International Journal of Agricultural Technology 2019 Vol. 15(1): 17-34
Available online http://www.ijat-aatsea.com
ISSN: 2630-0613 (Print) 2630-0192 (Online)

The contamination of toxins produced by naturally occurring fungi in nonpchemical rice products

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Jitjak, W. and Sanoamuang, N. (2019). The contamination of toxins produced by naturally occurring fungi in non-chemical rice products. International Journal of Agricultural Technology 15(1): 17-34.

Abstract A number of non-chemical products are increasing because customers are more aware of the sources and production processes of the products. One such product is rice, which is the main food source of Thai people. It can be seen that a variety of rice products are from non-chemical processes i.e. without chemical fertilizers and pesticides. However, these products could have naturally occurring toxins produced by fungi. Thus, the contamination of toxins naturally produced was investigated the fungi to ensure the trust of consumers that the products are safe to eat. Thirty-one rice products were randomly collected from retail stores in Muang District, Khon Kaen, Thailand and isolated fungi for molecular identification. The fungi found among the products were *Aspergillus syndowii*, *A. amstelodamii, A. tamari, A. niger, A. aculeatus, A. flavus, A. fumigatus*, *Fusarium equiseti, Rhizopus oryzae*, *R. microspores*, *Trichoderma asperellum, Dendryphiella* sp. and *Phoma multirostrata*. The toxins related to the fungi were quantified using a highly accurate method, High Performance Liquid Chromatography with Fluorescence Detector (HPLC/FLD) in comparison with toxin standards (aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, ochratoxin A, and zearalenone). The result suggested that among 31 samples with undetectable amounts, only one rice sample was contaminated with aflatoxin B1 (<1.5 ug/kg), total aflatoxin (0.66 ug/kg) and zearalenone (0.7 ug/kg).

Keywords: mycotoxins; organic; brown rice; food safety; local products

Introduction

Rice is the main staple food source of Thai people and is vital to the country. Furthermore, there are many varieties of rice grown in the country. In 2014-2015, the reported total area of rice paddy was about 62 million rai, with a production of 21 million tons. In the Northeast, the total rice area is 36 million rai. The area equates to more than 50% of the total area in the country. It was reported in 2015 that the area of rice cultivation is about 2.8 million rai, which

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shows a decrease of 26% compared to the previous year (Office of Agricultural Economics. 2014; Office of Agricultural Economics, 2013). Due to the reduction of rice cultivation area each year, one of the strategies announced by the Department of Agriculture is to omit using chemicals for food production (Pasanen *et al*., 1991) because it is harmful to consumers and also harmful to the surrounding environment (Viriyangkul, 2014). In addition to ensuring food security, food safety is also a continuing strategy of the government. Even in a country where there is an increase in food products without the use of chemicals, there may be contamination of naturally occurring toxins by microorganisms, especially fungi. Due to the high humidity of Thailand, the climate is suitable for the growth of fungus during the production process and the phase of postharvest. Poor management of these food products may result in the production of toxins by fungi which are naturally grown in post harvested crops and which contaminate products sold in the market.

The contamination of toxins from fungi in rice products is one of the factors in controlling the quality and safety of food (Benbrook, 2005). *Fusarium* spp., *Aspergillus* spp. and *Penicillium* spp. can produce many types of toxins, such as B1, B2, G1, G2, Fumonisin, Ochratoxin A, Trichothecenes, Deoxynivalenol (DON), and Zearalenone. Aflatoxins are commonly produced by *Aspergillus* fungi, Fumonisin, Deoxynivalenol (DON), Zearalenone and Trichothecenes and Ochratoxin A can be obtained from both *Aspergillus* and *Penicillium* (Benbrook, 2005). In Thailand, there are reports of fungal toxins in rice products, as follows: Panrapee *et al*. (2016) found that 12 samples out of 240 samples of brown rice and Alflatoxin B1 contents were higher than the European standard (2μg/kg). In Vietnam, one of the world's major rice producers, 25 samples were contaminated with toxin-producing fungi, *Aspergillus*, *Penicillium* and *Fusarium* with 2 examples detected with a very high amount of Ochratoxin A (Trung *et al*., 2001). However, there was no mention of these rice products being derived non-chemically or not. It is possible that there may be contamination of these toxins in the products sold in the market.

Local organic food products including organic rice products and other types of food products are becoming more popular. They are increasingly seen in the markets and sourced directly from local farmers. However, a specific investigation of the fungal toxins in these products has not clearly been conducted. In Khon Kaen, there are a number of non-chemical rice products in retail stores. Although these products are free from agricultural chemicals such as pesticides and chemical fertilizers, it is unclear whether or not toxins from natural fungus exist in the products. If there is, are they safe for consumption? Cirillo *et al*. (2003) found the contamination of Fumonisin B1, B2 and

Deoxynivalenol in both organic and conventional foods acquired from various retailers. Non-organic products are often found to contain more toxic amounts than organic products except in rice products, in whichhigher levels of toxic contamination and frequency are more common compared to other product types. Another research regarding organic wheat and rye in Germany found that there was a contamination of Deoxynivalenol at a very low level (Doll *et al*., 2002). Thus, it could be said that non-chemical and organic products are at-risk to have fungal toxins.

Based on the reports above regarding the toxins in food products, although it is safe from agricultural chemicals such as pesticides for insects and fungi, it is not yet certain that there will be no contamination of fungal toxins and toxin investigation in these products may be neglected in domestically-sold products sold. In Khon Kaen, non-chemical rice products can be found in many retail stores. They claim that these rice products are safe from chemicals. However, the issue of contamination of naturally occurring toxins is often overlooked in order to ensure the quality of the products. The toxicity can be lethal to consumers (Benbrook, 2005). The purpose of this research was to test the contamination of fungal toxins in non-chemical rice products available in the city of Khon Kaen.

Materials and methods

Sample collection

Rice products were interested in this study. Furthermore, 31 non-chemical rice products were randomly gathered from as many different shops and other sources as possible where these products could be found such as University's Agro outlet, organic shops, local rice shops and supermarkets. All types of rice cultivars and cultivation locations were recorded and labeled as shown in Table 2.

Fungal isolation

One hundred rice grains from each sample were randomly selected, surface-sterilized using 1% Clorox solution, washed with sterile water, and placed onto water agar for 3-7 days. The germinated fungi mycelia were selected, transferred onto potato dextrose agar medium and incubated at 25 ^{o}C for 3 days. Fungal mycelia growing on the agar medium were then transferred to potato dextrose broth for identification using morphology and molecular methods (Riker and Riker, 1963).

DNA isolation

The fungal mycelium on PDB were taken and washed in sterile distilled water for DNA isolation. The genomic DNA was extracted using the standard method (White *et al*., 1990). Of the collected colonies, 1 g were grounded in liquid nitrogen by using a sterile mortar and pestle in lysis buffer consisting of 200 mM Tris-HCl, pH 8.0, 250 mM NaCl, 25 mM EDTA, pH 8.0 and 2% sodium dodecyl sulfate for 700 µL with 2 µL ß-mercaptoethanol. Then, the mixture was transferred into tubes and incubated at 60 °C for 1 hr. After that the mixture of chloroform: isoamyl alcohol (24:1) for 700 µL were added and gently mixed before centrifuged at 12,000 rpm for 5 min at 4°C. Only supernatant was transferred to new tubes. Isopropanol, 0.7 times of collected supernatant volume was added then the tubes were kept at -20 °C for 20 min. The centrifugation at 12,000 rpm for 5min was to obtain DNA pellets from the tubes then 70% ethanol for 500 µL was used to wash the pellets twice and left at room temperature to be air-dried. The dried DNA pellets were dissolved in 50-µL TE buffer made up of 10 mM Tris-HCl and 1 mM EDTA then followed by the addition of RNase A, 2 µL before being incubated for 20 min at 37 ^{o}C and 1 µl of Proteinase K was respectively added before 20 min incubation. Chloroform: isoamyl alcohol (24:1) was added, centrifuged 12,000 rpm for 4 min again to clean the DNA solution. The supernatant was transferred to new tubes, and they were added with 3 µL of 3M sodium acetate and 150 µL of absolute ethanol and left at -20 °C for 30 min. The cleaned DNA pellets were obtained through centrifugation at 12,000 rpm for 10 min and the pellets were cleaned with 70% ethanol then dried at room temperature before re-suspended in TE buffer and kept at -20 °C.

Polymerase chain reaction

The desired genomic DNA of fungal specific regions informative for fungal classification and identification called internal transcribed spacer (ITS) was the target using primers ITS1-TCCGTAGGTGAACCTGCGG, ITS4-TCCTCCGCTTATTGATATGC and ITS5-GGAAGTAAAAGTCGTAACAAGG (White *et al.,* 1990). To amplify the ITS regions, the predenaturation temperature was 95 ^{o}C for 3 min followed by 35 cycles of 95 ^{o}C for 1 min, 55 ^{o}C (ITS1/ITS4), 57 ^{o}C (ITS4/ITS5) for 1 min then 72 ^{o}C for 2 min and 72 ^{o}C for 10 min (White *et al.* 1990). Then, 1 µL of PCR products was loaded in 1% agarose gel through the electrophoresis in TBE buffer (1 M Tris, 0.9 M boric acid, and 0.01 M EDTA, pH 8.3) for 1 h then stained with ethidium bromide solution then visualized in gel

documentation to determine whether the PCR yields were successful. The PCR products were in-gel purified and sequenced. The obtained sequenced were subject to database comparison (NCBI) through Blastn.

Table 1. DNA sequences of ITS region retrieved from the database used for phylogenetic analysis

Fungi species	Accession Number
Aspergillus aculeatus	KY315560, KY315561, KY320594, MF151167, MF920440, MG496018
Aspergillus amstelodami	KY828883, KY828887, KY828904, KY828915
Aspergillus flavus	MG991649, MG991651, MG991654, MG991655
Aspergillus fumigatus	MG991670, MG991672, MG991674, MG991675, MG991676
Aspergillus niger	MF152932, MF152933, MF152936, MH095994, MH174088
Aspergillus sydowii	KT826625, KX363451, KX958060, LN898717, LN898724, LN898728
Aspergillus tamarii	KY828882, MG857641, MG857642, MG857646, MG857652
Aspergillus terreus	KP131622
Coniothyrium glycines	JF740184, JF740185, KF251211
Dendryphiella sp.	JQ039898, KC832510, KC871034, KT796364
Fusarium equiseti	KP676595, KP676596, KP676597, KX463025, KX463028, KX463031
Fusarium falciforme	MF467480
Fusarium oxysporum	AY387702, AY387703, AY387704, AY387705
Fusarium solani	MG654678, MG654679, MG654681, MG654682
Mucor circinelloides	KP132470, KP132471, KP132472, KP132473
Phoma herbarum	AY293803, KU324835, KX766182
Phoma insulana	KJ207419,KJ207420,KR921746
Phoma multirostrata	KT928647, KU529840, KY310635, MG238549
Phoma putaminum	AM691000, AM691009, AM691010, AM691011
Rhizopus microsporus	KY828856, KY828894, MF197738, MG583908
Rhizopus oryzae	JX661043, KJ410025, MG583915, MG583920, MG583921, MG583995
Rhizopus stolonifer	JQ955583
Staurosphaeria lycii	MF434196, MF434197, MF434198
Trichoderma asperellum	KY381943, MG321328, MG971303
Trichoderma eijii	KJ783302, KJ783305, KJ783307
Trichoderma harzianum	EF568084
Trichoderma pseudolacteum	JX238469, JX238470, JX238472, JX238473
Trichoderma samuelsii	JN715593, JN715596
Trichoderma turrialbense	EU330944, EU330945, NR138448
Trichoderma vinosum	AY380904, DQ315446

Toxin extraction and quantification

The target toxins, aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, ochratoxin A, and zearalenone, derived from the collected rice samples, were extracted using appropriate organic solvents. These toxins were usually found in grains and the fungal species from the previous part. The procedures of toxin extracts were as follows. Ochratoxin A: 25 g of the sample were put in a container. Add 9 volumes of acetronitrile and 1 volume of water in the container. Aflatoxin G1, G2, B1, B2: 25 g of the sample were put in a container. Add methanol and 3% $NaHCO_3$ (1:1). Zearalenone: 20 g of the sample and 2 g of NaCl into a container. Add 9 volumes of acetronitrile and 1 volume of water in the container. After that, all toxin solutions were mixed with high speed for 2 minutes and filtered using filter paper and keep the solution in a new vial before being quantified using HPLC/FLD in comparison with toxin standards aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, ochratoxin A, and zearalenone (MERK-Supelco, USA). The settings of the HPLC conditions were performed as follows. Mobile phases used for orchratoxin A, all aflatoxins (G1, G2, B1, B2) and zearalenone were acetonitrile: 1% acetic acid (65:35), water: acetonitrile: methanol (72: 14: 14) and acetonitrile: water: methanol = (46: 46: 8) respectively. HPLC machines were Agilent 1100 Series for quantifying orchratoxin A and aflatoxins and Beckman 110B for zearalenone. The column for orchratoxin A and aflatoxins was C18 Size 4.6 x 150 mm, 5 µm with flow rate at 1 ml/min, 1 min run time and 3 min post time. The column temperature was at 40 ^oC with 20 µl of injection volume. The detector was fluorescence detector at excited 333 nm and emission 460 nm (orchratoxin A) and 440 (emission 440 nm). For zearalenone, the column was reverse phase, C18 (Waters Nova-Pak®, 3.9 mm x 150 mm, 4 um) with flow rate at 1 ml/min, 10 min run time, 3 min post time, colum temperature at 40 ^oC and equipped with the fluorescence detector at excited 274 nm, emission 440 nm. The injection volume was 50 µl.

Statistical analysis

Abundance rate

The abundance rates of the fungal species identified from the collected samples were calculated according to this formula: Abundance rate (A) = (Number of fungal species A ×100)/Number of all species.

Phylogenetic analysis

The blast results were preliminarily used to retrieve the DNA sequences for phylogenetic analysis. The pure fungal isolated were grouped and selected

as the representative of each group e.g. isolate 1_1, isolate 3_4, isolate 4_1, isolate 4_4, isolate 8_3, isolate 8_5, isolate 10_3, isolate 13_2, isolate 13_3, isolate 16_1, isolate 16_3, isolate 17_2, isolate 17_4, isolate 18_1, isolate 18_2, isolate 20_2, isolate 22_3, isolate 23_2, isolate 24_1, isolate 24_2, isolate 27_1, isolate 27_2, isolate 27_3, isolate 28_1, isolate 28_2, isolate 29_2 and isolate 30. The obtained unclear DNA sequences were trimmed according to chromatograms before submitted to phylogenetic analysis using MEGA 6.0 (Tamura *et al*, 2013) with fungal species retrieved from the database NCBI (GenBank) as shown in Table 1. The alignment was done using ClustalW and Neighbor-joining was performed to analyze the trees with following settings, Close-Neighbor-Interchange (CNI) algorithm (Nei and Kumar, 2000), Jukes-Cantor (Jukes and Cantor, 1969), search level at 1, gaps treated as missing data, 1000 bootstrap replications. Nodes of the trees with bootstrap value greater than 50 were used to ascertain the fungal species (Felsenstein, 1985).

Multiple correspondence analysis (MCA)

Results from fungal identification and toxin identification were brought to multiple correspondence analysis to elucidate the relations of all samples and features of each sample e.g. origins, cultivars, color, fungal abundance rates and identified toxins using XLSTAT.

Results

Rice samples and their characteristics

The collected samples consisted of 31 different products from Muang district, Khon Kaen. These products were various in origin: ranging from Khon Kaen toother provinces in the northeastern region of Thailand, including central and northern regions. The colors of the rice grains were also different depending on the rice cultivars e.g. brown rice, rice berry as purple grains, red (Sangyod cultivar) and white as color of the milled grains. All of the collected samples were derived from either organic or non-chemical cultivation as summarized in Table 2.

Fungal identification via inferences of phylogenetic trees

Due to the resulted phylogenetic trees using isolated fungal DNA sequences of ITS region amplified with ITS4 and ITS5 primers with Neighbor-Joining method, the findings suggested that different *Aspergillus* spp. which were *Aspergillus syndowii*, *A. amstelodamii, A. tamari, A. niger, A. aculeatus,*

A. flavus และ *A. fumigatus* (tree length = 0.26116075) as the most abundant group shown in Fig. 1 with nodal bootstrap scores greater than 50. In Fig. 2, the phylogenetic analysis yielded the tree length at 0.92949645 and suggested 2 *Rhizopus* species, *R. oryzae* and *R. microsporous* with 100 bootstrap scores. Additionally, fungus *Dendryphiela* sp. was also identified with supportive bootstrap at 100 (tree length = 0.20936572 as in Fig. 3). *Fusarium equiseti* was also found in the samples as illustrated in Fig. 4 (bootstrap value at 100 and tree length = 0.19049035). In Fig. 5, one of fungal phytopathogen, *Phoma multirostrata* was identified with bootstrap score at 57 and tree length at 0.07254042. An additional fungus identified from the collected samples *Trichoderma asperellum* as exhibited in Fig. 6 with tree length at 0.16592446 and bootstrap score at 95.

The resulting 6 phylogenetic trees with supportive bootstrap scores suggested the fungal isolates from the samples were *A. syndowii*, *A. amstelodamii, A. tamari, A. niger, A. aculeatus, A. flavus, A. fumigatus*, *F. equiseti, R. oryzae*, *R. microspores*, *T. asperellum, Dendryphiella* sp. and *P. multirostrata* as summarized and the abundance rates of each fungi from each sample are also concluded in Fig. 7. These identified fungal species were used to select relevant toxins related to the fungi for the next experiment.

The fungal species and their abundance rate were used to conduct multiple correspondence analysis together with other features e.g. origins, colors and cultivation techniques. The analysis suggested that sample 16 was the most different from the others followed by sample 5 and 15 because they were found with the most *T. asperellum* and *Dendryphiella* sp. (Fig. 8).

Fungal toxins in rice

As the results of fungal identification, most of the samples were found with *Aspergillus* spp. Thus, toxins total aftlatoxin, aflatoxin B1, aflatoxin B2, aflatoxin G1 and aflatoxin G2 were performed in those samples with *Aspergillus* spp. For ochratoxin A and zearalenone quantifications, they were conducted in the samples detected with *A. niger* and *F. equiseti* (Bánáti *et al.,* 2017). Due to the results in comparison with the toxin standards, it suggested only one sample out of 31 samples, sample 29, was found with Aflatoxin B1 (<1.5 ug/kg), total aflatoxin (0.66 ug/kg) and zearalenone (0.7 ug/kg) which were in the detection ranges of the analytic instrument. Moreover, multiple correspondence analysis also showed the most distant relationship of sample 29 among all collected samples as it was the only product with the toxins (Fig. 9).

Table 2. Collected rice products and their features

Sample	Characteristics			
	Color	**Origin**	**Cultivation method**	**Type**
1	Purple	Nongpok, Roi-ed	Organic	Rice berry
2	Brown	Kangkro, Chaiyaphoom	Organic	Jasmine
3	Brown	Naphang, Amnatcharoen	Organic	Rice berry
4	Purple	Donjiang, Maetaeng, Chaing Mai	Organic	Rice berry
5	Brown and Red	Pakdeeruamjai group, Pattalung	Non-chemical	Sung-yod
6	Purple	Chumpae, Khon Kaen	Non-chemical	Jasmine
7	Purple	Nakorn Pathom	Non-chemical	Rice berry
8	Light Purple	Nakorn Pathom	Non-chemical	Purple jasmine
9	Brown	Namkang organic group, Supanburi	Organic	Jasmine
10	Brown	Surin Rajamangkala Technology University, Surin	Organic	Jasmine
11	Brown	Sila, Muang, Khon Kaen	Organic	Jasmine
12	Purple	Sila, Muang, Khon Kaen	Organic	Rice berry
13	Purple	Banka, Ubonrat, Khon Kaen	Organic	Rice berry
14	Brown	Muang, Kalasin	Organic	Jasmine
15	Brown, Purple and Red	Nonghin, Coke-ko, Muang, Mahasarakham	Non-chemical	Jasmine
16	Brown	Nongwaeng, Ban Haed, Khon Kaen	Organic	Brown rice
17	Purple	Nongkung, Muang, Khon Kaen	Organic	Jasmine
18	Purple	Nongkung, Muang, Khon Kaen	Organic	Sticky rice
19	Purple	Nongbua, Udon Thani	Organic	Rice berry
20	Brown	Nongbua, Udon Thani	Organic	Rice berry
21	White	Chang Sam mo, Chayaphoom	Organic	Germinated jasmine
22	Brown	Naimuang, Muang, Petchbun	Organic	Hill rice
23	Red and Purple	Nongsalab, Om-goi, Chiang Mai	Organic	Buekeezou
24	Black	Naimuang, Muang, Petchbun	Organic	Sticky rice
25	White	Tabluang, Muang, Nakorn Pathom	Organic	Jasmine
26	Brown and Purple	Pua, Nan	Non-chemical	Jasmine
27	Purple	Maejan, Chiang Rai	Non-chemical	Rice berry
28	Red	Surin	Organic	Jasmine
29	Purple	Maetang, Chiang Mai	Organic	Homnil
30	Purple	Sila, Muang, Khon Kaen	Non-chemical	Rice berry
31	Black	Rairuang, Pua, Nan	Organic	Sticky rice

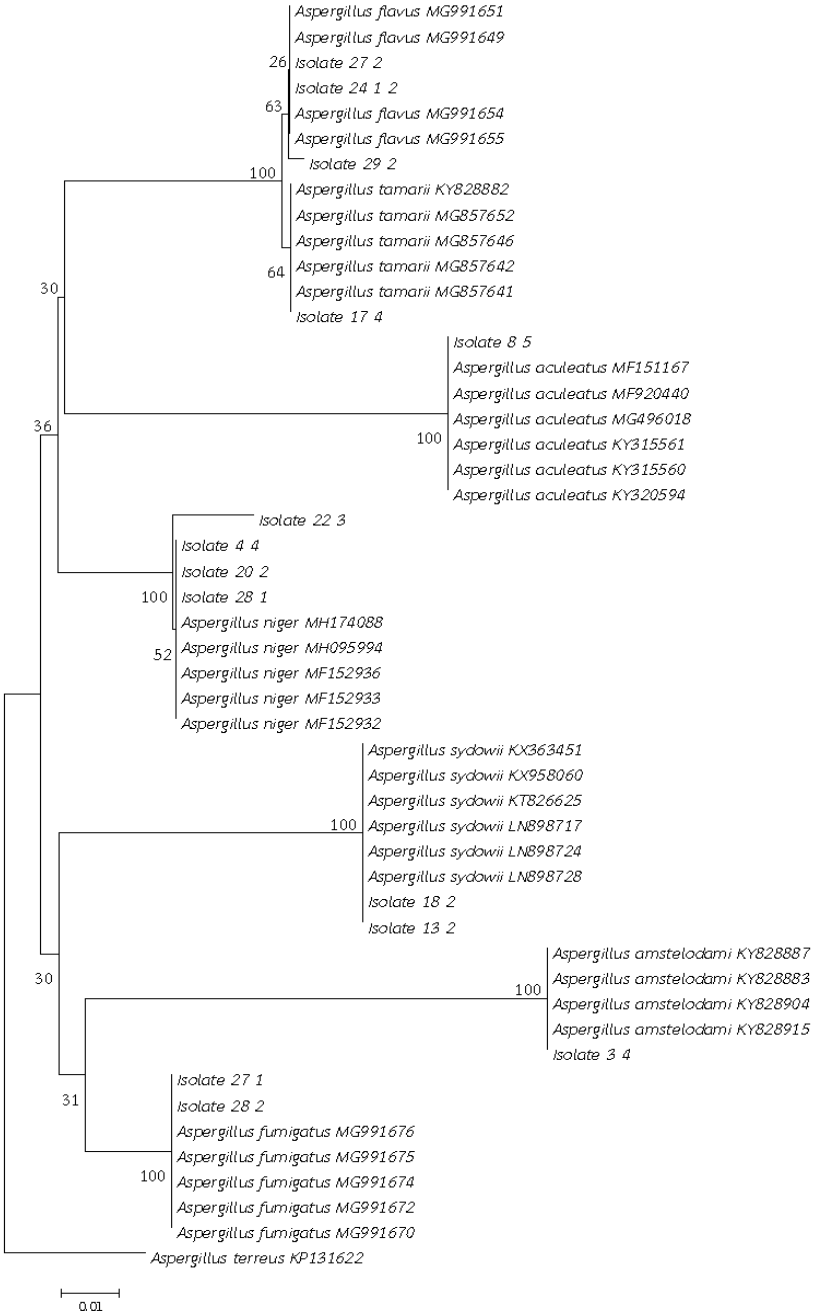

Figure 1. Phylogenetic trees of *Aspergillus* isolates. The tree suggests that the fungi species are *Aspergillus syndowii*, *A. amstelodamii, A. tamari, A. niger, A. aculeatus, A. flavus, A. fumigatus* with bootstrap values at 100, 100, 64, 100, 100, 63 and 100 respectively

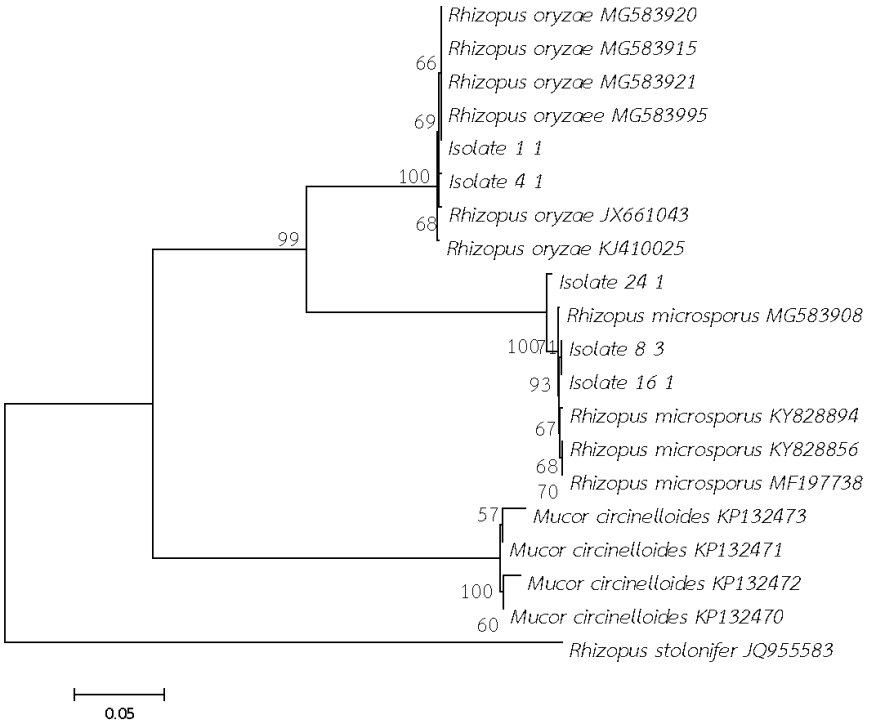

Figure 2. Phylogenetic tree of *Rhizopus* isolates. The tree suggests that the fungi species are *Rhizopus oryzae* and *R. microsporous* with bootstrap at 100 on both nodes

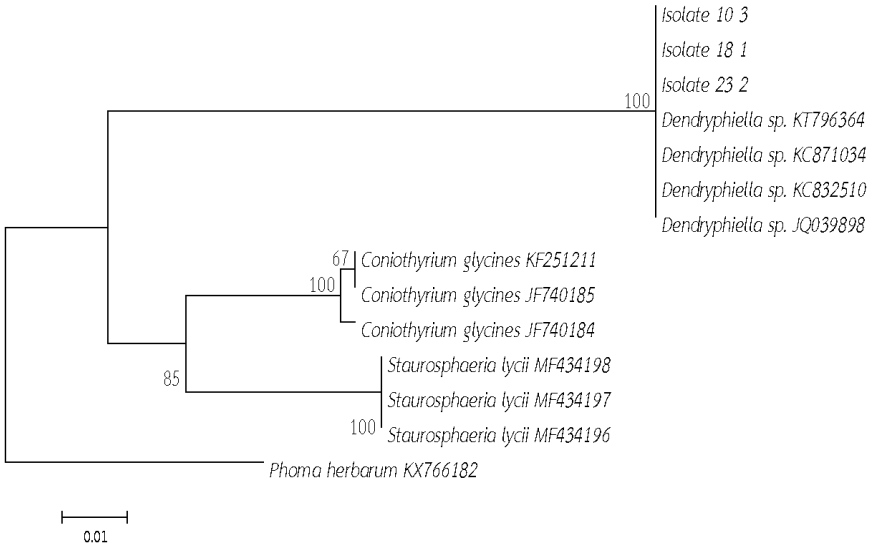

Figure 3. Phylogenetic tree of *Dendryphiela* with bootstrap value at 100

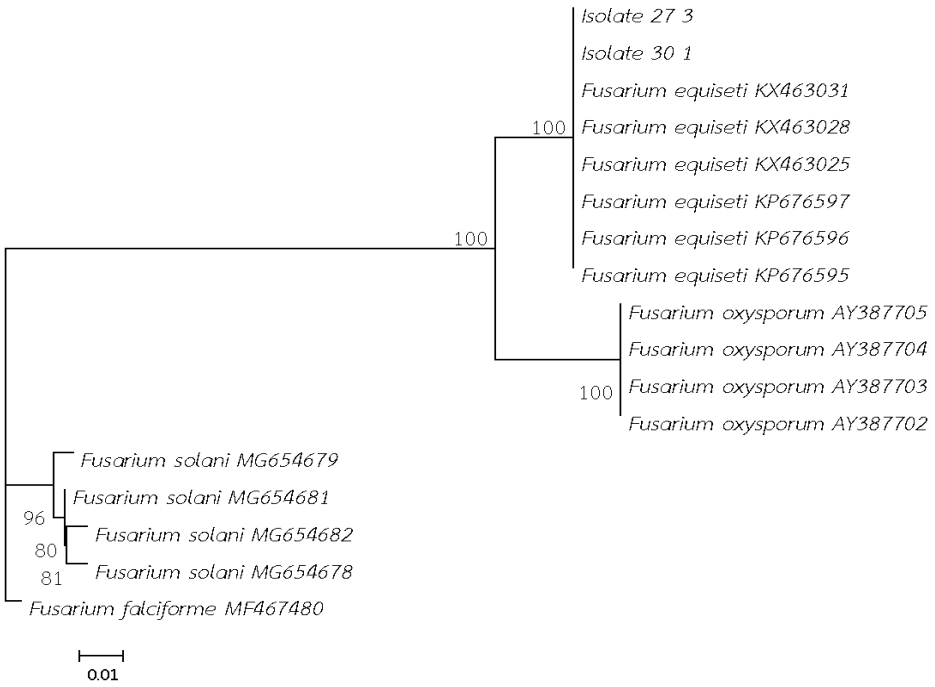

Figure 4. Phylogenetic tree of *Fusarium* spp. The tree suggested *F. equiseti* with bootstrap value at 100

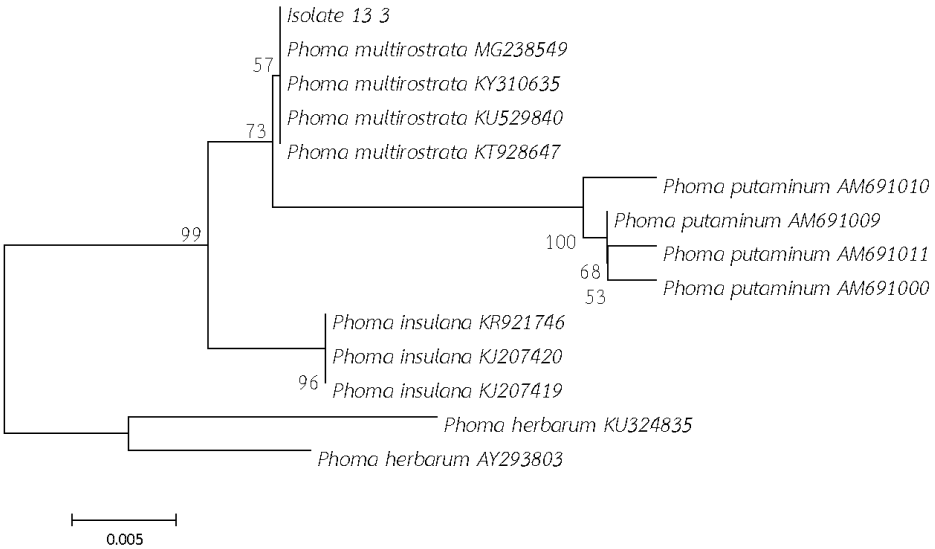

Figure 5. Phylogenetic tree of *Phoma*. The fungal species is *P. multirostrata* with bootstrap value at 57

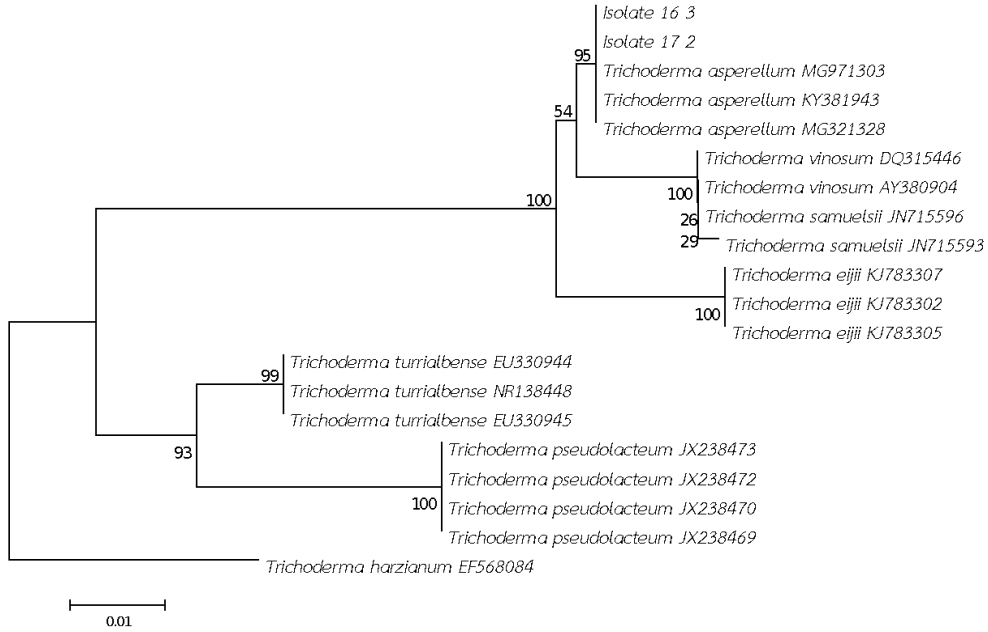

Figure 6. Phylogenetic tree of *Trichoderma*. The fungal species is *T. asperellum* with bootstrap value at 95

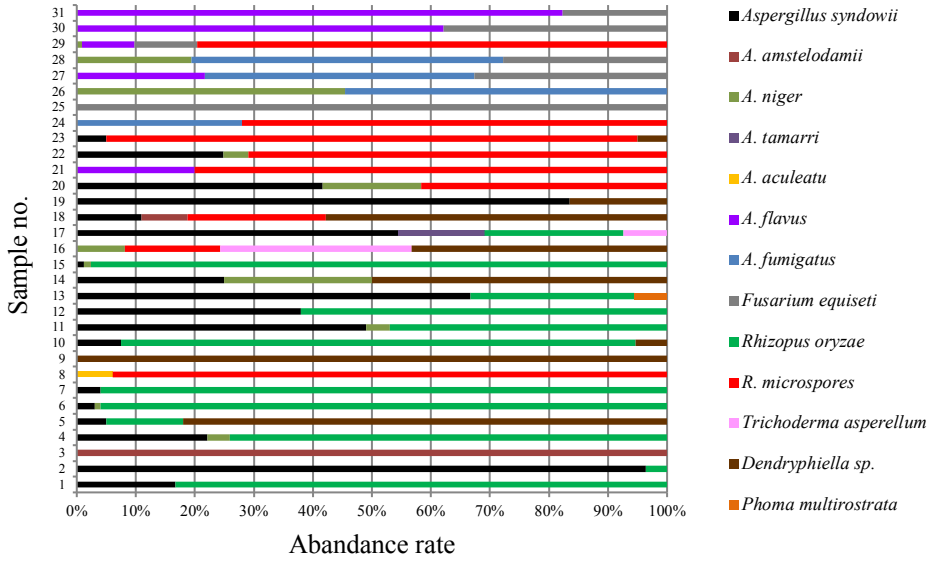

Figure 7. Fungal species abundance rate from the collected rice products

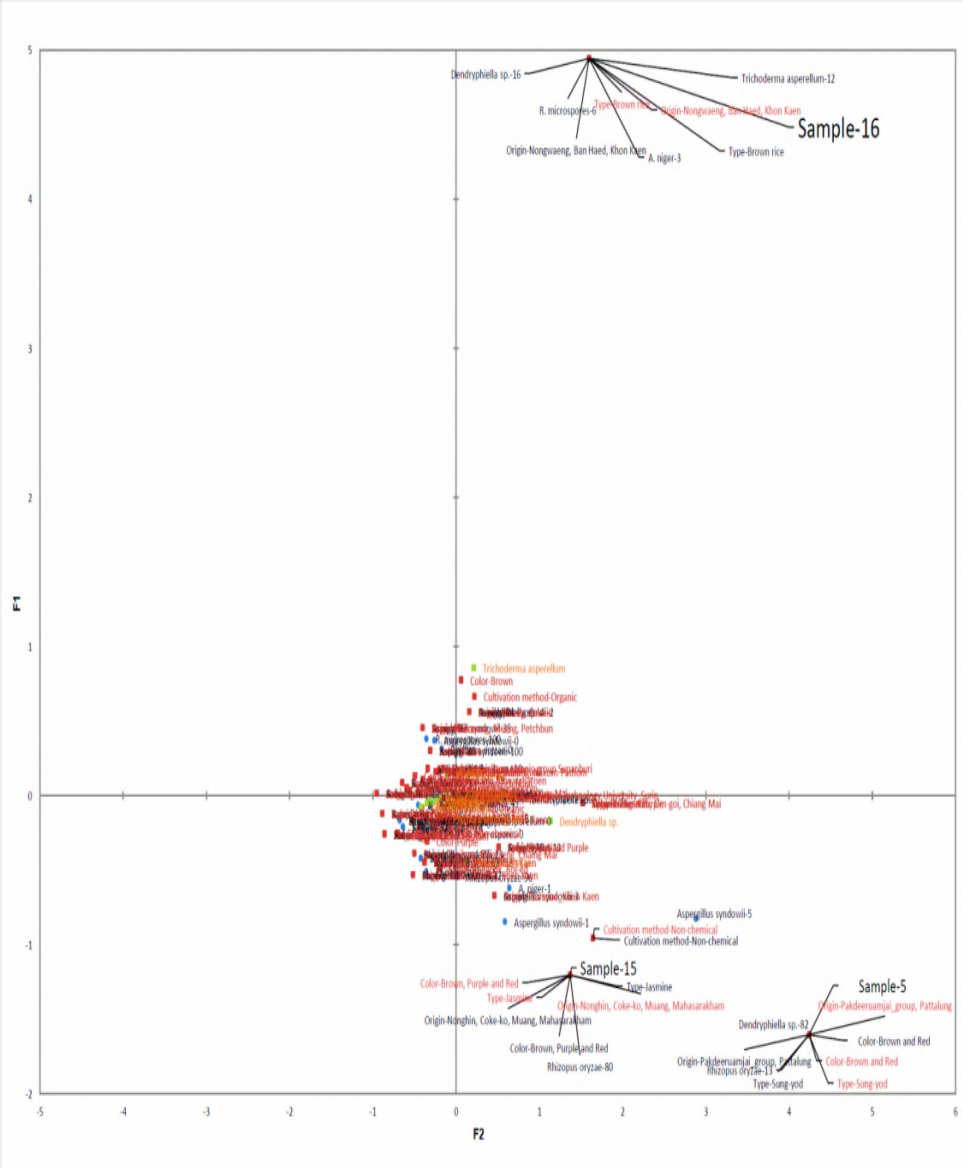

Figure 8. Multiple correspondence analysis of the collected samples towards fungal species identified in each sample. The sample 16 was the most different among 31 species followed by samples 5 and 15 respectively

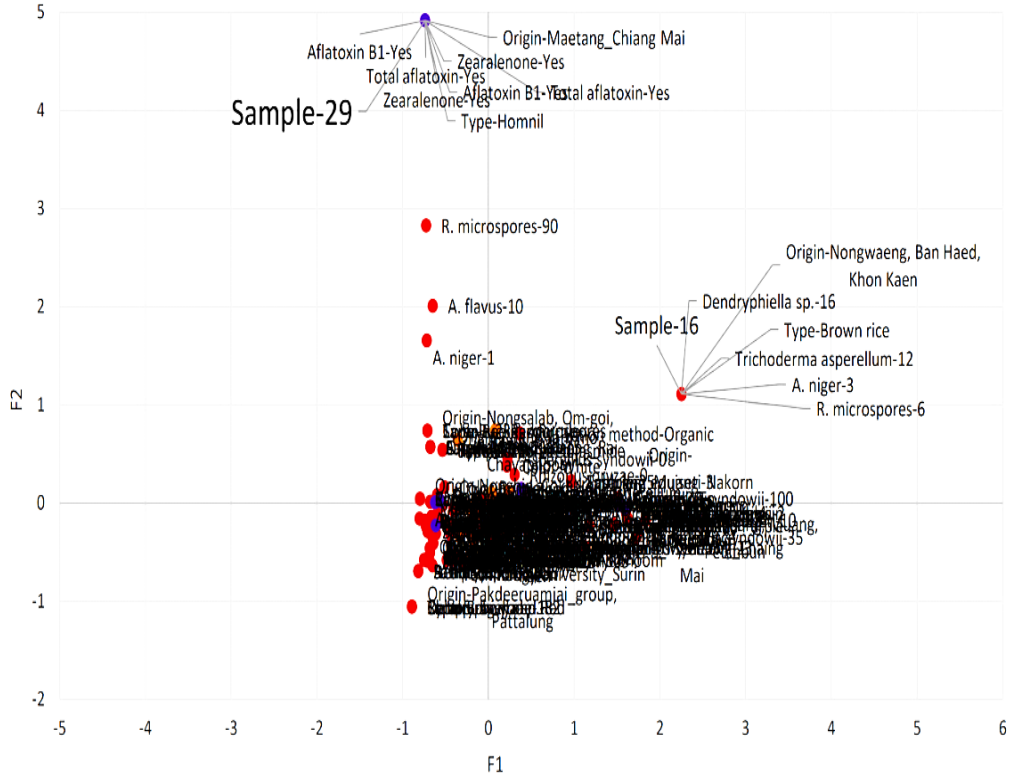

Figure 9. Multiple correspondence analysis collected samples. It suggests that sample 29 is the most different as it is the sample found with toxins

Discussion

Fungal species in these genera *Aspergillus, Fusarium* and *Penicillium* play significant roles as various toxin producers in cereals and grains regardless of farming systems (Brodal *el al*., 2016). A study comparing the incidence of *Fusarium* spp. in two wheat farming systems, organic and conventional ones, suggested no difference at all during 2010-2011 in Italy (Lazzaro *et al*., 2015). As suggested by the results in this tudy, different fungal species were found including *Aspergillus syndowii*, *A. amstelodamii, A. niger, A. aculeatus, A. flavus, A. fumigatus*, *Fusarium equiseti, Rhizopus oryzae*, *R. microspores*, *Trichoderma asperellum, Dendryphiella* sp. and *Phoma multirostrata*. Samples detected with *Aspergillus* were subjected to toxin quantification, aflatoxins and ochratoxin A, and those with *Fusarium* were brought for zearalenone quantification (Benbrook, 2005).

There were other fungal species that are not included for toxin quantification like *Rhizopus* spp*., Trichoderma asperellum* and *Dendryphiella* sp. These fungi are endophytes and commonly found in nature (Lapmak *et al*., 2009; De França *et al*., 2014 Cantabrana *et al*, 2015). Therefore, they are not considered as toxin-producing fungi for this study. Fungal genera *Aspergillus* and *Fusarium* are commonly found in cereals and grains. The experiment 1 accordingly suggested *Aspergillus* spp. as the most abundant genus. Moreover, *Rhizopus* spp. and *P. multirostrata* were also found as this group of fungi is also naturally occurring. Apart from these, *T. asperellum* was identified. Usually, this species of *Trichoderma* is widely use as the biological control agent in organic and non-chemical agriculture (El_Komy *et al*., 2015). For *Dendryphiella* sp., it was reported as an endophyte in rice (Lapmak *et al*., 2009). In conclusion, the fungi identified from the collected rice products are categorized into 3 groups which are 1) Toxin producing fungi e.g. *Aspergillus* and *Fusarium*, 2) biocontrol agent e.g. *T. asperellum* and 3) endophytic fungi e.g. *P. multirostrata*, *Rhizopus* spp. and *Dendryphiella* sp.

In regards to the first experiment that aimed to identify fungal species, it was taken into account which toxins should be quantified in all 31 collected samples depending on fungi. The rice samples were quantified for the following toxins due to the fungal species found in the samples: aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, ochratoxin A, and zearalenone. According to the quantification results, most of the toxins were not detected in the samples. However, among 31 samples, sample 29 was found with aflatoxin B1 (<1.5 ug/kg), total aflatoxin (0.66 ug/kg) and zearalenone (0.7 ug/kg). The levels of these toxins are relatively low. However, this cannot be ignored because even though the products are derived organically or non-chemically, the fungal-toxin contamination was still detected. Thus, there is a risk for the products to be tarnished with the toxins, which puts the consumer at risk as well. Therefore, food safety regarding storage and post-harvest management of the products should be more regulated.

Acknowledgements

This research project was supported by KKUIC individual project grant mainly for fungal identification and Thailand Toray Science Foundation-Granted Research Project for Year 2016 for fungal toxin identification. Moreover the author would like to thank Center of excellence on Agricultural Biotechnology (AG-BIO/PERDO-CHE) and its staff at Faculty of Agriculture, Khon Kaen University in order to provide facilities for molecular work. Additionally, the author would like to thank Miss Kanchita Kenluem, a master student who assisted the fungal genomic DNA extraction and special thanks to Mr. Kyle Daniel Craven for proofreading the manuscript.

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(Received: 24 July 2018, accepted: 5 December 2018)