DIVERSITY OF COPROPHILOUS FUNGI, ANTAGONISM AGAINST PLANT PATHOGENIC FUNGI, AND SECONDARY METABOLITES OF *Ascodesmis macrospora* AND *Sordaria fimicola*

INTRODUCTION

Coprophilous fungi can survive thermal and chemical effects in herbivore digestive tract. They can adapt to exist in the very rich substrate and show a distinct morphological feature. Herbivorous animals grazing on vegetation ingest many fungal spores along with their food. The high temperature and gastric juices in the gut of the animals evidently destroy most of the spores of the non-coprophilous species, but the spores of the coprophilous fungi are protected in various ways from the action of the gut enzymes. Once the dung is voided fungal spores on to the surrounding herbage where by good fortune they may be eaten by a herbivore, so continuing the cycle of events (Webster, 1970; Wicklow and Moore, 1974; Parker, 1979; Bell, 1983, 2005; Ebersohn and Eicker, 1992; Richardson and Watling, 1997; Richardson, 2001b).

Coprophilous fungi is a large group of saprobic fungi. Subramanian (1983) stated that Saccardo in his *Sylloge* enumerated 757 species belonging to 187 genera as coprophilous fungi and most of them were recorded from herbivore excreta. The number has increased as the results of the researches from different parts of the world. Further species were described by Cain (1934), Sutton (1980), Bell (1983, 2005), Seifert *et al.* (1983), Nag Raj (1993), Richardson and Watling (1997), Richardson (2001a, 2001b). Currently 1,200 species of coprophilous fungi from 260 genera have been recorded

worldwide (Kirk *et al.*, 2001), whilst approximately 130 species have been reported from dung in Thailand (Somrithipol, 2004).

Ascomycetes are commonly found on dung of herbivorous animals (Bell, 1983, 2005). Some are important sources of antibiotics, organic acids, enzymes, and other secondary metabolites of economic importance to pharmaceutical and agricultural enterprises, e.g. *Ascodesmis sphaerospora* and *Sporormiella vexans* (Hein *et al.*, 1998; Soman *et al*., 1999). However, coprophilous fungi have been little studied in Thailand. Van Brummelen (1967, 1969, 1977) reported several coprophilous ascomycetes from Thailand, including *Ascobolus siamensis*, *A. demangei*, *Saccobolus minimus*, *S. thaxteri*, *S. truncatus*, *S. succineus* and *Leptokalpion albicans* from various kinds of dung. Rogers *et al*. (1998) described *Podosordaria elephati* as a new species on elephant dung from Chachoengsao Province. And Manoch *et al*. (1999) reported 19 coprophilous ascomycetes from 12 dung samples from Huay Kha Khang Wild Life Sanctuary, Uthai Thani Province and Khao Yai National Park, Nakhon Ratchasima Province. Somrithipol (2004) summarized the approximately 26 genera including 36 species coprophilous ascomycetes known in Thailand.

Subramanian (1983) stated that Hyphomycetes are a normal component of the mycoflora of dung and animal excrements, including birds and mammals, both herbivores and carnivores. Bell (1983) mentioned three Hyphomycetes from dung : *Athrobotrys oligospora*, *Penicillium claviforme* and *Sepedonium* sp. A key to Hyphomycetes on dung was developed by Seifert *et al*. (1983). Several new genera of Hyphomycetes from dung were established by Subramanian and his associates. He noted that a great many Hyphomycete that occur on dung are anamorphs of the discomycetes and the pyrenomycetes.

Antagonistic activity test of *Trichoderma harzianum*, *Chaetomium cupreum* and *Talaromyces flavus* against plant pathogenic fungi *in vitro* and in the greenhouse have been reported (Chamswarng *et al*., 1996, 2001; Madi *et al*., 1997; Soytong, 2004; Dethoup *et al*., 2007). In addition, *Sordaria fimicola* has been recorded as antagonistic agent against soil-borne plant diseases caused by *Pythium aphanidermatum* and *Dematophora necatrix* (Watanabe, 1991). Dewan *et al*. (1994) reported that *S. fimicola,* isolated from wheat and rye-grass roots could reduce the mortality of these hosts after inoculation with the take-all fungus (*Gaeumannomyces graminis* var. *tritici*).

The studies on secondary metabolites of some coprophilous fungi have been reported (Soman *et al*., 1999; Che *et al*., 2001; Kuwahara *et al*., 2005). Arugosin F and xanthone from *Ascodesmis sphaerospora* on bison dung in Canada have been reported by Hein *et al*. (1998). They found that these compounds were active against *Bacillus subtilis* and *Staphylococcus aureus.* Arugosin F has been described previously as a metabolite from *Cyathus intermedius*. This compound differs from arugosin A-E and other arugosins, metabolites of *Aspergillus variecolor, A. rugulo*sa and *A. silvaticus* in the lack of prenyl and oxyprenyl substituents at position 2 and 7, respectively.

It is very interesting to study coprophilous fungi in Thailand, because these fungi 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. Hence, more investigations on coprophilous fungi need to be carried out in this tropical region for the discovery of new taxa, the use of some species as biological control agents against plant pathogenic fungi and the analysis for secondary metabolites of dung fungi is a very challenging topic for industrial, phamaceutical and agricultural enterprises.

Objectives

1. To isolate and identify coprophilous and other microfungi from dung of various wildlife and domestic animals from different locations in Thailand.

2. To study on diversity and distribution of coprophilous fungi associated with different dung types from various locations.

3. To maintain the herbarium specimen and pure cultures of coprophilous fungi at Kasetsart University Fungal Collection.

4. To study antagonistic activity test of *Ascodesmis macrospora* and *Sordaria fimicola* against nine species of plant pathogenic fungi *in vitro*.

5. To investigate the secondary metabolites of two species of coprophilous fungi, *Ascodesmis macrospora* and *Sordaria fimicola*.

LITERATURE REVIEW

1. Diversity and taxonomic study of coprophilous fungi

Cain (1956) reported three new species of *Tripterospora*, including *T. brevicaudata*, *T. erostrata* and *T. longicaudata* on hose and rabbit dung from Canada. *Tripterospora* differs from *Podospora* and *Sordaria* because *Tripterospora* produce ovoid non ostiolate ascocarps, and non-gelatinous appendage and sheath of ascospores.

Cain (1968) studied coprophilous Ascomycetes, Order Sphaeriales on cattle, horse, hare, deer and several hebivorous dung collected from different locations in Ontario, Canada. The morphological studies of the following species were reported: *Bombardia scortea, B. caerulea, B. coprophila, B. muskokensis, B. arachnoidea, Coniochaeta leucoplaca, C. discospora, C. scatigena, C. hansenii, C. multispora, C. philocoproides, C. saccardoi, Delitschia didyma, D. leporina, D. timagamensis, D. marchalii, D. vulgaris, D. araneosa, D. auerswaldii, D. winteri, D. griffithsii, D. leptospora, D. gigaspora, D. furfuracea, D. bisporula, Hypocopra amphisphaerioides, H. equorum, H. merdaria, Phomatospora hyalina, Pleophragmia leporum, P. ontariensis, Sordaria fimicola, S. humana, S. macrospora, S. bombardioides, S. minima, S. rabenhorstii, S. barbata, S. montanensis, S. septospora, S. hypocoproides, S. neglecta, S. kansensis, S. inaequilateralis, S. leporina, S. ontariensis, S. taenioides, S. fimiseda, S. appendiculata, S. cervina, S. tetraspora, S. minuta, S. curvula, S. coronifera, S. glutinans, S. dubia, S. linguiformis, S. decipiens, S. pleiospora, S. vestita, S. hyalopilosa, S. zygospora, S. anserina, S. pilosa, S. piriformis, S. perplexens, S. eminens, S. araneosa, S. setosa, S. curvicolla, S. similis, S. globosa, S.*

carbonaria, Sporormia pulchella, S. lata, S. pilosa, S. pilosella, S. minima, S. australis, S. intermedia, S. muskokensis, S. obliquisepta, S. dakotensis, S. leporina, S. ambigua, S. megalospora, S. longispora, S. vexans, S. heptamera, S. affinis, S. corynespora, S. Zygospermum setosum octomera, S. ontariensis, S. pascua, S. bipartis, S. splendens, S. venusta, S. herculea and *Z. insignis.*

In addition, Cain and Mirza (1970) described the characteristic of the genus *Apodospora* found from hare and rat dung. This fungus was recorded as the new genus in Family Sordariaceae. *Apodospora* produces dark brown perithecium with ostiole, ascus containing 8 ascospores. Ascospore one cell, dark or hyaline with gelatinous sheath and one germ pore at the end. The morphological characteristics of three species of *Apodospora* were recorded, including *A. simulans* (type species) from rat dung, *A. thescelina* and *A. viridis* from hare dung were recorded. However, Kirk *et al.* (2001) placed *Apodospora* in the Family Lasiosphaeriaceae, and 4 species were recorded as coprophilous fungi from North America and Europe.

Ahmed and Cain (1972) reported the revision of the two genera, *Sporormia* and *Sporormiella* found on several dung from Canada. They studied three species of *Sporormia*, including *S. fimetaria, S. fimicola* and *S. mirabilis*, and 35 species of *Sporormiella*, including *S. affinis, S. americana, S. antarctica, S. australis, S. bipartis, S. capybarae, S. chaetomioides, S. commulata, S. corynespora, S. dakotensis, S. heptamera, S. herculea, S. insignis, S. irregularis, S. kansensis, S. lageniformis, S. lata, S. leporina, S. longispora, S. megalospora, S. minima, S. muskokensis, S. ontariensis, S. ovina, S. pascua, S. pentamera, S. pilosa, S. pilosella, S. polymera, S. pulchella, S. pyriformis, S. scandinavica, S. schoterriana, S. splendens* and *S. vexans.*

Richardson (1972) studied coprophilous Ascomycetes from 137 dung samples, including sheep, horse, cattle, deer and hare from the UK. Most coprophilous fungi were found from herbivorus dung, such as *Lasiobolus ciliatus*, *Phomatospora coprophila*, *Ascophanus microsporus*, *Podospora curvula*, *Coprobia granulata* and *Ascobolus immersus*, whereas *Podospora appendiculata*, *Thelebolus stercoreus* and *Sporormia intermedia* were found on lagomorph dung. However, *Thelebolus nanus*, *Podospora vesticola*, *Ascobolus albidus* and *Saccobolus versicolor* were found from all dung. The results of this study showed the relation of growth rate between numerous of the species and richness of nutrition on dung. The investigation showed the discovery of rare species, for example *Trichobolus zukalii*, *A. carletonii*, *A. brassicae*, *Sporormia bipartis*, *S. vaxans*, *S. fimetaria*, *Zygospermella insignis*, *P. dagobertii* and *Mycorhynchus petchii*.

The genus *Podospora* is one of the most interesting genera of Ascomycetes found on hebivorous dung. Khan and Cain (1972) studied morphological characteristic of *Podospora* on several dung from East Africa. Five new species were recorded, including *Podospora caligata* on cow dung from Tanzania, *P. papillata* on elephant and zebra dung from Kenya, *P. deropodalis* on zebra dung from Kenya, *P. spinulosa* on cow, elephant and zebra dung from Kenya and Tanzania, and *P. multispora* on cow dung from Kenya. These species were found in moist chamber.

Podosordaria (Order Xylariales) belong to Class Ascomycetes, very close to *Podospora* and *Sordaria*. The fungus produces conspicuous stroma on dung, consisting the group of perithecia. Krug and Cain (1974) reported five new species of *Podosordaria*, including *P. crinita* on cow dung from America, *P. ianthina* on goat dung from Mexico, *P. phoenicea* on zebra dung from Kenya, *P. vinacea* on cow dung

from Mexico and *P. violacea* (*Sordaria violacea*) on cow, sheep and rabbit dung from Canada and the United States. In addition, ten species of *Podosordaria* were recorded, including *P. crinita, P. hircina, P. ianthina, P. leporina, P. mexicana, P. pedunculata, P. phoenicea, P. tulasnei, P. vinacea* and *P. violacea.*

Wicklow and Moore (1974) studied the effect of temperature and incubation period in moist chamber for the production of fruiting body of coprophilous fungi on rabbit dung in America. The results revealed the diversity of coprophilous fungi at different temperature. *Sporormiella intermedia* and *Thelebolus* spp. were found on dung at 10 ^{o}C. *Ascodesmis nigricans, Coprotus granuliformis* and *Podospora curvicolla* were found on dung at 24 ^{o}C, whereas *Sordaria fimicola* and *Sporormiella minima* were found at 10, 24 and 37.5 ^{o}C. The incubation period for the production of fruiting body of coprophilous fungi depended on the temperature, for example *Ascobolus mancus* and *Sordaria fimicola* produced ascoma and ascospores after 10 days incubation at 24 ^{o}C, whereas at 10 ^{o}C they produced ascoma and ascospores after 20 and 30 days incubation respectively. The optimum temperature for the production of ascoma and ascospore was at 37.5 ^{o}C. The ascospores of *Ascobolus*, *Sordaria*, *Sporormiella* and the basidiospores of *Coprinus* were formed at 10 days incubation, whereas ascospores of *Kernia* and *Podospora* were found after 15 days incubation at 37.5 ^{o}C.

Dickinson and Underhay (1977) studied the growth of hyphae and ascospore production of coprophilous fungi from cow dung *in vitro*. The experiment was conducted with the control optimum environment for ascospores production. The results indicated that the development of fruiting body and ascospore production depended on water content in dung samples. The succesion of coprophilous fungi on

dung were found starting with the Zygomycetes, Hyphomycetes, Ascomycetes and Basidiomycetes respectively.

Jeng and Krug (1977) reported coprophilous Discomycetes in Order Pezizales from Argentina and Venezuela. They devided into two families belonging to Ascobolaceae and Pyronemataceae. The Family Ascobolaceae included *Ascobolus americanus*, *A. crenulatus*, *A. immerses*, *A. lineolatus*, *A. scatigenus*, *Saccobolus depauperatus*, *S. germinatus*, *S. glaber*, *S. infestans*, *S. minimus*, *S. verrucisporus*, *Iodophanus carneus* and *I. venezuelensis*. The Family Pyronemataceae were recorded, including *Ascodesmis macrospora*, *A. nigricans*, *A. porcina*, *A. sphaerospora*, *Coprotus aurora*, *C. baeosporus*, *C. granuliformis*, *C. lacteus*, *C. leucopocillium*, *C. luteus*, *C. niveus*, *C. ochraceus*, *Fimaria trochospora*, *Lasiobolus ciliatus*, *L. microsporus*, *L. trichoboloides*, *Scutellinia scutellata* and *Thelebolus caninus*.

Parker (1979) studied coprophilous Ascomycetes from 163 dung samples of domestic and wild animals, including horse, cattle, goat, rabbit and deer in Illinois, U.S.A. A total of 41 genera of coprophilous Ascomycetes were reported in this study. *Lasiobolus ciliatus*, *Saccobolus depauperatus*, *Sporormiella teretispora* and *Tripterospora longicaudata* were found on horse dung. Three species were found on rabbit dung, including *Podospora appendiculata*, *P. dakotensis* and *P. tetraspora*. Four species were recorded on deer and rabbit dung, such as *Arnium leporinum*, *Ascobolus crenulatus*, *Saccobolus globuliferellus* and *Thelebolus stercoreus*, whereas *Iodophanus carneus* was found on many kinds of dung. Moreover, some species showed specificity with type of animal dung. *Ascobolus albidus*, *A. aglasporus*, *Coprotus glaucellus*, *C. sexdecimsporus*, *Saccobolus versicolor*, *Sporormiella intermedia* and *S. leporina* were only found on horse and deer dung. *Sordaria*

fimicola and *Podospora curvicolla* were found on horse and rabbit dung, whereas *Kernia nitida* was found on all type of animal dung samples, but did not found on rabbit dung.

Bell (1983) studied coprophilous fungi in New Zealand. Seventeen kinds of dung samples namely avian, bison, camel, cat, cattle, cervid, chamois, elephant, goat, horse, insect, lagomorph, lama, pig, possum, sheep and wallaby were collected from various locations in New Zealand. The keys for identification on the Zygomycetes, Fungi Imperfecti, Ascomycetes and Basidiomycetes were proposed with line drawings. The fungal succession was studied on herbivorous dung. Coprophilous Discomycetes producing apothecia were *Ascobolus*, *Ascozonus*, *Cheilymenia*, *Coprobia*, *Coprotus*, *Fimaria*, *Iodophanus*, *Lasiobolus*, *Peziza*, *Saccobolus*, *Thecotheus*, *Thelebolus* and *Trichobolus.* Coprophilous Plectomycetes producing cleistothecia included *Anixiella*, *Cleistothelebolus*, *Copromyces*, *Nigrosabulum*, *Preussia* and *Zopfiella*. Coprophilous Pyrenomycetes producing perithecia were *Apiosordaria*, *Bombarbiodea*, *Cercophora*, *Chaetomium*, *Coniochaeta*, *Delitschia*, *Hypocopra*, *Melanospora*, *Podospora*, *Poronia*, *Selinia*, *Sordaria*, *Sphaeronaemella*, *Sporormia*, *Sporormiella* and *Zygopleurage* etc. In addition, she studied the development of asci (uniseriate and biseriates) and characteristics of ascospores. Coprophilous Basidiomycetes were reported in this study, including *Bolbitius*, *Coprinus*, *Conocybe*, *Crucibulum*, *Lysurus* and *Psilocybe*, whereas coprophilous Zygomycetes were *Mucor*, *Phycomyces*, *Pilaira*, *Pilobolus* and *Piptocephalis*, *Thamnostylum*.

Seifert *et al*. (1983) stated that Hyphomycetes may be not true coprophilous fungi, but contaminants arriving from the air or soil, after the dung has been deposited.

However, they also stated that some Hyphomycetes are known only from dung, such as *Arthrobotrys*, *Basifimbria* and *Oedocephalum*.

Krug and Khan (1991) described a new taxon, *Dictyocoprotus mexicanus* in the Family Pyronemataceae, Order Pezizales. This fungus was found on rodent dung. It produces reticulate ascospores ornamentation that different from the genus *Coprotus*. Eight genera of coprophilous Discomycetes were recorded in China, including *Ascobolus*, *Cheilymenia*, *Coprotus*, *Iodophanus*, *Lasiobolus*, *Peziza*, *Pseudombrophila* and *Saccobolus* (Wang *et al*., 2000). *Pseudombrophila xiangchengensis* is a new record for China.

Bell and Mahoney (1995) studied *Podospora* from dung of various herbivorous animals in New Zealand. Eight species of *Podospora* with swollen agglutinated perithecial hairs were found, including *P. aloides*, *P. conica*, *P. curvuloides*, *P. dakotensis*, *P. glutinans*, *P. miniglutinans*, *P. tetraspora* and *P. vesticola*. In addition, Bell and Mahoney (1996) reported the study on perithecium development in *P. tetraspora* and *P. vesticola* from rabbit dung collected in New Zealand.

Richardson and Watling (1997) reported coprophilous fungi from hebivorous dung in England and classified to Ascomycetes, Basidiomycetes and Zygomycetes. Ascomycetes are divided into three major taxa based on the shape of ascocarp, including perithecium (pseudothecium), apothecium and cleistothecium (gymnothecium). Coprophilous Ascomycetes producing ascoma apothecia were *Ascobolus*, *Ascozonus*, *Ascophanus*, *Cheilymenia*, *Coprobia*, *Coprotus*, *Fimaria*, *Iodophanus*, *Lanzia*, *Lasiobolus*, *Orbilia*, *Peziza*, *Pezizella*, *Saccobolus*, *Thecotheus*, *Thelebolus* and *Trichobolus*, while perithecia or pseudothecia producing Ascomycetes

included *Anopodium*, *Apiosordaria*, *Arnium*, *Bombardioidea*, *Cercophora*, *Chaetomium*, *Coniochaeta*, *Delitschia*, *Gelasinospora*, *Hypocopra*, *Lophotrichus*, *Melanospora*, *Microascus*, *Nectria*, *Onygena*, *Podosordaria*, *Podospora*, *Poronia*, *Pyxidiophora*, *Schizothecium*, *Selinia*, *Sordaria*, *Sphaerodes*, *Sporormia*, *Sporormiella*, *Trichodelitschia*, *Viennotidia*, *Wawelia* and *Zygospermella*. Ascoma type of gymnothecium were *Actinodendron*, *Arachniotus*, *Arthroderma*, *Ctenomyces*, *Gymnoascus*, *Myxotrichum*, *Pseudogymnoascus*, whereas ascoma type of cleistothecium were *Aphanoascus*, *Arachnomyces*, *Copromyces*, *Preussia*, *Heleococcum*, *Kernia*, *Lasiobolidium*, *Mycoarachis*, *Orbicula*, *Pleuroascus*, *Pseudeurotium*, *Roumegueriella*, *Thielavia* and *Zopfiella*. Basidiomycetes were classified based on fruiting body, type and colour of spore print, characteristic of gill, size and shape of basidiospore. Coprophilous Basidiomycetes were *Agrocybe*, *Athelia*, *Bolbitius*, *Clitocybe*, *Conocybe*, *Coprinus*, *Cristella*, *Cyathus*, *Lepista*, *Leucocoprinus*, *Panaeolus*, *Platygloea*, *Pleurotellus*, *Psathyrella*, *Psilocybe*, *Sebacina*, *Sphaerobolu*, *Stropharia*, *Typhula* and *Volvariella*. Zygomycetes were classified based on sporangiophore, sporangium and sporangiospore. Coprophilous Zygomycetes comprised of *Absidia*, *Actinomucor*, *Ballocephala*, *Cunninghamella*, *Coemansia*, *Chaetocladium*, *Helicostylum*, *Kickxella*, *Mucor*, *Mycotypha*, *Phycomyces*, *Pilobolus*, *Piptocephalis*, *Rhizopus*, *Syncephalastrum*, *Syncephalis* and *Thamnidium.*

Lundqvist *et al.* (1999) reported *Podospora austrohemisphaerica*, a new heterothallic ascomycetes from dung of wild and domestic herbivores. Twenty-five isolates are cited, including 23 from Argentina, New Zealand and Australia and 2 from England. The species is characterized by *Phialophora* anamorph, perithecia with rigid neck hairs and large ascospores with gelatinous sheaths and multiple caudae both at the spore extremities and at the proximal end of the lower cell. Ascospore

germination, sexual compatibility, intraspecific variation, distribution and relationships to other species of *Podospora* were discribed.

Delgado *et al.* (2000) reported *Zygopleurage zygospora*, as a new record from calf dung in Venezuela. This fungus was classified to the Family Lasiosphaeriaceae, Order Sordariales, Class Ascomycetes. In addition, this fungus has been reported from Northern Europe, Canada and the United States.

Ramos *et al*. (2000) studied the isoenzyme analysis of *Saccobolus* in Argentina. Esterases were the only consituents in which differences between some geographical isolates were observed within the same species, resulting in a total of 15 band pattern. The results of isozyme analysis and morphological characteristic could identified the genus *Saccobolus* (Order Pezizales, Class Ascomycetes) at a total of 114 isolates into two sections belonging to the Section *Saccobolus* (*S. citrinus*, *S. longevisporus*, *S. succineus*, *S. platensis*, *S. saccoboloides* and *S. truncates*) and Section Eriobolus (*S. depauperatus*, *S. verrucisporus*, *S. infestans* and *S. seudodep*).

Wang (2000) reported eight species of *Podospora* on cow, sheep, goat and rabbit dung from Taiwan, including *P. anserina*, *P. argentinensis*, *P. communis*, *P. aff-conica*, *P. curvicolla*, *P. curvuloides*, *P. dakotensis*, *P. decipiens*, *P. dolichopodalis*, *P. fimiseda*, *P. formosana*, *P. globosa*, *P. hyalopilosa*, *P. inflatula*, *P. longicaudata*, *P. myriaspora*, *P. prethopodalis* and *P. setosa.*

Webster and Weber (2000) reported the development of subterranean rhizomorphs which are rare among Ascomycetes, and the production of mature

perithecial stromata of *Podosordaria tulasnei* (Family Xylariaceae) on rabbit pellets collected from the filed in the UK.

Richardson (2001a) studies the diversity and occurrence of coprophilous fungi in the UK using the moist chamber method. The results showed that the genus and species of coprophilous fungi varied with different types of dung, location of collecting site, and season of collecting period. A total of 425 samples of animal dung were used in this research, including sheep, cattle, deer, rabbit, and hare. *Coprinus stercoreus* (Basidiomycetes) was found on sheep, cattle, deer and rabbit, whereas *Coprinus miser*, *Ascobolus* spp., *Saccobolus versicolor, Thelebolus* spp., *Iodophanus carneus* and *Lasiobolus cuniculi* were found on all dung. Coprophilous Pyrenomycetes especially *Schizothecium*, *Podospora*, *Coniochaeta* and *Sporormiella* accounted for 50% of all records.

Richardson (2001b) reported diversity and species richness of coprophilous fungi from seven dung samples of capybara (1 sample), cattle (1), deer (1), horse (2) and sheep (2) collected from the State of Matto Grosso do Sul, Brazil. Thirty-two species of coprophilous fungi were recorded, including *Ascobolus immersus*, *Cercophora mirabilis, Coprinus cordisporus, C. curtus*, *C. heptemerus, C. pellucidus*, *C. radiatus*, *C. stercoreus*, *Coprinus* sp., *Coprotus lacteus*, *Cyathus stercoreus*, *Iodophanus carneus*, *Phomatospora minutissima*, *Pilobolus crystallinus*, *P. sphaerosporus, Podospora argentinensis, P. communis*, *P. inflatula*, *P. pauciseta*, *Podospora* sp., *Poronia oedipus*, *Ryparobius polysporus*, *Saccobolus citrinus*, *S. depauperatus, S. truncatus, S. verrucisporus, S. versicolor, Selinia africana, S. pulchra, Sporormiella cf. Megalospora, S. minima* and *Zygopleurage zygospora.*

Dokmetzian and Ranalli (2002) studied a natural mutant of *Ascobolus michaudii* (Ascomycotina, Pezizales) from Argentina. Mature apothecia of *A. michaudii* were obtained from cow dung collected in Ciudad Universitaria and developed in a moist chamber. Wild strains of *A. michaudii* produce 8-spored asci and uninucleate ascospores with thick longitudinal anastomosing lines in the exosporium. A natural mutant in heterozygous condition with ascospores showing a thin verrucosely ornated exosporium was isolated.

Adamonyte (2003) described *Trichia papillata* from moist chamber cultures of hare (*Lepus* sp.) and roe deer (*Capreolus capreolus*) dung collected from the southern Lithuania, a new species of coprophilous Myxomycetes. This species is characterise by angular peridial plates with a central papilla, double-crested sprirals on the elaters and yellow spore mass.

Hennebert (2005) studied the variability in conidiogenesis of the coprophilous Hyphomycetes, *Basifimbria aurea* isolated from horse dung and correlated species, including *Rhinotrichum subalutaceum* and *Stenocephalopsis subalutacea*.

Elshafie (2005) studied coprophilous fungal mycobiodata of the Sultanate in Oman. Forty-five species belonging to twenty-five genera are reported. Most of the genera and species are new records for Oman. Twenty-one species are new records for the Arabian Peninsula, four species are new records for Asia, several dung types are new substrates. The most common species were *Iodophanus carneus* and *Sporormiella minima*. More than 50% of the species were found in Al-Batinah and Sahalah regions. Percentages of fungal species found on dung of camels, goats and cows were 58, 53 and 36 respectively.

Bell (2005) reported coprophilous Ascomycetes from dung of wild and domestic animals in Australia. A total of 49 genera and 199 species were recorded, including *Apiosordaria*, *Arnium*, *Ascobolus*, *Ascozonus*, *Cercophora*, *Chaetomium*, *Cheilymenia*, *Coniochaeta*, *Coprotus*, *Delitschia*, *Fimetariella*, *Gymnoascus*, *Hypocopra*, *Iodophanus*, *Kernia*, *Lasiobolidium*, *Lasiobolus*, *Melanospora*, *Mycoarctium*, *Nigrosabalum*, *Nigrosabulum*, *Ophiostroma*, *Orbilia*, *Petriella*, *Peziza*, *Phomatospora*, *Pleuroascus*, *Podosordaria*, *Podospora*, *Poronia*, *Preussia*, *Pseudoarachniotus*, *Pulvinula*, *Saccoblus*, *Saccobolus*, *Selinia*, *Semidelitschia*, *Sordaria*, *Sphaeronaemella*, *Sporormia*, *Sporormiella*, *Spororomiella*, *Strattonia*, *Thecotheus*, *Trichobolus*, *Trichodelitschia*, *Zopfiella*, *Zygopleurage* and *Zygospermella*.

Manoch *et al.* (1999) reported the diversity of coprophilous fungi from dung in Thailand. Dung sample of wild animals, such as elephant, banteng, deer, barking deer, tapin and bird collected from Khao Yai National Park, Huay Kha Khang Wild Life Sanctuary and Dusit Zoological Garden were used to investigate dung microfungi. Manure samples of domestic animals and amphibion such as cow, rabbit, chicken and toad were collected from various locations. The results revealed the presence of dung fungi of different groups. The first colonizers were the Zygomycetes of the genera *Circinella*, *Mucor*, *Pilobolus* and *Syncephalastrum*. The second colonizers belonged to the Ascomycetes and Deuteromycetes of the genera *Acremonium*, *Arachniotus*, *Ascodesmis*, *Aspergillus*, *Cephaliophora*, *Chaetomium*, *Coniochaeta*, *Coprotus*, *Delitschia*, *Eupenicillium*, *Eurotium*, *Fusarium*, *Gelasinospora*, *Hamigera*, *Heterocephalum*, *Humicola*, *Lophotrichus*, *Neosartorya*, *Nigrospora*, *Papularia*, *Penicillium*, *Podospora*, *Saccobolus*, *Scopulariopsis*, *Sordaria*, *Talaromyces*, *Thielavia*, *Trichoderma* and *Zopfiella*. The last colonizer was the Basidiomycetes of the genus *Coprinus*.

In addition, Manoch *et al.* (2000a, 2000b) reported microfungi collected from soil and dung from different forest types of Huay Kha Khang Wildlife Sanctuary in Uthai Thani province. Alcohol and heat treatment techniques were employed to isolate Ascomycetes from soil and dung samples. The results showed that 45 genera of microfungi were recorded. Five genera in the Order Sordariales, including *Anixiella*, *Chaetomium*, *Gelasinospora*, *Sordaria* and *Thielavia* were isolated. Five species of the genus *Chaetomiun* were isolated from soil and dung, including *C. cupreum*, *C. globosum*, *C. minutum*, *C. trilaterale* and *C. venezuelenze.* Two genera of Order Hypocreales, including *Nectria* and *Neocosmospora* and three genera of Order Dothideales (Sporormiaceae), including *Preussia*, *Sporormiella* and *Westerdykella* were isolated. One genus of Discomycetes, *Ascodesmis* was isolated from toad dung. Eight genera of Plectomycetes, including *Arachniotus*, *Emericella*, *Eupenicillium*, *Eurotium*, *Hamigera*, *Monascus*, *Neosartorya* and *Talaromyces* were recorded from soil and dung. The moist chamber method was used to isolate coprophilous Pyrenomycetes from dung. After a few days incubation, several ascomata were observed on deer, elephant, rabbit, tapin and chicken dung. The results indicated that coprophilous Pyrenomycetes were most frequent on dung, comprising *Cercophora*, *Chaetomium*, *Coniochaeta*, *Delitschia tomentosa*, *Gelasinospora*, *Lophotrichus*, *Podospora communis*, *P. curvicolla*, *P. anserina*, *Podospora* spp., *Sordaria fimicola*, *Thielavia* and *Zopfiella*. Two genera of operculate Discomycetes, *Coprotus leucopocillus* and *Saccobolus citrinus* were found on deer and cow dung, whereas one species of *Podosordaria* (Order Xylariales) could develop stromata on rabbit dung collected from Queen Sirikit Botanic Garden, Mae Rim, Chiang Mai Province.

Somrithipol and Hywel-Jones (2002) reported a preliminary investigation on coprophilous fungi in Northeastern Thailand. A total of 146 isolates were found on 85 dung samples of sambar deer, barking deer and Asian elephant collected from Khao Yai National Park, Nakhon Ratchasrima Province. They studied the fungal succession of coprophilous fungi on dung and found that Zygomycetes occurred after 3 days incubation, including *Cunninghamella*, *Pilobolus* and *Syncephalastrum*. Four to seven days later, the apothecial forming Ascomycetes, such as *Ascobolus* and *Saccobolus* were found. Perithecia of Pyrenomycetes e.g. *Chaetomium*, *Delitschia*, *Podospora*, *Poronia*, *Sordaria*, *Sporormia*, *Zygopleurage* and *Zygospermella* appeared after 7-14 days incubation and often associated with basidiocarps of *Coprinus.*

In addition, Somrithipol (2004) summarized that a total of 71 genera, 130 species of coprophilous fungi have been recorded in Thailand. The noteworthy of coprophilous fungi are *Ascobolus*, *Saccobolus*, *Chaetomium*, *Delitschia*, *Podospora*, *Sordaria*, *Sporormia*, *Zygopleurage*, *Zygospermella* and *Poronia*.

2. Antagonistic activity test of *Sordaria fimicola* and *Ascodesmis macrospora* against plant pathogens

Sordaria fimicola was recorded as antagonistic agent against soil-borne plant diseases caused by *Pythium aphanidermatum* and *Dematophora necatrix* (Watanabe, 1991). Dewan *et al*. (1994) reported that *S. fimicola*, isolated from wheat and rye-grass roots could reduce the mortality of these hosts after inoculation with the take-all fungus (*Gaeumannomyces graminis* var. *tritici*).

S. fimicola was first reported on horse dung in France in 1893 (Lundqvist, 1972). It is the commonest species among coprophilous pyrenomycetes found on dung of wild and domestic animals in many countries (Lundqvist, 1972; Bell, 1983, 2005; Manoch *et al.*, 1999; Richardson, 2001). However, the numerous reports of *S. fimicola* were found from various soil samples (Domsch *et al*, 1993; Manoch *et al.*, 2001).

Ascodesmis sphaerospora was reported from dung of various animals including dog, raccoon, wild boar and jaguar (Obrist, 1961). This species was recorded to produce arugosin F, an antibacterial agent which can inhibit the growth of *Bacillus subtilis* and *Staphylococcus aureus*. However, there has been no report on antagonistic activity test of *Ascodesmis macrospora* against plant pathogens (Hein *et al*., 1998).

3. Secondary metabolites from coprophilous fungi

Coprophilous fungi play an important ecological role in controlling other organisms, as well as decomposing and recycling nutrients from animal dung. This group of fungi has been reported to produce various novel secondary metabolites. Interestingly, antagonistic interactions among coprophilous fungi often involve the production of chemical agents by one species that inhibit the growth of another (Gloer and Truckenbrod, 1988). Researches on bioactive secondary metabolites isolated from coprophilous Ascomycetes were summarized (Table 1, Figure 1)

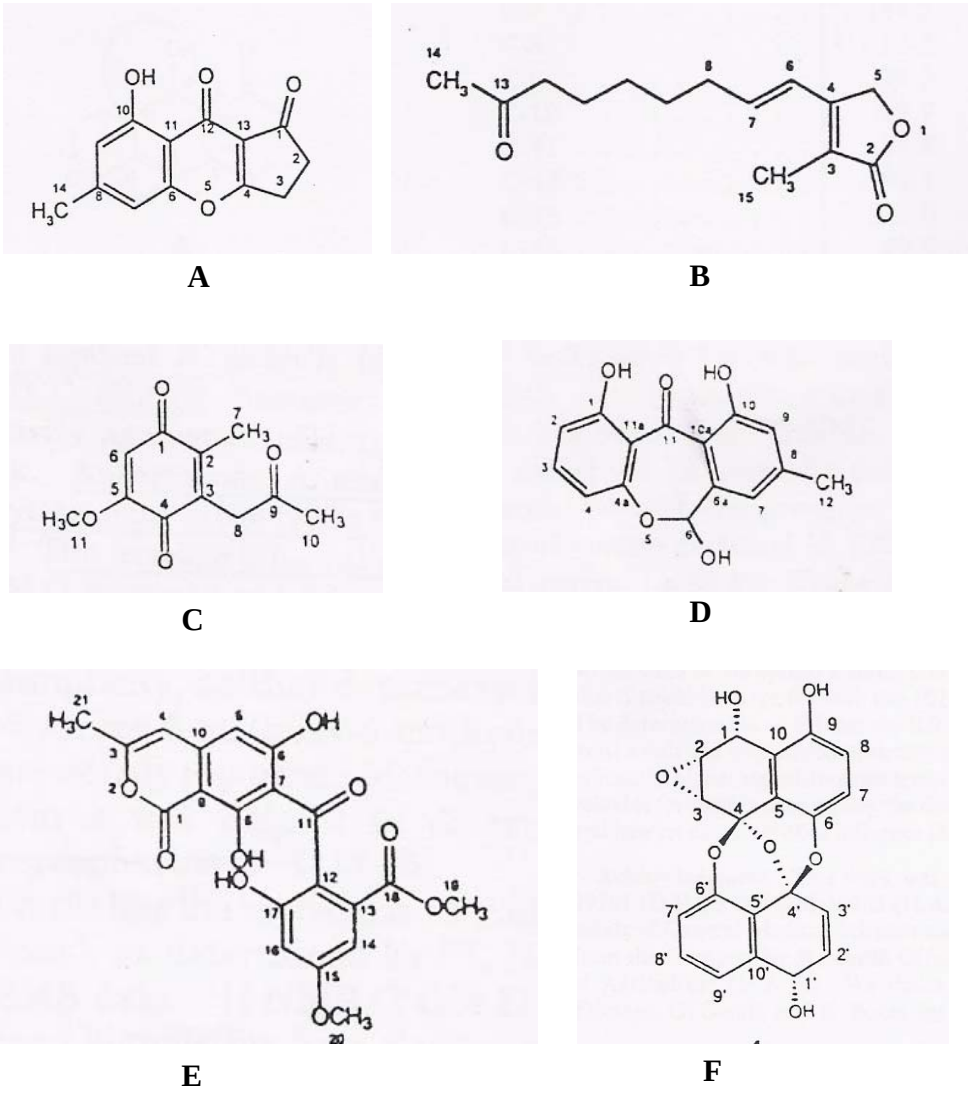
Wang *et al*. (1993) reported appenolides A (Figure 1B) isolated from *Podospora appendiculata* which colonized on deer dung, as antibacterial and antifungal compounds against *Candida albican* and *Bacillus subtilis*.

Wang *et al*. (1995a) could isolate coniochaetone-A (Figure 1A) from *Coniochaeta saccardoi* on lemming dung in the United States. This compound showed the antimicrobial activity by inhibiting the radial growth rates of *Ascobolus furfuraceaeus*, *Candida albicans* and *Sordaria fimicola in vitro*.

Hein *et al*. (1998) studied arugosin F (Figure 1D) and xanthone from *Ascodesmis sphaerospora* on bison dung in Canada were active against *Bacillus subtilis* and *Staphylococcus aureus.* Arugosin F has been described previously as a metabolite from *Cyathus intermedius*. This compound differs from arugosin A-E and other arugosins, metabolites of *Aspergillus variecolor, A. rugulo*sa and *A. silvaticus* in the lack of prenyl and oxyprenyl substituents at position 2 and 7 respectively.

Table 1 Secondary metabolites from coprophilous fungi

Coprophilous fungi	Compounds	References
Apiospora montagnei	Apiosporamide	Alfatafta *et al.* (1994)
	Dihydroisocoumarin cis-(3R,4R)- 4-hydroxymellein	Alfatafta *et al*. (1994)
Ascodesmis sphaerospora	Arugosin F	Hein *et al.* (1998)
	Xanthone	Hein *et al.* (1998)
Bombardioidea anartia	Bombardolides	Hein *et al.* (2001)
Cercophora areolata	4-acetyl-8-hydroxy-6-methoxy-5-methy-1- isocoumarins	Whyte *et al.* (1996)
	Cercophorins A, B, C	Whyte *et al.* (1996)
	Decarboxy-citrinone	Whyte *et al.* (1996)
	Roridin E	Whyte *et al*. (1996)
Chaetomium brasiliense	Chaetochalasin A	Oh *et al.* (1998)
Coniochaeta saccardoi	Coniochaetone A, B	Wang *et al.* (1995a)
Nigrosabulum globosum	Pseudodestruxins A, B	Che *et al.* (2001)
Petriella sordida	Petriellin A	Lee *et al.* (1995)
Podospora anserina	Anserinones A, B	Wang *et al*. (1997)
Podospora appendiculata	Appenolides A, B, C	Wang *et al*. (1993)
Podospora communis	Communiol A, B, C	Kuwahara *et al*. (2005)
Podospora decipiens	Podosporin A	Weber *et al.* (1988)
Polytolypa hystricis	Polytolypin	Gamble *et al.* (1995)
Poronia punctata	Punctatin A, C, D, F	Poyser *et al*. (1986)
	Punctatin B	Gloer and Truckenbrod (1988)
	Isoepoxydon	Gloer and Truckenbrod (1988)
Preussia isomera	Preussomerin A	Weber *et al.* (1990)
	Preussomerin A-F	Weber and Gloer (1991)
Sporormiella similis	Similius A, B	Weber *et al.* (1992)
Sporormiella teretispora	Terezines A-D	Wang *et al*. (1995b)
Sporormiella vexans	Sporovexins A, B, C	Soman *et al.* (1999)
	Preussomerin analog	Soman *et al*. (1999)
Unidentified species	Sonomoldes A, B	Morris *et al.* (1996)
Unidentified species	Coprophilin	Ondeyka *et al.* (1998)



A **B**

C **D**

E **F**

Figure 1 Bioactive secondary metabolites isolated from coprophilous fungi

A = coniochaetone A from *Coniochaeta saccardoi* (Wang *et al*., 1995a)

B = appenolides A from *Podospora appendiculata* (Wang *et al*., 1993)

C = anserinone A from *Podospora anserina* (Wang *et al*., 1997)

D = arugosin F from *Ascodesmis sphaerospora* (Hein *et al*., 1998)

E = cercophorin A from *Cercophora areolata* (Whyte *et al*., 1996)

F = preussomerin A from *Preussia isomera* (Weber *et al*., 1990)

On the other hand, preussomerin A isolated from *Preussia isomera* (Figure 1F) and petriellin A isolated from *Petriella sordida,* have been reported to show the antifungal activity against *Sordaria fimicola* and *Ascobolus furfuraceus* (Weber *et. al*., 1990; Lee *et al*., 1995). Polytolypin, a triterpenoide isolated from *Polytolypa hystricis* (Gambel *et al*., 1995), as well as spororvexins A-C and a derivative of preussomerin (Figure 1F) isolated from *Sporormiella vexans* (Soman *et al*., 1999) have also been reported to inhibit the growth of *Ascobolus furfuraceus* and *Candida albicans.*

Further more, many compounds have been found to show both antibacterial and antifugal activities. Apiosporamide, terezine A-D, cercophorin A-C and anserinones A-B (Table 1, Figure 1C) produced inhibition zone and reduced the growth rate of *Bacillus subtilis*, *Staphylococcus aureus* as well as inhibited growth of *Ascobolus furfuraceus*, *Candida albicans* and *Sordaria fimicola*. While apiosporamide, isolated from *Apiospora montagnei,* was found to be closely related to tenellin from *Beauveria tenella* (Alfatafta *et al*., 1994), terezine A-D were amino acid derived bioactive metabolites isolated from *Sporormiella teretispora* (Wang *et al*., 1995b). On the other hand, the isocoumarin derivative cercophorin A-C (Figure 1E) with antifungal and cytotoxic activity were isolated from *Cercophora areolata* (Whyte *et al*., 1996). Anserinones A-B, the antifungal and antibacterial benzoquinones were reported from *Podospora anserina* isolated from rat dung (Wang *et al*., 1997). Interestingly, N-alkoxypyridone, isolated from sclerotia of *Aspergillus leporis* from white-tailed jack rabbit (*Lepus townsendii* Bachman) dung, was found to possess insecticidal property (Wicklow, 1985).

MATERIALS AND METHODS

Materials

1. Isolation and morphological study of coprophilous fungi

1.1 isolating agar media: Gochenaur's glucose ammonium nitrate agar (GAN) and water agar (WA) (Appendix)

1.2 cultivating agar media: potato dextrose agar (PDA), cornmeal agar (CMA), malt extract agar (MEA), Czapek's solution agar (CZA) (Appendix)

1.3 mounting media: distilled water, lactophenol and Melzer's reagent

1.4 3% Potassium hydroxide (3% KOH)

1.5 65%, 70% and 95% ethyl alcohol

1.6 streptomycin

1.7 glass bowl or plastic box with damp cotton or tissue paper

1.8 forcep and fine needle

1.9 Petri dishes

1.10 beaker

1.11 test tube

1.12 distilled water

1.13 thermometer

1.14 slide and cover slip

1.15 emersion oil

1.16 Light microscope (BH-2 Olympus) with Normaski Interference Contrast and camera lucida drawing

1.17 stereo microscope (SZ-PT Olympus)

1.18 scanning electron microscope (JEOL JSM 6400)

2. Preservation of coprophilous fungi

2.1 dry dung samples

2.2 sterile soil (for soil culture)

2.3 sterile filter paper Whatman No.1 (for filter paper method)

2.4 sterile water (for water culture)

2.5 sterile liquid paraffin

2.6 refrigerator (–20 ^{o}C)

2.7 aluminiumfolie

2.8 paper bag

2.9 plastic bag

2.10 plastic box

2.11 vial, size 1 dram.

2.12 Petri dishes

2.13 forcep

2.14 electronic dry cabinet (WEIFO)

3. Antagonistic tests

3.1 pure cultures of nine plant pathogenic fungi (Table 4)

3.2 Petri dishes

3.3 cock borer

3.4 fine needle

4. Isolation and purification of the secondary metabolite from coprophilous fungi

4.1 Fungal cultivation

- Petri dishes and PDA
- cock borer
- Erlenmyer flask 250, 500, 1000 and 2000 ml
- cultivating agar medium: potato dextrose broth (PDB) (Appendix)
- orbital shaker

4.2 Preparation of the crude extract

- filtrate pump
- filter paper Whatman no.1
- separating funnel
- stand and clamp
- rotary evaporator

4.3 Isolation by column chromatography

- column glass
- TLC aluminium sheets 20x20 cm silica gel 60 F_{254} , Merck
- silica gel 60 F_{254}, Merck for column chromatography
- silica gel 60 GF_{254} , Merck for thin layer chromatography
- 20x20 cm glass plate
- sea sand
- cotton
- ethyl acetate (EtOAc)
- chloroform ($CHCl_3$)

- duteration of chloroform
- acetone ($(CH_3)_2CO$)
- petroleum ether (Petrol)
- methanol (CH_3OH)
- ethanol (CH_3CH_2OH)
- 98% formic acid (HCO_2H)
- distilled water
- microcapillary pipettes, calibrated, size 10 μL
- vials, size 4 dram
- volumetic flask, size 10, 50, 100 ml
- hot plate
- UV detector (λ = 254 nm)
- ultrasonic machine
- aluminiumfolie
- tank chamber

4.4 Structure elucidation of the compounds

- Proton Nuclear Magnetic Ressonance (^{1}H NMR)
- Carbon-13 Nuclear Magnetic Ressonance (^{13}C NMR)
- Correlation Spectroscopy (COSY)
- Heteronuclear Single Quantum Coherence (HSQC)
- Heteronuclear Multiple Bond Correlation (HMBC)
- High Resolution Mass Spectrometry (HRMS)

Methods

1. Collection of the dung samples

Sixty dung samples of thirteen wildlife and domestic animals including barking deer, buffalo, camel, cow, deer, eld's deer, elephant, guar, goat, horse, rabbit, rat and toad were collected from eighteen provinces during December 2001 to August 2004 (Tables 2, 3). They were placed in paper bags and brought to the laboratory. All samples were air dried for a few days in Petri dishes or glass bowls to eliminate bacteria and other soil fauna. After drying, the samples were kept in a paper bag, label and put in the box at Kasetsart University Herbarium for further study.

Table 2 Frequency of various dung samples collected from different provinces

No.	Location	Number of Dung Sample													Total
		barking deer	buffalo	camel	cow	deer	eld's deer	elephant	gaur	goat	horse	rabbit	rat	toad	
1.	Bangkok (BK)	-	1	-	-	-	-	-	-	-	-	1	13	7	22
2.	Chiang Mai (CM)	-	1	-	-	-	-	1	-	-	-	1	-	-	3
3.	Chiang Rai (CR)	-	-	1	1	-	-	1	-	-	-	-	-	-	3
4.	Chon Buri (CB)	1	-	-	-	-	1	1	-	-	-	-	-	-	3
5.	Kalasin (KL)	-	-	-	1	-	-	-	-	-	-	-	-	-	1
6.	Khon Kaen (KK)	-	-	-	1	-	-	-	-	-	-	-	-	-	1
7.	Krabi (KB)	-	-	-	1	-	-	1	-	1	1	-	-	-	4
8.	Loei (LE)	-	-	-	-	-	-	1	-	-	-	-	-	-	1
9.	Mae Hong Son (MS)	-	1	-	1	-	-	1	-	1	-	-	-	-	4
10.	Nakhon Ratchasima (NR)	1	-	-	1	1	-	1	-	-	-	-	-	-	4
11.	Phetchaburi (PT)	-	-	-	1	-	-	-	-	-	-	-	-	-	1
12.	Prachin Buri (PC)	-	-	-	-	-	-	-	-	-	-	1	-	-	1
13.	Ratchaburi (RT)	-	-	-	1	-	-	-	-	-	-	-	-	-	1
14.	Saraburi (SB)	-	1	-	1	-	-	-	-	-	-	-	-	-	2
15.	Surat Thani (ST)	-	-	-	1	-	-	-	-	-	-	-	-	-	1
16.	Trat (TR)	-	-	-	1	-	-	-	-	-	-	-	-	-	1
17.	Ubon Ratchathani (UB)	-	-	-	1	-	-	-	-	-	-	-	-	-	1
18.	Uthai Thani (UT)	1	-	-	-	1	1	1	1	-	-	1	-	-	6
	Total	3	4	1	12	2	2	8	1	2	1	4	13	7	60

Table 3 Dung samples from thirteen wildlife and domestic animals collected from various locations

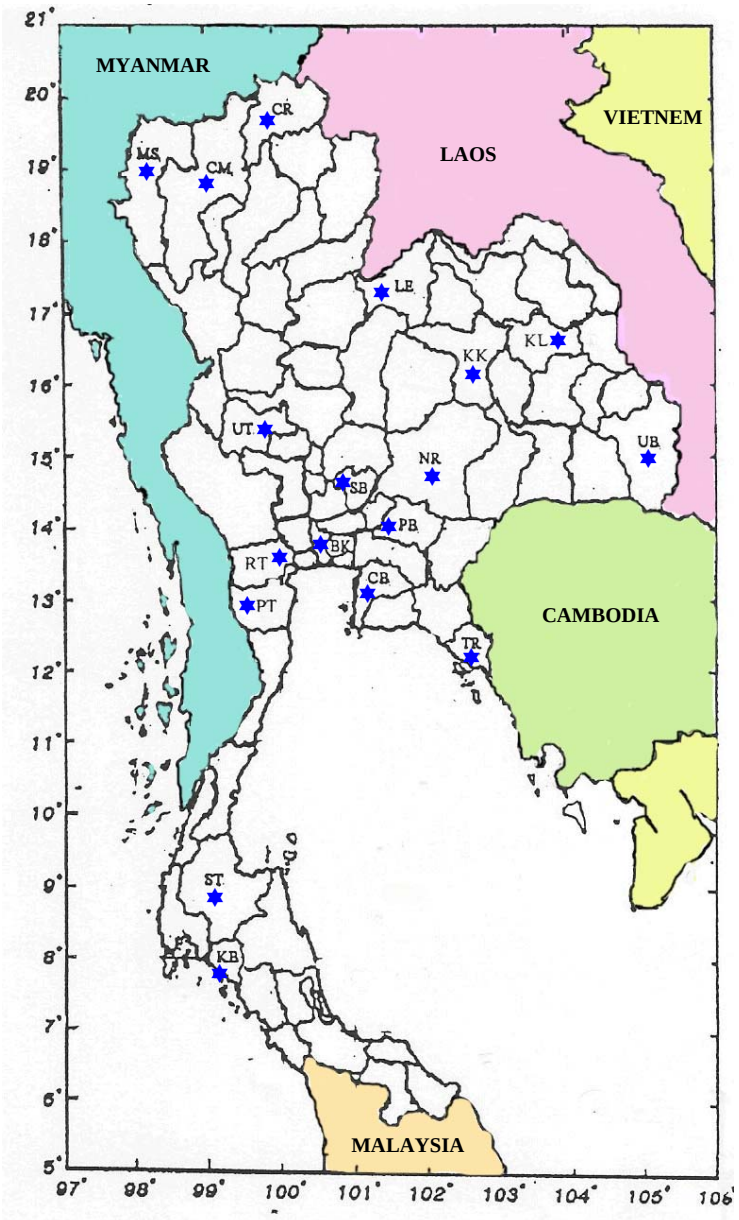
No.	Dung types*	KUH**	Location	Collecting date
1.	Barking deer	0002	Khao Yai National Park, Nakhon Ratchasima	16 June 2002
		0022	Hauy Kha Khang Wlid Life Sanctuary, Uthai Thani	2 May 2003
		0046	Khao Khaew Open Zoo, Chon Buri	17 Febuary 2004
2.	Buffalo	0007	Maung District, Saraburi	8 July 2002
		0012	Mae Tang District, Chiang Mai	7 November 2002
		0029	Dong Maung, Bangkok	24 June 2003
		0037	Maung District, Mae Hong Son	9 October 2003
3.	Camel	0047	Khao Khaew Open Zoo, Chon Buri	17 Febuary 2004
4.	Cow	0009	Pak Thong Chai District, Nakhon Ratchasima	25 October 2002
		0015	Nong Po District, Ratchaburi	5 December 2002
		0021	Maung District, Saraburi	14 April 2003
		0031	Maung District, Surat Thani	8 August 2003
		0032	Lunta District, Krabi	10 August 2003
		0038	Maung District, Mae Hong Son	9 October 2003
		0043	Maung District, Khon Kaen	11 November 2003
		0044	Horticultural Research Institute, Chiang Rai	16 January 2004
		0050	Sirinthorn District, Ubon Ratchathani	25 Febuary 2004
		0051	Ban Lard District, Phetchaburi	28 Febuary 2004
		0052	Khoa Goad District, Trat	16 March 2003
		0056	Maung District, Kalasin	16 April 2004
5.	Deer	0003	Khao Yai National Park, Nakhon Ratchasima	16 June 2002
		0023	Hauy Kha Khang Wlid Life Sanctuary, Uthai Thani	2 May 2003
6.	Eld's deer	0024	Hauy Kha Khang Wlid Life Sanctuary, Uthai	2 May 2003
		0048	Thani Khao Khaew Open Zoo, Chon Buri	17 Febuary 2004
7.	Elephant	0004	Khao Yai National Park, Nakhon Ratchasima	16 June 2002
		0010	Mae Sa District, Chiang Mai	7 November 2002
		0017	Phu Ka Dung District, Loei	31 December 2002
		0039	Maung District, Mae Hong Son	9 October 2003
		0025	Hauy Kha Khang Wlid Life Sanctuary, Uthai	2 May 2003
		0033	Thani Lunta District, Krabi	10 August 2003
		0045	Horticultural Research Institute, Chiang Rai	16 January 2004
		0049	Khao Khaew Open Zoo, Chon Buri	17 Febuary 2004

Table 3 (Cont'd)

No.	Dung types*	KUH**	Location	Collecting date
8.	Gaur	0026	Hauy Kha Khang Wlid Life Sanctuary, Uthai Thani	2 May 2003
9.	Goat	0034	Lunta District, Krabi	10 August 2003
		0040	Maung District, Mae Hong Son	9 October 2003
10.	Horse	0035	Lunta District, Krabi	10 August 2003
11.	Rabbit	0011	Queen Sirikit Botanic Garden, Chiang Mai	7 November 2002
		0013	Dong Maung District, Bangkok	14 November 2002
		0027	Hauy Kha Khang Wlid Life Sanctuary, Uthai Thani	2 May 2003
		0030	Tup-Larn District, Prachin Buri	26 June 2003
12.	Rat	0001	Bang Sue District, Bangkok	7 December 2001
		0005	Bang Sue District, Bangkok	18 June 2002
		0008	Bang Sue District, Bangkok	9 July 2002
		0014	Bang Sue District, Bangkok	14 November 2002
		0018	Bang Sue District, Bangkok	20 January 2003
		0028	Bang Sue District, Bangkok	15 May 2003
		0041	Bang Sue District, Bangkok	19 October 2003
		0053	Bang Sue District, Bangkok	4 April 2004
		0057	Bang Sue District, Bangkok	3 July 2004
		0036	Kasetsart University, Bangkok	26 September 2003
		0042	Kasetsart University, Bangkok	20 October 2003
		0054	Kasetsart University, Bangkok	4 April 2004
		0058	Kasetsart University, Bangkok	19 July 2004
13.	Toad	0006	Bang Sue District, Bangkok	18 June 2002
		0016	Bang Sue District, Bangkok	19 December 2002
		0019	Bang Sue District, Bangkok	2 Febuary 2003
		0020	Bang Sue District, Bangkok	16 March 2003
		0055	Bang Sue District, Bangkok	4 April 2004
		0059	Bang Sue District, Bangkok	19 July 2004
		0060	Bang Sue District, Bangkok	8 August 2004

* Barking deer (*Muntiacus feael*), Buffalo (*Bubalus bubalus*), Camel (*Camelus bactrianus*), Cow (*Bos taurus*), Deer (*Cervus unicolor*), Eld's deer (*Cervus eldi*), Elephant (*Elephas maximus*), Gaur (*Bos gaurus*), Goat (*Capra aegagrus*), Horse (*Equus caballus*), Rabbit (*Sylvilagus audubonii*), Rat (*Rattus rattus*), Toad (*Bufo* sp.)

** KUH = Kasetsart University Herbarium



CR = Chiang Rai CM = Chiang Mai MS = Mae Hong Son LE = Loei,
KK = Khon Kaen KL = Kalasin UB = Ubon Ratchathani NR = Nakhon Ratchasima
SB = Saraburi PC = Prachin Buri TR = Trat CB = Chon Buri
BK = Bangkok UT = Uthai Thani RT = Ratchaburi PT = Phetchaburi
ST = Surat Thani KB = Krabi

Figure 2 Map of Thailand indicating the collection sites from 18 provinces.

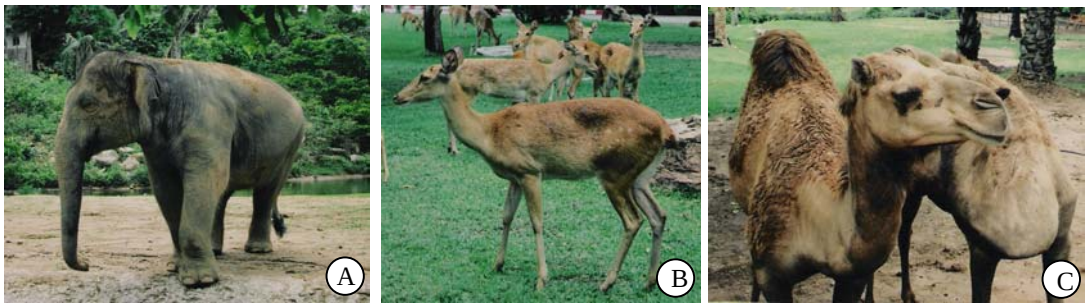


Figure 3 A. Elephant, B. eld's deers and C. camels at Khao Khaew Open Zoo, Chon Buri Province.

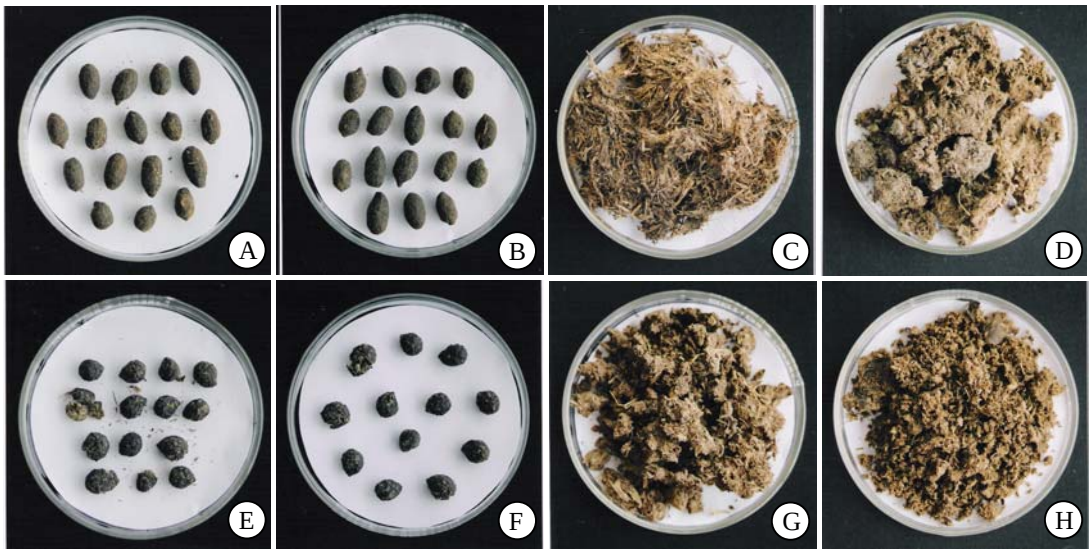


Figure 4 Air dried dung samples: A-B. goat, C. elephant, D. buffalo, E-F. rabbit and G-H. cow.

2. Isolation and identification of coprophilous fungi

2.1 Isolation of coprophilous fungi

The moist chamber, soil plate, dilution plate, alcohol and heat treatment techniques were employed to isolate coprophilous fungi.

2.1.1 **Moist chamber method** (Bell, 1983; Manoch *et al*., 1999)

Each excrement sample was placed in a moist chamber consisting of a glass bowl or plastic box lined with damp cotton or tissue-paper and placed by the window. They were incubated for 2-7 days or longer at 28 ^{o}C and observation was made under stereomicroscope. Transferred needle was used to transfer spores or fruiting structures on a slide and mount with a drop of distilled water. Melzer's reagent was used for Ascomycetes, then covered with cover slip and examined under light microscope with Normaski Interference Contrast. Photomicrographs were taken and camera lucida drawing were used.

Figure 5 Incubation of dung in a moist chamber.

A direct isolation method was carried out from the dung surface under a stereomicroscope (SZ-PT Olympus). The ascomata were transferred directly onto water agar (WA) and squashed to release the ascospores and incubated for 48 hours. If the ascospores did not germinate, a fine needle was used to transfer the fruiting body with the remaining ascospores from WA to a test tube containing 60-80 ^{o}C distilled water, 65% ethyl alcohol and 3% KOH for 30, 15 and 3-5 minutes respectively. The treated ascospores were spread on WA in a Petri dish and incubated for 12-24 hours. A hyphal tip from a single spore was transferred to a slant potato dextrose agar (PDA) and kept as pure culture at the Department of Plant Pathology, Faculty of Agriculture, Kasetsart University Fungal Collection (KUFC). Dry specimens of dung samples were kept in a herbarium at the Department of Plant Pathology, Faculty of Agriculture, Kasetsart University Herbarium (KUH).

2.1.2 **The soil plate method** (A modification of Warcup, 1950)

Small amount of dung samples (0.005-0.015 g) was placed in a sterile Petri dishes. About 10 ml of warm Gochenaur's glucose ammonium nitrate agar (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) (Gochenaur, 1964) was poured on the soil and the Petri dishes were rotated gently. The plates were placed in covered boxes and incubated in darkness at 28 ^{o}C for a few days. A hyphal tips were transferred onto PDA and maintained as pure culture for identification.

2.1.3 **The dilution plate methods** (Barron, 1968)

A 10 g of dung samples was added to 100 ml of sterile distilled water. Then, 10 ml of the suspension were mixed with 100 ml of sterile distilled water in a flask. Ten milliliter samples were transferred through a succession of 100 ml sterile distilled water until the desired dilution is reached. One-ml aliquots of the selected dilutions (usually 10^{-2}, 10^{-3}, 10^{-4}) was pipetted into Petri dishes for each selected dilution. The same procedures described in the previous method were followed.

2.1.4 **Alcohol treatment technique** (A modification of Warcup & Baker, 1963)

A small amount of dung sample (0.3 g) were placed in 65% ethanol in a sterile test tube for 10-15 min, after the liquid was decanted, bits of the treated soil dispensed into several sterile Petri dishes and the plate immediately poured with GAN. The same procedures described in the previous method were followed.

2.1.5 **Heat treatment technique** (A modification of Warcup & Baker, 1963)

The dung sample (0.3 g) was placed in a sterile test tube containing sterile distilled water and placed in a water bath at 60-80 ^{o}C for 15-20 minutes. Excess water was drained off and the dung sample was placed on GAN in a sterilized Petri-dishes. The same procedures described in the previous method were followed.

2.2 Identification of coprophilous fungi

Macroscopic features were studied including colony growth pattern, color, texture on different agar media. Fungal growth rate was measured on PDA, CMA, CZA and MEA. Microscopic characters were observed on a slide preparation using sterile distilled water and lactophenol as mounting media and examined under a light microscope (Olympus BH-2 with Normaski Interference Contrast). Camera lucida drawings were employed. Photomicrographs of fungal structure were taken under stereo, light and scanning electron microscopes.

The procedure for SEM photomicrographs (Sharples and Moss, 2000), ripen ascomata and ascospores of Eurotiales and Sphaeriales from dry culture agar media were transferred with a fine needle and placed onto double-strick scotch tape on aluminium stubs. The specimens were coated with gold for 5 -7 min. and examined in a JEOL JSM 6400 scanning electron microscope (Manoch *et al*., 2004, 2005).

Identification was based on morphological characteristics as observed under a stereo, light and scanning electron microscopes. Coprophilous fungi was identified following the researches done by Ahmed and Cain (1972); Khan and Cain (1972); Bell (1983, 2005); Jeng and Krug (1977); Khan and Krug (1989); Richardson (1972, 2001a); Bell and Mahoney (1995, 1996); Richardson and Watling (1997); Barr (2000); Wang (2000); Webster and Weber (2000); Dokmetzian and Ranalli (2002); Chang and Wang (2005) and Elshafie (2005).

2.3 Preservation of coprophilous fungi

2.3.1 **PDA slant method** (Smith and Onions, 1994)

Pure cultures of coprophilous fungi were maintained on PDA slants at 28 ^{o}C. Sub-culturing was carried out every 6 months.

2.3.2 **Liquid paraffin method** (Smith and Onions, 1994)

Pure cultures were maintained on PDA agar slant in a vial (1 dram). Liquid paraffin was placed in a vial and autoclaved twice. Covering the pure culture on agar slant with sterile liquid paraffin about 2/3 of a vial and stored at 28 ^{o}C in order to prevent dehydration and slows down metabolic activity and growth through reduced oxygen tension.

2.3.3 **Filter paper method** (Fong *et al*., 2000)

Fifteen pieces (0.5 x 0.5 cm^2) of sterile filter paper Whatman no. 1 were placed on PDA in sterile Petri-dishes. The mycelia were transferred on PDA and incubated for 7-14 days depend on the species. The filter papers with mycelium were transferred to new sterile Petri dishes by using sterile forcep and placed in an electric dessicator for 7-10 days. Dried filter papers covered with mycelium and fruiting bodies were kept in the aluminiumfoile in a sterile plastic bags, labeled and placed in a box and storage at -20 ^{o}C.

2.3.4 Soil culture (Smith and Onions, 1994; Manoch *et al*., 2005)

Loamy soil was placed in a vial about 2/3 full and autoclaved twice at 121 ^{o}C for 15 minutes. One ml of spore suspension in sterile water was added. The soil cultures were left to grew at room temperature and then left to dry while stored in a refrigerator at 4-10 ^{o}C.

3. Antagonistic tests

Three isolates of *Ascodesmis macrospora* and six isolates of *Sordaria fimicola* were selected to test for antagonistic activity against nine species of plant pathogenic fungi (Table 4). They were cultivated as dual cultures on PDA for 14 days at 28 ^{o}C. The young mycelium from the colony margin of *S. fimicola* and the specific plant pathogenic fungus was cut with sterile cork borer (0.8 cm diam.) and placed on PDA, 6 cm apart. They were incubated at room temperature (28 ^{o}C) for 14 days.

The inhibition levels were calculated by using the formula: G_1-G_2 / G_1 x 100, G_1 indicated colony radius of plant pathogenic fungi in the control and G_2 indicated colony radius of plant pathogenic fungi in the dual culture test (Intana *et al*., 2003). Each treatment was performed on 2 replicates.

Table 4 Species of plant pathogenic fungi from various diseased fruits and vegetables used for antagonistic activity test

Plant pathogenic fungi	**Host plant**
Alternaria alternata	*Pyrus pyrifolia* (pear)
Colletotrichum capsici	*Capsicum annuum* (chili)
Curvularia lunata	*Zae mays* (corn)
Fusarium oxysporum	*Psidium guajava* (guava)
Lasiodiplodia theobromae	*Mangifera indica* (mango)
Pestalotiopsis guepinii	*Psidium guajava* (guava)
Pythium aphanidermatum	*Cucumis sativus* (cucumber)
Rhizoctonia solani	*Oryza sativa* (rice)
Sclerotium rolfsii	*Vigna radiata* (mungbean)

4. Isolation and purification of the secondary metabolites from coprophilous fungi

Two coprophilous fungi *Ascodesmis macrospora* Obrist (KUFC 2456) and *Sordaria fimicola* (Roberge ex Desm.) Ces. & De Not (KUFC 2495) were investigated for their secondary metabolites. The process included:

4.1 Extraction and isolation of the compounds

4.1.1 Fungal culture

Forty 2L flasks, each containing 800 mL of potato dextrose broth (PDB), were individually inoculated with ten of 1 cm^3 agar plugs taken from stock culture of *A. macrospora* on PDA (thirty 2L flasks for *S. fimicola*). Flask cultures were incubated at 28-30 ^oC on rotary shakers at 150 rpm for 30 days (Figure 6).

4.1.2 Preparation of the crude extract

The fungal cultures were filtered, and the filtrate (32 L for *A. macrospora* and 24 L for *S. fimicola*) was extracted three times with equal volume of EtOAc at room temperature. The organic phase was evaporated under reduced pressure by a rotary evaporator to give a dark brown viscous mass of a crude ethyl acetate extract of *A. macrospora* (6.3 g) and *S. fimicola* (5.17 g) (Figure 6).

1) Ten of 1 cm2 agar plugs taken from stock culture of fungal material on potato dextrose agar (PDA) cultivated on potato dextrose broth (PDB) 800 ml per flask

2) Flask cultures were incubated at 28-30 ^{o}C on rotary shakers at 150 rpm for 30 days

3) Fungal mycelium and ascospores grew in the broth after 30 days incubation

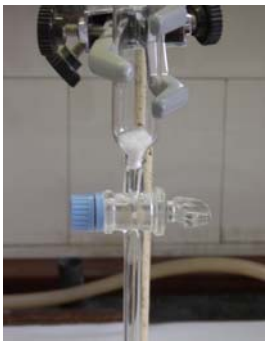
4) The fungal cultures were filtered by the vacuum pump

5) The filtrate (32 L for *A. macrospora* and 24 L for *S. fimicola*) was extracted with EtOAc (3x) at room temperature

6) The organic phase was evaporated under reduced pressure by a rotary evaporator

7) A dark brown viscous mass of a crude extract

Figure 6 Procedure for the preparation of crude extracts from the culture filtrate of *Ascodesmis macrospora* and *Sordaria fimicola*.



1) Setting the column with the stand and make sure the column (30 x 2.5 cm) was perfectly vertical. Put the wad of cotton at the bottom of the column

2) Filled the sea sand above the cotton (0.5 cm)

3) Filled the solvent, Petrol: $CHCl_3$ (5:5) about 2/4 of the column

4) Mixed 25 g of silica gel 60 with a mixture of Petrol: $CHCl_3$ (5:5) and then poured into the prepared column

5) Mixed 5 g of the silica gel 60 with the crude extract, added slowly and mixed thoroughly until all the crude extract is incorporated to the silica gel before added to the top of the column

6) Put the wad of cotton on the top of crude extract in the column. Filled the solvent Petrol: $CHCl_3$ (5:5) in the column andcollected the fractions every 100 mL

Figure 7 Procedure for applying the crude extract in the column chromatography.

4.1.3 Fractionation of the crude extract

The crude extract of fungi (6.3 g for *A. macrospora* and 5.17 g for *S. fimicola*) was applied to a Silica gel column (Figure 7) and eluted with Petrol: $CHCl_3$, $CHCl_3$: $(CH_3)_2CO$ and $CHCl_3$: CH_3OH. A 100 ml fractions were collected as follows Table 5.

Table 5 Fractionation of the crude extracts of *Ascodesmis macrospora* and *Sordaria fimicola*

Eluents	Fractions	
	A. macrospora	*S. fimicola*
Petrol: $CHCl_3$ (5:5)	1-50	1-29
Petrol: $CHCl_3$ (3:7)	51-92	30-57
Petrol: $CHCl_3$ (1:9)	93-117	58-138
$CHCl_3$: $(CH_3)_2CO$ (9:1)	118-157	139-168
$CHCl_3$: $(CH_3)_2CO$ (7:3)	158-181	-
$CHCl_3$: $(CH_3)_2CO$ (8:2)	-	169-178
$CHCl_3$: CH_3OH (9.5:5)	182-202	179-186

Each fraction was concentrated by rotatory evaporator to remove the solvent. The concentrated fractions were spotted onto the analytical TLC plate and then placed in the chromatographic tank with an appropriate solvent system (Figure 8). The analytical TLC plate was visualized by UV lamp (λ = 254 nm) and iodine vapor. Fractions were combined according to their composition as revealed by analytical TLC, as follows Table 4 and Table 5.

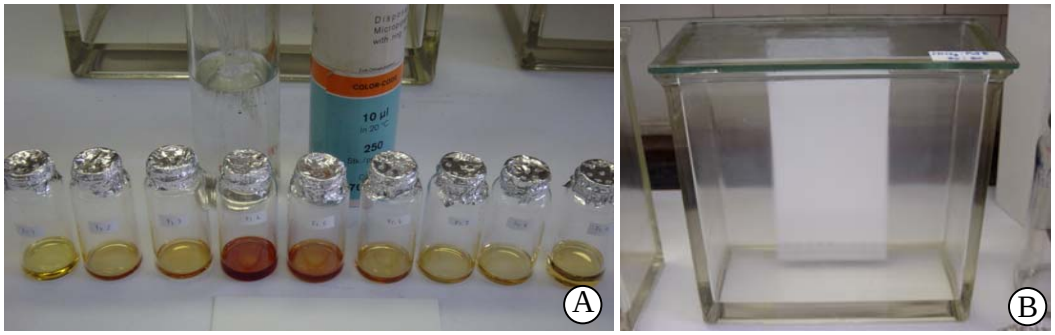


Figure 8 A. Concentrated fractions and B. Chromatographic tank.

4.2 Isolation and purification of the compounds

The compounds from the column fractions were purified by preparative TLC. The combined fractions were applied on preparative TLC plate and developed in an appropriate solvent system. The TLC plate was revealed by the UV lamp (λ = 254 nm), the separated bands were taken off the plate and the compounds were re-extracted by an appropriate solvents (Figure 9). Purity of the compounds was checked by analytical TLC.

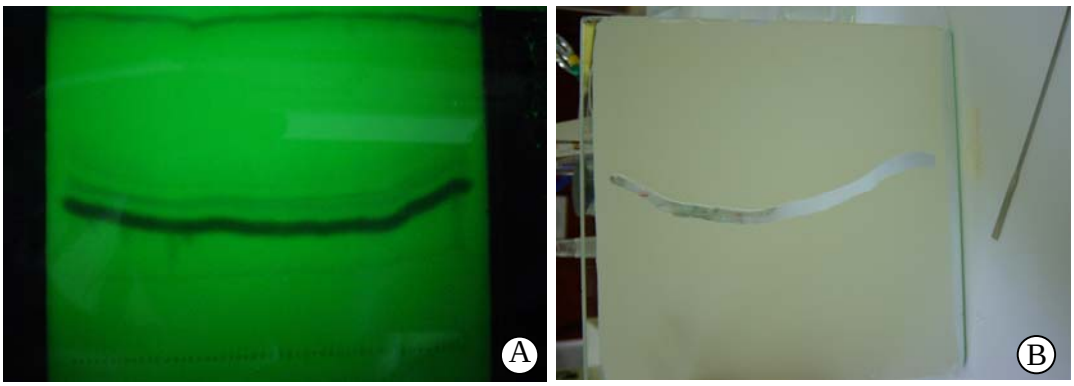


Figure 9 A. TLC plate shows dark band under UV lamp and B. separated compound by scraping.

4.3 Structure elucidation of the compounds

The structure of the compounds were established by spectroscopic methods (^{1}H, ^{13}C NMR, COSY, HSQC, HMBC, HRMS) as well as comparison of their NMR data with those in the literatures.

5. Place

The experiment on taxonomic study and antagonistic test against plant pathogenic fungi were conducted at Mycology Laboratory, Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok. For scanning electron photomicrographs were examined at Scientific Equipment Centre, Kasetsart University, Bangkok and the Scanning Electron Microscopic Section, School of Biomolecular Sciences, Liverpool John Moores University (JMU), Byrom Street, Liverpool. The isolation, purification and structure elucidation of the secondary metabolites were conducted at the Instituto de Ciencias Biomedicas de Abel Salazar (ICBAS), Universidade do Porto, Portugal.

6. Duration

The study was carried out during 2001 to 2005.

RESULTS AND DISCUSSION

I. Diversity and distribution of coprophilous fungi

Seventy hundred and five microfungi were isolated from 60 dung samples of 13 wildlife and domestic animals from 18 provinces comprising 57 genera 69 species. Four hundred and six isolates of Hyphomycetes (57.6 %) were found on various dung samples, comprising 24 genera, 28 spp. followed by 182 isolates of Ascomycetes (25.8 %), comprising 19 genera, 27 spp., 54 isolates of Agonomycetes (7.7 %), comprising 2 genera, 2 spp., 41 isolates of Zygomycetes (5.8 %), comprising 7 genera, 7 spp., 22 isolates of Coelomycetes (3.1 %), comprising 4 genera, 4 spp. and one Basidiomycetes (Table 6).

Hyphomycetes were most abundantly encountered on cow dung (106 isolates), followed by elephant (62 isolates), buffalo (43), deer (35), toad, barking deer (23), goat and horse (21) (Table 7).

Table 6 Frequency of coprophilous fungi and other microfungi found on 60 dung samples

Class	Number of			Percentage
	genera	species	isolates	(%)
Zygomycetes	7	7	41	5.8
Ascomycetes	19	27	182	25.8
Hyphomycetes	24	28	406	57.6
Coelomycetes	4	4	22	3.1
Agonomycetes	2	2	54	7.7
Basidiomycetes	1	1	-	-
Total	**57**	**69**	**705**	**100**

Table 7 Occurrence of coprophilous and other microfungi on 60 dung samples of 13 wildlife and domestic animals

Class	Number of isolate													Total isolate
	barking deer	buffalo	camel	cow	deer	eld's deer	elephant	gaur	goat	horse	rabbit	rat	toad	
Zygomycetes	1	5	2	16	2	2	2	3	3	1	2	2	-	**41**
Ascomycetes	5	15	5	43	20	7	20	8	13	8	10	17	11	**182**
Hyphomycetes	23	43	8	106	35	16	62	11	21	21	16	21	23	**406**
Coelomycetes	2	5	-	7	-	3	-	1	1	2	1	-	-	**22**
Agonomycetes	-	15	-	18	3	1	10	1	4	-	-	2	-	**54**
Basidiomycetes	-	-	-	-	-	-	nc	-	-	-	-	-	-	**-**
Total	**31**	**83**	**15**	**190**	**60**	**29**	**94**	**24**	**42**	**32**	**29**	**42**	**34**	**705**

Remark: nc = non cultivated

Ascomycetes were mostly found on cow dung, and 43 isolates were recorded followed by deer and elephant (20 isolates), rat (17), buffalo (15), goat (13), toad (11) and rabbit (10) (Table 7).

Fifty-four isolates of Agonomycetes were found on dung, comprising 2 genera : *Papulaspora* and *Rhizoctonia*. *Papulaspora immersa* was the most common species found on cow (18 isolates), buffalo (15), elephant (10), goat (4), deer (3), rat (2), eld's deer and gaur (1), whilst only one species of *Rhizoctonia* was found on cow dung (Table 7).

Twenty-two isolates of Coelomycetes were found on various dung samples, including 4 genera, 4 spp. : *Chaetomella raphigera*, *Colletotrichum gloeosporioides*, *Lasiodiplodia theobromae* and *Pestalotiopsis guepinii*. They occurred at high frequency on cow dung (7 isolates), followed by buffalo (5), eld's deer (3), barking deer, horse (2), gaur, goat and rabbit (1) (Table 7).

One species of *Coprinus* (Basidiomycete) was found on elephant dung, but this fungus failed to grow on agar media (Table 7).

The Zygomycetes are the normal flora on dung, and 41 isolates were found from 12 dung samples except toad dung (Tables 6, 7). Seven genera and seven species were identified : *Absidia corymbifera*, *Choanephora cucurbitarum*, *Cunninghamella elegan*s, *Mucor* sp., *Pilobolus crystallinus*, *Rhizopus oryzae*, *Rhizopus stolonifer* and *Syncephalastrum racemosum*. *Rhizopus oryzae* was the common species found on all dung samples followed by *Cunninghamella elegans* and *Rhizopus stolonifer* (Table 8). All Zygomycetes in this study were cultivated on artificial media in pure culture except *Pilobolus crystallinus*. *Rhizopus stolonifer* is a common plant pathogenic fungi causing fruit rot of jack fruit and it has been reported as pathogenic fungi causing gastrointestinal disease in cattle. *Rhizopus oryzae* was rarely reported from animal but important agent of human mucormycosis (De Hoog *et al*., 2002).

Table 8 Number of isolates of coprophilous Zygomycetes and other microfungi obtained from different dung types

Fungal species	**Number of isolate**													**Total species**
	barking deer	buffalo	camel	cow	deer	eld's deer	elephant	gaur	goat	horse	rabbit	rat	toad	
Absidia corymbifera *	-	1	1	2	-	-	-	-	-	-	-	-	-	**4**
Choanephora cucurbitarum	-	-	-	-	-	-	-	1	-	-	-	-	-	**1**
Cunninghamella elegan *	-	2	-	4	-	-	1	-	2	-	-	1	-	**10**
Mucor sp. *	-	-	-	1	-	-	-	-	-	-	-	-	-	**1**
Pilobolus crystallinus *	-	-	-	-	-	-	nc	-	-	-	-	-	-	-
Rhizopus oryzae *	-	1	1	3	2	1	-	2	1	1	1	1	-	**14**
Rhizopus stolonifer *	1	1	-	4	-	1	1	-	-	-	1	-	-	**9**
*Syncephalastrum racemosum**	-	-	-	2	-	-	-	-	-	-	-	-	-	**2**
Total	**1**	**5**	**2**	**16**	**2**	**2**	**2**	**3**	**3**	**1**	**2**	**2**	**-**	**41**
% Total isolate	**2.4**	**12.2**	**4.9**	**39**	**4.9**	**4.9**	**4.9**	**7.3**	**7.3**	**2.4**	**4.9**	**4.9**	**-**	**100**

Remark: * = Coprophilous Zygomycetes; nc = non cultivated

One hundred and eighty-two isolates of coprophilous Ascomycetes were found from 60 dung samples of 13 animals from 18 locations using various methods (Table 9). Nineteen genera and 27 species of Ascomycetes were identified comprising 10 genera, 11 species of Pyrenomycetes; 6 genera, 12 species of Plectomycetes and 3 genera, 4 species of Discomycetes (Table 9). All taxa were cultivated on PDA and CMA except *Podosordaria leporina* and *Saccobolus glaber* which failed to grow on agar media. The pure cultures are being maintained in a culture collection at the Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok (KPFC).

Chaetomium globosum was the most common species and 19 isolates were found from dung sample except elephant, buffalo, rat and toad, followed by *Sordaria fimicola* (16 isolates) and *Sporormiella minima* (10 isolates). It is interesting to note that *Ascobolus albidus*, *Ascodesmis macrospora*, *Ascodesmis sphaerospora*, *Cercophora silvatica*, *Gelasinospora brevispora*, *Podosordaria leporina*, *Podospora curvicolla*, *Saccobolus glaber* and *Zopfiella latipes* were found on only one type of dung (Table 9). The difference among fungal species has been shown to be dependent on the digestive processes, type of stomachs, feeding habitats and food preferences of the animals (Ebersohn and Eicker, 1992). The moist chamber method yielded the highest number of coprophilous Ascomycetes, followed by the soil plate method, alcohol treatment, heat treatment and dilution plate method. The results indicated that for the wildlife, deer and elephant dung yielded the highest number of fungal species (20 isolates, 11%), followed by rabbit (10 isolates, 5.5%). For domestic animal, most fungal species were found on cow dung (43 isolates, 23.6%), followed by buffalo (15 isolates, 8.2%) and goat (13 isolates, 7.1%.) (Table 9).

Table 9 Number of isolates of coprophilous Ascomycetes and other microfungi obtained from different dung types

Fungal species	Number of isolate													Total isolate
	barking deer	buffalo	camel	cow	deer	eld's deer	elephant	gaur	goat	horse	rabbit	rat	toad	
†*Ascobolus albidus* *	-	-	-	-	-	-	2	-	-	-	-	-	-	**2**
†*Ascodesmis macrospora* *	-	-	-	-	-	-	-	-	-	-	-	3	-	**3**
†*Ascodesmis sphaerospora* *	-	-	-	-	-	-	-	-	-	-	-	-	1	**1**
†*Cercophora silvatica* ***	-	-	-	-	-	-	3	-	-	-	-	-	-	**3**
†*Chaetomium crispatum* ***	-	-	-	2	-	-	1	-	-	-	-	-	-	**3**
†*Chaetomium cupreum* ***	-	-	-	2	1	1	1	-	-	-	1	-	-	**6**
†*Chaetomium globosum* ***	1	1	1	3	3	2	-	1	2	1	4	-	-	**19**
Emericella nidulans **	-	-	-	1	-	1	-	1	1	-	-	-	-	**4**
Emericella rugulosa **	-	-	-	1	-	-	-	-	-	-	-	1	-	**2**
Emericella variecolour **	-	-	-	2	-	-	-	-	-	-	-	1	1	**4**
Eupenicillium parvum **	-	1	1	4	1	-	3	-	1	1	-	2	2	**16**
Eurotium amstelodami **	1	1	-	4	-	-	2	1	-	2	1	1	2	**15**
†*Gelasinospora brevispora* ***	-	-	-	1	-	-	-	-	-	-	-	-	-	**1**
Hamigera avellanea **	-	-	-	2	-	-	1	-	1	-	-	-	-	**4**
Neosartorya fischeri **	-	2	-	8	4	1	1	2	1	-	-	2	1	**22**
Neosartorya fumigata **	-	-	-	1	-	-	-	-	-	-	-	1	-	**2**
†*Podosordaria leporina* ***	-	-	-	-	-	-	-	-	-	-	nc	-	-	**-**
†*Podospora curvicolla* ***	-	-	-	-	1	-	-	-	-	-	-	-	-	**1**
†*Podospora* sp. ***	-	-	-	-	2	-	-	-	-	-	-	-	-	**2**
†*Saccobolus glaber* *	-	-	-	-	-	-	nc	-	-	-	-	-	-	-
†*Sordaria fimicola* ***	2	1	1	2	4	2	-	1	2	-	1	-	-	**16**
†*Sporormiella minima* ***	-	1	-	2	1	-	-	-	2	-	1	2	1	**10**
Talaromyces bacillisporus **	-	4	-	3		-	1	1	1	1	2	1	-	**14**
Talaromyces flavus **	1	3	1	2	2	-	2	1	1	1	-	2	2	**18**
Talaromyces rotundus **	-	-	-	-	-	-	1	-	-	1	-	1	1	**4**
T. wortmanii **	-	-	1	-	-	-	1	-	-	1	-	-	-	**3**
Thielavia terricola ***	-	1	-	3	-	-	-	-	1	-	-	-	-	**5**
Xylaria sp. ***	-	-	-	-	-	-	1	-	-	-	-	-	-	**1**
†*Zopfiella latipes* ***	-	-	-	-	1	-	-	-	-	-	-	-	-	**1**
Total	**5**	**15**	**5**	**43**	**20**	**7**	**20**	**8**	**13**	**8**	**10**	**17**	**11**	**182**
% Total isolate	**2.8**	**8.2**	**2.8**	**23.6**	**11**	**3.9**	**11**	**4.4**	**7.1**	**4.4**	**5.5**	**9.3**	**6**	**100**

Remark: † = Coprophilous Ascomycetes, nc = non cultivated

* = Class Discomycetes, ** = Class Plectomycetes, *** = Class Pyrenomycetes

Teleomorphs of *Aspergillus* (*Emericella nidulans*, *Emericella rugulosa*, *Emericella variecolour*, *Eurotium amstelodami*, *Neosartorya fischeri*, *Neosartorya fumigata*) and *Penicillium* (*Eupenicillium parvum*, *Hamigera avellanea*, *Talaromyces bacillisporus*, *Talaromyces flavus*, *Talaromyces rotundus*, *Talaromyces wortmanii*) were recorded in this study (Table 9). *Neosartorya fischeri* was the dominant species for this group followed by *Talaromyces flavus*, *Eupenicillium parvum* and *Eurotium amstelodami* as well as *Talaromyces bacillisporus*, respectively. All of these genera were found to produce a wide variety of novel bioactive compounds such as fumitremorgin A-B, mitorubrins, vermiculine, vermicillin verrucologen and talaron that are potentially useful for pharmaceutical, industrial and agricultural applications (Langley, 1997; Peberdy, 1987; Powell *et al*., 1994; Samson *et al*., 2002; Suzuki, *et al*. 1999; Tjamos and Fravel, 1997).

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 10). 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. Table 10 showed that most Hyphomycetes were 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 10). All isolates could be cultivated on artificial media and maintained as a pure culture with specimen codes of KUFC.

Table 10 Number of isolates of coprophilous Hyphomycetes and other microfungi obtained from different dung types

Fungal species	Number of isolate													**Total**
	barking deer	buffalo	camel	cow	deer	eld's deer	elephant	gaur	goat	horse	rabbit	rat	toad	**isolate**
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**

Remark: * = Coprophilous Hyphomycetes

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 which derived by using the moist chamber technique (Table 10).

Paecilomyces lilacinus was the most common species and 44 isolates were found from dung sample except eld's deer dung follow by *Aspergillus fumigatus* (42), *A. niger* (37), *A. flavus* (30), and *Scopulariopsis brevicaulis* (29). It is 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 10).

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

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. We found *Oidiodendron griseum* on toad dung, *Cephaliophora irregularis* mostly on cow dung, *Arthrobotrys oligospora* on cow and deer dung, *Memnoniella echinata* on cow and horse dung, and

Nodulisporium gregarium on cow dung, which is 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-methoxy-dihydrofuscin 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* produces 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* can 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, is 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).

A total of 22 isolates of Coelomycetes were isolated from dung. Four genera and 4 species were identified including, *Chaetomella raphigera*, *Colletotrichum gloeosporioides*, *Lasiodiplodia theobromae* and *Pestalotiopsis guepinii* (Table 11). All Coelomycetes in this study have been reported as the plant pathogenic fungi and were found on organic debris (Sutton, 1980; Nag Raj, 1993).

Table 11 Number of isolates of Coelomycetes obtained from different dung types

Fungal species	**Number of isolate**													**Total isolate**
	barking deer	buffalo	camel	cow	deer	eld's deer	elephant	gaur	goat	horse	rabbit	rat	toad	
Chaetomella raphigera	-	1	-	2	-	1	-	-	-	-	-	-	-	**4**
Colletotrichum gloeosporioides	1	2	-	3	-	1	-	-	1	1	-	-	-	**9**
Lasiodiplodia theobromae	1	1	-	-	-	-	-	-	-	-	1	-	-	**3**
Pestalotiopsis guepinii	-	1	-	2	-	1	-	1	-	1	-	-	-	**6**
Total	**2**	**5**	**-**	**7**	**-**	**3**	**-**	**1**	**1**	**2**	**1**	**-**	**-**	**22**
% Total isolate	**9.2**	**22.7**	**0**	**31.8**	**0**	**13.6**	**0**	**4.5**	**4.5**	**9.2**	**4.5**	**0**	**0**	**100**

Fifty-four isolates of Agonomycetes were found on various dung samples, comprisng 2 genera, 2 spp. : *Papulaspora immersa* and *Rhizoctonia* sp. (Table 12). *P. immersa* was the most common species found frequently on cow dung (17 isolates), whilst only one species of *Rhizoctonia* was found on cow dung (Table 12). Subramanian (1983) stated that species of *Papulaspora* were recorded invariably and primarily on dung and excreta of animals but rarely, or not at all on other substrates. Domsch *et al*. (1993) mentioned that the original isolate of *P. immersa* was derived from horse, dog and rabbit dung in USA and Canada. De Hoog *et al*. (2000) reported *P. equi* as the pathogen causing eye infection in horses.

Table 12 Number of isolates of coprophilous Agonomycetes and other microfungi obtained from different dung types

Fungal species	**Number of isolate**													**Total isolate**
	barking deer	buffalo	camel	cow	deer	eld's deer	elephant	gaur	goat	horse	rabbit	rat	toad	
Papulaspora immersa *	-	15	-	17	3	1	10	1	4	-	-	2	-	**53**
Rhizoctonia sp.	-	-	-	1	-	-	-	-	-	-	-	-	-	**1**
Total	**-**	**15**	**-**	**18**	**3**	**1**	**10**	**1**	**4**	**-**	**-**	**2**	**-**	**54**
% Total isolate	**0**	**27.7**	**0**	**33.3**	**5.6**	**1.9**	**18.5**	**1.9**	**7.4**	**0**	**0**	**3.7**	**-**	**100**

Remark: * = Coprophilous Agonomycetes

One species of *Coprinus* (Basidiomycete) was observed on elephant dung from Loei Province. This fungus failed to grow on agar media.