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## The diversity of fungi associated with rice (*Oryza sativa* L.) from Nakhon Si Thammarat, Thailand

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Seephueak, P.<sup>1\*</sup>, Preecha, C.<sup>2</sup> and Seephueak, W.<sup>3</sup>

<sup>1</sup>Division of Plant Science, Faculty of Agriculture, Rajamangala University of Technology Srivijaya, Thung-Yai, Nakhon Si Thammarat. 80240, Thailand; <sup>2</sup>Division of Plant Science, Faculty of Agriculture, Rajamangala University of Technology Srivijaya, Thung-Song, Nakhon Si Thammarat. 80110, Thailand; <sup>3</sup>Division of Animal Science, Faculty of Agriculture, Rajamangala University of Technology Srivijaya, Thung-Yai, Nakhon Si Thammarat. 80240, Thailand.

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**Abstract** The diversity of fungi associated with rice (*Oryza sativa* L.) RD41 variety from Pak Phanang, Nakhon Si Thammarat province was investigated. Fungal samples were isolated from soil, stem, and leaves at each growth stage of rice plants and grains. A total of 39 species were identified, including 24 genera. Thirty-one species were found on soil and stem, 22 species were found on leaves and 14 species were found on grains. Among these, 4 species, *Bipolaris oryzae*, *Curvularia lunata*, *Fusarium solani* and *Nigrospora oryzae* were the dominant species found in all substrates. The diversity of fungi that were found at each growth stage of rice showed that in the maximum tiller stages had the highest fungi diversity which was following flowering, panicle formation, maturity and the transplanting stage which were 36, 35, 33, 32 and 30 species, respectively. Only one species, *C. lunata* was a dominant among fungi found of all growth stages of rice.

**Keywords:** Biodiversity, Dominant species, Occurrence, Substrates

### Introduction

Rice (*Oryza sativa* L.) is one of the most important primary foods in the world, especially in Asia. Rice is consumed by more than three billion people worldwide each day (Bawa *et al.*, 2018). In 2018, Thailand was the second largest exporter of rice after India, exporting 11 million tons (Thai Rice Exporters Association, 2018). Several important factors that have an effect on rice production namely biotic factors such as fungi, bacteria, viruses, viroid, phytoplasma, nematodes, insects, weeds and abiotic factor such as rainfall, temperature and humidity (John *et al.*, 2007; Yamaguchi *et al.*, 2008).

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\* **Corresponding Author:** Seephueak, P.; **Email:** [spornsil@gmail.com](mailto:spornsil@gmail.com)

Moreover, Bawa *et al.* (2018) reported that when there are favourable environmental conditions for rice cropping, farmers can cultivate two or even three rice crops a year. Growing the same crop repeatedly causes a reduction in yield and enlargement of microorganism populations (John *et al.*, 2007). In addition, intensive chemical inputs such as fertilizers and pesticides may influence microbial diversity and cause an environment imbalance (Naik *et al.*, 2009).

Soil, plant roots and green parts of plants are a source for diversity of microorganisms which play a major role in enhancement of or harm to the plant, especially in the case of rice. Previously, Naik *et al.* (2009); Lapmak *et al.* (2009); Yuan *et al.* (2010); Leewijit *et al.* (2016) studied diversity of fungi in rice and reported that there were many types of fungi on rice that were found, particularly endophytic fungi. Endophytic fungi are mutualist and the plant does not show one disease symptom. It has the benefit for plants that it promotes the growth of plants and controls plant diseases. Endophytic fungi were found in rice genera *Aspergillus*, *Chaetomium*, *Fusarium*, *Penicillium* and *Trichoderma* (Niharika *et al.*, 2012; Leewijit *et al.*, 2016). Moreover, fungi that have caused important rice diseases were found in rice blast disease (*Magnaporthe oryzae*; Anamorph: *Pyricularia oryzae*), brown spot of rice disease (*Bipolaris oryzae*, *Curvularia lunata*) and narrow brown spot disease (*Cercospora oryzae*). The negative side effects of intensive rice cultivation may be reduced by the use of alternative agricultural practices. For this strategy to be successful, the role of microorganisms in these processes must be taken into account.

In this study, fungi from soil and parts of rice were studied in order to understand the role of communities because fungi have the potential for use in intergrated pest management or biological control for sustainable crop production. Additionally, the fungal samples would be screened for production of growth promoting factors which can be used as applications for the enhancement of yields of rice and control of plant diseases.

## **Materials and methods**

### ***Study sites and sampling design***

The study was conducted at Chamao, Pak Phanang District (latitude 8.14-9.00°N and longitude 100.02-100.04°E), Nakhon Si Thammarat province. Pak Phanang district is located on the Gulf of Thailand in southern Thailand. Soil and parts of rice plants include stems, leaves and grains were isolated from the rice field cultivar RD41. The samples were based growth stage of rice each

sample was divided in to 5 stages; 1) transplanting 2) maximum tiller number 3) panicle formation 4) flowering and 5) maturity. Whole, grains were collected only one time when harvested.

### ***Incubation, observation and isolation of fungi***

Dilution-plate, moist chamber and baiting technique were used to study the fungi. In dilution-plate technique, 10 g of all samples were chopped with a sterilized knife and blended for 3 min in 90 ml of sterile water. From this initial suspension, 1 ml of  $1 \times 10^5$  serial dilutions was pipetted into glucose ammonium nitrate agar (GANA) with 400 ppm streptomycin sulfate, which was cooled at 45 °C and poured into Petri dishes. The dishes were incubated at room temperature for 24-48 hours and then examined for fungal growth. For moist chamber technique; stem, leaves (cut into 4 cm long sections) and grains were treated and incubated in moistened Petri dish at room temperature (28-32 °C). The fungi present on the samples were examined after 24 hours of being incubated and examined daily for up to 7 days. The fungal colonies were lifted from the stem and leaf surface by cellophane tape and mounted on slides using lactophenol. For the baiting technique, soil samples were mixed in the plastic bags, approximately 30 g were placed into empty sterile Petri dishes and baited with sterilized sorghum. Baited samples were incubated at room temperature (28-32 °C) in a dark room. The plates were inspected daily for one week. The presence of fungi was detected by stereo microscopic examination. Mycelia of colonized sorghums were transferred to PDA plate. Identification was based on morphology following examination using stereo and compound microscopes.

### ***Definition and statistical analyses***

Fungal species were recorded as either present or absent from substrates at each growth stage of the rice. The number of substrate samples on which fungal species were found was designated at the occurrence of a fungus and was used to calculate the percentage occurrence of a species on substrate of rice at each growth stage using the following formula (Seephueak *et al.*, 2010): percentage occurrence of taxon A = (occurrence of taxon A / occurrence of all taxa in at each growth stage) x 100%. Fungal species diversity at each stage of rice growth and each substrate of rice was calculated using the Shannon-Wiener index (H) and Simpson's index (D) (Wang *et al.*, 2008; Seephueak, 2012).

## Results

### *Fungal diversity from the rice field*

Examination of diversity of fungi from rice field RD 41 variety from soil stem, leaves at each of the growth stages of rice and grains is shown in table 1. A total of 39 fungal taxa were found comprising 36 mitosporic species and one species each of ascomycetes, basidiomycetes and zygomycetes. Fungal diversity from rice showed variations in fungal types between substrates and growth stages of rice. A total of 39 fungal species were found in the rice filled habitat. Thirty-one species were found on soil and stem, 22 species were found on leaves and 14 species were found on grains. Eleven species, *Aspergillus padwickii*, *Bipolaris oryzae*, *Cercospora oryzae*, *Chaetomium globosum*, *Cladosporium elatum*, *C. oxysporum*, *C. tenuissimum*, *Curvularia lunata*, *Fusarium solani*, *Nigrospora oryzae* and *N. sphaerica* were found with the highest frequencies in all substrates. Whereas, *C. lunata*, *F. solani* and *N. oryzae* were the dominant species ( $\geq 10\%$  occurrence) found all substrates (Table 1).

For the fungal diversity at each growth stage of rice there were found 36 fungal species in maximum tiller number stage followed by flowering, panicle formation, maturity and transplanting stages were found 35, 33, 32, and 30 species, respectively (Table 2). Fungal species that were found most frequently at all substrates and all growth stages were *B. oryzae*, *C. lunata* and *N. oryzae* and only one species, *C. lunata* was a dominant species ( $\geq 10\%$  occurrence) and it was found in all growth stages of the rice (Table 3).

### *Diversity of fungi from soil in the rice field*

Thirty-one species of fungi were found from soil in the rice field. Thirteen species were the dominant species comprising *Aspergillus fumigatus*, *B. oryzae*, *C. cladosporioides*, *C. lunata*, *F. solani*, *Gongronella butleri*, *N. oryzae*, *Penicillium* sp., *P. digitatum*, *P. fusiculosum*, *P. janthinellum*, *R. solani* and *T. harzianum* (Table 1).

The diversity of fungi that were found in soil at each growth stage of rice shown in Table 4. Twenty-seven species were found on flowering stage, 24 species found on the panicle formation stage, 22 species found on the maximum tiller number and the maturity stage and 16 species found on the transplanting stage. Eleven species, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *B. oryzae*, *C. lunata*, *F. solani*, *Gongronella butleri*, *N. oryzae*, *P. fusiculosum*, *P. janthinellum* and *Trichoderma harzianum* were found most frequently at all

growth stage of rice. Three species, *C. lunata*, *P. fumiculosum* and *T. harzianum* were the dominant species ( $\geq 10\%$  occurrence) from soil at found all growth stages of the rice.

#### ***Diversity of fungi on rice stem***

A total of 31 fungal species were found on the stem of rice. Fourteen species, *A. hansfordii*, *A. flavus*, *A. fumigatus*, *A. niger*, *B. oryzae*, *C. cladosporioides*, *C. elatum*, *C. lunata*, *F. solani*, *N. oryzae*, *N. sphaerica*, *P. fumiculosum*, *P. oryzae* and *V. apiculata* were dominant species ( $\geq 10\%$  occurrence) (Table 1).

For the fungi were found on stem at each growth stage of rice showed 21 species were found on maximum tiller number stage, 17 species found on panicle formation stage, 16 species were on flowering stage, 15 species found on transplanting and 13 species found on the maturity stage. Six species, *A. hansfordii*, *A. flavus*, *A. niger*, *C. lunata*, *F. solani* and *N. oryzae* were found the most frequency on stem at all growth stages of rice. Only one species, *C. lunata* was the dominant species ( $\geq 10\%$  occurrence) found from stem at all growth stages of rice (Table 5).

#### ***Diversity of fungi on rice leaves***

A total of 22 fungal species were found on rice leaves. Eight species, *A. hansfordii*, *B. oryzae*, *C. cladosporioides*, *C. elatum*, *C. lunata*, *F. solani*, *N. oryzae* and *P. oryzae* were the dominant species ( $\geq 10\%$  occurrence) (Table 1). For fungal growths on leaves at each growth stage of rice, there were found 17 species in the maximum tiller number and the panicle formation stage, 16 species were found on the transplanting and the maturity stage and 15 species were found on the flowering stage. Ten species had the highest frequency on rice leaves at all growth stages of rice. They were *A. hansfordii*, *A. padwickii*, *B. oryzae*, *C. oryzae*, *C. globosum*, *C. elatum*, *C. lunata*, *N. oryzae*, *P. oryzae* and *R. oryzae*. Five species, *B. oryzae*, *C. elatum*, *C. lunata*, *N. oryzae* and *P. oryzae* were the dominant species found on leaves at all growth stages of rice (Table 6).

#### ***Diversity of fungi on rice grains***

A total of 14 fungal species were found on rice grains. All species were dominant fungi ( $\geq 10\%$  occurrence) consist of *A. padwickii*, *B. oryzae*, *C. oryzae*, *C. globosum*, *C. elatum*, *C. oxysporum*, *C. tenuissimum*, *C. lunata*, *D. australiensis*, *F. solani*, *F. semitectum*, *N. oryzae*, *N. sphaerica* and *P. oryzae* (Table 1).

**Table 1.** Occurrence (%) of fungi from soil, stem, leaves and grains in the rice field

Fungal taxa	Part of the rice			
	Soil	Stem	Leaves	Grains
<b>Ascomycetes</b>				
<i>Chaetomium globosum</i>	2.42	1.18	7.36	14.29
<b>Basidiomycetes</b>				
<i>Rhizoctonia solani</i>	13.59	-	-	-
<b>Mitosporic fungi</b>				
<i>Acremonium hansfordii</i>	-	11.97	12.22	-
<i>A. luzulae</i>	-	1.33	-	-
<i>Alternaria alternata</i>	3.15	7.41	-	-
<i>A. padwickii</i>	3.98	6.92	8.61	21.43
<i>Aspergillus flavus</i>	1.61	13.13	3.60	-
<i>A. fumigatus</i>	11.69	10.89	2.43	-
<i>A. niger</i>	1.46	11.05	2.43	-
<i>Bipolaris oryzae</i>	13.53	14.59	13.55	35.71
<i>Cercospora oryzae</i>	4.54	8.08	9.79	28.57
<i>Cladosporium cladosporioides</i>	10.35	11.27	13.33	-
<i>C. elatum</i>	4.13	12.87	13.55	21.43
<i>C. oxysporum</i>	1.48	7.63	7.52	28.57
<i>C. tenuissimum</i>	3.39	3.46	3.92	21.43
<i>Colletotrichum graminicola</i>	5.88	1.33	-	-
<i>Curvularia lunata</i>	31.43	31.95	43.38	50.00
<i>Dactylaria hawaiiensis</i>	-	7.24	-	-
<i>Drechslera australiensis</i>	-	6.51	4.94	28.57
<i>Fusarium solani</i>	16.16	15.53	12.23	21.43
<i>F. semitectum</i>	4.97	4.20	2.43	21.43
<i>Gongronella butleri</i>	11.95	-	-	-
<i>Nigrospora oryzae</i>	14.91	15.16	18.84	21.43
<i>N. sphaerica</i>	0.83	12.38	3.60	35.71
<i>Penicillium</i> sp.	16.40	8.00	-	-
<i>P. digitatum</i>	13.86	-	-	-
<i>P. fumiculosum</i>	20.79	15.13	-	-
<i>P. janthinellum</i>	11.10	-	-	-
<i>P. oxalicum</i>	8.85	-	-	-
<i>P. rubrum</i>	6.94	-	-	-
<i>Pyricularia oryzae</i>	-	15.13	45.73	57.14
<i>Rhinocladiella simillis</i>	-	2.35	8.46	-
<i>Rhynocosporium oryzae</i>	2.73	8.07	9.95	-
<i>Sarocladium oryzae</i>	-	3.81	-	-
<i>Stachybotrys dichroa</i>	4.30	3.81	-	-
<i>T. harzianum</i>	28.10	-	-	-
<i>Veronaea apiculata</i>	3.47	12.02	-	-
<i>V. coprophila</i>	-	4.86	9.76	-
<b>Zygomycetes</b>				
<i>Rhizopus stolonifer</i>	9.30	-	-	-
<b>Total</b>	<b>31</b>	<b>31</b>	<b>22</b>	<b>14</b>

**Table 2.** Diversity indices of fungi from soil, stem, leaves and grains at different growth stages of the rice

Sources	Growth stage of the rice					Total	Index D	Index H
	Transplanting	Maximum tiller number	Panicle formation	Flowering	Maturity			
soil	16	22	24	27	22	31	0.9539	2.9311
stem	15	21	17	16	13	31	0.9486	2.6240
leaves	16	17	17	15	16	22	0.9218	2.5368
grains	-	-	-	-	14	14	0.9336	2.5668
<b>Total</b>	<b>30</b>	<b>36</b>	<b>33</b>	<b>35</b>	<b>32</b>	<b>39</b>		

**Fungal diversity at each growth stage of rice**

First stage, transplanting stage of rice were found 5 species that consisted of *A. fumigatus*, *A. flavus*, *B. oryzae*, *C. lunata* and *N. oryzae* and were the most common type in found at all substrates. *B. oryzae*, *C. lunata* and *N. oryzae* were the dominant species. The next stage, the maximum tiller stage, found 7 species, *A. niger*, *B. oryzae*, *C. oryzae*, *C. lunata*, *F. solani*, *N. oryzae* and *Rhynocosporium oryzae* showed the most frequency. *B. oryzae*, *C. oryzae*, *C. lunata*, *F. solani* were the dominant species.

For the panicle formation stage, *A. fumigatus*, *A. flavus*, *B. oryzae*, *C. globosum*, *C. lunata*, *F. solani*, *N. sphaerica* and *N. oryzae* were the most frequent and *A. flavus*, *B. oryzae* and *C. lunata* were the dominant species. In the flowering stage, *B. oryzae*, *C. oxysporum*, *C. lunata*, *F. solani* and *N. oryzae* were the most frequency and *C. lunata* was the dominant species. Finally, the maturity stage, *B. oryzae*, *C. oryzae*, *C. lunata*, *F. solani* and *N. oryzae* were the most frequency and *C. lunata* was the dominant species (Table 3).

**Table 3.** The most common frequency and the dominant fungi found at each growth stage of the rice

Transplanting stage	Maximum tiller stage	Panicle formation Stage	Flowering stage	Maturity stage
<i>A. fumigatus</i>	<i>A. niger</i>	<i>A. fumigatus</i>	<i>B. oryzae</i>	<i>B. oryzae</i>
<i>A. flavus</i>	<i>B. oryzae</i> *	<i>A. flavus</i> *	<i>C. oxysporum</i>	<i>C. oryzae</i>
<i>B. oryzae</i> *	<i>C. oryzae</i> *	<i>B. oryzae</i> *	<i>C. lunata</i> *	<i>C. lunata</i> *
<i>C. lunata</i> *	<i>C. lunata</i> *	<i>C. globosum</i>	<i>F. solani</i>	<i>F. solani</i>
<i>N. oryzae</i> *	<i>F. solani</i> *	<i>C. lunata</i> *	<i>N. oryzae</i>	<i>N. oryzae</i>
	<i>N. oryzae</i>	<i>F. solani</i> ,		
	<i>R. oryzae</i>	<i>N. sphaerica</i>		
		<i>N. oryzae</i> *		

\* dominant species

**Table 4.** Occurrence (%) of fungi from soil at each growth stages of the rice

Fungal species	Growth stages of the rice				
	Transplanting	Maximum tiller number	Panicle formation	Flowering	Maturity
<b>Ascomycetes</b>					
<i>Chaetomium globosum</i>	-	-	4.17	7.41	-
<b>Basidiomycetes</b>					
<i>Rhizoctonia solani</i>	25.00	22.73	-	11.11	9.09
<b>Mitosporic fungi</b>					
<i>Alternaria alternata</i>	-	-	8.33	7.41	-
<i>A. padwickii</i>	-	-	12.50	7.41	-
<i>Aspergillus flavus</i>	6.25	13.64	16.67	7.41	9.09
<i>A. fumigatus</i>	18.75	22.73	4.17	3.70	9.09
<i>A. niger</i>	18.75	9.09	12.50	7.41	4.54
<i>Bipolaris oryzae</i>	25.00	18.18	12.50	7.41	4.54
<i>Cercospora oryzae</i>	-	18.18	-	-	4.54
<i>Cladosporium cladosporioides</i>	12.50	22.73	-	7.41	9.09
<i>C. elatum</i>	-	-	4.17	7.41	9.09
<i>C. oxysporum</i>	-	-	-	7.41	-
<i>C. tenuissimum</i>	-	-	4.17	3.70	9.09
<i>Colletotrichum graminicola</i>	-	-	8.33	7.41	13.64
<i>Curvularia lunata</i>	31.25	31.82	33.333	25.93	31.82
<i>Fusarium solani</i>	25.00	27.27	8.33	11.11	9.09
<i>F. semitectum</i>	-	-	8.33	7.41	9.09
<i>Gongronella butleri</i>	25.00	18.18	8.33	3.70	4.54
<i>Nigrospora oryzae</i>	18.75	27.27	8.33	11.11	9.09
<i>N. sphaerica</i>	-	-	4.17	-	-
<i>Penicillium</i> sp.	31.25	42.86	4.17	3.70	-
<i>P. digitatum</i>	37.50	27.27	-	-	4.54
<i>P. fusiculosum</i>	18.75	31.82	16.67	18.52	18.18
<i>P. janthinellum</i>	12.50	13.64	8.33	7.41	13.64
<i>P. oxalicum</i>	-	31.82	4.17	3.70	4.54
<i>P. rubrum</i>	-	18.18	8.33	3.70	4.54
<i>Rhynocosporium oryzae</i>	-	9.09	-	-	4.54
<i>Stachybotrys dichroa</i>	-	13.64	4.17	3.70	-
<i>Trichoderma harzianum</i>	43.75	31.82	20.83	25.93	18.18
<i>Veronaea apiculata</i>	-	13.64	-	3.70	-
<b>Zycomycetes</b>					
<i>Rhizopus stolonifer</i>	25.00	13.64	4.17	3.70	-
<b>Total</b>	<b>16</b>	<b>22</b>	<b>24</b>	<b>27</b>	<b>22</b>



**Table 5.** Occurrence (%) of fungi on stem at each growth stages of the rice

Fungal species	Growth stages of the rice				
	Transplanting	Maximum tiller number	Panicle formation	Flowering	Maturity
<b>Ascomycetes</b>					
<i>Chaetomium globosum</i>	-	-	5.88	-	
<b>Mitosporic fungi</b>					
<i>Acremonium hansfordii</i>	13.33	14.29	17.65	6.67	7.69
<i>A. luzulae</i>	-	-	-	6.67	-
<i>Alternaria padwickii</i>	13.33	9.52	11.77	-	-
<i>A. alternata</i>	-	-	-	7.41	-
<i>Aspergillus flavus</i>	13.33	19.05	11.77	13.33	7.69
<i>A. fumigatus</i>	20.00	28.57	5.88	-	-
<i>A. niger</i>	20.00	28.57	-	6.67	-
<i>Bipolaris oryzae</i>	26.67	14.29	17.65	6.67	7.69
<i>Cercospora oryzae</i>	-	14.29	11.77	6.67	7.69
<i>Cladosporium cladosporioides</i>	26.67	14.29	-	-	15.38
<i>C. elatum</i>	-	23.81	11.77	13.33	15.38
<i>C. oxysporum</i>	-	23.81	-	6.67	7.69
<i>C. tenuissimum</i>	-	17.29	-	-	-
<i>Colletotrichum graminicola</i>	-		-	6.67	-
<i>Curvularia lunata</i>	26.67	28.57	47.06	26.67	30.77
<i>Dactylaria hawaiiensis</i>	26.67	9.52	-	-	-
<i>Drechslera australiensis</i>	26.67	-	5.88	-	-
<i>Fusarium solani</i>	20.00	23.81	11.77	6.67	15.38
<i>F. semitectum</i>	-	-	-	13.33	7.69
<i>Nigrospora oryzae</i>	20.00	23.81	17.65	6.67	7.69
<i>N. sphaerica</i>	-	-	11.77	-	7.69
<i>Penicillium sp.</i>	33.33	28.57	-	-	-
<i>P. fumiculosum</i>	40.00	-	-	-	-
<i>Pyricularia oryzae</i>	-	28.57	47.06	-	
<i>Rhinocladiella simillis</i>	-	-	11.77	-	-
<i>Rhynocosporium oryzae</i>	-	28.57	11.77	-	-
<i>Sarocladium oryzae</i>	-	19.05	-	-	-
<i>Stachybotrys dichroa</i>	-	19.05	-	-	-
<i>Veronaea apiculata</i>	26.67	19.05	-	6.67	7.69
<i>V. coprophila</i> *	-	-	17.65	6.67	-
<b>Total</b>	<b>15</b>	<b>21</b>	<b>17</b>	<b>16</b>	<b>13</b>

**Table 6.** Diversity of fungi on leaves at each growth stage of the rice

Fungal species	Growth stages of the rice				
	Transplanting	Maximum tiller number	Panicle formation	Flowering	Maturity
<b>Ascomycetes</b>					
<i>Chaetomium globosum</i>	6.25	11.77	5.88	6.67	6.25
<b>Mitosporic fungi</b>					
<i>Acremonium hansfordii</i>	12.50	11.77	17.65	6.67	12.50
<i>Alternaria padwickii</i>	6.25	5.88	11.77	6.67	12.50
<i>Aspergillus flavus</i>	6.25	-	11.77	-	-
<i>A. fumigatus</i>	6.25	-	5.88	-	-
<i>A. niger</i>	6.25	5.88	-	-	-
<i>Bipolaris oryzae</i>	12.50	11.77	17.65	13.33	12.50
<i>Cercospora oryzae</i>	12.50	11.77	11.77	6.67	6.25
<i>Cladosporium cladosporioides</i>	-	-	-	13.33	-
<i>C. elatum</i>	12.50	17.65	11.77	13.33	12.50
<i>C. oxysporum</i>	-	11.77	-	13.33	12.50
<i>C. tenuissimum</i>	6.25	-	-	13.33	6.25
<i>Curvularia lunata</i>	43.75	29.41	47.06	46.67	50.00
<i>Drechslera australiensis</i>	-	5.88	5.88	6.67	6.25
<i>Fusarium solani</i>	-	23.53	11.77	13.33	12.50
<i>F. semitectum</i>	6.25	5.88	-	-	-
<i>Nigrospora oryzae</i>	31.25	17.65	17.65	13.33	12.50
<i>N. sphaerica</i>	-	-	11.77	-	6.25
<i>Pyricularia oryzae</i>	43.75	41.18	47.06	46.67	50.00
<i>Rhinocladiella simillis</i>	18.75	11.77	11.77	-	-
<i>Rhynocosporium oryzae</i>	12.50	5.88	11.77	13.33	6.25
<i>Veronaea coprophila</i>	-	11.77	17.45	13.33	6.25
<b>Total</b>	<b>16</b>	<b>17</b>	<b>17</b>	<b>15</b>	<b>16</b>

## Discussion

### *Diversity of fungal on rice field*

Diversity of fungi from soil, stem, leaves at different growth stage of rice and grains were shown in various conditions. This research found fungal species similar to the previously published reports for isolation of fungi from soil and parts of rice (Fisher and Petrini, 1992; Tian *et al.*, 2004; Niharika *et al.*, 2012; Leewijit *et al.*, 2016). Soil and rice sample were collected from RD 41 varieties. The total number of fungal isolates were identified as 39 species. Most of the fungi which were isolated from soil and stem covered 31 species. Whereas, the maximum tiller number stage of rice was found with the highest of fungal diversity at 36 species, which were more than previous studied by Niharika *et al.* (2012) reported the number of fungi from soil in rice field were found 15 species and 11 species detected by Leewijit *et al.* (2016). The occurrence of fungi from rice were dependent of the isolation technique and media used. In this study, 3 methods were used to isolate dilution pour plate, baiting and moist chamber and use GANA media. Seephueak (2012) reports that there are many more fungi isolated by dilution pour plate from plant litter on GANA media more than use of PDA media.

Moreover, fungal growth associated with rice was reported in different researches carried out on the areas and substrates (John *et al.*, 2007; Ibiam *et al.*, 2006; Ashfaq *et al.*, 2015). Lapmak *et al.* (2009) studied diversity of fungi associated with brown rice from Pattalung province of Thailand and found 10 genera that consisted of *Acremonium*, *Aspergillus*, *Bipolaris*, *Colletotrichum*, *Curvularia*, *Drehslera*, *Fusarium*, *Geotrichum*, *Nigrospora* and *Penicillium* while, *Colletotrichum* was the most common genus. Beside, Taligoola *et al.* (2004) reported 6 fungal species associated with rice grains in Uganda such as *Aspergillus*, *Penicillium*, *Eurotium*, *Fusarium*, *Cladosporium* and *Cochliobolus*. Furthermore, *Penicillium*, *Aspergillus* and *Alternaria* were found on rice in Korea (Park *et al.*, 2005).

Also, the growth stage of the plant can differentially affect the diversity of colonization and species of fungi. The environmental conditions under which the rice plants grew might be a reason for variation in fungal colonization (Naik *et al.*, 2009). Fungal colonization differed among the rice varieties (Naik *et al.*, 2009) and depended on which plant tissue such as bark, stem and leaves (Fisher *et al.*, 1994; Bayman *et al.*, 1997), midrib and laminar tissue (Rodrigues, 1994), vein, midrib and pseudostem (Photila *et al.*, 2001) and root (Bayman *et al.*, 1997) was used. Fungi are diverse in different tissue types might be a factor for

utilizing the substrate along with factors as chemistry and tissue physiology (Rodrigues, 1994).

### ***Occurrence and the dominant of fungal species***

This study has shown that the most high frequency of fungi found from rice were of the genera *Aspergillus*, *Fusarium*, *Penicillium*, *Cladosporium*, *Nigrospora* and *Curvularia*, with were found on all substrates and at all growth stages of of rice.

The dominant species of fungi at each substrate are different. Three species, *Curvularia lunata*, *F. solani* and *N. oryzae* were the dominant species found at all substrates. Three species, *C. lunata*, *P. funiculosum* and *T. harzianum* were the dominant species found from soil at all growth stages of rice. One species, *C. lunata* was the dominant species found on rice stem. Five species, *B. oryzae*, *C. elatum*, *C. lunata*, *N. oryzae* and *P. oryzae* were the dominant species found on rice leaves. Whereas, 14 fungal species were the dominant species found on rice grains. This study reports the similar findings to those reported by Kaewchai *et al.* (2009); Niharika *et al.* (2012); Leewijit *et al.* (2016) who reported fungal association with leaves of Hom Kradung-Nga rice were found *Chaetomium* sp., *Penicillium*, *Aspergillus* sp., *Trichoderma* sp., *Fusarium* sp. and *Colletotrichum* sp. Whereas, Yuan *et al.* (2007) and Naik *et al.* (2009) found *C. globosum*, *Penicillium* and *F. oxysporum* that were the dominant species found from roots and leaves. Additionally, Leewijit *et al.* (2016) reported the dominant species isolated from rice leaves as *C. cupreum*, *Colletotrichum* spp., *C. lunata*, *F. solani*, *Penicillium* spp. and *Trichoderma* sp.

In the study of the fungal diversity from soil in rice field by Niharika *et al.* (2012), it was found that the important fungi were *A. flavus*, *A. fumigatus*, *A. niger*, *A. nidulans*, *A. terreus*, *P. chrysogenum*, *P. frequentans*, *P. funiculosum*, *T. viride*, *T. harzianum*, *F. oxysporum*, *F. solani*, *Curvularia clavata*, *C. lunata* and *Rhizopus stolonifer*. Similar to this study was the study where there were found the the dominant species on soil *A. fumigatus*, *A. niger*, *B. oryzae*, *C. lunata*, *F. solani*, *G. butleri*, *N. oryzae*, *P. funiculosum*, *P. janthinellum* and *T. harzianum*.

In this study, *C. lunata* was the domanint species found on all substrates and on all growth stages of rice. *Curvularia* species have been reported to be pathogenic to brown leaf spot of rice disease (Meethongkham and Soyong, 2013; Tann and Soyong, 2017; Bawa *et al.*, 2018). Moreover, *Curvularia* is plant pathogenic to maize and wheat. Khanum and Khanzada (1989) and Utobo

*et al.* (2011) have reported *C. lunata* in different rice varieties and have labelled it as seedborne.

For rice grains, many seed borne fungi associated with them have been reported. As the result, there are 14 fungal species that were found from rice seed. *P. oryzae* showed the highest number of the dominant species followed by *C. lunata* which were 57.14 and 50.00 %. Javaid and Anjum (2006) and Butt *et al.* (2011) reported that some seedborne fungi may be pathogenic such as *Fusarium moniliforme* (bakanae disease of rice). According to this research, there were found *C. lunata* and *P. oryzae* that caused brown spot and blight disease of rice. Moreover, Rodolfi *et al.* (2006) reported that *Penicillium*, *Fusarium* and *Aspergillus* were detected from various rice cultivars. Especially, *Aspergillus* and *Penicillium* were easily found on the rice grains (Muthomi *et al.*, 2012). Whereas, Ashfaq *et al.* (2015) reported 18 different fungal species that did belong to 8 genera and were isolated from rice seed for example *C. lunata* which had the highest percent frequency followed by *Aspergillus flavus*, *A. phoenicis* and *Phoma* sp.

However, the study that found many species of endophytic fungi found fungal diseases associated with rice, particularly endophytic fungi that was found to be a source of high diversity which plays an important role in the enhancement of the plant. The plant and fungi have an interaction that influences plant growth because host plant and fungi is mutualism and the plant does not get infected (Wongcharoen, 2014). Endophytic fungi can control plant disease and enhance plant productivity and survive in the host plant and it can stimulate in other words promote the growth of the plant. The results showed that some endophytic fungi were found such as genera *Aspergillus*, *Fusarium*, *Trichoderma* and *Penicillium* which is according to previous reported by Naik *et al.* (2009); Yuan *et al.* (2010); Kim *et al.* (2013); Leewijit *et al.* (2016).

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