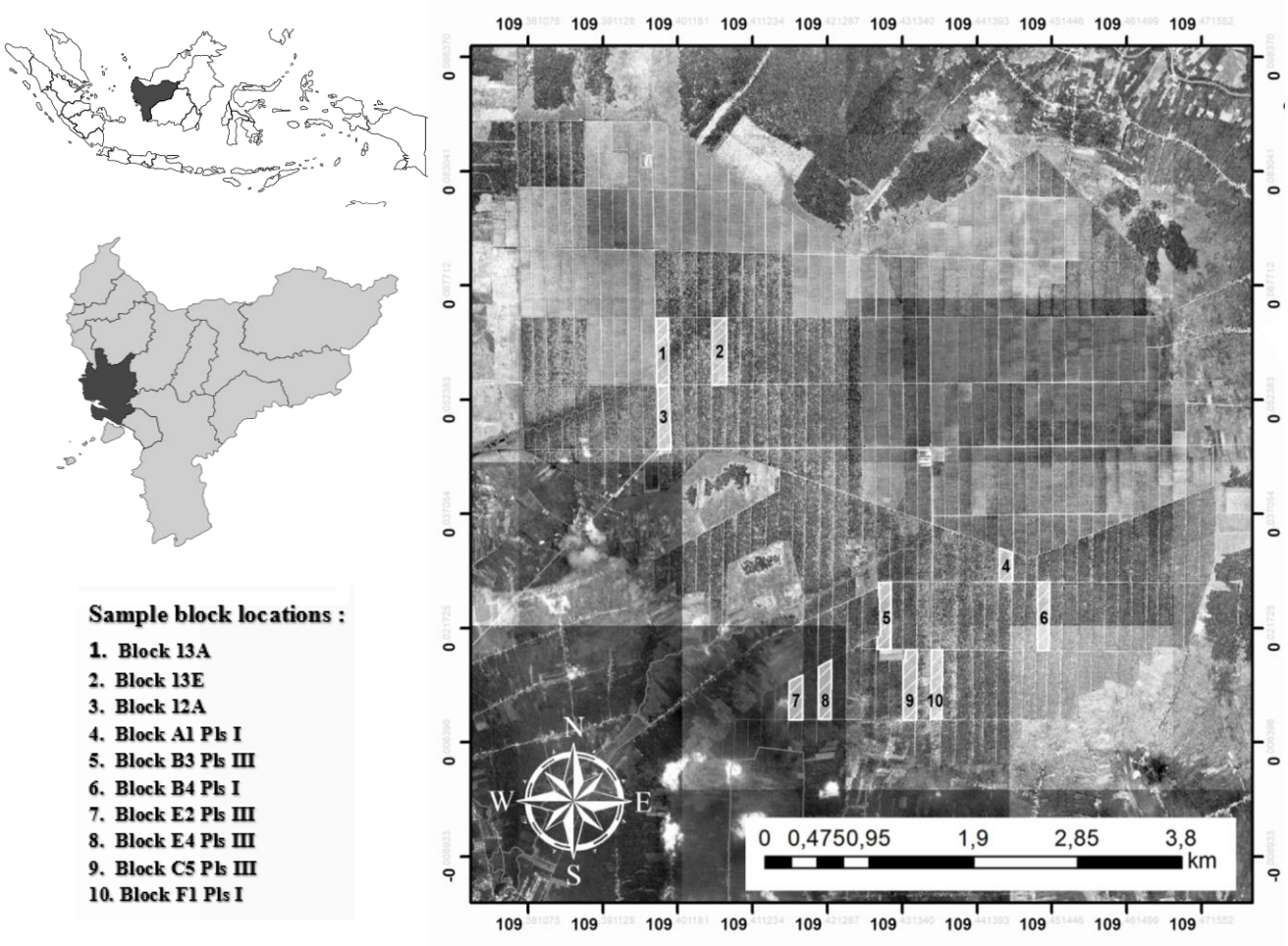

Figure 1. Map of the peat soil sampling locations in Kubu Raya District, West Kalimantan Province, Indonesia

Measurements of peat pH were carried out in three replications. Isolation was carried out by serial dilution on potato-dextrose agar (PDA) and Martin agar medium, identified based on colony morphological characteristics and characteristics of conidiophores and conidia (Domsch and Gams 1972). The isolated fungi were tested for their antagonistic ability against *Ganoderma* by dual culture method. Disc-shaped pieces of antagonistic candidate isolates and 3-day old *Ganoderma* isolates of 5-mm diameter were placed opposite at a distance of 4 cm in a petri dish, incubated at room temperature, and were observed for 7 days. The percentage of inhibition of antagonistic fungi against *Ganoderma* was calculated by the formula (100 × (CT)/C), where C is the radius of the *Ganoderma* mycelium leading to the petri dish edge and T is the *Ganoderma* radius leading to the antagonist (Ahlem et al. 2012; Ting and Jioe 2016). Isolates with inhibition of ≥ 60% were maintained in a PDA medium at pH 5.5 for further testing. PDA pH 5.5 was obtained by adding HCl and measuring until it reached pH 5.5 before the medium was autoclaved.

The effect of pH degrees on antagonistic fungal biomass growth in potato-dextrose broth (PDB)

PDB (HiMedia) was dissolved in distilled water and adjusted to pH 2, 3, 4, 5, 6, and 7 by adding HCl and KOH, according to the required pH adjustment. A total of 20 mL of PDB was placed into a 100 mL bottle and autoclaved for 15 minutes at 121°C. After it cooled down, a 5-mm diameter of 3-day old antagonistic fungal isolate disc was inoculated and shaken at 150 rpm for 7 days. On the 7th day, culture was harvested by filtering, using Whatman No. 1 filter paper that had previously been stored in an oven at 80°C for 24 hours and weighed. The filtering results substrates were then dried in an oven at 80°C and weighed every 24 hours until their weight was relatively constant. The final weight of biomass was calculated by subtracting the weight of the dry substrates with the initial filter-paper weight (Senthamizhlselvan et al. 2010).

The effect of pH degrees on sporulation and mycelial biomass growth in PDA

The testing of the effect of pH on sporulation and growth of mycelium biomass was carried out following the method of Senthamizhlselvan et al. (2010) with modification. PDB was dissolved at 2× concentration in McIlvaine buffer (McIlvaine 1921) at pH 2, 3, 4, 5, 6, and 7, and then adjusted by adding HCl and KOH, according to the required pH adjustment (Steyaert et al. 2010). An equivalent volume of water agar was prepared at 2× concentration and then both were sterilized. Both solutions were then combined immediately before pouring into 10-cm Petri dishes. After it cooled down, antagonistic isolates with a 5-mm diameter and aged 3 days were inoculated in the middle of a petri dish and incubated for 7 days. The growth of the colony diameter was observed every day. On the 7th day, conidium and mycelium were harvested. The conidium was harvested by adding 10 mL of sterile distilled water to the culture and the conidia were slowly scraped off. The conidium was then filtered using sterile cheesecloth. The amount of conidium was calculated using a hemocytometer by observing the number of conidia in the smallest plot under a light microscope at 400× magnification. Mycelium biomass was harvested by melting the agar culture that was previously added to 10 mL of 0.1 N HCl in a water bath at 90°C. The mycelium was then filtered using Whatman No.1 filter paper, which was previously stored in an oven at 80°C for 24 hours and weighed. Mycelium was then dried in an oven at 80°C and weighed every 24 hours until their weight was relatively constant. The weight of mycelium was calculated by subtracting the weight of the dry mycelium with the initial filter-paper weight.

The effect of pH degrees on conidia germination

Conidium was harvested from 3-day old antagonistic isolate culture by adding 10 mL sterile distilled water and then the conidium was filtered using sterile cheesecloth, following which 0.1-mL conidium suspension was placed on a thin layer of water agar media and their pH was adjusted using McIlvaine buffer (Mc Ilvaine 1921) at pH 2, 3, 4, 5, 6, and 7, and incubated at room temperature. After 24 hours, the conidium was stained with lactophenol cotton blue, and the number of germinated conidia was observed under a light microscope at 400× magnification.

The effect of pH degrees on the antagonistic ability against *Ganoderma*

For each antagonistic fungi species obtained in antagonism screening tests, one representative sample of isolates was taken randomly to test its ability as a *Ganoderma* antagonist in low pH. The effect of the degree of pH on the ability of antagonism was observed by the dual culture method between antagonistic isolates and *Ganoderma* on a PDA medium that was prepared at pH 2, 3, 4, 5, 6, and 7, using a McIlvaine buffer (McIlvaine 1921). Disc-shaped pieces of *Ganoderma*-antagonistic isolates and 3-day old *Ganoderma* isolates 5 mm in diameter were placed opposite at a distance of 4 cm on a PDA medium in a petri dish, incubated at room temperature and observed for 7 days. The percentage of inhibition of antagonistic fungi against *Ganoderma* was calculated by the formula (100 × (CT)/C), where C is the radius of the *Ganoderma* mycelium leading to the Petri dish edge and T is the *Ganoderma* radius that leads to the antagonist (Ahlem et al. 2012).

RESULTS AND DISCUSSION

The antagonistic isolates origin environment

Out of the peat soils taken from 10 blocks of the observed samples, 27 fungal isolates were obtained that had the ability of *Ganoderma* antagonists, consisting of four fungi isolates from the *Gliocladium* genera and 23 from the *Trichoderma* genera. As reported by Supriyanto et al. (2020), these 10 samples of oil palm plantation blocks in the peatlands were the ones attacked by *Ganoderma* with varying intensities, ranging from 4.62% to 69.81%. Candidates for the *Ganoderma*-antagonist fungi were taken from the infected area because peatland characteristics

were varied across different locations. Thus, if the *Ganoderma*-antagonist fungi were taken from another area, there is a possibility that it would not be suitable for use in these locations. Three blocks, namely 12A, 13A, and 13E, were intensively maintained with twice-a-year fertilization, including NPK, Borate, and $CuSO_4$ fertilizers. Meanwhile, 7 other blocks were maintained intensively until the age of around 8 years; however, the maintenance was less intensive subsequently. The pH degrees of peatlands ranged from 2.2 to 3.4 (Table 1).

The effect of low pH degrees on the growth of antagonistic fungal mycelium

Based on the dry weight of mycelium (mycelium biomass), there are variations in the growth of antagonistic fungal isolates due to media pH treatment, both in PDB and PDA. In PDB medium wherein the pH was adjusted from 2 to 7, there were 77.8% antagonistic fungal isolates showing growth response patterns as convex curves. The response patterns of 4 isolates (12AJ4, 12AJ7, F1J24, and 13EJ35) were like sigmoid curves and 2 isolates (13AJ10 and E4J11) were similar to concave curves. 37% isolates reached a maximum of mycelium biomass at pH 3, 37% isolates at pH 4, and 26% isolates at pH 5 and pH 6 (Table 2). Meanwhile, in the PDA medium, there were 41% isolates whose maximum biomass reached pH 4, pH 3, and pH 2, and the other isolates reached pH 5 (25.9%) and pH 6 and pH 7 (33.3%) (Table 2 and 3). The results also showed that in the PDB medium, 74% isolates had maximum growth at pH 3 and pH 4, whereas on PDA medium, only 67% of isolates were able to grow at acidic pH (pH 4, 3, and 2). Except for B3J19, 13EJ15, E4J8, 12AJ7, and E4J13 isolates, all isolates produced maximum biomass at lower pH in the PDB medium than in PDA medium. This is probably because, in a liquid medium, the fungal mycelium is believed to be freely dispersed evenly throughout the medium and form macroscopic aggregates (Veiter et al. 2018). Therefore, absorption of nutrients and oxygen occurs faster (Zhou et al. 2018). Meanwhile, in a solid medium, fungal mycelium can only grow on the surface of the medium (Canovas et al. 2017; Gomes et al. 2018).

The results of the analysis of variance of the mycelium biomass with a confidence level of 95% also showed that there were significant differences among the isolates in response to changes in the pH degrees of PDB and PDA medium. Five isolates' biomass was significantly different from other isolates in the pH 2 PDB medium; these were B3J19, B3J9, 13EJ15, 13AJ6, and 12AJ7. At the same pH, 8 isolates, 13AJI0, C5J16, E4J8, E4J11, 13EJ15, B4J28, B3J19, and F1J6, in PDA had higher biomass than in PDB. Growth variation among the isolates was also observed in antagonistic fungal colony diameter on PDA medium, where a greater growth variation was found at pH 2, 6, and 7, as compared to pH 3, 4, and 5. Most isolates had an ideal range of mycelium growth at pH 3 to pH 5 (Figure 2).

Table 1. Peatland pH degrees of *Ganoderma*-antagonistic fungi origin that were tested in this research

Isolate code	Species	Inhibition against *Ganoderma* (%)	Blocks	Peat pH ($CaCl_2$)	
				At depth 0-15 cm	At depth 50-60 cm
12AJ4	*Trichoderma harzianum*	76.0	12A	2.4	-
12AJ7	*Trichoderma harzianum*	70.0	12A	2.4	-
13AJ10	*Trichoderma harzianum*	72.7	13A	-	2.3
13AJ4	*Gliocladium viride*	76.9	13A	2.9	-
13AJ6	*Trichoderma harzianum*	71.4	13A	2.9	-
13EJ4A	*Gliocladium viride*	75.0	13E	2.9	-
13EJ15	*Trichoderma harzianum*	730	13E	-	2.4
13EJ35	*Trichoderma harzianum*	80.0	13E	-	2.4
13EJ4	*Gliocladium* sp.	80.95	13E	2.9	-
13EJ8	*Trichoderma harzianum*	75.0	13E	2.9	-
B3J19	*Trichoderma viride*	70.0	B3 Pls III	3.1	-
B3J3	*Trichoderma harzianum*	80.0	B3 Pls III	3.1	-
B3J5	*Trichoderma harzianum*	80.0	B3 Pls III	3.1	-
B3J9	*Trichoderma harzianum*	70.0	B3 Pls III	3.1	-
B4J20	*Trichoderma viride*	70.0	B4 Pls I	3.0	-
B4J28	*Trichoderma harzianum*	83.0	B4 Pls I	3.0	-
B4J9	*Trichoderma viride*	80.0	B4 Pls I	-	2.6
C5J10	*Trichoderma harzianum*	75.0	C5 Pls III	3.4	-
C5J12	*Trichoderma harzianum*	77.0	C5 Pls III	3.4	-
C5J16	*Trichoderma harzianum*	100.0	C5 Pls III	3.4	-
C5J18	*Trichoderma harzianum*	68.0	C5 Pls III	3.4	-
E4J11	*Trichoderma harzianum*	76.0	E4 Pls III	-	2.8
E4J13	*Trichoderma harzianum*	76.0	E4 Pls III	3.3	-
E4J8	*Trichoderma harzianum*	85.0	E4 Pls III	3.3	-
F1J24	*Trichoderma harzianum*	70.0	F1 Pls I	2.6	-
F1J4	*Trichoderma harzianum*	75	F1 Pls I	-	2.3
F1J6	*Gliocladium viride*	65. 0	F1 Pls I	2.6	-

Table 2. Dry weight of mycelium variation of *Ganoderma*-antagonistic fungi isolates grown in PDB medium in the pH range 2-7 for 7 days

Isolate code	Species	Dry weigh of mycelium (mg)					
		pH 2	pH 3	pH 4	pH 5	pH 6	pH 7
12AJ4	*T. harzianum*	6.6ab	82.2a	97.8i	76.7a	72.2ghi	75.6def
12AJ7	*T. harzianum*	13.2bc	81.1a	62.2cdef	77.8a	93.3i	92.2f
13AJ10	*T. harzianum*	7.0ab	73.3a	70.0cdefgh	4.4a	5.6a	25.6ab
13AJ4	*G. viride*	4.2a	91.1a	85.6fghi	55.8a	92.2i	74.4def
13EJ4A	*T. harzianum*	8.2ab	71.7a	71.7cdefgh	47.5a	64.2efgh	65.8cdef
13AJ6	*G. viride*	15.4c	116.7a	76.7cdefghi	78.9a	72.2ghi	61.1cdef
13EJ15	*T. harzianum*	23.2d	82.2a	80.0efghi	67.8a	68.9fghi	74.4def
13EJ35	*T. harzianum*	3.9a	56.7a	36.7ab	42.2a	41.1bcde	56.7bcde
13EJ4	*Gliocladium sp.*	5.3a	64.2a	57.5bcde	93.3a	50.8cdefg	60.8cdef
13EJ8	*T. harzianum*	5.2a	64.2a	58.3bcde	54.2a	58.3efgh	49.2bcde
B3J19	*T. viride*	32.2e	32.2a	55.6bcd	58.9a	53.3cdefg	61.1cdef
B3J3	*T. harzianum*	3.7a	56.7a	73.3cdefgh	43.3a	31.1bcd	8.9a
B3J5	*T. harzianum*	1.2a	94.4a	54.4abc	38.9a	24.4ab	31.1abc
B3J9	*T. harzianum*	26.2de	81.1a	83.3fghi	83.3a	52.2cdefg	64.4cdef
B4J20	*T. viride*	3.3a	67.5a	87.5ghi	75.8a	65.0efgh	61.7cdef
B4J28	*T. harzianum*	1.8a	121.7a	75.0cdefghi	809.2b	74.2ghi	70.8def
B4J9	*T. viride*	2.0a	106.7a	54.4abc	17.8a	24.4ab	60.0cdef
C5J10	*T. harzianum*	4.0a	73.3a	65.0cdefg	78.3a	66.1efgh	78.3ef
C5J12	*T. harzianum*	4.5a	66.7a	67.8cdefgh	44.4a	62.2efgh	53.3bcde
C5J16	*T. harzianum*	8.2ab	112.0a	90.0hi	85.3a	82.7hi	78.0ef
C5J18	*T, harzianum*	2.5a	389.3b	551.3j	566.7b	564.0j	559.3g
E4J11	*T. harzianum*	2.2a	55.0a	53.3abc	70.0a	65.0efgh	56.7bcde
E4J13	*T. harzianum*	3.3a	31.7a	33.3a	30.8a	55.8defg	40.8bcd
E4J8	*T. harzianum*	5.6a	72.2a	78.9defghi	64.4a	57.8efgh	46.7bcde
F1J24	*T. harzianum*	4.9a	67.8a	80.0efghi	50.0a	57.8efgh	77.8ef
F1J4	*T. harzianum*	0.4a	64.4a	57.8bcde	102.2a	44.4bcdef	71.1def
F1J6	*G. viride*	3.3a	46.7a	67.8cdefgh	43.3a	28.9abc	43.3bcde

Note: Means followed by the same letters were not significantly different at 5% level of significant

Table 3. Dry weight of mycelium of *Ganoderma*-antagonistic fungi isolates from peatlands grown in PDA medium in the pH range 2-7 for 7 days

Isolate	Species	Dry weigh of mycelium (mg)					
		pH 2	pH 3	pH 4	pH 5	pH 6	pH 7
12AJ4	*T. harzianum*	27.8abc	36.7abc	61.1bcdef	51.1abcde	65.6cdefgh	77.8efghij
12AJ7	*T. harzianum*	46.7cdefgh	70.0defgh	56.7bcdef	53.3abcde	53.3bcdef	56.7cdefg
13AJ10	*T. harzianum*	57.5defghi	39.2abc	35.0ab	61.7bcdef	70.0efgh	94.2hij
13AJ4	*G. viride*	0,0a	22.2a	41.1ab	35.6ab	63.3bcdefgh	52.2bcdef
13EJ4A	*T. harzianum*	51.1cdefghi	85.6ghi	87.8fgh	105.6hi	35.6ab	61.1cdefgh
13AJ6	*G. viride*	47.8cdefgh	73.3efgh	93.3gh	66.7cdef	111.1j	43.3bcd
13EJ15	*T. harzianum*	68.9ghi	58.3bcdefgh	65.0bcdefg	41.7abc	38.3abc	53.3bcdef
13EJ35	*T. harzianum*	28.9bcd	28.1ab	36.7ab	48.3abcd	48.0abcde	51.3bcdef
13EJ4	*Gliocladium sp.*	46.7cdefgh	86.7ghi	80.8efgh	109.2hi	88.3ghij	107.5j
13EJ8	*T. harzianum*	0,0a	75.6fghi	65.6bcdefg	54.4abcdef	56.7bcdef	48.9bcde
B3J19	*T. viride*	78.9i	34.4abc	47.8abc	54.4abcdef	38.9abc	63.3cdefghi
B3J3	*T. harzianum*	30.0bcde	42.2abcd	48.9abcd	26.7a	43.3abcde	37.8abcd
B3J5	*T. harzianum*	48.3cdefgh	32.5abc	63.5bcdefg	80.8efgh	100.8ij	95.8ij
B3J9	*T. harzianum*	44.4cdefgh	44.4abcde	56.7bcdef	58.9bcdef	50.0bcdef	35.6abc
B4J20	*T. viride*	37.3cdef	51.3abcdef	46.0abc	122.0i	78.0fghi	40.7bcd
B4J28	*T. harzianum*	71.1hi	77.5fghi	46.7abc	60.8bcdef	55.8bcdef	42.5bcd
B4J9	*T. viride*	8.9ab	35.6abc	36.7ab	67.8cdef	61.1bcdefg	66.7cdefghi
C5J10	*T. harzianum*	40.0cdefg	35.8abc	37.5ab	30.8ab	40.8abcd	5.8a
C5J12	*T. harzianum*	26.7abc	87.8hi	80.0defgh	123.3i	162.2k	84.4fghij
C5J16	*T. harzianum*	58.9efghi	56.7bcdefg	96.7h	167.8j	106.7j	87.8ghij
C5J18	*T, harzianum*	50.8cdefghi	62.5cdefgh	50.8abcde	70.0cdefg	77.5fghi	20.0ab
E4J11	*T. harzianum*	64.4fghi	62.2cdefgh	20.0a	56.7abcdef	61.1bcdefg	71.1defghi
E4J13	*T. harzianum*	44.4cdefgh	103.3i	82.5efgh	97.5ghi	69.2defgh	80.8efghij
E4J8	*T. harzianum*	60.7fghi	48.7abcdef	40.0ab	48.7abcd	20.7a	34.0abc
F1J24	*T. harzianum*	25.6abc	43.3abcde	56.7bcdef	41.1abc	47.8abcde	54.4cdefg
F1J4	*T. harzianum*	47.5cdefgh	70.8defgh	75.8cdefgh	75.8defg	90.0hij	53.3bcdef
F1J6	*G. viride*	79.2i	52.5abcdef	41.7ab	85.0fgh	35.8ab	83.3efghij

Note: Means followed by the same letters were not significantly different at 5% level of significance

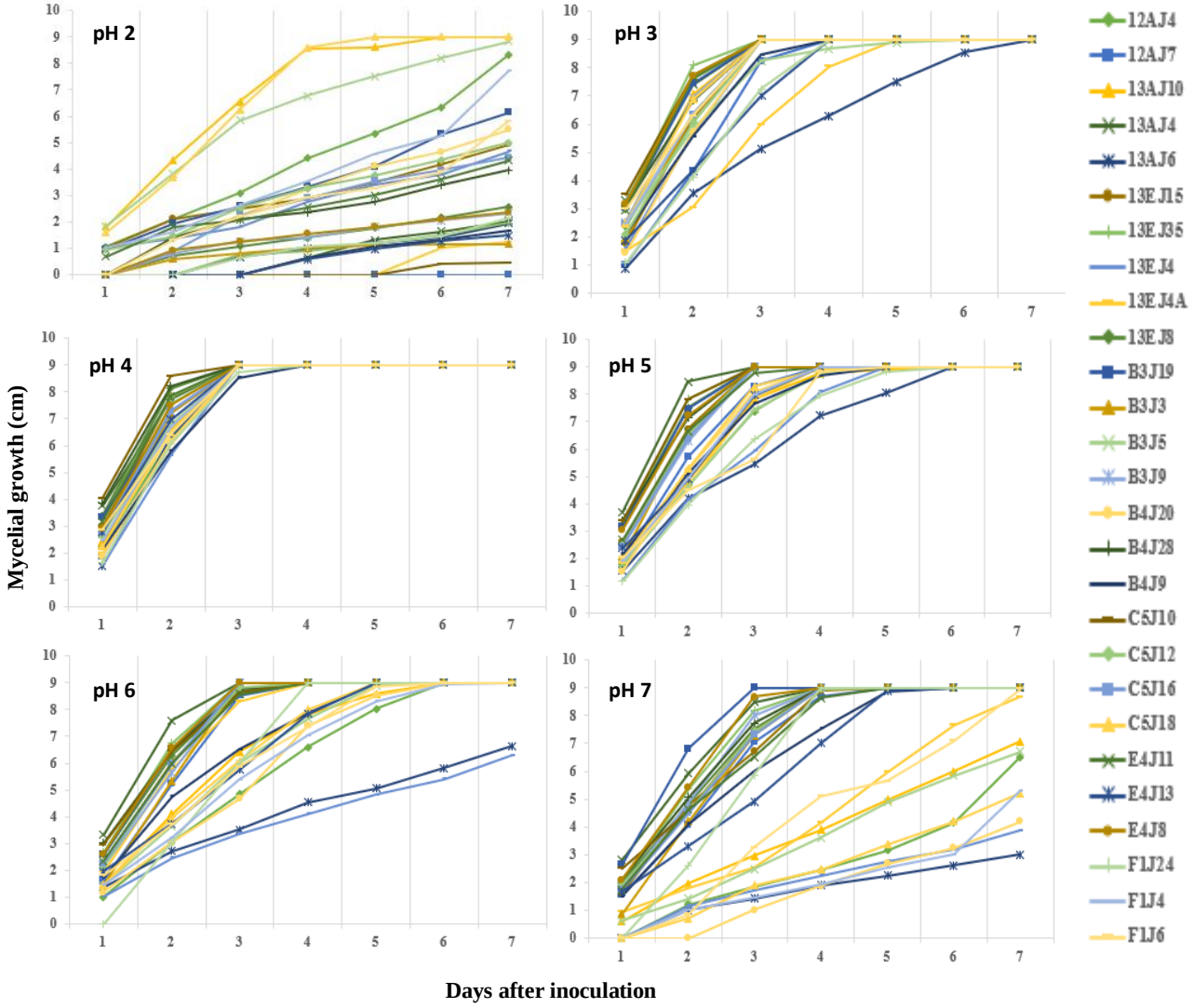

Figure 2. Patterns of the mycelial growth response of 27 isolates of *Ganoderma*-antagonistic fungi from peatlands grown in PDA medium with a pH of 2-7 for 7 days of observation

Variations in the growth of mycelium isolate from *Ganoderma*-antagonists from peatlands at low pH degrees occur at the species level. In the PDB medium, all *Gliocladium viride* isolates were weak at pH 2. However, except for the 13AJ4 isolate, all others could produce maximum biomass at pH 4, 3, and 2. In *Trichoderma harzianum*, only 3 isolates were able to produce significantly higher biomass at pH 2, namely 13EJ15, B3J9, and 13AJ6 isolates. Most of the isolates could also produce maximum biomass at pH 4, 3, and 2, except for 6 isolates: 12AJ7, B3J9, C5H10, E4J13, E4J11, and F1J4. Meanwhile, in *Trichoderma viride*, only one isolate, namely B3J19, could grow to pH 2. However, all *T. viride* isolates were able to produce maximum biomass at pH 4, 3, and 2.

In the PDA medium, all *G. viride* isolates were able to grow at pH degrees until pH 2, except for 13AJ4 isolate, which was unable to grow at pH 2. The maximum biomass of isolates was at pH 5, except isolate 13AJ4, which occurred at pH 6. In *T. harzianum*, almost all isolates were able to grow at pH 2, except for 13EJ8 isolate, which was unable to grow at pH 2. However, there were 10 isolates (50% of *T. harzianum*) whose optimum growth was achieved at pH 4, 3, and 2, namely 12AJ7, 13EJ8, B3J3, B4J28, C5J10, E4J8, E4J11, E4J13, F1J24, and 13EJ15; while for other isolates, the optimum biomass was reached at pH 5, 6 and 7. Moreover, in *T. viride*, except for B3J19 isolate, wherein maximum biomass was reached at pH 2, all isolates produced the highest mycelium biomass at pH 5. Variation in growth ability among the isolates in a species living in a specific acidic environment seems to be a common phenomenon, even to the genetic level, as has been reported by Hujslova et al. (2014) and Thanh et al. (2019).

The effect of low pH degrees on the sporulation and germination of conidia

As much as 84,5% of the isolates were optimally sporulated at pH 3 and 4 (63% occurred at pH 3; 21.5% at pH 4). Only 3.7% isolates' optimal sporulation occurred at pH 5, 6, and 7. Four isolates were not able to sporulate at

pH 2, namely 12AJ7, 13AJ4, C5J10, and B3J3. The increase in pH degrees of the medium caused an increase in sporulation until a certain pH and then the sporulation decreased with increasing pH degrees, except for isolates 12AJ7, 13AJ4, C5J10, and F1J4 (Table 4). The same pattern was found in the observation of optimum conidium germination of isolates where as many as 88.9% of the optimum conidium germination isolates occurred at pH 3 and 4 (25.95 % occurred at pH 3 and 62.9% at pH 4). 55.6% isolates were unable to germinate at pH 2 and there were only 2 whose germination reached about 50% at pH 2, namely13EJ8 and B3J5. For almost all isolates except F1J24, the percentage of the conidia germination increased with increasing pH until pH 3 and 4, then decreased with increasing pH degrees of the medium. At pH 6, the conidium germination for almost all isolates dropped dramatically to below 10%. At pH 7, nearly 75% of isolates were unable to germinate and germination of the 25% other isolates decreased to below 2% (Table 5).

There was variation in the ability to produce conidia in *G. viride*, *T. harzianum*, and *T. viride* from peatlands in West Kalimantan when grown in a medium with a low pH. In the *G. viride*, except for 13AJ4 isolate where the maximum conidia production occurred at pH 5, all isolates produced maximum conidia at pH 4 and pH 3. In *T. harzianum*, 3 isolates were unable to form conidium at pH 2, namely 12AJ4, B3J5, and F1J4, while other isolates formed conidium but in small amounts. The highest amount of conidia at pH 2 was obtained from 13EJ15 isolate. Most of the isolates produced the maximum number of conidia at pH 4 and pH 3, except for 13AJ6, C5J12, and C5J16, which produced the maximum number of conidia at pH 5 and pH 6. Further, 5 isolates could produce the maximum conidium number at pH 3, namely 12AJ4, 13AJ10, B3J9, C5J10, and F1J4. In *T. viride*, no isolate could sporulate at pH 2 and they produced maximum sporulation at pH 4. However, there was one isolate that was different from the others because the conidium production was small at all degrees of the given pH treatment, namely, isolate B4J9. Morphologically, this isolate also tends to show a brighter conidia color, which is yellowish-green. Variation was also found in the ability to germinate conidium of *G. viride*, *T. harzianum*, and *T. viride*. In *G. viride*, all isolates had optimum conidia germination at pH 3 to 5; however, the conidium could not germinate at pH 2 and pH 7. Variation only occurred at the optimum pH of conidia germination, where 13EJ4A and F1J6 isolates occurred at pH 3, while 13AJ4 and 13EJ4 isolates occurred at pH 4.

Variation was also found in the ability to germinate conidium of *G. viride*, *T. harzianum*, and *T. viride*. In the *G. viride*, all isolates had optimum conidium germination at pH 3 to pH 5. However, they were unable to germinate at pH 2 and pH 7. Notably, a variation only occurred at the optimum pH degrees of conidium germination where the 13EJ4A and F1J6 isolates occurred at pH 3, while the 13AJ4 and 13EJ4 isolates occurred at pH 4. The conidium of 10 *T. harzianum* isolates germinated optimally at pH 4, namely 13EJ15, B3J5, B4J28, C5J10, C5J12, C5J16, C5J18, E4J8, E4J11, and F1J4, while 8 isolates, namely 12AJ4, 12AJ7, 13AJ10, 13AJ6, 13EJ35, B3J3, B3J9, and F1J24, germinated optimally at pH 3. There were only two isolates, 13EJ8 and E4J13, whose optimal conidium germination occurred at pH 5. At pH 2 and pH 6, most isolates were unable to germinate, or even if they were able, the percentage dropped drastically. There were 5 isolates whose conidium were able to germinate at pH 2, namely 13EJ8, 13EJ15, B3J5, C5J18 and E4J8, whereas others were not able to germinate. There were only 8 isolates that grew at pH 6, namely 13EJ8, 13EJ15, B4J28, B3J5, B3J9, C5J12, C5J18, and E4J8, while the remaining isolates were unable to grow. All isolates germinated at pH 7. At *T. viride*, all isolates had optimum conidium germination at pH 3 to pH 5, i.e. B3J19 occurred at pH 3, B4J9 isolate at pH 4, and B4J20 isolate at pH 5. At pH 2 and pH 6, only B3J19 isolate germinated, while at pH 7, all isolates were unable to germinate.

These results are to some extent different from the results of previous studies in which *Trichoderma* was generally unable to produce spores optimally at pH < 4. Onilude and Seyi-Amole (2018) found that optimum sporulation of *T. harzianum* from Nigeria was achieved at pH 4, *Trichoderma* spp. from Punjab, India, optimum at pH 6 (Roy et al. 2015), and 4 *Trichoderma* species from Uttar Pradesh, India, optimum at pH 4.6 to 7.6 (Zehra et al. 2017). The results of this research are also different than the results of Srivastava et al. (2014) and Mishra and Khan (2015) where optimum sporulation *T. harzianum* and *T. viride* (from Kanpur and Bhopal, India) occurred at pH 5.5-7.5 and 5-9 respectively. These results indicate that the optimum pH range for conidium sporulation and germination of *Trichoderma* and *Gliocladium* is likely to be strongly influenced by the area of origin of the isolate. In this research, the optimum sporulation ability achieved at pH 3 and 4 by most isolates was thought to be the result of adaptation to the peatland environment below pH 4. However, Steyaert et al. (2010) concluded that sporulation at low pH is also influenced by lighting factors. Fungal biomass was not necessarily correlated with fungal sporulation (Gao 2016). Thus, in low pH level situations, it is necessary to learn more about other environmental factors that influence conidium sporulation and germination in those isolates.

The effect of low pH degrees on the ability as an antagonist against *Ganoderma*

Based on Pearson's correlation analysis, it is known that there is no strong correlation between the pH degrees of peatlands from isolates and the ability of antagonism of each isolate to *Ganoderma*. The value of r 0.36 (*p-value*: 0.12) at a depth of 0-15 cm and 0.21 (*p-value*: 0.37) at a depth of 50-60 cm. It is well known that the ability of *Trichoderma* spp. and other antagonistic fungi are affected by a certain environmental pH condition. Therefore, it is important to gather information about the effect of pH on its growth and antagonistic activity in vitro in the desired environmental pH situation. In this research, 3 isolate samples were taken randomly to represent *G. viride* (13AJ4 isolate), *T. harzianum* (13EJ15 isolate) and *T. viride* (B3J19 isolate) species and tested their antagonistic ability

Table 4. Variation in conidium production of *Ganoderma*-antagonist isolates from peatlands grown in PDA medium in the pH range of 2-7 for 7 days

Isolate	Species	Number of conidium (×10^8/mL)					
		pH 2	pH 3	pH 4	pH 5	pH 6	pH 7
12AJ4	*T. harzianum*	2.99ab	16.00a	11.30ab	12.00abcd	8.75cdef	0.41ab
12AJ7	*T. harzianum*	0.00a	6.61a	17.80ab	6.72abc	8.73cdef	10.40cde
13AJ10	*T. harzianum*	0.02a	12.60a	2.70a	0.05a	0.18a	0.13a
13AJ4	*G. viride*	0.00a	5.47a	3.35a	13.90abcde	7.59bcde	7.45abcd
13EJ4A	*T. harzianum*	0.60a	2.54a	9.79ab	0.64ab	0.21a	0.00a
13AJ6	*G. viride*	0.09a	6.89a	6.06ab	8.73abcd	21.11hij	6.90abcd
13EJ15	*T. harzianum*	21.11d	54.80a	155.00def	14.60bcde	13.30efg	14.90def
13EJ35	*T. harzianum*	0.42a	23.00a	68.20bc	16.60cdef	7.20abcde	20.40f
13EJ4	*Gliocladium sp.*	21.90d	25.80a	31.10ab	18.20cdef	8.13bcde	1.53abc
13EJ8	*T. harzianum*	0.01a	20.60a	181.00ef	26.30efg	14.70efgh	1.71abc
B3J19	*T. viride*	0.01a	74.10a	186.00ef	186.00h	15.70fghi	1.99abc
B3J3	*T. harzianum*	0.00a	64.50a	201.00fg	19.90cdef	17.30ghij	18.30ef
B3J5	*T. harzianum*	12.20bcd	26.70a	114.00cd	7.89abc	1.93abc	0.64ab
B3J9	*T. harzianum*	0.01a	139.00a	403.00i	22.10def	23.60jkl	20.60f
B4J20	*T. viride*	5.92abc	131.00a	208.00fg	5.94abc	8.02bcde	0.29ab
B4J28	*T. harzianum*	0.73a	46.50a	106.00cd	12.90abcde	10.30bcde	9.45bcd
B4J9	*T. viride*	0.00a	1.37a	2.46a	0.92ab	0.24a	0.01a
C5J10	*T. harzianum*	0.00a	35.50a	32.90ab	18.60cdef	29.3l	18.10ef
C5J12	*T. harzianum*	1.32a	14.90a	20.90ab	29.00fg	10.10defg	13.80def
C5J16	*T. harzianum*	1.82a	18.40a	23.90ab	35.30g	28.30kl	18.20ef
C5J18	*T. harzianum*	15.40cd	26.00a	31.20ab	18.60cdef	13.90efg	0.53ab
E4J11	*T. harzianum*	1.29a	71.40a	137.00de	12.70abcde	9.38def	1.52abc
E4J13	*T. harzianum*	0.00a	104.00a	114.00cd	6.71abc	4.06abcd	4.50abc
E4J8	*T. harzianum*	0.58a	77.80a	249.00g	17.30cdef	22.30ijk	22.00f
F1J24	*T. harzianum*	9.58a	10.30a	16.80ab	12.80abcde	0.89ab	0.43ab
F1J4	*T. harzianum*	12.20bcd	16.80a	7.42ab	10.40abcd	13.10efg	0.ab
F1J6	*G. viride*	1.25a	23.30a	16.70ab	0.64ab	0.25a	0.17a

Note: Means followed by the same letters were not significantly different at 5% level of significance.

Table 5. Variation in the percentage of conidial germination of *Ganoderma*-antagonist isolates from peatlands in the pH range 2-7 for 7 days.

Isolate	Species	Conidial germination (%)					
		pH 2	pH 3	pH 4	pH 5	pH 6	pH 7
12AJ4	*T. harzianum*	0.00a	76.57abc	99.28f	81.98bcd	0.14a	0.00a
12AJ7	*T. harzianum*	0.00a	97.32bcd	96.67ef	97.11d	0.88a	0.00a
13AJ10	*T. harzianum*	0.00a	96.64cde	97.32ef	97.22d	1.92a	0.00a
13AJ4	*G. viride*	0.00a	98.32de	90.73bcde	79.37bcd	2.01a	0.00a
13EJ4A	*T. harzianum*	0.00a	87.60bcde	100f	94.63d	1.08a	0.00a
13AJ6	*G. viride*	1.61a	95.29cde	95.99def	99.47d	3.63a	0.00a
13EJ15	*T. harzianum*	2.02a	86.00bcde	95.86def	64.64b	6.85a	0.00a
13EJ35	*T. harzianum*	0.000a	99.01e	96.91ef	98.90d	1.01a	1.35a
13EJ4	*Gliocladium sp.*	3.72a	95.15cde	97.20ef	97.97d	55.81c	0.00a
13EJ8	*T. harzianum*	48.04cd	61.15a	79.17a	81.98bcd	8.15a	0.00a
B3J19	*T. viride*	27.89bc	95.91cde	95.33def	30.78a	2.20a	1.23a
B3J3	*T. harzianum*	0.00a	98.39de	97.79ef	83.08bcd	36.63b	0.00a
B3J5	*T. harzianum*	54.52d	77.23abcd	94.84def	70.21bc	6.66a	0.00a
B3J9	*T. harzianum*	0.00a	96.88cde	87.10bc	90.63cd	3.20a	0.00a
B4J20	*T. viride*	0.00a	69.54ab	85.32b	84.94bcd	0.00a	0.00a
B4J28	*T. harzianum*	0.00a	100e	100f	100d	6.38a	0.00a
B4J9	*T. viride*	2.78a	93.36cde	99.42f	95.58d	1.56a	2.62b
C5J10	*T. harzianum*	0.00a	76.57abc	99.28f	81.97bcd	0.14a	0.00a
C5J12	*T. harzianum*	0.00a	94.02cde	99.81f	98.55d	5.08a	0.60a
C5J16	*T. harzianum*	0.29a	80.40abcde	92.54cdef	77.61bcd	0.98a	0.34a
C5J18	*T. harzianum*	13.99ab	70.33ab	94.89def	87.64bcd	3.92a	0.00a
E4J11	*T. harzianum*	0.00a	96.93cde	100f	64.81b	0.00a	0.00a
E4J13	*T. harzianum*	0.00a	92.93cde	95.00def	97.32d	1.49a	0.00a
E4J8	*T. harzianum*	29.38bc	89.60bcde	95.17def	87.03bcd	3.80a	0.00a
F1J24	*T. harzianum*	1.45a	95.46cde	89.21bcd	93.60cd	0.00a	0.00a
F1J4	*T. harzianum*	0.00a	94.66cde	100f	83.64bcd	1.61a	0.60b
F1J6	*G. viride*	0.41a	89.25bcde	100f	42.91a	1.23a	0.18a

Note: Means followed by the same letters were not significantly different at 5% level of significance

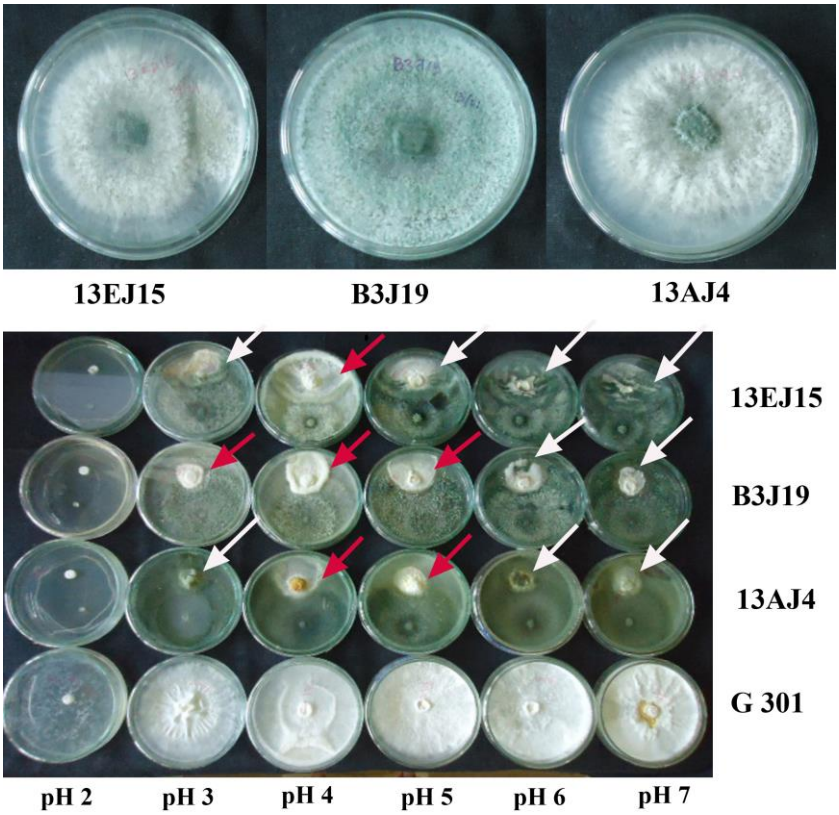

Figure 3. Three examples of antagonistic fungal isolates from peatlands used in the antagonism test (*above*) and the results of their antagonism test against *Ganoderma* G301 in the pH range 2-7 (*bottom*) on the 7th day after inoculation. The antagonistic fungal mycelium grows past the *Ganoderma* mycelium (*white arrow*) and the antagonist mycelium fungus is unable to pass through the *Ganoderma* mycelium (*red arrow*)

against *Ganoderma* on a medium regulated at pH 2, 3, 4, 5, 6, and 7, each isolate gave a different response. The highest percentage of inhibition against *Ganoderma* in all isolates occurred at pH 3 and 4. However, all isolates lost their ability at pH 2 because they were unable to grow. At pH 2, *Ganoderma* was also unable to grow as a result of no interaction. Based on visual observations of colony interactions, the influence of the pH level of the medium also causes differences in interactions between antagonistic fungi and *Ganoderma* (Figure 3).

The results of the antagonism observations were different from those of some previous studies. Usually, the ability of the antagonism of *Trichoderma* spp. was declined at acidic pH. Optimum inhibition of *Trichoderma* against *Fusarium* sp., the cause of rot seedling on red onion in Sri Lanka, occurred at pH 6 (Abeyratne and Deshappriya 2018). Also, inhibition of *T. viride* against *Sclerotium rolfsii* and *Rhizoctonia solani* occurred at pH 5.5 to pH 6 (Bagwan 2010). Media pH degrees affect the ability of fungal antagonism possibly through its influence in regulating enzyme secretion and its stability. Regulating the secretion of these enzymes plays an important role when fungal acting as a competitor for nutrition and living space as well as a mycoparasites. The β-glucosidase enzyme was activated below pH 5, while xylosidase was activated at pH 6 (Naher et al. 2014). Asran-Amal et al. (2010) also identify pH 6-7 as the optimal pH range for the activity of chitinase enzymes released by *Trichoderma* sp. Therefore, further research is needed to determine the effect of low pH degrees on the mechanism of the antagonism more clearly.

Observation of mycelium biomass and colony diameter showed that most of the *Ganoderma*-antagonist fungal isolates from West Kalimantan peatlands were able to grow at acidic pH (pH 4, 3, and 2). This ability was alleged to be the result of a process of natural selection in the peatlands environment, which occurs continuously such that genetic variation is enabled. Some previous research results indicate that based on the dry weight of the biomass, *Trichoderma* species have a variation in optimum pH range, depending on the native habitat of the isolates. These variations of optimum pH were from a pH range of 4.6 to 6.8 (Jackson et al. 1991), between 5.5 to 7.5 (Singh et al. 2014), or 3.5 to 5 (Onilude and Seyi-Amole 2018). Taylor et al. (2017) stated that fungal genomes are remarkably fluid and variable. Variation in wild populations is a rich resource for associating genetic variation with phenotypic variation. The sources of genetic variation, including single nucleotide mutations and recombination, gene gain and loss, gene family expansion and contraction, horizontal gene transfer, loss of heterozygosity, genome rearrangements, epigenetic

modifications, and other sources. Genetic variation in the fungal population could potentially lead to differences in physiology function and response to the environments (Lind et al. 2017; Longnya et al. 2020). In general, genetic variation in the fungal population was the result of long-term adaptations to their ecological environments and environmental heterogeneity has played an important role in these processes (Xu et al. 2014). The variations in the peatland environments, especially those related to pH and other chemical environmental characteristics were believed to trigger these genetic variations. However, soil age and the C accumulation rate were found to play important roles in shaping the fungal community structure (Zhang et al. 2017). Also, in West Kalimantan peatlands, changes in water content, bulk density and porosity of the soil were positively correlated with the fungal communities in peatlands (Nusantara and Aspan 2017). Therefore, physical characteristics of peat such as bulk density, soil temperature, water table depth, water content, and soil porosity were also believed to play an important role in the occurrence of fungal genetic variation.

Based on these results, most of the isolates tested in this research were believed to be acid-tolerant fungi, as proposed by Gross and Robbins (2000) and Hujslova et al. (2014). These fungi, in addition to being able to grow in acidic environments, were also still able to grow in an environment that tends to be neutral. This shows that most of the antagonistic fungi isolates from the peatland were indigenous peat fungi that were in accordance with the conditions of a low pH level so that their activities were not disturbed by low pH degrees.

The results of this research confirm previous studies about the presence of acid-tolerant fungi (Gross and Robbins 2000; Hujslova et al. 2014; Mardanov et al. 2016; Thanh et al. 2019). For example, the study of Mardanov et al. (2016), in which strains of *Penicillium* were taken from wastes of the ore mining deposit in Siberia that were known to be able to grow in a pH 1.9, and 4 acid-tolerant *Trichoderma* species reported by Gross and Robbins (2000), which have the optimum growth pH range varies from 3.7 to 4.7, 2 to 7, 3.4 to 4.7 and 3.1 to 5. The results of this research also indicate that most of the fungal isolates from peatlands could act as biological control agents against *Ganoderma* on peatlands because they could grow at pH 3 to 4, which is the pH of the peatlands environment in this research site. Most of the isolates were able to grow and produce abundant conidium and their conidia were able to germinate normally at the same pH degrees and were able to act as an antagonist for the pathogenic fungi *Ganoderma* that is devastating to oil palm plantations in peatlands.

ACKNOWLEDGEMENTS

The author would like to thank the Directorate General of Research and Development, Ministry of Research and Higher Education, Indonesia for providing funding for this research according to contract No. 107/ADD/SP2H/LT/DRPM/VIII/2017.

REFERENCES

Abeyratne GDD, Deshappriya N. 2018. The effect of pH on the biological control activities of a *Trichoderma* sp. against *Fusarium* sp. isolated from the commercial onion fields in Sri Lanka. Trop Plant Res 5 (2): 121-128.

Ahlem H, Mohammed E, Badoc A, Ahmed L. 2012. Effect of pH, temperature and water activity on the inhibition of *Botrytis cinerea* by *Bacillus amyloliquefaciens* isolates. Afr J Biotechnol 11 (9): 2210-2217.

Alexander A, Abdullah S, Rossall S, Chong KP. 2017. Evaluation of the efficacy and mode of action of biological control for suppression of *Ganoderma* boninense in oil palm. Pak J Bot 49 (3): 1193-1199.

Andriesse JP. 1988. Nature and Management of Tropical Peat Soils. Food and Agriculture Organization of the United Nations, Roma. http://www.fao.org/3/x5872e/x5872e06.htm#4.3.3%20Acidity.

Asran-Amala, A, S.M. Moustafa-Mahmoud, K.K. Sabet, O.H. El Banna. 2010. In vitro antagonism of cotton seedlings fungi and characterization of chitinase isozyme activities in *Trichoderma harzianum*. Saudi J Biol Sci 17: 153-157.

Assis K, Chong KP, Idris AS, Ho CM. 2016. Economic loss due to *Ganoderma* disease in oil palm. In: Proceeding of Indonesia Conference on Mathematics, Statistics and Scientific Computing, Kuala Lumpur, 11-12 February 2016.

Azahar TM, Jawahir CM, Mazliham S, Boursier P. 2011. Temporal analysis of basal stem rot disease in oil palm plantations: an analysis on peat soil. IJET-IJENS 11 (03): 96-101.

Bagwan NG, 2010. Influence of temperature and pH on antagonistic potential of *Trichoderma viride* in vitro. Intl J Plant Protec 3 (2): 165-169.

Bakker MG, Manter DK, Sheflin AM, Weir TL, Vivanc JM. 2012. Harnessing the rhizosphere microbiome through plant breeding and agricultural management. Plant Soil 360: 1-13.

Cánovas D, Studt L, Marcos AT, Strauss J. 2017. High-throughput format for the phenotyping of fungi on solid substrates. Sci Rep 7: 4289. DOI:10.1038/s41598-017-03598-9

Chong KP, Dayou J, Alexander A. 2017. Detection and Control of *Ganoderma* boninense in Oil Palm Crop. Springer, Switzerland.

Cooper RMA, Flood JB, Rees RW. 2011. *Ganoderma boninense* in oil palm plantations: current thinking on epidemiology, resistance and pathology. The Planter 87 (1024): 515-526.

Darmono TW. 2000. *Ganoderma* in oil palm in Indonesia: Current status and prospective use of antibodies for the detection of infection. In: Flood J, Bridge PD, Holderness M (eds.). *Ganoderma* diseases of perennial crops. CABI Publishing, New York.

Direktorat Jenderal Perkebunan, 2017. Statistik Perkebunan Indonesia 2016-2018, Direktorat Jenderal Perkebunan, Jakarta. [Indonesian]

Domsch KH, Gams W. 1972. Compendium of Soil Fungi, Volume I. Academic Press, London.

Fee CG. 2011. Management of *Ganoderma* diseases in oil palm plantations. The Planter 87 (1022): 325-339.

Gao L. 2016. An improved method to optimize the culture conditions for biomass and sporulation of mycoparasitic fungus *Trichoderma viride* TV-1. J Yeast Fungal Res 7 (1): 1-6. DOI: 10.5897/JYFR2015.0162

Gomes E, Da Silva R, Pereira JC, Ladino-Orjuela G. 2018. Fungal growth on solid substrates: a physiological overview. In: Pandey A, Larroche C, Soccol CR (eds.). Current Developments in Biotechnology and Bioengineering, Elsevier, Amsterdam.

Gross IS, Robbins IE, 2001. Acidophilic and acid-tolerant fungi and yeasts. Hydrobiologia 433: 91-109.

Hujslova M, Kubatova A, Kostovcik M, Blanchette RA, de Beer ZW, Chudíckova M, Kolarik M. 2014. Three new genera of fungi from extremely acidic soils. Mycol Progress 13: 819-831.

Jackson AM, Whipps JM, Lynch JM. 1991. Effects of temperature, pH and water potential on growth of four fungi with disease biocontrol potential. World J Microb Biot 7: 494-501.

Lind AL, Wisecaver JH, Lameiras C, Wiemann P, Palmer JM, Keller NP, Rodrigues F, Goldman GH, Rokas A. 2017. Drivers of genetic diversity in secondary metabolic gene clusters within a fungal species. Plos Biol 15 (11): e2003583. DOI: 10.1371/journal. pbio.2003583.

Longya A, Talumphai S, Jantasuriyarat C. 2020. Morphological characterization and genetic diversity of rice blast fungus, *Pyricularia oryzae*, from Thailand using ISSR and SRAP markers. J Fungi 6: 38. DOI:10.3390/jof6010038

Mardanov AV, Glukhova LB, Gruzdev EV, Beletsky AV, Karnachuk OV, Ravin NV. 2016. The complete mitochondrial genome of the acid-tolerant fungus *Penicillium* ShG4C. Genom Data 10: 141-14.

McIlvaine TC. 1921. A buffer solution for colorimetric comparison. J Biol Chem 49: 183-186.

Miettinen J, Shi C, Liewolerant SC. 2016. Land cover distribution in the peatlands of Peninsular Malaysia, Sumatra and Borneo in 2015 with changes since 1990. Glob Ecol Conserv 6: 67-78.

Mishra PK, Khan FN. 2015. Effect of different growth media and physical factors on biomass production of *Trichoderma viride*. People's J Sci Res 8 (2): 11-16.

Muniroh MS, Nusaibah SA, Vadamalai G, Siddique Y. 2019. Proficiency of biocontrol agents as plant growth promoters and hydrolytic enzyme producers in *Ganoderma* boninense infected oil palm seedlings. Curr Plant Biol 20: 100116. DOI: 10.1016/j.cpb.2019.100116.

Musa H, Nusaibah SA, Khairulmazmi A. 2018. Assessment on *Trichoderma* spp. mixture as a potential biocontrol agent of *Ganoderma* boninense infected oil palm seedlings. J Oil Palm Res 30 (3): 403-415.

Naher L, Siddiquee S, Yusuf UK, Mondal MMA. 2015. Issues of *Ganoderma* spp. and basal stem rot disease management in oil palm. Am J Agric Sci 2 (3): 103-107.

Naher L, Yusuf UK, Ismail A, Hossain K. 2014. *Trichoderma* spp.: A biocontrol agent for sustainable management of plant diseases. Pak J Bot 46 (4): 1489-1493.

Notohadiprawiro RMT, 1985. Selidik cepat ciri tanah di lapangan. Ghalia Indonesia, Jakarta. [Indonesian]

Nusantara RW, Aspan A. 2017. Differentiation of soil organisms at different types of peatland in West Kalimantan, Indonesia. Bonorowo Wetlands 7 (1): 26-30.

Onilude AA, Seyi-Amole DO. 2018. Mycelia growth and spore yield of *Trichoderma harzianum*in batch and fed-batch cultures: Influence of pH and temperature. Intl J Curr Microbiol App Sci 7 (4): 627-635. DOI: 10.20546/ijcmas.2018.704.070

Pilotti CA, 2005. Stem rots of oil palm caused by *Ganoderma* boninense: Pathogen biology and epidemiology. Mycopathologia 159: 129-137.

Priwiratama H, Prasetyo AE, Susanto A. 2014. Pengendalian Penyakit Busuk Pangkal Batang Kelapa Sawit secara Kultur Teknis. J Fitopatol Indones 10 (1): 1-7. DOI: 10.14692/jfi.10.1.1 [Indonesian]

Rakib RM, Bong CJ, Khairulmazmi A, Idris AS, Jalloh MB, Ahmed OH. 2017. Association of copper and zinc levels in oil palm (Elaeis guineensis) to the spatial distribution of *Ganoderma* species in the plantations on peat. J Phytopathol 165: 276-282.

Rakib RM, Bong CJ, Khairulmazmi A, Idris AS, Jalloh MB, Idris AS. 2014. Occurrence and spatial distribution of *Ganoderma* species causing upper and basal stem rot in oil palm. J Food Agric Environ 12 (2): 360-364.

Roy MK, Hembram S, Debnath A. 2015. Effect of different media and Ph on growth and sporulation of different native *Trichoderma* spp. The Bioscan 10 (4): 1833-1837.

Senthamizhlselvan P, Alice J, Sujeetha RP, Jeyalakshmi C. 2010. Growth, sporulation and biomass production of native entomopathogenic fungal isolates on a suitable medium. J Biopesticides 3 (2): 466-469.

Singh A, Shahid M, Srivastava M, Pandey S, Sharma A, Kumar V. 2014. Optimal physical parameters for growth of *Trichoderma* species at varying pH, temperature and agitation. Virol Mycol 3: 127.

Srivastava M, Singh V, Shahid M, Singh A, Kumar V. 2014. Determination of biochemical and physiological aspects of a biocontrol agent *Trichoderma harzianum* Th azad. Intl J Adv Res 2 (3): 841-849.

Steyaert JM, Weld RJ, Stewart A. 2010. Ambient pH intrinsically influences *Trichoderma* conidiation and colony morphology. Fungal Biol-UK 114: 198-208.

Supriyanto, Purwanto, Poromarto SH, Supyani. 2020. The relationship of some characteristics of peat with oil palm basal stem rot (BSR) caused by *Ganoderma* in peatlands. In: Proceeding The 4th International Conference on Climate Change: "Climate Change: The Risk to Sustainable and Environmental Issue". Yogyakarta, Indonesia, 18-19 November 2019.

Susanto A, Rahmadi HY, Priwiratama H, Yenni Y, Suprianto E, Purba AR. 2011. Seleksi ketahanan berbagai persilangan kelapa sawit terhadap *Ganoderma boninense*. Jurnal Penelitian Kelapa Sawit 19 (1): 43-53. [Indonesian]

Taylor JW, Branco S, Gao C, Hann-Soden C, Montoya L, Sylvain I, Gladieux P. 2017. Sources of fungal genetic variation and associating it with phenotypic diversity. Microbiol Spectrum 5 (5): FUNK-0057-2016. DOI: 10.1128/microbiolspec.FUNK-0057-2016

Thanh VN, Thuy NT, Huong HTT, Hien DD, Hang DTM, Anh DTK, Hüttner S, Larsbrink J, Olsson L. 2019. Surveying of acid-tolerant thermophilic lignocellulolytic fungi in Vietnam reveals surprisingly high genetic diversity. Sci Rep 9: 3674. DOI: : 10.1038/s41598-019-40213-5

Ting ASY, Jioe E. 2016. In vitro assessment of antifungal activities of antagonistic fungi towards pathogenic *Ganoderma* boninense under metal stress. Biol Control 96: 57-63.

Veiter L, Rajamanickam V, Herwig C. 2018. The filamentous fungal pellet—relationship between morphology and productivity. Appl Microbiol Biotechnol 102: 2997-3006.

Wong LC, Bong CFJ, Idris AS. 2012. *Ganoderma* species associated with basal stem rot disease of oil palm. Am J Applied Sci 9 (6): 879-885.

Xu L, Lu Y, You Q, Liu XL, Grisham MP, Pan YB, Que YX. 2014. Biogeographical variation and population genetic structure of Sporisorium scitamineum in Mainland China: Insights from ISSR and SP-SRAP markers. Sci World J 2014: 296020. DOI: 10.1155/2014/296020

Zehra A, Dubey MK, Meena M, Upadhyay RS. 2017. Effect of different environmental conditions on growth and sporulation of some *Trichoderma* species. J Environ Biol 38: 197-203.

Zhang Z, Zhou X, Tian L, Ma L, Luo S, Zhang J, Li XJ, Tian CJ. 2017. Fungal communities in ancient peatlands developed from different periods in the Sanjiang Plain, China. PLoS One 12 (12): e0187575. DOI: 10.1371/journal.pone.0187575

Zhou Y, Han LR, He HW, Sang B, Yu DL, Feng JT, Zhang X. 2018. Effects of agitation, aeration and temperature on production of a novel glycoprotein GP-1 by *Streptomyces kanasenisi* ZX01 and Scale-up based on volumetric oxygen transfer coefficient. Molecules 23: 125. DOI:10.3390/molecules23010125.