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Diversity and Distribution of Hyphomycetes from Dung in Thailand

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ABSTRACT

This investigation on hyphomycetes from dung samples of wildlife and domestic animals, including deer, barking deer, eld's deer, elephant, guar, rabbit, camel, goat, horse, buffalo, cow, mouse and toad, was conducted from eighteen provinces in Thailand. Different isolation methods such as the moist chamber, Warcup's direct plating, dilution plate, and heat and alcohol treatments were used. Identification of the fungal isolates was based on morphological characteristics of colony growth on agar media and examination of the spores and fruiting bodies using stereo and light microscopes. Four-hundred and six isolates comprising 24 genera and 28 species of hyphomycetes were recorded. They were most numerous on cow dung, followed by elephant, buffalo and deer dung. Coprophilous hyphomycetes included *Arthrobotrys oligospora*, *Cephaliophora irregularis*, *Nodulisporium gregarium* and *Oidiodendron griseum*. Potential plant pathogenic fungi included *Alternaria alternata*, *Curvularia lunata*, *Exserohilum rostratum*, *Fusarium oxysporum*, *F. solani*, *Nodulisporium gregarium* and *Thielaviopsis* sp. Coprophilous hyphomycetes representing new records for Thailand were *Nodulisporium gregarium*, *Oidiodendron griseum* and *Pithomyces karoo*.

Key words: diversity, distribution, taxonomy, coprophilous fungi, Fungi Imperfecti

INTRODUCTION

The hyphomycetes are anamorphic fungi with a worldwide distribution on various substrates, comprising 1,800 genera and 9,000 species (Kirk *et al.*, 2001). They are classified based on the morphology of conidiophores and conidia (Carmichael *et al.*, 1980; Barnett and Hunter, 1998). The hyphomycetes are important sources of antibiotics, organic compounds, enzymes and other secondary metabolites useful in the pharmaceutical industry, agriculture, fermentation and other processes (Wei *et al*., 1994; Tanaka *et al*., 1997; Frisvad *et al*., 1998). Many species of the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Pithomyces*, *Memnoniella* and *Stachybotrys* have been recorded as mycotoxigenic fungi capable of producing several mycotoxins which cause human, animal and plant diseases (Moss, 2002a, 2002b, 2003; Bennett and Klich, 2003; Houbraken *et al*., 2006).

A key to hyphomycetes on dung was developed by Seifert *et al*. (1983). Bell (1983) mentioned three hyphomycetes from dung: *Athrobotrys oligospora*, *Penicillium claviforme* and *Sepedonium* sp. Subramanian (1983) stated

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that hyphomycetes were a normal component of the mycoflora of dung and animal excrements, including birds and mammals, both herbivores and carnivores. He also stated that Saccardo in his *Sylloge* enumerated 757 species belonging to 187 genera as coprophilous. The number has increased as the results of the researches from different parts of the world. Several new genera of hyphomycetes from dung were established by Subramanian and his associates. He noted that many hyphomycete that occurred on dung were anamorphs of the discomycetes and the pyrenomycetes. He also stated that the decomposition of dung and excreta of animals was part of the recycling process in nature in which not only the true coprophilous fungi, but also the hyphomycetes were involved in this process. Some of them are adapted for both survival within the animal gut and subsequent fruitings on dung.

Prior to this investigation, the hyphomycetes on dung in Thailand were poorly known with the only significant records contributed by Manoch *et al*. (1999) and Somrithpol (2004). These studies were limited in areas surveyed and also time of collecting. Somrithpol (2004) summarized 19 species from 24 genera belonging to the hyphomycetes from wildlife and domestic dung in Thailand.

The present study involved study on 1) morphology of hyphomycetes found on various dung samples, and 2) diversity and distribution of hyphomycetes associated with different dung types from various locations in Thailand.

MATERIALS AND METHODS

Sixty dung samples from thirteen wild and domestic animals were collected from eighteen provinces of Thailand during June 2002 to July 2004 (Table 1). Each excrement sample was placed in a moist chamber by a window. They were incubated for 2 - 7 days at 28°C for fungal growth and sporulation. A direct isolation method was carried out by using a fine sterile needle to pick up the fungal hyphae and spores from the dung surface under a stereomicroscope (SZ-PT Olympus), and transferred onto water agar (WA) in a Petri dish and incubated for 48 h. A hyphal tip was transferred to a slant potato dextrose agar (PDA) and kept as a pure cultures at the Department of Plant Pathology, Faculty of Agriculture, Kasetsart University Fungal Collection (KUFC). Dry dung samples were kept in a herbarium at the Department of Plant Pathology, Faculty of Agriculture, Kasetsart University Herbarium (KUH) (Table 1). Warcup's direct plating, dilution plate, heat treatment and alcohol treatment methods and Gochenaur's glucose ammonium nitrate agar (Gochenaur, 1964) (1 g NH_4NO_3, 1 g K_2HPO_4, 0.5 g $MgSO_4.7H_2O$, 0.03 g rose bengal, 1 g yeast extract, 5 g glucose, 15 g agar, 4 ml streptomycin solution, 1 l distilled water) as well as 2% malt extract agar were employed to isolate hyphomycetes from the dung samples. Identification was based on morphological characteristics, such as colony growth pattern, color, texture on different agar media and spore ornamentation which was examined by light microscopy. An Olympus BH-2 with Normaski Interference Contrast was employed.

RESULTS AND DISCUSSION

Four-hundred and six isolates comprising 24 genera and 28 species of coprophilous hyphomycetes were isolated from 60 dung samples of 13 animals from 18 provinces in Thailand (Table 2). The moist chamber and Warcup's direct plating and dilution plate methods yielded the highest fungal species of hyphomycetes, whilst alcohol and heat treatment methods showed fewer species. Morphological characteristics of hyphomycetes on various dung samples from different locations were summarized in Table 3.

Table 1 Dung samples from thirteen wildlife and domestic animals collected from various locations.

Dung type	Number of sample	Location/ Kasetsart University Herbarium (KUH)/ Collecting date
Barking deer	3	Nakhon Ratchasima, Khao Yai National Park, KUH 0002,16/6/2002; Uthai Thani, Hauy Kha Khang Wlid Life Sanctuary, KUH 0022, 2/2/2003; Chonburi, Khao Khaew Open Zoo, KUH 0046, 17/2/2004
Buffalo	4	Saraburi, Maung District, KUH 0007, 8/7/2002; Chiang Mai, Mae Tang District, KUH 0012, 7/11/2002; Bangkok, Dong Maung, KUH 0029, 24/6/2003; Mae Hong Son, Maung District, KUH 0037, 9/10/2003
Camel	1	Chonburi, Khao Khaew Open Zoo, KUH 0047, 17/2/2004
Cow	12	Nakhon Ratchasima, Pak Thong Chai District, KUH 0009, 25/10/2002; Ratchaburi, Nong Po District, KUH 0015, 5/12/2002; Saraburi, Maung District, KUH 0021, 14/4/2003; Surat Thani, Maung District, KUH 0031, 8/8/2003; Krabi, Lunta Island, Lunta District, KUH 0032, 10/8/ 2003; Mae Hong Son, Maung District, KUH 0038, 9/10/2003; Khon Kaen, Maung District, KUH 0043, 11/11/2003; Chiang Rai, Horticulture Research Institute, KUH 0044, 16/1/2004; Ubol Ratchathani, Sirinthorn District, KUH 0050, 25/2/2004; Phetchaburi, Ban Lard District, KUH 0051, 28/2/2004; Trat, Goad Island, Khoa Goad District, KUH 0052, 16/3/2004; Kalasin, Maung District, KUH 0056, 16/4/2004
Deer	2	Nakhon Ratchasima, Khao Yai National Park, KUH 0003, 16/6/2002; Uthai Thani, Hauy Kha Khang Wlid Life Sanctuary, KUH 0023, 2/5/2003
Eld's deer	2	Uthai Thani, Hauy Kha Khang Wlid Life Sanctuary, KUH 0024, 2/5/2003; Chonburi, Khao Khaew Open Zoo, KUH 0048, 17/2/2004
Elephant	8	Nakhon Ratchasima, Khao Yai National Park, KUH 0004, 16/6/2002; Chiang Mai, Mae Sa District, KUH 0010, 7/11/2002; Loei, Phu Ka Dung National Park, KUH 0017, 31/12/2002; Mae Hong Son, Maung District, KUH 0039, 9/10/2003; Uthai Thani, Hauy Kha Khang Wlid Life Sactuary, KUH 0025, 2/5/2003; Krabi, Lunta Island, KUH 0033, 10/8/2003; Chiang Rai, Horticulture Research Institute, KUH 0045, 16/1/2004; Chonburi, Khao Khaew Open Zoo, KUH 0049, 17/2/2004
Gaur	1	Uthai Thani, Hauy Kha Khang Wlid Life Sanctuary, KUH 0026, 2/5/2003
Goat	2	Krabi, Lunta Island, Lunta District, KUH 0034, 10/8/2003; Mae Hong Son, Maung District, KUH 0038, 9/10/2003
Horse	1	Krabi, Lunta Island, KUH 0035, 10/8/2003
Rabbit	4	Chiang Mai, Queen Sirikit Botanic Garden, KUH 0011, 7/11/2002; Bangkok, Dong Maung, KUH 0013, 14/11/2002; Uthai Thani, Hauy Kha Khang Wlid Life Sactuary, KUH 0027, 2/5/2003; Prachin Buri, Tub-Larn National Park, KUH 0030, 26/6/2003
Rat	13	Bangkok, Bang Sue District, KUH 0001, 7/12/2001; KUH 0005, 18/6/2002; KUH 0008, 9/7/2002, KUH 0014, 14/11/2002; KUH 0018, 20/1/2003; KUH 0028, 15/5/2003; KUH 0041, 19/10/2003; KUH 0053, 4/4/2004; KUH 0057, 3/7/2004; Kasetsart University, Bangkok, KUH 0036, 26/9/2003; KUH 0042, 20/10/2003; KUH 0054, 4/4/2004; KUH 9958, 19/7/2004
Toad	7	Bangkok, Bang Sue District, KUH 0006, 18/6/2002; KUH 0016, 19/12/2002; KUH 0019, 2/2/2003; KUH 0020, 16/3/2003; KUH 0055, 4/4/2004; KUH 0059, 19/7/2004; KUH 0060, 8/8/2004

Table 2 shows most hyphomycetes found on domestic animal dung, including cow dung (106 isolates, 26 spp.), followed by buffalo dung (43, 13) and goat dung (21, 13). However, for wildlife, elephant dung yielded the hightest number of hyphomycetes (62 isolates, 19 spp.), followed by deer (35, 16) and barking deer (23, 12) (Table 2). All isolates could be cultivated on artificial media and maintained as a pure culture with specimen codes of KUFC.

Dominant genera of hyphomycetes found on all dung samples using various isolation methods were *Aspergillus* spp. (144 isolates), including *A. candidus* (2), *A. clavatus* (7), *A. flavus* (30), *A. fumigatus* (42), *A. niger* (37) and *A. terreus* (26). The two isolates of *A. candidus* from deer dung and rat droplets were derived by using the moist chamber technique (Table 2).

Paecilomyces lilacinus was the most common species and 44 isolates were found from the majority of dung samples except eld's deer dung. Other common species found were *Aspergillus fumigatus* (42 isolates), *A. niger* (37 isolates), *A. flavus* (30 isolates), and *Scopulariopsis brevicaulis* (29 isolates). It was interesting to note that *Nigrospora oryzae*, *Nodulisporium gregarium*, *Oidiodendron griseum*, *Stachybotrys atra* and *Thielaviopsis* state of *Ceratocystis paradoxa* were found on only one type of dung, e.g. cow, toad and elephant (Table 2).

Seifert *et al*. (1983) stated that hyphomycetes might be not true coprophilous fungi, but contaminants arriving from the air or soil, after the dung was deposited. However, they also stated that some hyphomycetes were known only from dung, such as *Arthrobotrys*, *Basifimbria* and *Oedocephalum*.

Table 2 Number of isolates of coprophilous Hyphomycetes obtained from different dung types.

Fungal species	Number of isolate													Total isolate
	barking deer (3*)	buffalo (4)	camel (1)	cow (12)	deer (2)	eld's deer (2)	elephant (8)	gaur (1)	goat (2)	horse (1)	rabbit (4)	rat (13)	toad (7)	
Acremonium spp.	1	-	-	3	1	-	-	-	-	-	-	-	-	**5**
Alternaria alternata	-	2	-	8	-	-	-	-	1	1	-	-	-	**12**
Arthrinium phaeospermum	1	-	-	4	4	-	2	-	1	-	-	-	-	**12**
Arthrobotrys oligospora	-	-	-	1	1	-	-	-	-	-	-	-	-	**2**
Aspergillus candidus	-	-	-	-	1	-	-	-	-	-	-	1	-	**2**
Aspergillus clavatus	-	-	-	1	1	-	3	-	-	-	-	1	1	**7**
Aspergillus flavus	1	5	1	8	3		4	1	1	1	1	2	2	**30**
Aspergillus fumigatus	2	6	1	10	2	4	8		1	1		4	3	**42**
Aspergillus niger	-	5	1	6	4	1	4	1	1	2	3	4	5	**37**
Aspergillus terreus	1	4	2	4	1	-	2	-	-	4	2	3	3	**26**
Cephaliophora irregularis	-	-	-	4	-	-	-	-	4	-	-	-	-	**8**
Cladosporium cladosporioides	4	-	-	7	-	-	3	-	-	-	-	-	-	**14**
Curvularia lunata	-	-	-	2	-	1	-	-	1	-	-	1	-	**5**
Exserohilum rostratum	-	-	-	-	-	-	2	-	-	-	-	-	-	**2**
Fusarium oxysporum	2	2	1	10	2	-	4	-	-	1	-	1	-	**23**
Fusarium solani	-	1	-	8	3	4	7	1	1	-	1	-	-	**26**
Memnoniella echinata	-	-	-	1	-	-	-	-	-	1	-	-	-	**2**
Myrothecium verrucaria	2	3	-	5	-	-	2	-	1	1	-	-	-	**14**
Nigrospora oryzae	-	-	-	1	-	-	-	-	-	-	-	-	-	**1**
Nodulisporium gregarium	-	-	-	1	-	-	-	-	-	-	-	-	-	**1**
Oidiodendron griseum	-	-	-	-	-	-	-	-	-	-	-	-	1	**1**
Paecilomyces lilacinus	4	4	1	7	5	-	6	4	4	1	4	2	2	**44**
Penicillium spp.	-	4	-	2	1	1	2	2	3	2	1	1	1	**20**
Phialophora spp.	-	-	-	2	-	1	1	-	-	-	-	-	-	**4**
Pithomyces karoo	-	-	-	1	-	1	-	-	-	-	-	-	-	**2**
Scopulariopsis brevicaulis	1	5	-	2	4	3	7	-	-	2	4	1	-	**29**
Scytalidium lignicola	-	-	-	2	1	-	1	-	-	-	-	-	-	**4**
Stachybotrys atra	-	-	-	-	-	-	-	-	-	-	-	-	1	**1**
Thielaviopsis state of *Ceratocystis paradoxa*	-	-	-	-	-	-	1	-	-	-	-	-	-	**1**
Trichoderma hamatum	2	1	-	4	-	-	1	-	1	1	-	-	3	**13**
Trichoderma harzianum	2	1	1	2	1	-	2	2	1	3	-	-	1	**16**
Total	23	43	8	106	35	16	62	11	21	21	16	21	23	406
% Total isolate	**5.7**	**10.6**	**2**	**26**	**8.6**	**3.9**	**15.3**	**2.7**	**5.2**	**5.2**	**3.9**	**5.2**	**5.7**	**100**

* indicates the number of samples

Table 3 Morphological characteristics of Hyphomycetes isolated from various dung samples collected in different locations.

Fungal species	Descriptions	Ref.*
Acremonium sp. KUFC 2873, on barking deer dung Uthai Thani	Colonies on PDA, reaching 3.5 cm diam. in 7 days at 28 °C. Mycelium white to pale pink, reverse uncoloured. Conidiophores mostly simple orthotropic. Phialides arising from submerged tip without a conspicuous collarette, wall hardly thicker than that of the subtending hyphae, 15-20 µm long, tapering from 2.0-3.0 µm, near the base to 0.8-1.3 µm. Conidia in strongly slimy heads, ellipsoidal, smooth, thick-walled, 3-4 × 1-1.2 mm.	D
Alternaria alternata KUFC 2898, on cow dung Mae Hong Son	Colonies on PDA, reaching 9 cm in diam. in 7 days at 28 °C. Mycelium black. Conidiophores arising singly, golden brown, up to 50-55 µm long, 3-5 µm thick. Conidia formed in long, chains, obclavate or ovoid, with a short cylindrical beak, golden brown, up to 8 transverse and several longitudinal septa, 25-55 × 10-16 µm.	E1
Arthrinium phaeospermum KUFC 2890, on deer dung Uthai Thani (Figure 1A)	Colonies on PDA, reaching 9 cm diam. in 7 days at 28 °C, with the large numbers of white mycelium mats. Conidiophore mother cells lageniform, 5-7 × 3-6 µm. Conidiophores erect ascending, cylindrical, smooth, colourless. Conidia lenticular, dark brown with a hyaline band at the junction of the two sides, 8-10 × 5-8 µm.	D, E1, Sl
Arthrobotrys oligospora KUFC 2894, on deer dung Nakhon Ratshasima (Figure 1B)	Colonies on PDA, reaching 9 cm diam. in 7 days at 28 °C. Mycelium hyaline to pale pink. Conidiophores arising from fasciculate aerial hyphae, unbranched, 300-400 µm long, bearing several conidiferous nodes where the conidia are borne on short blunt denticles. Conidia hyaline, 2-celled, obovoid, broadest in longer apical call, 25-28.5 × 16-19 µm.	B, E3, Sl
Aspergillus candidus KUFC 2897, on deer dung Nakhon Ratchasima	Colonies on Czapek's solution agar (CZA), reaching 2.5-3 cm diam. in 14 days at 28 °C. Mycelium white to cream, dense, reverse uncolored. Conidial heads radiate, stipes 200-500 × 4-10 µm, smooth walled. Vesicles globose, 20 -35 µm wide. Metulae colorless, 7-10 × 5-8 µm. Phialides 6-9 × 2-3 µm. Conidia subglobose, thin walled, smooth, colorless, 3-4 µm.	R
Aspergillus clavatus KUFC 2900, on cow dung Saraburi	Colonies on CZA, reaching 2.5-3 cm diam. in 7 days at 28 °C. Mycelium blue green. Conidial heads clavate, 300-400 µm, Conidiophores thin wall smooth, colorless, gradually enlarging at the apex into a clavate. Vesicles colorless, 200-250 × 40-50 µm, unisereate. Phialides 8-10 × 2-3 µm. Conidia smooth-walled, ellipsoidal, pyriform, 3-4 × 2-3 µm.	R
Aspergillus flavus KUFC 2931, on barking deer dung Uthai Thani	Colonies on CZA, reaching 4-4.5 cm diam. in 7 day at 28 °C. Mycelium yellow-green. Conidial heads typically radiate, 400-500 µm, smaller heads occasionally up to 300 × 50 µm. Conidiophores, uncolored, coarsely roughened. Vesicles subglobose or globose. Metulae colorless, 6-10 × 4-5 µm. Phialides 6-12 × 3-5 µm. Conidia globose to subglobose, 3.5-4 diam, sometime elliptical, 4.5-5 × 3.5-4 µm. Sclerotia subglobose, dark red-brown.	R
Aspergillus fumigatus KUFC 2908, on barking deer dung Uthai Thani	Colonies on CZA, reaching 4-4.5 cm in diam. in 7 days at 28 °C. Mycelium bluish green. Conidial heads columnar, compact. Conidiophores smooth, 200-300 µm in length by 5-8 µm in diam. Vesicles up to 20 to 30 mmin diam., unisereate. Phialides crowed, 6-8 × 2-3 µm. Conidia green in mass, echinulate, globose, 2.5-3 diam. finely roughened.	R
Aspergillus niger KUFC 2929, on buffalo dung Chiang Mai	Colonies on CZA, reaching 3-3.5 cm diam. in 14 days at 28 °C. Mycelium black or deep brownish black. Conidial heads black, radiate or in age often splitting into two or more loose columns. Conidiophores walls smooth, brownish shades. Vesicles globose, biseriate. Metulae brownish, 20-30 × 5-6 µm. Phialides 7-8 × 3-3.5 µm. Conidia globose, irregularly roughened, 4-5 µm in diam.	R
Aspergillus terreus KUFC 3001, on barking deer dung Nakhon Ratchasima	Colonies on CZA, reaching 3-3.5 cm diam. in 7 days at 28 °C. Mycelium cinnamon. Conidial heads compact columnar, smooth-walled. Conidiophores smooth-walled, colorless, 100-300 × 4-7 µm. Vesicles, hemispherical, 12-20 µm in diam., biseriate. Metulae 5-7 × 2-3 µm. Phialides 5-7 × 1.5-2.5 µm. Conidia globose to slightly elliptical, smooth-walled, 1.5-2.5 µm in diam.	R
Cephaliophora irregularis KUFC 3034, on cow dung Krabi (Figures 1C, 1D)	Colonies on PDA, reaching 9 cm diam. in 7 days at 28 °C (Figure 1A). Mycelium yellow to pale brown. Conidiophores clavate, up to 100 µm long, 5-10 µm thick. Conidia pyriform or turbinate, colourless to pale brown 1- to 2- septate, 25-40 × 15-30 µm, protuberant hilum.	E1, Sl
Cladosporium cladosporioides KUFC 3053, on barking deer dung Chonburi	Colonies on PDA, reaching 5 cm in diam. in 7 days at 28 °C. Mycelium effuse, olive green, velvety, reverse olivaceous-black. Conidiophores 200-250 × 2-6 µm. Conidia formed in long branched chains, mostly 0-1 septate, ellipsoidal to lemon-shaped, smooth-walled, olivaceous-brown, 1-celled, 4-8 × 2-4 µm.	E1,D
Curvularia lunata KUFC 3069, on cow dung Surat Thani	Colonies on PDA, reaching 7 cm diam. in 7 days at 28 °C. Mycelium brow, to black, cottony or velvety. Conidiophores often geniculate, brown. Conidiogenous cells terminal, sympodial, cylindrical. Conidia solitary, simple, often curved, clavate, obovoid or pyriform with 3 transverse septa, pale or dark brown, 20-30 × 10-14 µm.	E1
Exserohilum rostratum KUFC 3079, on elephant dung Krabi	Colonies on PDA, reaching 8-9 cm diam. in 7 days at 28 °C. Mycelium brown, cottony or velvety. Conidiophores 200-320 × 6-7 µm, septate, cylindrical, olivaceous brown, paler towards the apex, simple, geniculate. Conidia straight to slightly curved, ellipsoidal to narrowly obclavate, brown or olivaceous, thick-walled, 15-220 × 10-27 µm.	S2

Table 3 Cont'd.

Fungal species	Descriptions	Ref.*
Fusarium oxysporum KUFC 3076, on barking deer dung Nakhon Ratshasima	Colonies on PDA, reaching 4.5-6.5 cm diam. in 7 days at 28 °C. Mycelium white or peach, but usually with a purple or violet tinge. Sporodochia with an orange slime of macro-conidia. Microconidia abundant, simple, mostly 0-septate, ellipsoildal to cylindrical, 5-10 × 2-3.5 µm. Macroconidia fusiform, curved, 3-5 septate, 25-47 × 3.0-4.5 µm. Chlamydospores terminal and intercalary in hyphae, smooth-walled, 5-15 µm diam.	D
Fusarium solani KUFC 3100, on buffalo dung Chiang Mai	Colonies on PDA, reaching 3.2 cm diam. in 7 days at 28 °C. Mycelium cream to buff. Microconidia useually abundant, produced on elongate, 10-15 × 2-4 µm. Macroconidia fusiform, curved, blunt apical and basal cells pedicellate, 3-5 septate, 27-46 × 3.0-4.5 µm. Chlamydospores terminal and intercalary in hyphae, 7-18 µm diam.	D
Memnoniella echinata KUFC 3089, on cow dung Saraburi (Figure 2A)	Colonies on PDA, reaching 2-2.5 cm diam. in 14 days at 28 °C. Mycelium pale yellow with dark spot of conidia. Conidiophores gray, 50-80 × 3-5 µm. Phialides mostly in groups of 5-8 × 3-5 µm. Conidia sphaerical of flattened dorsiventrally, 3-4 µm diam.	E1
Myrothecium verrucaria KUFC 3112, on barking deer dung Chonburi	Colonies on PDA, reaching 7 cm diam. in 14 days at 28 °C. Mycelium white to cream, forming diffuse or coalescent olivaceous to black sporodochia. Phialides 3-6 in a whorl, 12-15 × 1.5-2 µm. Conidia broadly ellipsoidal, the apical end pointed and the basal truncate, bearing an apical, 6-10 × 2-4.5 µm.	D
Nigrospora oryzae KUFC 3096, on cow dung Saraburi	Colonies on PDA, reaching 6-7 cm diam. in 7 days at 28 °C. Mycelium at first white, later brown when sporulation. Conidiophores flexuous, brown, smooth. Conidiogenous cells monoblastic, ampulliform, colourless, 3-5 µm in diam. Conidia solitary, spherical or broadly ellipsoidal, black, shining, smooth, 0- septate, 10-12.5 µm in diam.	E1
Nodulisporium gregarium KUFC 3098, on cow dung Trat	Colonies on PDA, reaching 6 cm diam. in 7 days at 28 °C. Mycelium effuse, grey, brown, often velvety. Conidiophores often synnematous, up to 2 mm. high, individual threads pale to mid brown or olivaceous brown, verrucose, much branched towards the apex. Conidiogenous cells 10-18 × 2.5-3 µm. Conidia 4.5-6.5 × 2-3 µm.	E1
Oidiodendron griseum KUFC 3097, on toad dung Bangkok	Colonies on PDA, reaching 6-6.5 cm diam. in 7 days at 28 °C. Mycelium grey to olivaceous brown, often raised in the middle, wrinkled. Conidiophores olivaceous to blackish brown, smooth, 100-180 × 1.5-2.5 µm. Conidia oblong or ellipsoidal, pale greyish green, smooth, 2.5-3.5 × 1.5-2 µm. E	D
Paecilomyces lilacinus KUFC 3177, on barking deer dung Uthai Thani	Colonies on PDA, reaching 5-7 cm diam. in 7 days at 28 °C. Mycelium vinaceous shades. Conidiophores mostly arising solitarity from the horizontal mycelium, stalks 3.5-5 µm wide, rough-walled, with densely clustered metulae and phialides. Conidia ellipsoidal to fusiform, slightly roughned, 2.5-3.0 × 2.0-2.5 µm.	
Penicillium sp. KUFC 3117, on buffalo dung Surat Thani	Colonies on CZA, 1.5-1.8 cm diam. in 7 days at 28 °C. Mycelium blue green, reverse yellow to orange. Conidiophores 80-150 × 2-3 µm, smooth-walled with 3-4 metulae in a whorl, Metulae 15-18 × 2-2.5 µm. Phailides flask-shaped, 10-12 × 2-2.5 µm. Conidia globose, smooth-walled, greenish, 2.5-3.0 µm in diam.	P
Phialophora sp. KUFC 3189, on cow dung Chiang Rai	Colonies on PDA, reaching 7-8 cm diam. in 7 days at 28 °C. Mycelium olivacoues brown, partly immersed. Conidiophores mononematous, branched, olivaceous brown, smooth. Conidiogenous cells monophialidic, ampulliform, with well defined collarettes. Conidia simple, straight, ellipsoidal, colourless to pale brown, smooth, 0-septate, 3-5 × 1.5-2 µm.	E1, D
Pithomyces karoo KUFC 3214, on cow dung Saraburi (Figure 2B)	Colonies on PDA, reaching 9 cm in diam. in 7 days at 28 °C. Mycelium effuse, greyish olive or black. Conidia very variable in shape, eillipsoidal, pyriform, clavate, with 2-3 transverse septa and 1 longitudinal septum, dark brown, verrucose, 15.5 –28 × 10-15 µm.	E2
Scopulariopsis brevicaulis KUFC 3158, on barking deer dung Nakhon Ratchasima	Colonies on PDA, reaching 4-5 cm in diam. in 7 days at 28 °C. Mycelium buff to brown. Annellides arising in groups of 2-3 on short stipes, 10-25 µm long, colourless or very pale. Conidia brown in mass but very pale when viewed singly, subspherical or obovoid, truncate at the base, smooth when young, coaesely verrucose when mature, 5.5-8 × 5-7.5 µm.	D
Scytalidium lignicola KUFC 3165, on cow dung Trat	Colonies on PDA, reaching 5-6 cm in diam. in 7 days at 28 °C. Mycelium dark blackish brown, immersed, smooth, some narrow, cylindrical, colourless 2-6 µm thick, swollen cells 10 µm thick. Conidiophores synnematous, unbranched, straight, colourless or brown, smooth. Conidia catenate, simple, brown, smooth, 0 septate, 8-10 × 2-3 µm.	E1
Stachybotrys atra KUFC 3207, on toad dung Bangkok	Colonies on PDA, reaching 5-6 cm in diam. in 7 days at 28 °C. Mycelium black, immersed. Conidiophores at first hyaline but soon becoming olivaceous brown to black, up to 100 µm long, 3.5-5 µm thick. Phialides mostly 12-13 × 4.5-6 µm in the broadest part. Conidia broadly ellipsoidal, dark brown to black, verrucose, 8.5-10 × 6-10 µm.	E1
Thielaviopsis state of *Ceratocrytis paradoxa* KUFC 3208, on elephant dung Nakhon Ratchasima (Figures 2C, 2D)	Colonies on PDA attaining 9 cm in diam. in 7 days at 28 °C (Figure 3A). Mycelium dark brown. Conidiophores colourless to pale brown, up to 52-60 × 4-4.5 µm. Phialides up to 200-300 µm. Phialoconidia in chains, at first cylindrical and colourless, becoming ellipsoidal and mid golden brown, 7-12 × 4-6 µm (Figures 3B-3C). Arthroconidia catenate, ellipsoidal, smooth, dark browm, with longitudinal slit, 12-25 × 10-15 µm.	E1

Table 3 Cont'd.

Fungal species	Descriptions	Ref.*
Trichoderma hamatum KUFC 3218, on barking deer dung Chonburi	Colonies on PDA, reaching 9 cm diam. in 7 days at 28 °C. Mycelium greyish green from a cover of mostly curled sterile conidiophore ends. Branches and phialides are particularly broad, 3-4 µm wide, Conidia short-cylindrical, green, smooth-walled, 3.5-5 × 2-3 µm.	D
Trichoderma harzianum KUFC 3306, on barking deer dung Uthai Thani	Colonies on PDA, reaching 9 cm diam. in 7 days at 28 °C. Mycelium greyish green. Branches and phialides are particularly broad, 3.5-4 µm wide Conidia subglobose to short-oval, measuring 3-3.2 × 2.5-2.8 µm.	D

* D = Domsch *et al*. (1993), E1 = Ellis (1971), E2 = Ellis (1976), E3 = Ellis and Ellis (1998), P = Pitt (1979), R = Raper and Fennell (1965), S1= Seifert *et al*. (1983), S2 = Sivanesan (1987)

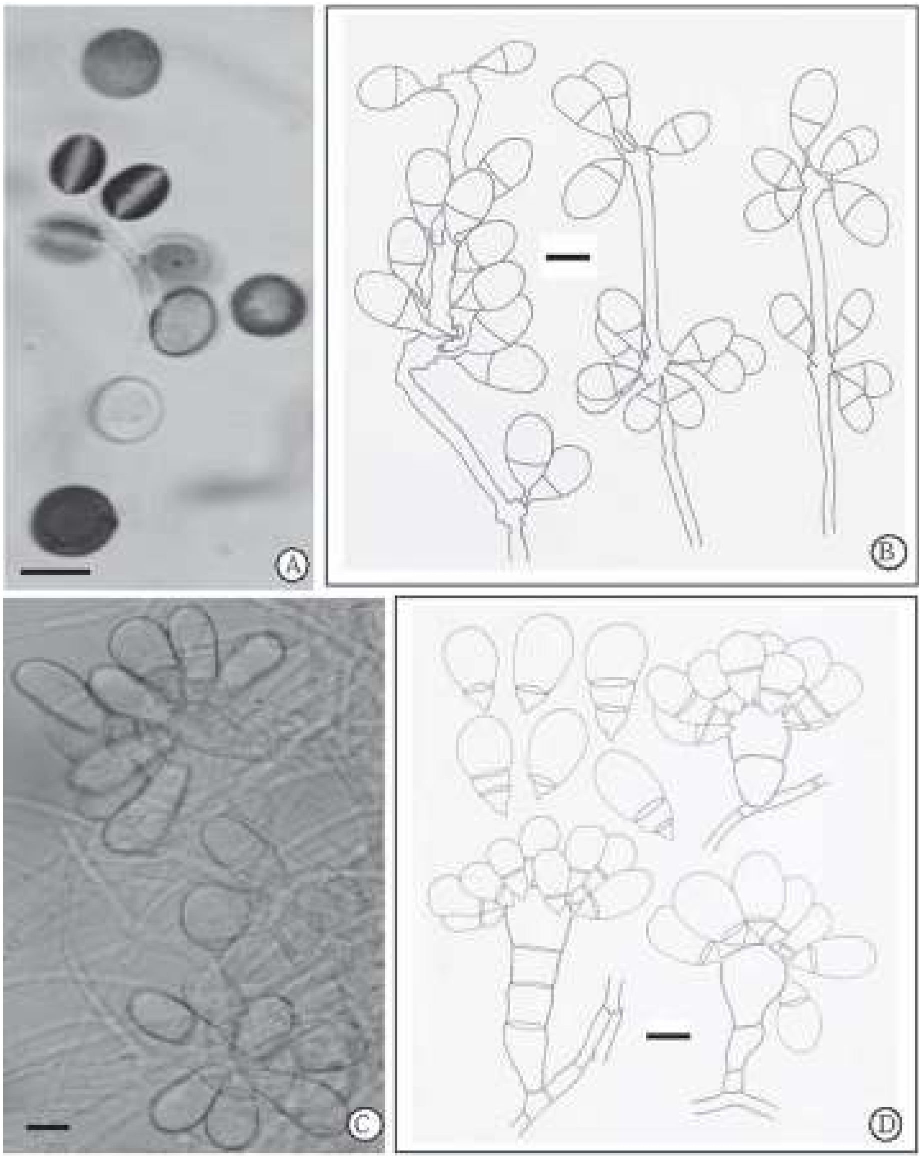

Figure 1 Light photomicrographs and camera lucida drawings of conidiophores and conidia A. *Arthrinium phaeospermum* (KUFC 2890), B. *Arthrobotrys oligospora* (KUFC 2894), C-D. *Cephaliophora irregularis* (KPFC 3034) (bars: A, B, D = 10 µm; C = 20 µm).

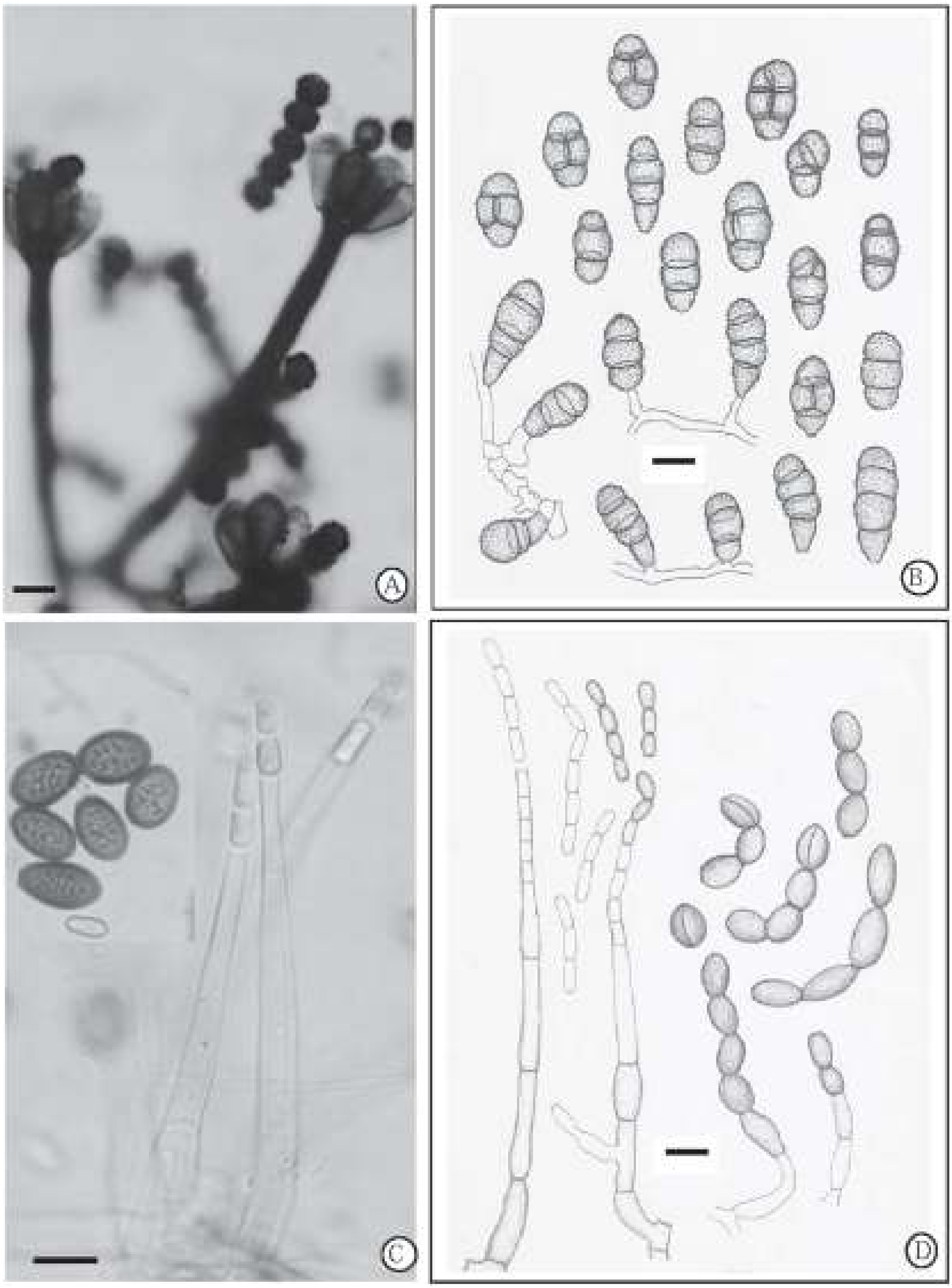

Figure 2 Light photomicrographs and camera lucida drawings of conidiophores and conidia. A. *Memnoniella echinata* (KUFC 3089), B. *Pithomyces karoo* (KUFC 3214), C-D. *Thielaviopsis* state of *Ceratocystis paradoxa* (KPFC 3208) (bars: A = 5 µm; B, D = 10 µm; C = 15 µm).

Seifert *et al*. (1983) reported *Arthrinium phaeospermum* on millipede pellets, *Arthrobotrys oligospora* on sheep, frog, bird and horse dung, *Cephaliophora irregularis* on ass, mouse and monkey dung, *Cladosporium cladosporioides* on horse, *Memnoniella echinata* on cow and rabbit dung, *Nodulisporium* sp. on cow, *Oidiodendron* spp. on earthworm casts and horse dung. *Oidiodendron griseum* on toad dung, *Cephaliophora irregularis* was mostly found on cow dung, *Arthrobotrys oligospora* on cow and deer dung, *Memnoniella echinata* on cow and horse dung, and *Nodulisporium gregarium* on cow dung, which was similar to the report of Seifert *et al*. (1983).

Manoch *et al*. (1999) recorded two isolates of *C. irregularis* on rabbit and toad dung and five isolates of *A. candidus* from deer, banteng, bird and chicken dung from Huay Khang Wild Life Sanctuary, Uthaithani province.

Manoch *et al*. (2001) reported *Nodulisporium* sp. on corn leaves and other graminaceous hosts as well as endophytic fungus on the leaves of the terrestrial orchid, *Lusidia discolor*. *Nodulisporium* is the anamorph state of *Hypoxylon* (Ju and Rogers, 1996) and *Biscogniauxia* (Hanlin, 1998).

Studies on hyphomycete secondary metabolites causing human and animal diseases have been published by many researchers. de Hoog *et al*. (2000) reported *Arthrinium phaeospermum* as the cause of cutaneous infection and onychomycosis. The fungus produces 3–nitropropionic acid which is toxic to the nervous membrane (Wei *et al*, 1994). *Scopulariopsis brevicaulis* is the anamorph of *Microascus brevicaulis* Abbott. It has been reported as frequently involved in onychomycoses in human and animal (De Hoog *et al*., 2000). Tanaka *et al*. (1997) reported nigrosporins A and B from *Nigrospora oryzae*. These compounds represent new phytotoxic and antibacterial metabolites. Isaka *et al.* (1999) reported antimalarial activity of macrocyclic trichothecenes from *M. verrucaria* BCC 112 isolated from soil in Kanchanaburi province, Thailand.

Yoganathan *et al.* (2003) reported two new compounds, 10-methoxydihydrofuscin and fuscinarin, and one known compound, fuscin from the soil fungus *Oidiodendron griseum*. These compounds can compete effectively with macrophage inflammatory protein (MIP)-1 alpha for binding to human CCR5, an important anti HIV-1 target that interferes with HIV entry into cells.

Paecilomyces lilacinus can produce indole-3-acetic acid which stimulates the growth of barley seedlings (Domsch *et al*., 1993). Samson *et al.* (2002) reported secondary metabolites from *F. oxysporum* such as fusaric acid, moniliformin, naphthoquinone pigment and nectriafurone *F. solani* produced mycotoxin, fusaric acid and naphthoquinone pigments. *Memnoniella echinata* can produce griseofulvins and the profiles of toxic compounds.

Klich (2002) reported patulin and cytochalasin-E from *Aspergillus clavatus*; ochratoxin A from *A. niger*; patulin, citrinin, citreoviridin and gliotoxin from *A. terreus*. *A. fumigatus* is a human and animal pathogen, responsible for systemic mycoses usually resulting from invasion of the lungs or respiratory tract. It was reported from a wide range of substrates in both indoor and outdoor environments including soil, plants, seeds, sludge, wood chips, compost, cotton and even penguin excreta. *A. flavus* is the commonest species which is the most carcinogenic mould producing aflatoxin B_1 toxigenic to the male rats (Moss, 2002a).

Moss (2003) stated that *Stachybotrys atra* was originally implicated in stachybotryotoxicosis of farm animals, especially horses fed on contaminated mouldy hay, and occasionally with the people handling such hay.

Exserohilum rostratum was recorded as the pathogen causing nasal phaeohyphomycosis, keratitis, subcutaneous and invasive infections in human and several animal infections (de Hoog *et al*., 2000). However, Chandramohan and Charudattan (2001) studied the biological control of seven grasses using a mixture of *Drechslera gigantea*, *Exserohilum longirostratum* and *E. rostratum*, isolated from large crabgrass (*Digitaria sanguinalis*), crowfootgrass (*Dactyloctenium aegyptium*) and Johnsongrass (*Sorghum halepense*), respectively in Florida. The results indicated that all seven grasses including crowfootgrass, guineagrass, Johnsongrass, large crabgrass, southern sandbur, *Texas panicum* and yellow foxtail were susceptible to each of the pathogens and the pathogen mixture. *E. rostratum* was recorded from corn leaf in Thailand (Manoch *et al*., 2001).

Domsch *et al*. (1993) stated that *Phialophora* is a heterogeneous assemblage of anamorphs of unrelated ascomycetes, including *Pyrenopeziza*, *Mollisia*, *Ascocoryne*, *Coniochaeta* and *Gaeumannomyces*. Manoch *et al*. (1999) reported *Coniochaeta* from deer dung, but no anamorphic state.

Ellis (1976) reported *Pithomyces karoo* from *Avena*, *Gnidia* and *Rhigozum* in S. Africa and from wheat rhizosphere in Australia. Houbraken *et al*. (2006) stated that *Pithomyces karoo* and *P. quadratus* could produce atlenuene and some alternariols. *P. karoo* represents a new record of hyphomycetes in Thailand.

Domsch *et al*. (1993) stated that *Trichoderma hamatum*, in contrast to other *Trichoderma* species, was totally ineffective against *Armillaria mellea*. Antifungal activity of volatile and non-volatile metabolites from *T. hamatum* was reported. *T. harzianum* is common on soil and rhizosphere of various plants (Domsch *et al*., 1993). In Thailand, *T. harzianum* was reported as a biological agent against control various plant diseases caused by *Pythium aphanidermatum*, *Phytophthora* spp., *Rhizoctonia solani* and *Sclerotium rolfsii* (Chamswarng and Tanangsnakool, 1996; Chamswarng *et al*., 2001). This fungus was reported to produce secondary metabolites such as azaphilone, harzianolide, butenolide and harzianopyridone (Vinale *et al*., 2006).

CONCLUSIONS

A total of 406 isolates, comprising 28 species of 24 genera of hyphomycetes were recorded from 60 dung samples of 13 animals collected from 18 provinces. Isolates were most frequent on cow dung among domestic animals and elephant dung for wild animals. The moist chamber, Warcup's direct plating and dilution plate methods yielded the highest numbers of species of hyphomycetes, whilst alcohol and heat treatment methods showed the fewer species.

Most common hyphomycetes on animal dung were *Paecilomyces lilacinus*, *Aspergillus fumigatus*, *A. niger*, *A. flavus*, *Scopulariopsis brevicaulis*, *Fusarium oxysporum* and *F. solani*. Five taxa including *Nigrospora oryzae*, *Nodulisporium gregarium*, *Oidiodendron griseum*, *Stachybotrys atra* and *Thielaviopsis* state of *Ceratocystis paradoxa* were found on only one type of dung.

Interesting species of hyphomycetes representing new records for Thailand were *Nodulisporium gregarium*, *Oidiodendron griseum* and *Pithomyces karoo*. True coprophilous hyphomycetes are *Arthrobotrys oligospora*, *Aspergillus candidus*, *Cephaliophora irregularis* and *Oidiodendron griseum*.

Six species of plant pathogenic fungi were found on animal dropings, including *Alternaria alternata*, *Curvularia lunata*, *Exserohilum rostratum*, *Fusarium oxysporum*, *F. solani*, *Nodulisporium gregarium* and *Thielaviopsis* sp.

Pathogenic hyphomycete causing diseases to human and animal were *Aspergillus clavatus*, *A. fumigatus*, *A. flavus*, *A. niger*, *Penicillium* spp., *Pithomyces karoo*, *Memnoniella echinata* and *Stachybotrys atra*.

Hyphomycetes known to be capable of produing secondary metabolites were *Acremonium*, *Arthrinium phaeospermum*, *Aspergillus clavatus*, *Memnoniella echinata*, *Myrothecium verrucaria*, *Oidiodendron griseum*, *Paecilomyces lilacinus* and *Stachybotrys atra*.

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