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TITLE: *Neosartorya* species: Diversity, Morphology, Phylogeny, Antagonistic Tests Against Plant Pathogenic Fungi and Secondary Metabolites of *N. pseudofischeri*

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THESIS

NEOSARTORYA SPECIES: DIVERSITY, MORPHOLOGY, PHYLOGENY, ANTAGONISTIC TESTS AGAINST PLANT PATHOGENIC FUNGI AND SECONDARY METABOLITES OF *N. PSEUDOFISCHERI*

AMNAT EAMVIJARN

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
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Amnat Eamvijarn 2013: *Neosartorya* species: Diversity, Morphology, Phylogeny, Antagonistic Tests Against Plant Pathogenic Fungi and Secondary Metabolites of *N. pseudofischeri.* Doctor of Philosophy (Plant Pathology), Major Field: Plant Pathology, Department of Plant Pathology. Thesis Advisor: Professor Leka Manoch, Ph.D. 204 pages.

Two hundred and forty-nine soil samples were collected from 16 provinces in Thailand during October 2008 to May 2009. The alcohol, heat treatment methods and Gochenaur's glucose ammonium nitrate agar were employed. Identification of fungal isolates was based on morphological characteristics as colony growth pattern, color and texture on standard media. *Aspergillus* anamorphic state and ascospore ornamentation were examined under light, stereo and scanning electron microscopes. Camera lucida drawing was made. Two hundred and forty-eight isolates of *Neosartorya* species were found including 7 known species, 1 new species and 3 unidentified species comprising *Neosartorya fischeri, N. glabra, N. laciniosa, N. pseudofischeri, N. siamensis* sp. nov. (KUFC 6349), *N. spinosa, N. takakii*, *N. tatenoi*, *Neosartorya* sp.1 (KUFC 6341), *Neosartorya* sp.2 (KUFC 6513) and *Neosartorya* sp.3 (KUFC 6579). *Neosartorya siamensis* was a new species, whereas *N. laciniosa* and *N. pseudofischeri* were new records to Thailand. *Neosartorya spinosa, N. fischeri, N. glabra,* and *N. siamensis* were the dominant species found in forest and agricultural soil. The validation of new species as well as the other four *Neosartorya* spp. were supported by analyses of the β-tubulin gene sequences.

In vitro Antagonistic activity test of 9 *Neosartorya* species against 9 species of plant pathogenic fungi showed that *Neosartorya fischeri* inhibited 53.9-58.3% of radial growth of *Fusarium oxysporum, Alternaria brassicicola, Colletotrichum capsici* and *Curvularia oryzae*. *Neosartorya tatenoi* provided of 62.2 and 51.1% growth inhibition of *Phytophthora palmivora* and *Pythium aphanidermatum*. However, 9 *Neosartorya* spp. could not inhibit *Rhizoctonia oryzae* and *Sclerotium rolfsii.* The efficacy of 6 *Neosartorya* spp. crude extracts revealed that *Neosartorya fischeri* at 1,000 ppm completely inhibited mycelial growth of *Pythium aphanidermatum, Phytophthora palmivora* and *S. rolfsii*, whereas the other 5 *Neosartorya* species and *N. fischeri* at 10,000 ppm strongly suppressed 100% mycelial growth of 8 species of plant pathogenic fungi, but failed to control *Lasiodiplodia theobromae*.

For inhibitory activity of 6 *Neosartorya* crude extracts on weed seed germination and growth inhibition, *Neosartorya pseudofischeri* at 10,000 ppm showed the strongest germination and growth inhibition on *Phaseolus lathyroides* L. at 91.6% and 81.0% respectively, whereas *Neosartorya siamensis* sp. nov. (KUFC 6349) at 10,000 ppm showed the strongest seed germination and growth inhibition on *Mimosa pigra* L. and *Corchorus* sp. at 98.7% , 86.3% and 100% , 99.3% respectively.

For secondary metabolites investigation, four known compounds including cadinene, eurochevalierine, brasiliamide B, and pyripyropene A and three new compounds including a 1,4-diacetyl-2,5-dibenzylpiperazine 3,7′′-oxide, pseudofischerine, and 2,4-dihydroxy-6-methylbenzoic acid were isolated from the fungus *Neosartorya pseudofischeri* KUFC 6422. Eurochevalierine displayed *in vitro* growth inhibitory activity in six human cancer cell lines.

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Amnat Eamvijarn

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LIST OF ABBREVIATIONS

α	=	alpha
APOAF	=	Annexin V-FITC Apoptosis detection kit
ATCC	=	The American Type Culture Collection
β	=	beta
$CHCl_3$	=	chloroform
CH_2Cl_2	=	dichloromethane
$(CH_3)_2CO$	=	acetone
CH_3OH	=	methanol
CF	=	culture filtrates
cm	=	centimeter
cm^2	=	square centimeter
^{13}C NMR	=	Carbon-13 Nuclear Magnetic Resonance
COSY	=	Correlation Spectroscopy
CYA	=	Czapex yeast autolysate agar
CZA	=	Czapek's agar
diam	=	diameter
DSMZ	=	Deutsche Summlung von Mikroorganismen and Zellkulturen
ECACC	=	European Collection of Cell Culture
EtOAc	=	ethyl acetate
$FeCl_3$	=	ferric chloride
g	=	gram
G_1	=	colony radius of plant pathogenic fungi in the control
G_2	=	colony radius of plant pathogenic fungi in the dual culture test
GAN	=	glucose ammonium nitrate agar
HCl	=	hydrochloric acid
HCOOH	=	formic acid
HMBC	=	Heteronuclear Multiple Bond Correlation
^1H NMR	=	Proton Nuclear Magnetic Resonance
HRMS	=	High Resolution Mass Spectroscopy

LIST OF ABBREVIATIONS (Continued)

HSQC	=	Heteronuclear Single Quantum Coherence
ITS	=	Internal Transcribed Spacer
KUFC	=	Kasetsart University Fungal Collection
MEA	=	malt extract agar
MEM	=	minimum essential medium
Me_2CO	=	acetone
MeOH	=	methanol
mg	=	milligram
MHz	=	megahertz
ml	=	milliliter
mm	=	millimeter
mM	=	millimolar
MTT	=	Optimization of the Tetrazolium Dye
µg	=	microgram
µm	=	micrometer or micron
NJ	=	neighbor-joining
OMA	=	oatmeal agar
PCR	=	polymerase chain reaction
PDA	=	potato dextrose agar
PDB	=	potato dextrose broth
petrol	=	petroleum ether
PI	=	propidium iodide
ppm	=	part per million
RFLP	=	restriction fragment length polymorphism
RPMI	=	Roswell Park Memorial Institute medium
SEM	=	Scanning electron microscope
Si Gel	=	silica gel
Stat. Anam.	=	state of anamorph
TLC	=	thin layer chromatography

LIST OF ABBREVIATIONS (Continued)

TMV	=	Tobacco mosaic virus
TPH	=	total petroleum hydrocarbon
TUNEL	=	terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling
UV	=	ultra violet
VEGF	=	vascular endothelial growth factor
WA	=	water agar

NEOSARTORYA SPECIES: DIVERSITY, MORPHOLOGY, PHYLOGENY, ANTAGONISTIC TESTS AGAINST PLANT PATHOGENIC FUNGI AND SECONDARY METABOLITES OF *N. PSEUDOFISCHERI*

INTRODUCTION

The genus *Neosartorya* is a member of Trichocomaeae in Eurotiales (Malloch and Cain, 1972). The anamorphic state belongs to *Aspergillus* section *Fumigati* characterized by uniseriate, columnar conidial heads, flask-shaped vesicle in shades of blue-green to dark green (Raper and Fennell, 1965). They produce thermoresistant ascospores and are commonly found in soils but can also be found in house dust, marine sediment and sponge, healthy plant tissue, air, foods and organic materials (Hong *et al.*, 2005, 2008; Horie *et al.*, 1992, 1995a, b, 2001, 2003; Manoch et al., 2004; Samson *et al.*, 2007; Someya *et al.*, 1999; Takada and Udagawa 1985; Udagawa *et al*., 1991, 1996; Yaguchi *et al*., 1994a, 2010). Ascospore ornamentation is a significant morphological feature for distinguishing species within the genus best seen under electron microscope (Yaguchi *et al.*, 2010). The genus *Neosartorya* currently comprises 36 species and anamorphic 11 species (Hong *et al*., 2005, 2006; Horie *et al.*, 2003; Pitt, 2000; Samson *et al*., 2007; Yaguchi *et al.*, 2010). Several *Neosartorya* species have been reported as pathogenic or mycotoxigenic agents to human and animals causing invasive aspergillosis, osteomyelitis, endocarditis and mycotic keratitis (Balajee *et al*., 2006; Brakhage and Langfelder, 2002; Coriglione *et al.*, 1990; Järv *et al*., 2004; Kwon-Chung and Kim, 1974; Peterson, 1992) and many mycotoxins produced by these species may cause serious health hazard (Fujimoto *et al.*, 1993, Frisvad and Samson, 1990; Larsen *et al*., 2007).

On the other hand, many species of *Neosartorya* produce novel bioactive compounds, which have potential for pharmaceutical (Jayasuriya *et al.*, 2009; Kijjoa *et al.*, 2011), and agricultural use including biodegradation, bioinsecticide and bioherbicide (Chaillan *et al*., 2004; Ozoe *et al*., 2004; Phattanawasin *et al*., 2007; Taewoo *et al*., 2011).

Wong *et al*. (1993) reported that *N. fischeri* produced fiscalins which effectively inhibit the binding of substance P to the human neurokinin receptor. Asami *et al.* (2008) reported that *Neosartorya* sp. isolated from plant rhizosphere, We Fung Chi Cascade region, Taiwan, produced a new angiogenesis inhibitor azaspirene. Azaspirene treatment reduced the number of tumor-induced blood vessels and suppressed Raf^{-1} activation induced by VEGF without affecting activation of kinase insert domain-containing receptor/fetal liver kinase 1. Jayasuriya *et al*. (2009) found that *N. glabra* produced Glabramycin A-C which revealed an antibacterial activity. Glabramycin C showed strong antibiotic activity against *Streptococcus pneumoniae* and modest antibiotic activity against *Staphylococcus aureus.* Kijjoa *et al*. (2011) also reported *N. glabra* which produces sartoryglabrins A-C having *in vitro* growth inhibitory activity on three human tumor cell lines. Shin *et al.* (2006) found that *N. spinosa* can be used for the complete enzymatic recovery of ferulic acid from corn residues. Ozoe *et al*. (2004) reported that *N. quadricincta* produces a derivative of dihydroisocoumarin which inhibited [^{3}H]EBOB binding by 65%. Phattanawasin *et al*. (2007) reported chloroform and ethyl acetate extracts of *Aspergillus fischeri* TISTR from Thailand showed good inhibitory activity on *Mimosa prigra* (Giant Sensitive Tree) and *Echinochloa crus-galli* (barnyard grass). Tan *et al*. (2012) studied *N. fischeri* strain 1008F, a marine-derived fungus produced two new compounds inhibiting the replication of *Tobacco mosaic virus*, human gastric and hepatic cancers.

Moreover, molecular genetic differences based on partial β-tubulin, calmodulin and actin gene sequences and secondary metabolite production can be used to identify fungal species in the genus *Neosartorya* (Hong *et al.*, 2006, 2008; Samson *et al.*, 2007; Yaguchi *et al.*, 2010).

In Thailand, six species of *Neosartorya* have been reported including *Neosartorya delicata, N. fischeri*, *N. multiplicata, N. quadricincta, N. spinosa* and *N. takakii* from soil and termite mould (Kanjanamaneesathian, 1988; Manoch *et al.,* 2005, 2007)*,* and *N. paulistensis* and *Neosartorya* sp. reported as endophytic and marine derived fungi

(Kokaew, 2011; Buaruang *et al.*, 2012) and also *N. fischeri* and *N. glabra* found from aninal dung (Jeamjitt, 2007; Sudsanguan, 2012).

Thus, it is very interesting to study the genus *Neosartorya* on various topics of soil in Thailand, especially diversity, taxonomy, phylogeny, antagonistic test against plant pathogenic fungi and the secondary metabolites production. Because these fungi were poorly known with the only significant records contributed by Kanjanamaneesathian (1988) and Manoch *et al.* (2005, 2007). Their studies were limited in areas surveyed and taxonomy. Hence, more investigations on *Neosartorya* species need to be carried out in this tropical region for the discovery of new taxa, the utilization of some species as biological control agents against plant pathogenic fungi and the analysis for secondary metabolites on *N. pseudofischeri* KUFC 6422 is a very challenging topic for industrial, pharmaceutical and agricultural enterprises.

OBJECTIVES

1. To isolate *Neosartorya* spp. from soil at different locations in Thailand

2. To identify *Neosartorya* spp. based on their morphological characteristics

3. To study molecular phylogenetic relationships of *Neosartorya* spp.

4. To maintain pure cultures of *Neosartorya* species in the Kasetsart fungal culture collection (KUFC) at the Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok

5. To isolate and identify secondary metabolites of *N. pseudofischeri* (KUFC 6422)

6. To study *in vitro* antagonistic activity of nine species of *Neosartorya* against nine species of plant pathogenic fungi

7. To study germination and growth inhibitory activity of *Neosartorya* crude extract on weed, *Phaseolus lathyroides* L., *Mimosa pigra,* and *Corchorus* sp.

LITERATURE REVIEW

1. Diversity and taxonomy study of *Neosartorya*

1.1 Diversity and distribution of *Neosartorya* in Thailand

Kanjanamaneesathian (1988) studied on thermophilic and thermoresistant fungi from soils, dungs and agricultural wastes from several parts of Thailand. The results showed that *Aspergillus fumigatus* and *Neosartorya fischeri* were found in these substrates.

Manoch and Chana (1995) reported six isolates of *Neosartorya* comprising *Neosartorya fischeri, N. glabra, N. spinosa* and 3 unidentified *Neosartorya* spp. isolated from several soil and dung samples from Kanchanaburi, Chachoengsao, Chiang Mai, Pattani and Phrae provinces.

Manoch *et al.* (2005, 2007) studied the fungal diversity and distribution from soil in hotsprings, agriculture soil and other habitats collected from 13 provinces including Bangkok, Buri Ram, Chon Buri, Chiang Mai, Chiang Rai, Krabi, Kanchanaburi, Mae Hong Son, Si Sa Ket, Surat Thani, Trat, Ubon Ratchathani and Uthai Thani provinces. Isolation methods using soil plate, dilution plate, alcohol, heat treatments and glucose ammonium nitrate agar (GAN) and streptomycin were employed. *Neosartorya fischeri* was found from Chon Buri, Mae Hong Son and Si Sa Ket provinces. *Neosartorya multiplicata* and *N. quadricincta* were isolated from agricultural soil in Chiang Rai and Krabi respectively.

Bussarakam (2002) reported *Neosartorya fischeri* from rhizosphere soil of terrestrial orchid *Goodyera procera* from Chiang Mai province, Northern Thailand.

Jeamjitt (2007) studied diversity of coprophilous fungi from sixty dung samples of thirteen wild and domestic animals. The results revealed that twenty two

isolates of *Neosartorya fischeri* were collected from buffalo, eld's deer, cow, deer, elephant, gaur, goat, rat and toad dung samples.

Kokaew (2011) studied the diversity of endophytic fungi from Thai forests. The results showed that *Neosartorya paulistensis* and *N. spinosa* were found on *Dracaena conferta* (Dracaenaceae) and *Neosartorya* sp.1 KUFC 5825 was isolated from *Schefflera actinophylla* (Araliaceae).

Buaruang *et al*. (2012) reported a marine-derived fungus, *Neosartorya paulistensis* isolated from marine sponge (*Chondrilla australiensis*) collected from coral reef at Mu Koh Lan, Chonburi province, in the Gulf of Thailand.

Sudsanguan (2012) studied on species and distribution of coprophilous fungi in central and northeastern Thailand. Alcohol, heat treatment methods and GAN were used to isolate dung fungi. *Neosartorya fischeri* and *N. glabra* were found from cow, elephant and toad dungs.

1.2 Diversity and morphological study of *Neosartorya*

Raper and Fennell (1965) studied the genus of *Aspergillus* and divided it into 18 groups. The *Aspergillus fumigatus* group is characterized by conidial heads that are typically columnar, often compactly so, in shades of green from light blue-green to very dark green; vesicles flask-shaped; typically fertile over the upper half to three-fourths; sterigmata in a single series, crowded; conidiophores smooth walled, particularly in the terminal area; conidia globose to subglobose, rarely elliptical, echinulate to delicately roughened; cleistothecia present in some species, at first in shades of white to cream, buff to yellow or orange in age; ascospores colorless, bivalve in pattern with equatorial crests and with convex surfaces diversely ornamented. Seven species producing cleistothecia were reported including *Aspergillus fischeri, A. fischeri* var. *glaber*, *A. fischeri* var. *spinosus, A. quadricinctus, A. aureoles, A. stramenius* and *A. auratus.*

Malloch and Cain (1972) reported the name Eurotiaceae Clem and Shear was shown to be a later synonym for the Trichocomaceae and the name Aspergillaceae Link was illegitimate when applied to Ascomycete perfect state.

Kwon-Chung and Kim (1974) reported a new heterothallic fungus, *Neosartorya fennelliae* (anamorphic state: *Aspergillus fennelliae*), isolated from eye balls of laboratory rabbits. The conidial heads and conidia were similar to *N. fischeri.* The strains can posses both mating types, *A* and *a*, of allelic pair and produce the teleomorph. Although interspecific crossing does not occur with any known member (Figure 1 A).

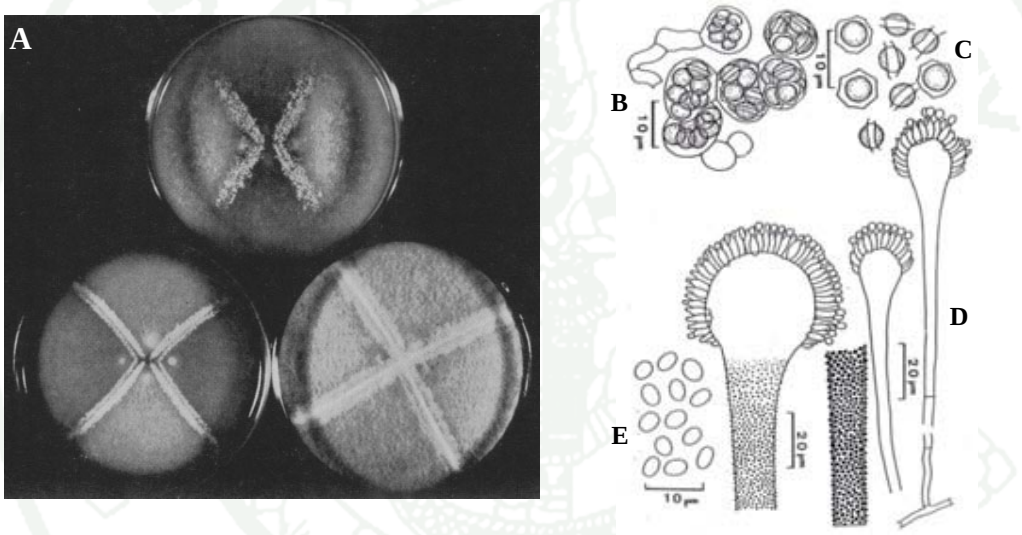


Figure 1 Morphological characteristic of heterothallic *Neosartorya* spp.
Neosartorya fennelliae (A): A. interspecific crossing (Kwon-Chung and Kim, 1974)
N. spathulata (B-E): B. Asci, C. Ascospores, D. Anamorph, E. Conidia
(Takada and Udagawa, 1985)

Table 1 Anamorph links of *Neosartorya* spp.

Teleomorph	Anamorph	References
Neosartorya assulata	*Aspergillus assulatus*	Hong *et al.*, 2008
N. aurata	*A.s igneus*	Kozakiewicz, 1989
N. aureola	*A. aureoluteus*	Malloch and Cain, 1972
N. australensis	-	Samson *et al.*, 2007
N. botucatensis	*A. botucatensis*	Horie *et al.*, 1995a
N. coreana	*A. coreanus*	Hong *et al.*, 2006
N. delicata	*A. delicatus*	Kong, 1997
N. denticulata	*A. denticulatus*	Hong *et al.*, 2008
N. fennelliae	*A. fennelliae*	Kwon-Chung and Kim, 1974
N. ferenczii	-	Samson *et al.*, 2007
N. fischeri	*A. fischeranus*	Malloch and Cain, 1972
N. galapagensis	*A. galapagensis*	Hong *et al.*, 2008
N. glabra	*A. neoglaber*	Kozakiewicz, 1989
N. hiratsukae	*A. hiratsukae*	Udagawa *et al.*, 1991
N. indohii	*A. indohii*	Horie *et al.*, 2003
N. laciniosa	*A. laciniosus*	Hong *et al.*, 2006
N. multiplicata	*A. multiplicatus*	Yaguchi *et al.*, 1994a
N. nishimurae	*A. nishimurae*	Takada *et al.*, 2001
N. otanii	*A. otanii*	Takada *et al.*, 2001
N. papuensis	-	Samson *et al.*, 2007
N. paulistensis	*A. paulistensis*	Horie *et al.*, 1995a
N. pseudofischeri	*A. thermomutatus*	Peterson, 1992
N. primulina	*A. primulinus*	Udagawa *et al.*, 1993
N. quadricincta	*A. quadricingens*	Malloch and Cain, 1972
N. shendawii	-	Yaguchi *et al.*, 2010
N. spathulata	*A. spathulatus*	Takada and Udagawa, 1985
N. spinosa	*A. spinosus*	Kozakiewicz, 1989
N. stramenia	*A. paleaceus*	Malloch and Cain, 1972

Table 1 (Continued)

Teleomorph	Anamorph	References
N. sublevispora	*A. sublevisporus*	Someya et al., 1999
N. takakii	*A. takakii*	Horie *et al.*, 2001
N. tatenoi	*A. tatenoi*	Horie *et al.*, 1992
N. tsunodae	-	Yaguchi *et al.*, 2010
N. tsurutae	*A. tsurutae*	Horie *et al.*, 2003
N. udagawae	*A. udagawae*	Horie *et al.*, 1995b
N. wacupii	-	Samson *et al.*, 2007

Takada and Udagawa (1985) reported a novel heterothallic fungus, *Neosartorya spathulata* (anamorphic state: *Aspergillus spathulatus*) was found from agricultural soil from *Alocasia macrorrhiza* field, Tai-Ping Village, Ilan Prefecture, Formosa. This species is characterized by light yellowish cleistothecia, bivalved ascospores with nearly smooth convex surfaces, and the production of two types of conidiophores (Figures 1 B-E).

In Japan, Udagawa *et al.* (1991) studied on the spoilage outbreak in commercial foods of heat resistant molds, an interesting *Neosartorya* was isolated from aloe beverage. *Neosartorya hiratsukae* (anamorphic state: *Aspergillus hiratsukae*) is characterized by its very restricted growth on CZA and ascospores with low, closely appressed equatorial crests and with convex surfaces bearing numerous close-anastomosing ridges arranged in a fine reticulate ornamentation (Table 1, Figures 2 A-D).

In the course of a survey of mycotoxigenic strains of *Aspergillus fumigatus* (Horie *et al.*, 1992) a new *Neosartorya tatenoi* (anamorphic state: *Aspergillus tatenoi*) was isolated from soil in a sugarcane plantation in Brazil. This fungus differs from other known species of the genus in having convex walls of ascospores, ornamented with distinctly reticulate ridges (Table 1, Figures 2 E-F).

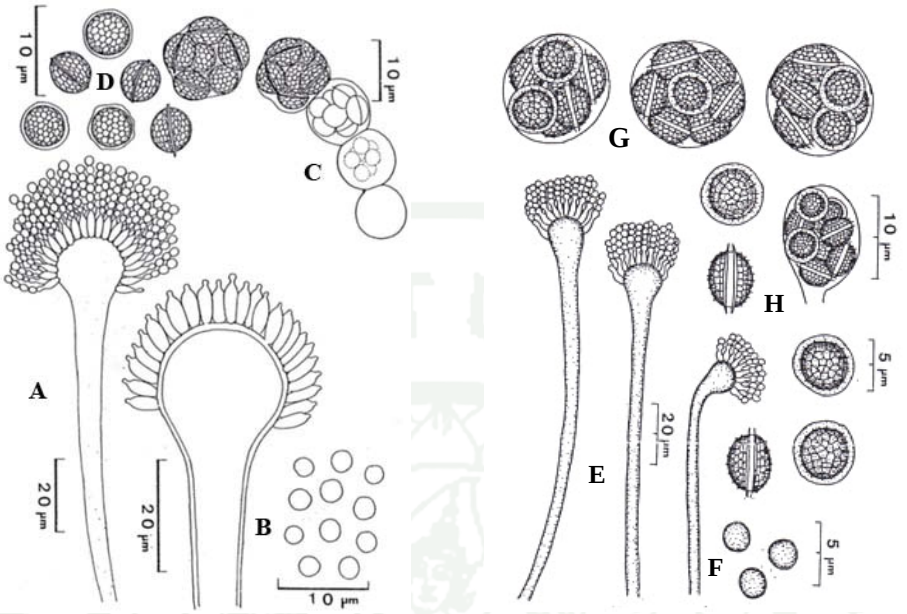


Figure 2 Morphological characteristic of homothallic; *Neosartorya hiratsukae* (A-D): A. Anamorph, B. Conidia, C. Asci, D. Ascospores (Udagawa *et al.,* 1991) *N. tatenoi* (E-H): E. Anamorph, F. Conidia, G. Asci, H. Ascospores (Horie *et al.,* 1992)

Peterson (1992) re-examined a *Neosartorya* strain isolated during an autopsy from fungal lesion occurring in human neck vertebrae. A new *Neosartorya pseudofischeri* (anamorphic state: *Aspergillus thermomutatus*) is characterized by its convex surfaces of ascospores ornamented with raised flaps of tissue, in shape resembling triangular projections or long ridge lines (Table 1, Figures 3 A-F).

Heat resistance fungus, *Neosartorya fischeri* was isolated mainly from soil samples that were exposed to temperatures of 60, 70 and 80˚C and sporadically from samples heated to 90˚C (Jasenská *et al.*, 1993).

An unusual *Neosartorya* was isolated from Houli, Taichung, in Taiwan. *Neosartorya multiplicata* (Table 1, Figures 3 G-J) (anamorph state: *Aspergillus*

multiplicatus) is characterized by its restricted growth on CZA, white ascomata, nearly globose ascospores with ribbed surface ornamentation of several linear ridges, and limited production of conidia on common media (Yaguchi *et al.*, 1994a, b).

Horie *et al*. (1995a) studied on thermophillic and thermotolerant fungi collected from soil planted with corn and sugar cane in Sao Paulo State, Brazil, two new species of homothallic *Neosartorya*, *N. botucatensis* (anamorphic state: *Aspergillus botucatensis*) characterized by its having ascospores with long spines on the convex surfaces (Figures 3 K-N). *N. paulistensis* (anamorphic state: *A. paulistensis*) characterized by its ascospore walls with spinose and verruculose ornamentation (Figures 3 O-R).

Neosartorya udagawae (anamorphic state: *A. udagawae*) is characterized by dull green colony on MEA, broadly lenticular ascospores with two equatorial or several irregular crests and tuberculate convex surfaces (Table 1, Figures 4 A-D). *N. aureola* (Table 1, Figures 4 E-H) is also reported as a new record from Brazilian soil (Horie *et al*., 1995b).

Udagawa *et al.* (1996) reported ascomycetous microfungi from 58 house dust samples from detached and apartment dwellings around Kobe city and revealed that species of *Neosartorya* were common ascomycetous propagules in the house dust. *Neosartorya* species accounted for 8.3% of the total identified isolated, of which *N. pseudofischeri* was the main constituent followed by *N. glabra* and *N. quadricincta* respectively.

Hua-Zhong (1997) reported a survey of microfungi isolated from Yunnan province, China. A new *Neosartorya delicata* Kong is characterized by its growth rapidly on CZA and prominent ridges spreading to the equatorial crests, and delicate ornamentation (Table 1).

Figure 3 Morphological characteristic of *Neosartorya pseudofischeri* (A-F): A, B. Anamorph, C. Conidia, D. Ascomatal initials, E. Asci, F. Ascospores (Peterson, 2992)

N. multiplicata (G-J): G. Anamorph, H. Ascomatal initials, I. Asci, J. Ascospore (Yaguchi *et al.,* 1994a)

N. botucatensis (K-N): K. Anamorph, L. Conidia, M. Asci, N. Ascospores; *N. paulistensis* (O-R): O. Anamorph, P. Conidia, Q. Asci, R. Ascospores (Horie *et al.,* 1995a)

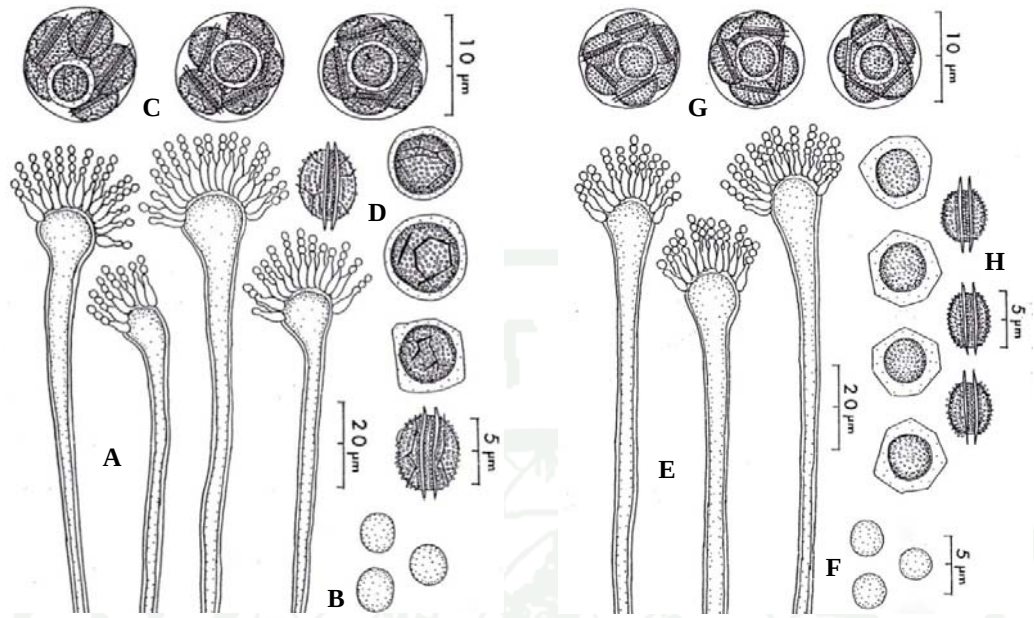


Figure 4 Morphological characteristic of homothallic; *Neosartorya udagawae* (A-D): A. Anamorph, B. Conidia, C. Asci, D. Ascospores (Horie *et al.,* 1995b)
N. aureola (E-H): E. Anamorph, F. Conidia, G. Asci, H. Ascospores (Malloch and Cain, 1972)

Someya *et al.* (1999) found a new species of *Neosartorya, N. sublevispora* (anamorphic state: *Aspergillus sublevisporus*) is characterized by lenticular ascospores with two low equatorial crests. The ornamentation of ascospores is composed of two closely appressed crests and small even-sized echines on their convex surfaces (Table 1).

Horie *et al.* (2001) reported the study of *Neosartorya* isolated from grassland soil in Roraima State, Brazil. A new species of *Neosartorya*, *N. takakii* Horie, Abliz & Fukushima is described and illustrated. *N. takakii* (anamorphic state: *Aspergillus takakii*) differs from the other known species of the genus in having lenticular ascospores with two distinct equatorial crests and with roughly circularly arranged projections on the convex walls (Table 1).

Two new heterothallic species of *Neosartorya* isolated from soil in Kenya. *Neosartorya nishimurae* (anamorphic state: *Aspergillus nishimurae*) Takada, Horie &

Abliz is characterized by its rapid growth on CZA and MEA, lenticular ascospores with broad equatorial crests and echinulate convex walls (Table 1, Figures 5 A-D). *N. otanii* (Table 1, Figures 5 E-H) (anamorphic state: *Aspergillus otanii*) Takada, Horie & Abliz is characterized by its rapid growth on CZA and MEA, lenticular ascospores with widely separated equatorial crests and tuberculate or lobate-reticulate convex walls (Takada *et al.*, 2001).

Horie *et al.* (2003) reported soil fungi from tropical rainforest soil in Amazonian area, Brazil and isolated by a soil plate method of CZA. Eight known *Neosartorya* spp. were obtained from 110 soil samples: *Neosartorya aureola, N. botucatensis, N. fischeri, N. glabra, N. pseudofischeri, N. quadricincta, N. spinosa, N. tatenoi* and two new *Neosatorya* species including *N. indohii* and *N. tsurutae*. *N. indohii* (anamorphic state: *Aspergillus indohii*) is characterized by its broadly lenticular ascospores with two conspicuously serrate-incised equatorial crests and tuberculate convex surfaces (Table 1, Figures 5 I-K). *N. tsurutae* (anamorphic state: *Aspergillus tsurutae*) is characterized by broadly lenticular ascospores with four equatorial crests and rugulose-ruminate convex surfaces (Table 1, Figures 5. L-N).

Samson *et al.* (2007) studies and revised the taxonomy of *Aspergillus* section *Fumigati* and its teleomorph *Neosartorya.* A key was created for identification of 24 species including *Neosatorya assulata*, *N. aurata, N. aureola, N. australensis, N. coreana, N. denticulata, N. fennelliae, N. ferenczii, N. fischeri, N. galapagensis, N. glabra, N. hiratsukae, N. laciniosa, N. multiplicata, N. nishimurae, N. papuensis, N. pseudofischeri, N. quadricincta, N. spathulata, N. spinosa, N. stramenia, N. tatenoi, N. udagawae* and *N. wacupii*. The main of characteristics used for identification include colony diameter and color on specific media; shape, size and ascospores ornamentation under scanning electron microscope; anamorph and the ability to produce the teleomorph in culture (homothallic or heterothallic strain) were studied.

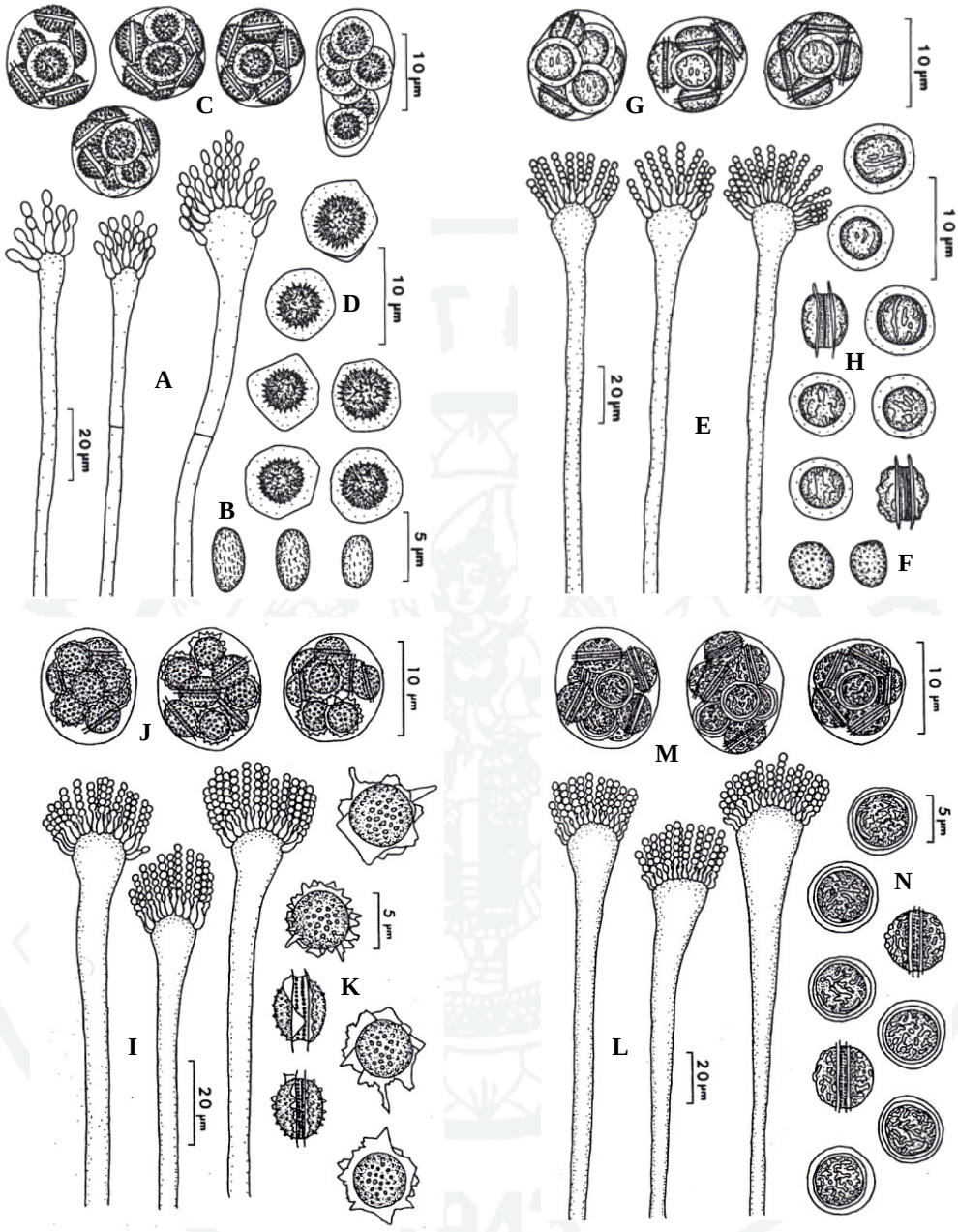


Figure 5 Morphological characteristic of homothallic; *Neosartorya nishimurae* (A-D): A. Anamorph, B. Conidia, C. Asci, D. Ascospores; *N. otanii* (E-H): E. Anamorph, F. Conidia, G. Asci, H. Ascospores (Takada *et al.,* 2001) *N. indohii* (I-K): I. Anamorph, J. Asci, K. Ascospores; *N. tsurutae* (L-N): L. Anamorph, M. Asci, N. Ascospores (Horie *et al.,* 2003)

Hong *et al.* (2006, 2008) studied on soil-borne *Apergillus* and *Penicillium* in Korea, Suriname, Netherlands and Ecuador, and many *Neosartorya* strains were encountered. Five new species of *Neosartorya* compring *N. assulata, N. coreana*, *N. denticulate, N. laciniosa* and *N. galapagensis* were isolated from soil planted with tomato and perilla and *Acer pseudoplatanus*. *Neosartorya assulata* has large, round flaps on convex surfaces of ascospores with two distinct equatorial crests. *N. coreana* has rugose to weak reticulate ascospores with two often bent crests, but without equatorial rings of small projections. *N. denticulata* has denticulate ascospores with a prominent equatorial furrow. *N. laciniosa* has microtubulate ascospores with two bent crests and two distinct equatorial rings of small projections. *N. galapegensis* has ascospores with two wide conspicuous equatorial crests and with microtubulate convex surfaces.

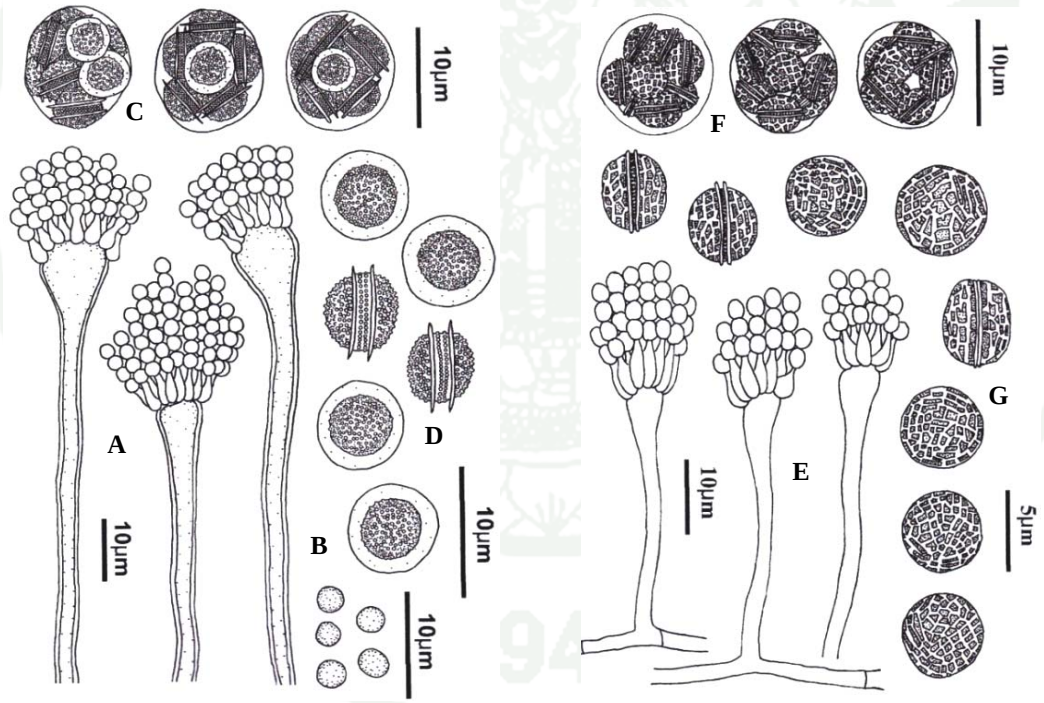


Figure 6 Morphological characteristic of homothallic; *Neosartorya shendaweii* (A-D): A. Anamorph, B. Conidia, C. Asci and ascospores, D. Ascospores; *N. tsunodae* (E-G): E. Anamorph, F. Asci and ascospores, G. Ascospores (Yaguchi *et al.,* 2010)

Heat resistance of *Neosartorya fischeri* isolated from an apple nectar processing plants, was studied in three different juices (apple, pineapple and papaya). The optimum heat activation temperature and time to kill the ascospores of *N. fischeri* was 85˚C for 10 min (Salomão *et al.*, 2007).

Yaguchi *et al.* (2010) studied on pathogenic and mycotoxigenic fungi in Xinjiang, China and in Pernambuco, Brazil. Two new *Neosartorya* species were reported; *N. shendaweii* is characterized by its ascospores with widely separated equatorial crests and tuberculate and verrucose convex surfaces (Table 1, Figures 6 A-D). *N. tsunodae* is characterized by its unique ascospores with low equatorial crests, an evident furrow as a deep depression, and finely reticulate convex surfaces (Table 1, Figures 6 E-G).

2. Secondary metabolites and molecular study of the genus *Neosartorya*

Fujimoto *et al.* (1993) reported a new toxic metabolite named fischerin (1, 4–dihydroxy-3, 5-disubstitute-2(1H)-pyridone) from *Neosartorya fischeri* var. *fischeri* which caused lethal peritonitis in mice.

Wong *et al.* (1993) reported three new compounds, named fiscalin A, B and C, were found in culture broth produced by a *Neosartorya fischeri*. These compounds inhibit the binding of radiolabeled substance P ligand to the human neurokinin (NK-1) receptor, with Ki values of 57, 174, and 68 μM, respectively. Detailed spectroscopic and amino acid analyses led to the elucidation of structures for the three fiscalins. The structures contain an indolyl moiety linked to an athranilic acid derived tricyclic system. The absolute configuration of fiscalin A was determined by X-ray crystallography and chiral amino acid analysis. The presence of fiscalins was detected directly in crude cellular extracts using LC-MS methods.

EcoRI restriction fragment length polymorphism (RFLP) and Southern hybridization were studied to identify the genomic relationship between *Aspergillus fumigatus* and *Neosartorya fischeri* and between the different varieties of *N. fischeri* (*N.*

fischeri var. *glabra* and *N. fischeri* var. *spinosa*). The result revealed RFLP patterns and Southern hybridization could differentiate most of *N. fischeri* var. *fischeri* and *N. fischeri* var. *spinosa* strains but could not exhibit RFLP patterns of *A. fumigatus*. By using the endonucleases *EcoRI* , HindIII and BglII it was possible to distinguish *N. fischeri* var. *fischeri* and *A. fumigatus* (Girardin *et al.*, 1995).

Proksa *et al.* (1998) stated that a new metabolite, named neosartorin (Figure 7.1) was identified in the mycelium of *Neosartorya fischeri* isolated from Vah river sediments in Slovakia and grown on glucose/glycerol medium. Mycelium obtained after filtration of fermentation beer was extracted with EtOAc and dried in vacuo, after which it was chromatographed on column of silica gel.

Ugwuanyi and Obeta (1999) stated that *Neosatorya fischeri, N. fischeri* var. *spinosa* and *N. quadricincta* isolated from Nigerian soil produced pectinolytic and cellulolytic enzymes to macerate mango (*Mangifera indica*) and African mango (*Irvingia gabonensis*).

Asami *et al.* (2002) discoverd new angiogenesis inhibitors from screening of microbial metabolites; azaspirene (Figure 7.2) was isolated from the fungus *Neosartorya* sp. Angiogenesis is recognized as a critical process in the growth and metastasis of tumor cells and many pathological conditions. Azaspirene inhibits the endothelial migration induced by vascular endothelial growth factor.

Souza-Motta *et al.* (2003) reported the hydrolysis of inulin by filamentous fungi especially *Aspergillus* isolated from the sunflower's rhizosphere, which is a source of fungi able to hydrolyse inulin, that might be used in biotechnological processes. Species of *Aspergillus, Chaetomium, Cunninhamella, Emericella, Eupenicillium, Fusarium, Myrothecium, Neosartorya, Neocosmospora, Penicillium and Thielavia* are being related by first time as inulinase producers.

Chaillan *et al.* (2004) stated that the screening of aerobic culturable hydrocarbon (HC)-degrading microorganism isolated from petroleum-polluted soils and cyanobacterial mats from Indonesia resulted in the collection of 33 distinct species. Twenty-one fungi were identified belonging to *Aspergillus, Penicillium, Fusarium, Amorphoteca, Neosartorya, Paecilomyces, Talaromyces and Graphium*. All strains were cultivated axenically in synthetic liquid media with crude oil as sole carbon and energy source. After incubation, the detailed chemical composition of the residual oil was studied by gravimetric and gas-chromatographic techniques.

Ozoe *et al.* (2004) reported the purpose of discovering GABA receptor-directed insecticides in natural products, so fungal culture extracts were screened for their ability to inhibit the specific binding of the competitive antagonist [^{3}H]EBOB to housefly head membranes. The screening efforts led to the isolation of a derivative of dihydroisocoumarin (Figure 7.3) from the culture of *Neosartorya quadricincta*. This compound 2.2 μM inhibited [^{3}H]EBOB binding by 65%. This ligand might prove to be a lead compound for the identification of novel insecticides cating at the insect GABA receptor.

Balajee *et al.* (2006) examined the genetic diversity of clinical isolates identified as *Aspergillus fumigatus* using restriction enzyme polymorphism analysis and sequence-based identification. Analysis of 50 clinical isolates from geographically diverse locations recorded the presence of at least three distinct species: *Aspergillus lentulus, A. fumigatus* and *A. udagawae* (teleomorph state: *Neosartorya udagawae*).

A polyphasic taxonomic approach can help determine species variability and consequently, the delimitation of species or genus. For phenotypic analyses, differences in morphology was determined using macro- and micro-morphology and scanning electron microscopy (SEM) methods and growth temperatures were studied. For phylogenetic analyses, β-tubulin and calmodulin gene sequences were used. Secondary metabolite profiles were analysed for *Aspergillus* taxonomy. However, the intermediate pattern of ascospore ornamentation in the group of *Neosartorya glabra* (smooth

surfaces), *N. spinosa, N. botucatensis* and *N. paulistensis* (echinulate surfaces) were analysed by using polyphasic taxonomy. Phenotypic and genotypic characters of strain of *N. spinosa*, *N. botucatensis* and *N. paulistensis* had identical partial β-tubulin and calmodulin gene sequences and could not be differentiated on morphological characteristics. The study of secondary metabolites showed that *N. spinosa*, *N. botucatensis* and *N. paulistensis* produced aszonalenin, 2-pyrovoylaminobenzamide and pseurotin (Hong *et al.*, 2006).

Shin *et al.* (2006) reported the recovery of ferulic acid from biomass via biological methods is of interest for a number of reasons. The discovery of a filamentous fungus *Neosartorya spinosa* NRRL 185 capable of producing a full complement of enzymes to release ferulic acid and the development of an enzymatic process for a complete recovery of ferulic acid from corn bran and corn fibers. The enzymatic ferulic acid recovery accompanied a significant release of reducing sugar (76-100%), suggesting much broader applications of the enzymes and enzyme mixtures from this organism.

Table 2 Secondary metabolites produced by *Neosartorya* species

Species	Secondary metabolites
Neosartorya assulata	indole alkaloids and apolar metabolites
N. aurata	helvolic acid, yellow unidentified compounds
N. aureola	fumagillin, tryptoquivaline, tryptoquivalone, pseurotin A and viriditoxin
N. australensis	wortmannin-like, aszonalenin-like
N. coreana	aszonalenin
N. denticulata	gliotoxin, viriditoxin
N. fennelliae	asperfuran, aszonalenin, fumigaclavine, viridicatumtoxin, gliotoxin-like, fumigatins and aszonalenin-like
N. fischeri	terrein, fumitremorgins A & C, tryptoquivaline A, trypacidin, TR-2, verruculogen, sarcin, aszonalenin, fischerin, neosartorin, fiscalins, helvolic acid

Table 2 (Continued)

Species	Secondary metabolites
N. galapagensis	gregatins
N. glabra	asperpentyn, avenaciolide, wortmannin-like compound
N. hiratsukae	avenaciolide
N. laciniosa	aszonalenin, tryptoquivalide, tryptoquivalone
N. multiplicata	helvolic acid
N. papuensis	wortmannin-like
N. pseudofischeri	asperfuran, cytochalasin-like compound, fiscalin-like compound, pyripyropene, gliotoxin
N. quadricincta	quinolactacin, aszonalenin
N. spinosa	aszonalenin, 2-pyrovoylaminobenzamide, pseurotin
N. spathulata	xanthocillins, aszonalenin
N. stramenia	quinolactacin, avenaciolide
N. tatenoi	aszonalenin
N. udagawae	fumigatin, fumigallin, tryptoquivaline, tryptoquivalone
N. warcupii	wortmannin-like, aszonalenin-like, chromanols-like, tryptoquivaline-like and tryptoquivalone-like

Source: Hong *et al.* (2008); Samson *et al.* (2007)

Phattanawasin *et al*. (2007) reported the activity of chloroform and ethyl acetate extracts of *Aspergillus fischeri* TISTR which showed good inhibitory activity on *Mimosa pigra* (Giant Sensitive Tree) and *Echinochloa crus-galli* (barnyard grass). Bioassay-directed fractionation of the active extracts led to the isolation of five known compounds (Figures 7.4-7.8), (+)-terrein, (-)-6-hydroxymellein, butyrolactone I, two diketopiperazines (*cyclo*-(*S*-Pro-*S*-Leu) and *cyclo*-(*S*-Pro-*S*-Val). Compounds 5-8 were reported for the first time in this fungus. Their structural determinations were based on analyses of the spectroscopic data and their weed growth inhibitory effects were assessed. Terrein showed the strongest inhibition, especially root growth of both weeds, at every

tested concentration whereas (-)-6-hydroxymellein revealed the strongest effect on *M. pigra*.

Samson *et al.* (2007) revised the taxonomy of *Aspergillus* section *Fumigati* with its teleomorph genus *Neosartorya* (Table 1).The species concept is based on phenotypic (morphology and extrolite profiles, Table 2) and molecular (β-tubulin and calmodulin gene sequences) characteristics in a polyphasic approach. Four new taxa are proposed: *Neosartorya australensis, N. ferenczii, N. papuensis* and *N. warcupii*.

Salomão *et al.* (2007) isolated heat resistant *Neosartorya fischeri* from an apple nectar processing plant. This fungus was studied in three different juices (apple, pineapple and papaya). The optimum heat activation temperature and time for the ascospores of the *N. fischeri* to grow was 85˚C for 10 min.

Simon *et al.* (2007) reported the bioethanol produced from lignocellulosic biomass such as yellow pine sawdust may significantly reduce the level of oil consumption in the USA in the near future. *Neosartorya spinosa* produced a series of lignocellulolytic enzymes that hydrolyze cellulose and hemicelluloses in the biomass substrate into monomeric sugars. The optimal cultivation period of the microbes was determined by analysis of the daily enzyme activity and protein concentration of the cellulolytic enzyme solution.

Alcazar-Fuoli *et al.* (2008) studied 28 *Aspergillus* strains belonging to section *Fumigati* that were isolated from clinical samples in Spain. Based on sequencing of partial sequences of the β-tubulin and rodlet A genes was used to classify into six different clades including *Aspergillus viridinutans, A. lentulus, A. fumigatiaffinis, Neosartorya hiratsukae* and *N. pseudofischeri.*

Asami *et al.* (2008) reported the discovery of a novel inhibitor of angiogenesis from the fungus, *Neosartorya* sp., isolated from soil sample, produced a new angiogenesis inhibitor, which designed azaspirene. Azaspirene (Figure 7.2) treatment

reduced the number of tumor-induced blood vessels and suppressed Raf[1] activation induced by VEGF without affecting activation of kinase insert domain-containing receptor/fetal liver kinase 1.

Igbinigie *et al.* (2008) stated in the investigation of root zone of *Cynodon dactylon* (Bermuda grass) from the surface of coal dumps in the Witbank coal mining area of South Africa indicated that a number of fungal species may be actively involved in the biodegradation of hard coal. In an extensive screening program of over two thousand samples, *Neosartorya fischeri*, was isolated and identified. This is the first report of *N. fischeri*-mediated coal biodegradation and, in addition to possible application in coal biotechnology, the finding may enable development of sustainable technologies in coal mine rehabilitation.

Čerňansky *et al.* (2009) reported the production of volatile derivatives of arsenic using pure culture of *Neosartorya fischeri* isolated from a mining site highly contaminated with arsenic (Pezinok, Slovakia). Arsenic was used in its trivalent and pentavalent forms to evaluate the effect of arsenic valency on its biovolatilization. *N. fischeri* volatilized 23% and 24% of arsenic in trivalent and pentavalent forms respectively.

Jayasuriya *et al*. (2009) reported the discovery and antibacterial activity of Glabramycin A-C from *Neosartorya glabra* for the treatment of drug-resistant bacteria. Glabramycin C showed strong antibiotic activity against *Streptococcus pneumoniae* and modest antibiotic activity against *Staphylococcus aureus*.

Shen *et al.* (2009) stated that identification of two marine fungi and the inhibitory effects of their crude extracts on *Tobacco Mosaic Virus* and two tumor cell lines. Crude extracts was obtained by extracting with MeOH and evaporated in vacuo. The extract was water-soluble fraction which was dissolved in water, and the other fraction was water insoluble. The fungi were identified by means of morphology and molecular methods using internal transcribed spacer (ITS) regions of the ribosomal DNA. The inhibitory

effects on Tobacco mosaic virus was evaluated by indirect enzyme linked immunosorbant assay, and the anti-tumor activity was tested by methyl thaialyl tetrazolium method. The fungi were identified as *Penicillium oxalicum* and *Neosartorya fischeri*. There crude extracts inhibited *Tobacco mosaic virus* and two tumor cell lines. The active fraction named 0312F1 inhibited *Tobacco mosaic virus* and was insoluble in water, whereas the fraction inhibited tumor cell lines was water-soluble. The active fraction named 0312F1 inhibited *Tobacco mosaic virus* was different from that named 1008F1 inhibited *Tobacco mosaic virus*. The active fraction named 0312F1 inhibited tumor cell lines was the same as that named 10008F1. Furthermore, the inhibitory activity of water-soluble fraction named 0312F1 against BEL7404 cell line was much higher than that against SGC-7901 cell lines, whereas the inhibitory activity of active fraction named 1008F1 against SGC-7901 cell lines was much higher.

In addition, five new *Neosartorya* species, *N. denticulata, N. assulata* and *N. galapagensis* were analysed using β-tubulin (benA), calmodulin and actin gene sequences showed that the taxa grouped separately from the known species and confirmed the phenotypic differences (Hong *et al.*, 2008; Yaguchi *et al.*, 2010).

Alves-Prado *et al.* (2010) reported a microbial screening of xylanase producer was carried out in Brazilian Cerrado area in Selviria city, Mato Grosso do Sul State, Brazil. About 15 fungal strains were isolated from soil sample at 35˚C. Between this isolated microorganisms, a fungus *Neosartorya spinosa* as good xylanase producer were identified. A fungus identified as *N. spinosa* (strain P2D16) was cultivated on solid-state fermentation using as substrate source wheat bran, wheat bran plus sawdust, corn straw, corncob, cassava bran, and sugar cane bagasse. Wheat bran and corncobs are substrates showing the better xylanase production after 72 h of fermentation.

Misidentification of fungal species within the section *Fumigati* has been increasingly reported by clinical laboratories. A multiplex polymerase chain reaction (PCR) was developed using β-tubulin and rodlet A partial gene sequence. β-tubulin was able to differentiate among 13 individual species: *Aspergillus fumigatus, A.*

fumigatiaffinis, A. novofumigatus, Neosartorya aurata, N. aureola, N. hiratsukae, N. fennelliae, N. fischeri, N. pseudofischeri, N. spathulata, N. stramenia, N. tatenoi and *N. udagawae.* Rodlet A was able to distinguish *A. viridinutans, N. hiratsukae* and *N. udagawae* (Serrano *et al.*, 2011).

Arunrattiyakorn *et al.* (2011) obtained α-Mangostin, a prenylated xanthone isolated from an EtOAc the hull of *Garcinia mangostana* L. (mangosteen) was individually metabolized by *Neosartorya spathulata*. This fungus was isolated from root of *Tiliacora triandra* (Colebr.) Diels. Incubation of α-mangostin with *N. spathulata* gave magostin 3-sulfate (Figure 7.9) which exhibited significant anti-mycobacterial activity against *Mycobacterium tuberculosis.*

Hamayun *et al.* (2011) reported the investigation of gibberellins production and growth promoting capacity of a novel endophytic fungal strain of *Neosartorya* CC-8, which was isolated from the roots of Chinese cabbage (*Brassica rapa* L. spp. perkinensis). The culture filtrates (CF) of fungus CC-8 significantly promoted plant length and biomass of Chinese cabbage. The CF of CC-8 produced higher amounts of GA3, GA4, GA7 and GA9 than wild type *Fusarium fujikuroi*, a well known gibberellins producing fungus.

Littera *et al.* (2011) reported the arsenic removal from aqueous solutions by biomass of fungal strain, *Neosartorya fischeri*. The biosorption capacity of fungal biomass was studied within the As (V) concentration range of approximately 0.2 to 5.0 mgL^{-1} at two different pH values (pH 5 and 7).With increasing initial arsenic concentration, the biosorption capacity of both fungal strains increased almost linearly and achieved the sorption capacity of 0.317 and 0.124 mg g^{-1} for biomass of *N. fischeri* and *N. niger*, respectively. The effect of biomass treatment with $FeCl_3$ and HCl on As(III) and As(V) uptake was also studied. The optimum biosorption pH as well as the effect of biomass treatment was found to be dependent on the fungal strain used. Treatment with $FeCl_3$ and HCl did not result in any significant increase in arsenic uptake. To the

contrary, treatment with feric oxyhydroxide was found to be very effective and virtually 100% of the arsenic was removed from the samples of contaminated natural water.

Mukasa-Mugerwa *et al.* (2011) reported the sporadic of *Cynodon dactylon* was observed to occur directly on the surface of hard coal in dumps of the Witbank. Microorganism analysis of plants and rhizosphere material from the dumps revealed the presence of arbuscular mycorrhizal fungi and the coal solubilising fungus, *Neosartorya fischeri*. Studies established to replicate the dump environment revealed increased coal degradation in the formof humic acid production and an increase in small size particles as a result of *Cynodon dactylon* growth in association with arbuscular mycorrhizal fungi and *N. fischeri.*

Taewoo *et al.* (2011) reported a new petroleum hydrocarbon-degrading fungus, isolated from an oil contaminant soil identified as *Neosartorya* sp. This isolate was able to degrade total petroleum (TPHs) without a lag phase, but degradation rates decreased with increasing initial TPH concentration. Dual lag phase of TPH degradation indicated the ability to adapt its metabolic activity to utilize different type of hydrocarbons as an electron donor.

Uribe-Alvalez *et al.* (2011) reported an asphalt degrading fungus *Neosartorya fischeri* which they investigated from a natural asphalt lake. This fungus grew in purified asphaltenes as the only source of carbon and energy so it metabolized 15.5% of the asphaltenic carbon to 13.2% of CO_2 and produced extracellular laccase activity but it is not detected when it grow in rich medium.

Buttachon *et al.* (2012) reported seven new indole alkaloids including indoloazepinone (Figures 7.10-7.16): sartorymensin, two quinazolinone: tryptoquivaline O and *3′-(4-Oxaquinazolin-3-yl) spiro[1H-indole-3,5′-oxalane]-2,2′-dione*, and four new pyrazinoquinazolinone derivatives: *epi*-fiscalin C, *epi*-fiscalin A, neofiscalin A and *epi*-neofiscalin A. Seven known compounds comprising 4-dihydroxy-3-methylacetophenone, tryptoquivaline, tryptoquivalines L, H, F, fiscalins A and C were

isolated from the culture of the fungus *Neosartorya siamensis* (KUFC 6349) collecting from forest soil at Samaesarn Island, Chonburi Province, Thailand. Eight compounds were evaluated for their *in vitro* growth inhibitory activity on the human U373 and Hs683 glioblastoma, the A549 non-small cell lung cancer, the MCF-7 breast cancer and the SKMEL-28 melanoma cell lines. Sartorymensin displayed a moderate *in vitro* growth inhibitory activity on the five cell lines.

Forty-eight isolates of endophytic fungi were isolated from a traditional medicinal herb: *Macleaya cordata* (Papaveraceae), mainly distributed in China. *Neosartorya fischeri* was the first report as endophytic fungi. The results of antibacterial activity of *N. fischeri* against eight bacteria by using thin layer chromatography-bioautography showed growth reduction of *Agrobacterium tumefaciens, Bacillus subtilis, Staphylococcus aureus, Staphylococcus haemolyticus, Salmonella typhimurium*, and *Xanthomonas vesicatoria* (Shan *et al.*, 2012).

Tan *et al.* (2012) reported a marine-derived fungus *Neosartorya fischeri* strain 1008F producing two new compounds named fischeacid and fischexanthone, together with eight known compounds from the culture. Bioassays indicated that AGI-B4 and 3,4-dihydroxybenzoic acid showed potent inhibitory effect on the replication and Tobacco mosaic virus (TMV), and AGI-B4 also possessed an inhibition of the cell proliferation of human gastric cancer cell line SGC-7901 and hepatic cancer cells BEL-7404.

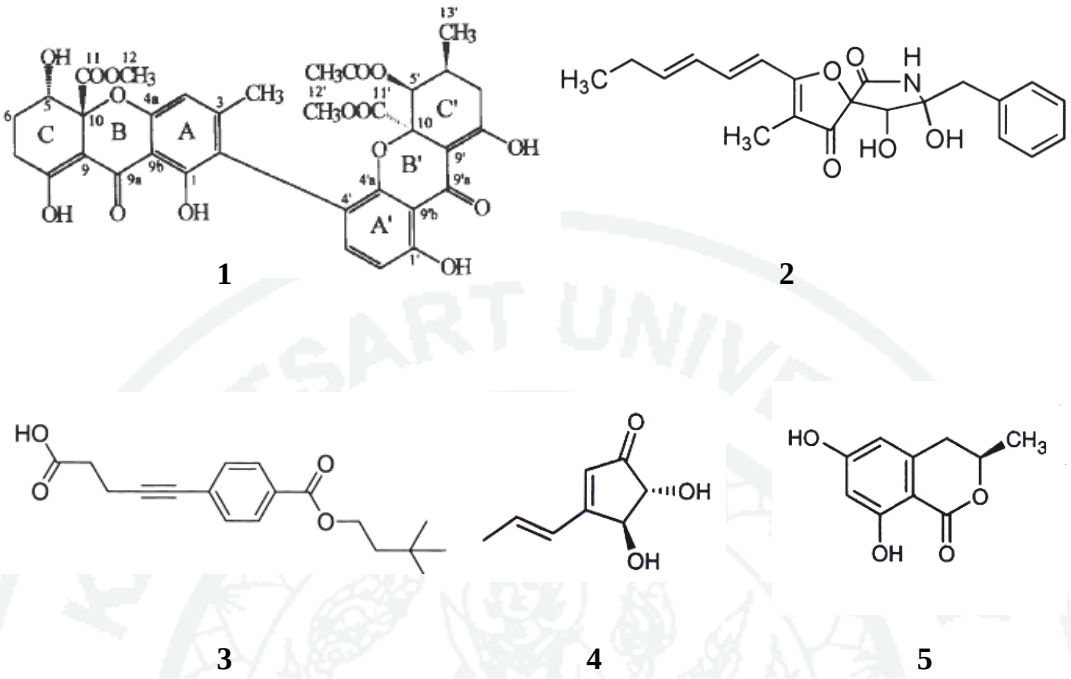


Figure 7 Bioactive secondary metabolites isolated from *Neosartorya* spp.

1 = neosartorin from *N. fischeri* (Proksa *et al.*, 1998)

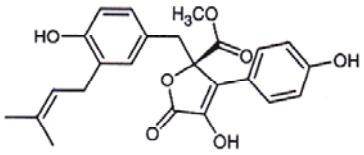
2 = azaspirene from *Neosartorya* sp. (Asami *et al.,* 2002)

3 = a derivative of dihydroisocoumarin from *N. quadricincta* (Ozoe *et al.*, 2004)

4 - 8 = (+)-terrein (4), (-)-6-hydroxymellein (5), butyrolactone I (6) diketopiperazines (*cyclo*-(*S*-Pro-*S*-Leu) (7) and *cyclo*-(*S*-Pro-*S*-Val) (7) from *Aspergillus fischeri* (Phattanawasin *et al*., 2007)

9 = magostin 3-sulfate from *N. spathulata* (Arunrattiyakorn *et al.*, 2011)

10 – 16 = sartorymensin (10), trptoquivaline O (11) and *3′-(4-Oxaquinazolin-3-yl) spiro[1H-indole-3,5′-oxalane]-2,2′-dione* (12), *epi*-fiscalin C (13), *epi*-fiscalin A (14), neofiscalin A (15) and *epi*-neofiscalin A (16) (Buttachon *et al*., 2012)



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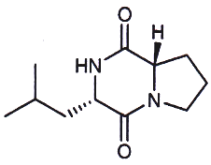
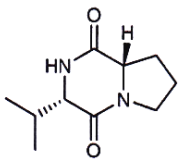


7



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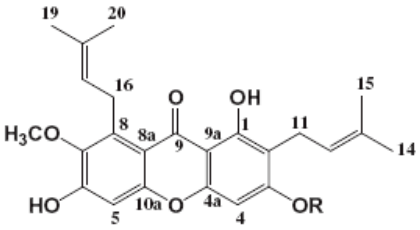

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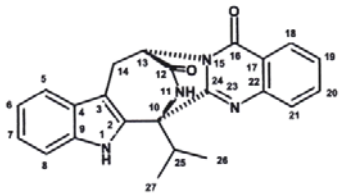
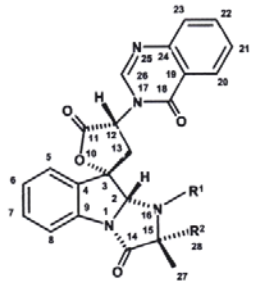
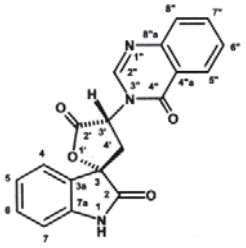


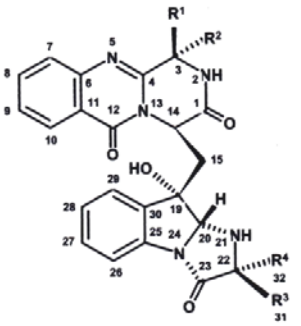
10



11 (R^1=CHO, R^2=H)



12



13 (R^1=CH$(CH_3)_2$, R^2=H, R^3=R^4=Me)

14 (R^1=CH$(CH_3)_2$, R^2=H, R^4= R^3 =Me)

15 (R^1=R^3=H, R^2 =CH$(CH_3)_2$, R^3=R^4=Me)

16 (R^1=CH$(CH_3)_2$, R^2= R^3=H, R^4=Me)

Figure 7 (Continued)

MATERIALS AND METHODS

Materials

1. Isolation and identification of *Neosartorya*

1.1 Materials for collecting soil samples

1.1.1 shovel 1.1.2 permanent marker 1.1.3 plastic bags
1.1.4 camera 1.1.5 rubber band 1.1.6 paper note

Table 3 Two hundred and forty-nine soil samples collected from various locations in 16 provinces

Date	Collection site	Number of soil sample
5/10/08	Amphoe Khlong Luang, Pathum Thani	2
9/10/08	Amphoe Mueang, Uttaradit	2
18/10/08	Amphoe Ban Khai and Field Crops Research Institute, Rayong	22
11/11/08	Phu Luang wildlife Sanctuary, Loei	42
22/11/08	Samaesarn Isand, Chonburi	31
13/12/08	Phetchabun Research Station, Phetchabun	8
13/12/08	Rice Research Center, Phitsanulok	5
14/12/08	Field Crops Research Center, Nakhon Sawan	11
28/1/09	Amphoe Khueang Nai, Ubon Ratchathani	4
30/1/09	Maesa Elephant Camp and Doi Suthep temple, Chiang Mai	6
27/2/09	Amphoe Kamphaeng Saen, Nakhon Pathom	5
8/3/09	Amphoe Wisetchaichan, Angthong	37
2/3/09	Khlong Lan waterfall, Kamphaeng Phet	3
21/4/09	Mu Ko Angthong National Park, Surat Thani	59
27/5/09	Field Crops Resarch and Khao Yai National Park, Nakhon Ratchasima	16
28/5/09	Field Crops Research Center, Khon Kaen	10

1.2 Laboratory materials

1.2.1 fine needles
1.2.2 Petri dishes
1.2.3 hot air oven
1.2.4 autoclave
1.2.5 alcohol lamp
1.2.6 65%, 70% and 95% ethyl alcohol
1.2.7 glass slides and cover slips
1.2.8 lactophenol mounting media and emersion oil
1.2.9 stereo microscope (SZ-PT Olympus)
1.2.10 light microscope (Carl Zeiss Scope.A.1, BH-2 Olympus)
1.2.11 camera lucida
1.2.12 Scanning Electron Microscope (JEOL JSM 6400)

2. Preservation of *Neosartorya*

2.1 sterilized soil
2.2 sterilized filter paper (Whatman No.1)
2.3 liquid paraffin
2.4 aluminum foil
2.5 vial, size 1 dram.
2.6 desiccator, electronic dry cabinet (WEIFO)

3. **Media** (Listed in Appendix)

3.1 Glucose ammonium nitrate agar (GAN)
3.2 water Agar (WA)
3.3 potato dextrose agar (PDA)
3.4 potato dextrose broth (PDB)
3.5 malt extract agar (MEA)

3.6 Czapek Agar (CZA)

3.7 Czapek Yeast Autolysate Agar (CYA)

4. Phylogeny study of *Neosartorya*

4.1 Centrifuge

4.2 Hot water bath

4.3 spatulas

4.4 Pipettes

4.5 Pipette tips (10, 200 and 1,000 µl)

4.6 mortar and pestle

4.7 liquid nitrogen

4.8 Micro centrifuge tubes

5. Isolation and purification of the secondary metabolite from *Neosartorya*

5.1 cooked rice

5.2 Petri dishes

5.3 cork borer

5.4 Erlenmeyer flask 250, 500, 1,000 and 2,000 ml

5.5 filtrate pump

5.6 paper filtrate (Whatman No.1)

5.7 rotary evaporator (Buchi)

5.8 column chromatography

5.9 TLC aluminium sheets 20 x 20 cm, Merck

5.10 Silica gel 60 F_{245} (0.063-0.200 mm), Merck for column chromatography

5.11 Silica gel 60 F_{245}, Merck for thin layer chromatography

5.12 20 x 20 glass plates

5.13 sea sand

5.14 cotton

5.15 ethyl acetate (EtOAc)

5.16 chloroform ($CHCl_3$)

5.17 acetone ($(CH_3)_2CO$)

5.18 petroleum ether (Petrol)

5.19 methanol (CH_3OH)

5.20 formic acid (HCOOH)

5.21 dichloromethane (CH_2Cl_2)

5.21 distilled water

5.22 microcapillary pipettes, calibrated size 10 µl

5.23 vials, 4 dram

5.24 volumetric flask

5.25 hot plate

5.26 UV transilluminator

5.27 Ultrasonic machines

5.28 Aluminium foil

5.29 Tank chamber

6. Structure elucidation of the compounds and *in vitro* cytostatic activity in human cancer cells by secondary metabolites of *Neosartorya pseudofischeri*

6.1 Structure elucidation of the compounds

6.1.1 Proton Nuclear Magnetic Resonance (^{1}H NMR)

6.1.2 Carbon-13 Nuclear Magnetic Resonance (^{13}C NMR)

6.1.3 Correlation Spectroscopy (COSY)

6.1.4 Heteronuclear Single Quantum Coherence (HSQC)

6.1.5 Heteronuclear Multiple Bond Correlation (HMBC)

6.1.6 High Resolution Mass Spectroscopy (HRMS)

6.1.7 Spectrophotometer

6.1.8 X-ray crystallography

6.2 *In vitro* cytostatic activity in human cancer cells by secondary metabolites of *Neosartorya pseudofischeri*

6.2.1 Human U373 (ECACC code 89081403) glioblastoma cancer cell

6.2.2 Hs683 (ATCC code HTB-138) glioblastoma cancer cell

6.2.3 A549 (DSMZ code ACC107) Non-Small-Cell Lung Cancer Cells (NSCLC)

6.2.4 MCF-7 (DSMZ code ACC115) breast cancer

6.2.5 SKMEL-28 (ATCC code HTB-72) melanoma cancer cell

6.2.6 OE21(ECACC 96020201) esophageal cancer cell

6.2.7 Minimum essential medium (MEM, Invitrogen)

6.2.8 Roswell Park Memorial Institute medium (RPMI, Invitrogen)

6.2.9 fetal calf serum (Invitrogen)

6.2.10 glutamine (Invitrogen)

6.2.11 gentamicin (Invitrogen)

6.2.12 penicillin-200 (Invitrogen)

6.2.13 streptomycin (Invitrogen)

6.2.14 Etoposide (Sigma Aldrich)

6.2.15 Carboplatin (Sigma Aldrich)

6.2.16 Computer-assisted phase-contrast videomicoscopy

6.2.17 Annexin V-FITC Apoptosis detection kit (APOAF, BD Phramingen)

6.2.18 terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL lebeling)

6.2.19 propidium iodide

6.2.20 CellLab Quanta SC flow cytometer (Beckman Cuulter)

Methods

1. Isolation of *Neosartorya*

1.1 Soil samples collection

Two hundred and forty-nine soil samples were collected from agricultural fields, non-agricultural fields, forest and along the roadside (Table 3, 5, 6, Figure 9). These were labelled with location, date, and collector name and brought back to the laboratory for fungal isolation.

1.2 Isolation of *Neosartorya*

1.2.1 Alcohol treatment method (A modification of Warcup and Baker, 1963)

Three miligrams of soil samples were placed in 65% ethanol for 10-20 min. The liquid was decanted, bits of the treated soil were dispensed into several sterile Petri dishes. About 10 ml of warm GAN containing rose bengal and streptomycin was added and the Petri dish was gently rotated to disperse the soil particles before the agar solidified. The plates were then placed in covered boxed for incubation in darkness at room temperature. Hyphal tips were transferred onto PDA and maintained as pure cultures for identification.

1.2.2 Heat treatment method (A modification of Warcup and Baker, 1963)

One gram of soil samples was placed in a sterile test tube in a water bath at 60-80˚C for 20-30 min. Excess water was drained off and soil particles were placed into Petri dishes, and plates were immediately poured with GAN containing rose bengal and streptomycin. The same procedure described in 1.2.1 were followed.

1.3 Single ascospore isolation (Intana, 2003)

PDA plus 100 ppm streptomycin was poured in a Petri dish. A sterile glass rod was used to spread 0.5 ml ascospores suspension (10^3 ascospores / ml) on a solidified agar media and incubated for 24 h at room temperature. Ascospore germination was examined under a light or compound microscopes and a piece of agar containing a single ascospore was transferred to slant PDA.

2. Identification of *Neosartorya* species (Raper and Fennell, 1965; Horie *et al.* 2003., Samson *et al.*, 2007)

2.1 Macroscopic examination

Morphological characteristic of colonies were determined such as growth pattern, color, texture on different media, including CZA, CYA and MEA for 7 to 14 days, at 28˚C. Diameters of colonies were measured in millimeters, most effectively by transmitted light and from the reverse side.

Colony characteristics were examined under a stereo microscope and naked eyes. The microscope was used for assessing texture of colonies and the appearance of anamorph and conidial heads. For judgement of conidia and colony colors, Rayner's "A Mycological Colour Chart" (Rayner, 1970) has been used.

2.2 Microscopic examination

Microscopic characteristics were examined on a slide preparation using sterile distilled water and lactophenol as mounting media and examined under a light microscope (Carl Zeiss Scope.A.1). Camera lucida drawings were made. Photomicrographs of fungal structure were taken under stereo, light and scanning electron microscopes.

Study on ornamentation of ascospore was conducted using Scanning Electron Microscopy. Matured ascomata and ascospores of *Neosartorya* from dry culture agar media were transferred with a fine needle and placed onto double-stick transparent adhesive 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, 2004)

Identification was based on examination of the morphological characteristics under stereo, light and scanning electron microscopes. *Neosartorya* were identified following the research done in previous reports (Raper and Fennell, 1965; Hong *et al*., 2006, 2008; Horie *et al*., 2003; Peterson, 1992; Samson *et al*., 2007; Yaguchi *et al*., 2010).

3. Preservation of *Neosartorya*

Pure cultures were maintained on PDA slants, PDA slants covered with liquid paraffin, filter paper, autoclaved barley grain, sterilized soil and 15% glycerol at the culture collection, Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok.

3.1 PDA slant method (Manoch *et al*., 2005; Smith and Onions, 1994)

Pure cultures of *Neosartorya* spp. were maintained on PDA slants at 28˚C. Subculturing was carried out every 6 months.

3.2 Liquid paraffin method (Smith and Onions, 1994)

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

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 dish. The mycelia were transferred on PDA and incubated for 7-14 days depended on the species. The filter papers with fungal mycelium were transferred to new sterile Petri dish by using sterile forceps and placed in a desiccator or electric desiccator (35˚C) for 7-10 days. Dried filter papers covered mycelial mass and ascomata were kept in an aluminium foil, labelled and placed in a box for storage at -20˚C.

3.4 Autoclaved barley grain (Kokaew, 2011)

Barley grains were autoclaved at 121˚C for 15 minutes and placed on PDA in sterile Petri dishes. Mycelium plug of *Neosartorya* were transferred to PDA for 7-14 days. Barley grains with mycelium were transferred to vial by using sterile forceps, labeled and placed in the box and storage at -20˚C.

3.5 Soil culture (Smith and Onions, 1994)

Loamy soil was placed in a vial about 2/3 full and autoclaved twice at 121˚C for 15 min. One ml of spore suspension in sterile water was added the soil cultures were left to grow at room temperature and the left to dry while stored in a refrigerator at 4-10˚C.

3.6 15% glycerol (Kokaew, 2011)

Fifteen percent of glycerol in vial was autoclaved at 121˚C for 15 minutes. *Neosartorya* plug were transferred to 15% glycerol and kept in the box and storage at 4˚C.

4. The molecular study of *Neosartorya* spp.

4.1 DNA extraction and sequencing analysis

DNA was prepared using the GenTorukun kit (Takara Bio Inc., Ltd., Otsu, Japan) from approximately 100 µl volume of fungal suspension cultured at 37˚C for 5 days on potato dextrose agar (PDA) slants. The β-tubulin was sequenced directly from PCR products using primer pair Bt2a and Bt2b (Glass and Donaldson, 1995). The PCR products were sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystem, Foster City, CA, USA) on and ABI PRISM 3130ABI Genetic Analyser (Applied Biosystem), according to the manufacturer's instructions.

4.2 Molecular phylogenetic analyses

DNA sequences were edited using ATGC Ver. 4 sequence assembly software (Genetyx Co., Tokyo, Japan). The alignments of the sequences and phylogenetic analysis based on neighbor-joining (NJ) method (Saitou and Nei, 1987) were performed using Clustal X software (Thompson *et al*, 1997). The distances between sequences were calculated using Kimura's two-parameter model (Kimura, 1980). A bootstrap was conducted with 1,000 replications (Felsenstein, 1985).

5. Test of antagonism against plant pathogenic fungi by *Neosartorya*

Neosartorya spp. were subcultured on PDA for 14-21 days or until ascospores became mature. Ascospore suspension was prepared by flooding the culture surface with sterile water and then ascomata of *Neosartorya* spp. were scraped from medium surface by sterile spatula. The concentration was determined with haemacytometer before adjusted with sterile water to 10^6 ascospores / ml for antagonistic test in this study (Intana, 2003).

5.1 *In vitro* growth inhibition and overgrowing of mycelia of plant pathogenic fungi

Nine species of *Neosartorya* were selected to test for antagonistic activity against nine species of plant pathogenic fungi (Table 4). The young mycelium from the colony margin of *Neosartorya* spp. and the specific plant pathogenic fungi were cut with sterile cork borer (0.5 mm diam.) and placed as a dual culture on PDA, 7 cm apart. All Petri dishes were incubated at room temperature (28˚C) for 14 days. The inhibition levels were calculated by using the formula: G_1-G_2 / G_1 x 100 where G_1 = colony radius of plant pathogenic fungi in the control and G_2 = colony radius of plant pathogenic fungi in the dual culture test (Intana, 2003). Each treatment was performed with three replicates.

5.2 *In vitro* antagonistic activity test of nine *Neosartorya* crude extracts against nine species of plant pathogenic fungi

One gram of dark brown crude extract of *Neosartorya* was dissolved in ten ml of ethyl acetate (100,000 ppm). Then the stock solution was serially diluted to four concentrations (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}). Each concentration of crude extract was added to nine ml of warm PDA. Mixed, and poured into Petri dishes. The young mycelia of the various plant pathogenic fungi (Table 3) were transferred to the PDA plates containing various concentrations of the crude extract solution. All Petri dishes were incubated at 28˚C. The colony diameters were recorded at 7 days. The inhibition levels were calculated by comparison with a control. Each treatment was performed with two replicates.

Table 4 Nine species of plant pathogenic fungi from various diseased plants used for antagonistic test

Plant pathogenic fungi	Host plant	Diseases
Alternaria brassicicola	*Brassica albograbra* (Chinese kale)	Leaf spot
Colletotrichum capsici	*Capsicum frutescens* (chili)	Anthracnose
Curvularia oryzae	*Oryza sativa* (rice)	Leaf spot
Fusarium oxysporum f.sp. *cubense*	*Musa sapientum* (banana)	Fusarium wilt
Lasiodiplodia theobromae	*Citrus maxima* (pomelo)	Fruit rot
Phytophthora palmivora	*Durio zibethinus* (durian)	Root rot
Pythium aphanidermatum	*Brassica albograbra* (Chinese kale)	Damping-off
Rhizoctonia oryzae	*Oryza sativa* (rice)	Sheath blight
Sclerotium rolfsii	*Solanum tuberosum* (potato)	Stem rot

6. Inhibitory activity of *Neosartorya* crude extracts for weed germination and growth

One gram of dark brown crude extract of *Neosartorya* was dissolved in ten ml of ethyl acetate (100,000 ppm). Then the stock solution was serially diluted to three concentrations (10^{-1}, 5^{-3}, 10^{-3}). Five ml of each concentration of crude extract was pipette onto filter paper (Whatman No.1) containing in steriled glass bottle chambers. After allowing ethyl acetate to evaporate in glass bottle chambers for five hours, 10 weed seeds were placed and covered with lids. In the control, the same amount of ethyl acetate was used instead. All of the glass bottle chambers were placed at 28˚C for five days. The lengths of root and shoot were measured. Three replications were conducted for each treatment. The percent growth inhibitory activity was calculated as follows: 100 - $[(L_{treatment} - L_{control}) \times 100]$ where $L_{treatment}$ is the everage of the root and shoot length of ten seedling in each glass bottle chambers treated with *Neosartorya* crude extracts, and $L_{control}$ is the everage of the root and shoot length of ten seedling in the control.

7. Analytical secondary metabolites of *Neosartorya pseudofischeri* (Dethoup, 2007)

7.1 General Experimental

7.1.1 Merck Si Gel 60 (0.2-0.5 mm; 70-230 mesh) was used for column chromatograph

7.1.2 Analytical and preparative TLC was performed on silica gel-60 (GF_{254}; Merck), 0.25 thickness. The plates were activated at 110˚C in the oven for 1 hour. All TLC plates were visualized under UV 254 nm or developed with iodine vapor.

7.1.3 Melting points were recorded on a Bock Monoscope and are uncorrected.

7.1.4 Rotations were determined on a Polax-2L instrument.

7.1.5 ^{1}H and ^{13}C NMR spectra were recorded at ambient temperature in DMSO on a Bruker DRX instrument operating at 500 and 125 MHz respectively, ^{1}H (200 MHz) and ^{13}C (50 MHz) NMR spectra were measured on a Bruker CxP spectrometer. The solvents used were deuterated chloroform (Merck) or hexadeuterated dimethylsulfoxide (Merck).

7.1.6 EI mass spectra were measured on a Hitachi Perkin-Elmer RMV-GM instrument. For HR mass spectra were measured on CONCEPT II, 2 sectors mass spectrometer. The accelerating voltage was 8 KV.

7.1.7 X-ray diffraction studied were performed with a Stoe IPOS image plant equipped with Mo Kα radiation. The structure was solved using SHELX 597 and refined with SHELXL 97. A perspective view of the molecule was obtained with ORTEP.

7.1.8 The solvents used were commercial grade of Vidrolab 2 which distilled prior to use and analytical reagent grade of brand Merck and Lab-Scan.

7.1.9 All solvents were evaporated either by reduced pressure using “Buchi evaporator” nitrogen gas.

7.1.10 The weight was measured on the balance Mettler AE 200.

7.2 Isolation and Purification of the Secondary Metabolites from *Neosartorya pseudofischeri* (KUFC 6422).

7.2.1 Fungal materials

Neosartorya pseudofischeri Peterson was isolated from a soil sample collected on rose apple plantation, Angthong province, Thailand in March 2009 and identified on the basis of the description in Raper and Fennell (1965) and with standard test. A sample with accession number KUFC 6422 has been deposited in the Department of Plant Pathology, Faculty of Agriculture, Kasetsart University and at the Medical Mycology Research Center, Chiba University, Japan.

7.2.2 Extraction and isolation of the constituents

7.2.2.1 Preparation of the crude extract

Neosartorya pseudoficheri was cultured for 7 days in 5 Petri dishes (90 mm diam.) containing 25 ml of PDA. Twenty 1000 ml Erlenmeyer flask containing 300 g cooked rice were autoclaved at 121˚C for 15 min, inoculated with 2 ml of mycelium fungal of which cultured in PDB and incubated at 28˚C for 30 days. To each flask containing the moldy rice was added 500 ml of EtOAc (20 L total), after which the contents were left to macerate for 3 days and then filtrated using filter paper (Whatman No.1). Evaporation of the combined filtrates to a volume of 1,000 ml at reduced pressure followed by addition of anhydrous sodium sulphate, filtration and evaporation of the filtrate at reduced pressure furnished 33.5 g of dark brown crude EtOAc extract which was extracted with $CHCl_3$ (3 x 500 ml). The $CHCl_3$ extracts were combined and concentrated at reduced pressure to afford 24 g of dark brown mass.

7.2.2.2 Fractionation of the crude extract

Twenty grams of the crude ethyl acetate extract was chromatographed over a 0.2-0.5 mm Merck Si gel column (250 g, 5.0 x 80 cm) and eluted with mixtures of $CHCl_3$-petrol and $CHCl_3$-Me_2CO; 250 ml/fractions were collected as follows:

Fractions	Eluents (ratio)
1-88	$CHCl_3$-petrol (1:1)
89-198	$CHCl_3$-petrol (7:3)
199-257	$CHCl_3$-petrol (9:1)
258-277	$CHCl_3$
278-293	$CHCl_3$-Me_2CO (9:1)
294-366	$CHCl_3$-Me_2CO (7:3)

The fractions were analysed by analytical TLC and combined, according to their compositions, as follows:

Fractions	Isolated compounds
1-20 (6.58 g)	not purified
21-24 (823.2 mg)	not purified
25-26 (198.9 mg)	not purified
27-31 (195.6 mg)	not purified
32-40 (215.9 mg)	not purified
41-48 (107.5 mg)	not purified
49-57 (84.0 mg)	not purified
58-65 (326.8 mg)	not purified
66-82 (338.8 mg)	not purified
83-95 (221.1 mg)	not purified
96 (356.5 mg)	not purified
97 (553.2 mg)	not purified
98 (427.7 mg)	not purified
99-112 (708.2 mg)	**cadinene (1**, 120 mg)
113-123 (202.0 mg)	not purified
124-134 (968.0 mg)	**eurochevalierine (2,** 150 mg**)**
135-148 (1.56 g)	**eurochevalierine (2,** 136 mg**)**
	brasiliamide B (3, 86 mg**)**

Fractions	Isolated compounds
149-175 (660 mg)	**1,4-diacetyl-2,5-dibenzylpiperazine 3,7″-oxide (4,** 35 mg**)**
175-184 (226.9 mg)	not purified
185-191 (113.3 mg)	not purified
192-214 (1.3 g)	**pseudofischerine (5,** 30 mg**)**
215-223 (410 mg)	**pseudofischerine (5,** 55 mg**)** **pyripyropene A (6,** 18 mg**)**
224-229 (190.9 mg)	not purified
230-235 (115.5 mg)	not purified
236-239 (51 mg)	not purified
240-247 (108.4 mg)	not purified
248-256 (110 mg)	not purified
257-264 (104.1 mg)	not purified
265-273 (109.3 mg)	not purified
274-279 (65.9 mg)	not purified
280 (702.9 mg)	not purified
281-334 (1.0 g)	**2-4-dihydroxy-6-methylbenzoic acid (7,** 8.5 mg)
335-342 (74.2 mg)	not purified
343-352 (73.6 mg)	not purified
353-365 (88.3 mg)	not purified
366-373 (143.1 mg)	not purified

7.2.3 Isolation and Purification of the compounds

Fractions 99-112 were combined (708.2 mg) and recrystalized from a mixture of petrol $CHCl_3$ to yield of cadinene (1, 120 mg).

Fractions 124-134 were combined (968.0 mg) and purified by TLC (Si Gel, $CHCl_3$:Me_2CO:HCO_2H, 95:5:0.1) to yield of eurochevalierine (2, 150 mg**)**

Fractions 135-148 were combined (1.56 g) and applied over a column chromatography of Si Gel (30 g, 2.0 x 40 cm) and eluted with a mixture of $CHCl_3$ and petrol; 100 ml/subfractions were collected as follows:

Subfractions	**Eluents**
1-19	$CHCl_3$-petrol (3:7)
20-37	$CHCl_3$-petrol (1:1)
38-90	$CHCl_3$-petrol (7:3)
91-104	$CHCl_3$-petrol (9:1)

Subfraction 39-43 were combined (764.2 mg) and applied over a column chromatography of Si Gel (18 g, 1.5 x 30 cm) and eluted with a mixture of $CHCl_3$ and petrol; 100 ml/subfractions were collected as follows:

Subfractions	**Eluents**
1-50	$CHCl_3$-petrol (1:1)
51-59	$CHCl_3$-petrol (4:1)
31-37	$CHCl_3$-petrol (7:3)
91-104	$CHCl_3$-petrol (9:1)

Subfractions 31-37 were combined (170 mg) and purified by TLC (Si Gel, $CHCl_3$:Me_2CO:HCO_2H, 9:1:0.1) to yield, besides an additional of eurochevalierine (2, 76 mg), a less polar component (23 mg) which was applied to column of Sephadex LH-20 (10 g, 1.5 x 30 cm) and eluted with a 1:1 mixtures of CH_2Cl_2 and MeOH and after evaporation of the solvent yielded of brasiliamide B (3, 18 mg).

Subfractions 44-50 were combined (220 mg) and purified by TLC (Si Gel, $CHCl_3$:Me_2CO:HCO_2H, 9:1:0.1) to give eurochevalierine (2, 60 mg) and brasiliamide B (3, 68 mg).

Fractions 149-175 were combined (692.1 mg) and chromatographed over a Si Gel column (30 g, 2.0 x 40 cm) and eluted with mixtures of $CHCl_3$-petrol and $CHCl_3$-Me_2CO; 100 ml/subfractions were collected as follows:

Subfractions	**Eluents**
1-11	$CHCl_3$-petrol (1:1)
12-64	$CHCl_3$-petrol (7:3)
63-82	$CHCl_3$-petrol (9:1)

Subfractions 35-56 were combined (140 mg) and purified by TLC (Si Gel, $CHCl_3$:Me_2CO:HCO_2H, 95:5:0.1) to give 1,4-diacetyl-2,5-dibenzylpiperazine 3,7″-oxide (4, 35 mg).

Fractions 192-214 were combined (1.3 g) and chromatographed over a Si Gel column (20 g, 1.5 x 30 cm) and eluted with mixtures of $CHCl_3$-petrol and $CHCl_3$-Me_2CO, 100 ml/subfractions were collected as follows:

Subfractions	**Eluents**
1-17	$CHCl_3$-petrol (1:1)
18-83	$CHCl_3$-petrol (7:3)
84-96	$CHCl_3$-petrol (9:1)

Subfractions 48-56 were combined (49 mg) and purified by TLC (Si Gel, $CHCl_3$:Me_2CO:HCO_2H, 9:1:0.01) to give 30 mg of pseudofischerine (5).

Fractions 215-223 were combined (410 mg) and chromatographed over a Si Gel column (28 g, 2.0 x 30 cm) and eluted with mixtures of $CHCl_3$-petrol, 100 ml/subfractions were collected as follows:

Subfractions	**Eluents**
1-4	$CHCl_3$-petrol (1:1)
5-69	$CHCl_3$-petrol (7:3)
70-95	$CHCl_3$-petrol (9:1)

Subfractions 34-80 were combined (230.5 mg) and purified by TLC (Si Gel, $CHCl_3$:Me_2CO:HCO_2H, 7:3:0.01) to give 55 mg of pseudofischerine (5) and 18 mg of pyripyropene A (6).

Fractions 281-334 were combined (1.0 g) and applied to the Sephadex LH-20 column (10 g, 1.5 x 30 cm) and and eluted with a 1:1 mixtures of CH_2Cl_2 and MeOH; 5 ml subfractions were collected. Subfractions 22-26 were combined and after evaporation of the solvent yielded of white solid of 2,4-dihydroxy-6-methylbenzoic acid (7, 8.5 mg).

7.3 Structure elucidation of the compounds

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

7.4 *In vitro* cytostatic activity in human cancer cells by secondary metabolites of *Neosartorya pseudofischeri*

7.4.1 Determination of *in vitro* growth-inhibitory activity by the MTT colorimetric assay

The histological types and origins of the six human cancer cell lines are described in the Table 31 legend. U373 cells were cultured in MEM (Invitrogen), and Hs683, A549, MCF-7, OE21, and SKMEL-28 cells were cultured in RPMI (Invitrogen). All media were supplemented with 5% heat-inactivated fetalcalf serum (Invitrogen), 4 mM glutamine, 100 µg/ml gentamicin, and 200 U/ml penicillin-200 µg/ml streptomycin (Invitrogen). Etoposide (>95%) and carboplatin (>95%) were purchased from Sigma-Aldrich.

The overall growth levels of the human cancer cell lines were determined using the colorimetric MTT (3-4, 5-dimethylthiazol2yl-diphenyl tetrazolium bromide; Sigma) assay as described previously (Hayot *et al.*, 2002; Joseph *et al*., 2002). Each experiment was carried out in six replicates.

7.4.2 Computer-assisted phase contrast videomicroscopy recording (quantitative videomicroscopy)

U373 GBM and A549 NSCLC cell viability, growth, and division were characterized in vitro with computer-assisted phase-contrast videomicroscopy as described previously (Balde *et al.*, 2010; Delbrouck *et al.*, 2002; Gao *et al.*, 2010). Cells were monitored for 72 h, and movies were constructed from the time-lapse image sequences, which enabled rapid screening for cell viability.

In both control and treated conditions, cell growth levels were evaluated by the ratio between the numbers of cells counted in the last and first frames of the image sequences. The global growth ratio is defined by the ratio between the two growth levels obtained in the treated and control conditions. All the cell counts were performed in triplicate using an interactive computer tool (Debeir *et al.*, 2008)

7.4.3 Flow cytometry determination of apoptosis and cell cycle kinetics

Analysis of apoptosis and cell cycle kinetics were carried out, according

to our previously published protocol (Mijatovic *et al.*, 2006), with the APOAF detection kit according to the manufacturers instructions (BD Pharmingen). TUNEL labeling of U373 GBM cells was performed for 1 h at 37˚C followed by propidium iodide (PI) staining at 5 µg/ml in the presence of RNAse. Stained cells were analyzed on a CellLab Quanta SC flow cytometer (Beckman Coulter).

8. Place

The experiments of taxonomic study, antagonistic test and preparation of crude extracts were conducted at Mycology laboratory, Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand. The isolation, purification and structure elucidation of the secondary metabolites were conducted at Instituto de Biomedicas de Abel Salazar (ICBAS), Universidade do Porto, Portugal under the guidance of Prof. Dr. Anake Kijjoa. For phylogenetic analysis were conducted with the help of Assoc. Prof. Dr. Takashi Yaguchi at Medical Mycology Research Center (MMRC), Chiba University, Japan.

RESULTS AND DISCUSSION

1. Diversity and distribution of *Neosartorya*

Two hundred and forty eight isolates of the genus *Neosartorya* were isolated from 249 soil samples collected from agricultural, non-agricultural and forest soils from 16 provinces in Thailand. Eleven species of *Neosartorya* were identified comprising 7 known, 1 new and 3 unidentified species. *N. spinosa* (79 isolates) was most frequently found followed by *N. glabra* (45), *N. siamensis* sp. nov. (45), *N. fischeri* (41), *N. tatenoi* (26), *N. laciniosa* (5), *N. takakii* (3), and one isolate each of *N. pseudofischeri*, *Neosartorya* sp.1 (KUFC 6341), *Neosartorya* sp.2 (KUFC 6513) and *Neosartorya* sp.3 (KUFC 6579) (Tables 4, 5; Figure 8).

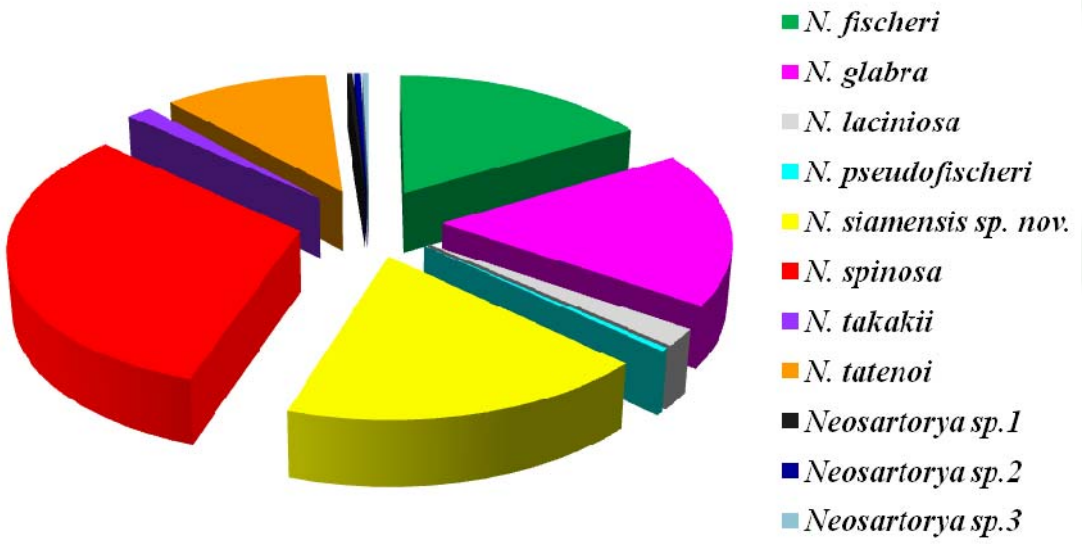


Figure 8 Occurrence of *Neosartorya* species from various soil samples at different locations

Neosartorya spinosa and *N. fischeri* were commonly found in forest soil in Southern and Northeastern Thailand, whereas few isolates were obtained from agricultural and non-agricultural soils (Tables 6, Figures 8, 9). *N. glabra* occurred at high frequently in agricultural soil, whereas few isolates were obtained from non-agricultural

and forest soils (Tables 6, Figures 8, 9). One hundred and thirty-five and one hundred and thirteen isolates of *Neosartorya* spp. were derived from alcohol and heat treatment methods, respectively (Tables 6). Raper and Fennell (1965) reported that heat activation of ascospores of *N. glabra* may, in part, account for the apparent "resistance" of this organism to heating. All of *Neosartorya* species produce heat-resistant ascospores that are frequently encountered in different food products (Gómez *et al.,* 1994; Samson, 1989; Tournas, 1994). *Neosartorya spinosa* isolates were obtained most frequently from Surat Thani, Loei, Rayong, Chonburi, Nakhon Ratchasima, Angthong, Chiang Mai, Kamphaeng Phet, Khon Kean, Phetchabun and Ubon Ratchathani provinces, respectively. *N. glabra* was predominately found from agricultural soil in Angthong, but *N. siamensis* sp. nov. was commonly found in forest soil in Surat Thani province.

Twenty-six isolates of *N.tatenoi* were found from non-agricultural soil in Chiang Mai, Phetchabun and Uttaradit, agricultural soil in Nakhon Pathom, Pathum Thani and Rayong, forest soil in Kamphaeng Phet, Surat Thani and Nakhon Ratchasima provinces, respectively. Five isolates of *N. laciniosa* were found from nonagricultural, agricultural and forest soils in Chiang Mai, Loei, Kamphaeng Phet, Nakhon Ratchasima and Rayong provinces. However, three isolates of *N. takakii* were obtained from agricultural and forest soils in Loei, Phetchabun and Surat Thani provinces. In addition, one isolate each of *N. pseudofischeri* and *Neosartorya* sp.3 (KUFC 6579) were found from agricultural soil in Angthong, whereas a single isolate of *Neosartorya* sp.1 (KUFC 6341) and *Neosartorya* sp.2 (KUFC 6513) were found from forest soil at Phu Luang Wildlife Sanctuary in Loei and coastal forest soil at Samaesarn Island in Chonburi provinces (Table 5).

In Thailand, six species of *Neosartorya* were previously reported including *Neosartorya delicata*, *N. fischeri*, *N. glabra, N. multiplicata*, *N. quadricincta* and *N. spinosa* (Jeamjitt, 2007; Kokaew, 2011; Manoch *et al.,* 2005, 2007). In this study, *N. siamensis* Manoch & Eamvijarn sp. nov. was a new finding for the genus *Neosartorya*, whereas *N. laciniosa* and *N. pseudofischeri* were new records to Thailand. Three unidentified species comprising *Neosartorya* sp.1 (KUFC 6341), *Neosartorya* sp.2 (KUFC 6513) *Neosartorya* sp.3 (KUFC 6579) might be new species.

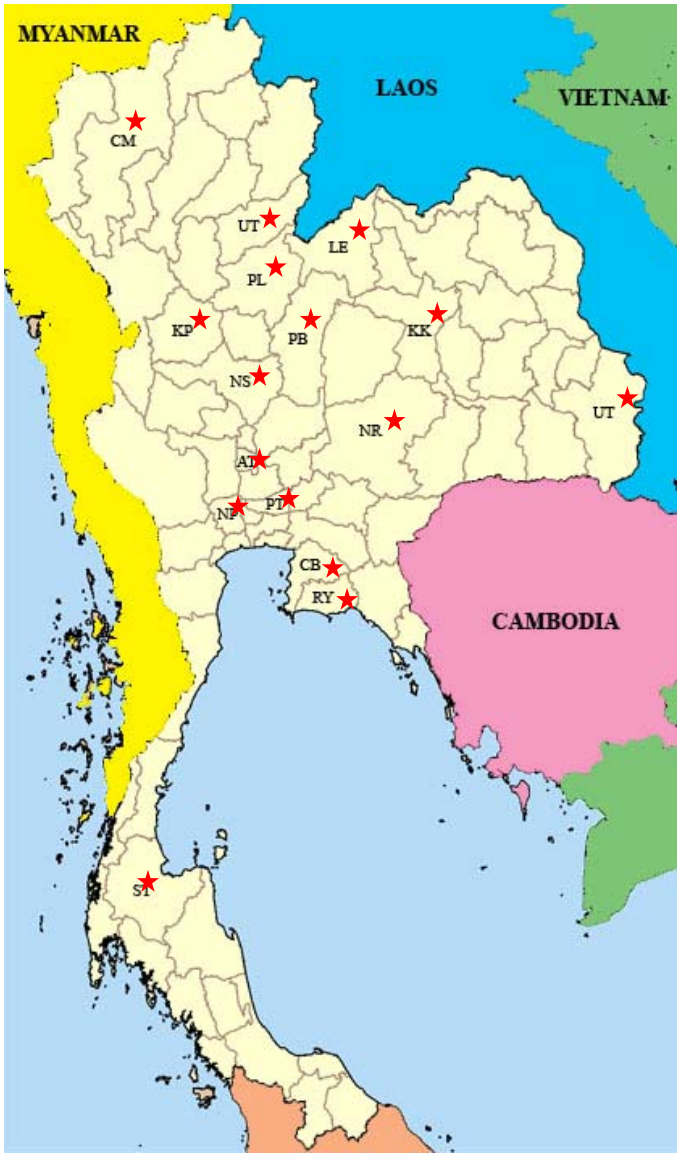
Table 5 Frequency of *Neosartorya* species isolated from various soil samples at different locations

Fungal species	Accession number (KUFC)	Total isolates
Neosartorya fischeri	6317, 6344, 6346, 6357, 6358, 6359, 6371, 6372, 6382, 6386, 6406, 6407, 6445, 6447, 6448, 6455, 6456, 6459, 6461, 6463, 6464, 6466, 6468, 6471, 6475, 6476, 6483, 6485, 6486, 6501, 6508, 6522, 6544, 6545, 6546, 6549, 6552, 6553, 6561, 6568, 6569	41
Neosartorya glabra	6311, 6360, 6361, 6367, 6368, 6370, 6373, 6374, 6375, 6376, 6378, 6379, 6380, 6381, 6383, 6384, 6385, 6387, 6390, 6391, 6392, 6394, 6395, 6398, 6399, 6400, 6410, 6413, 6420, 6430, 6435, 6441, 6467, 6474, 6477, 6514, 6518, 6519, 6521, 6525, 6527, 6528, 6533, 6536, 6538	45
Neosartorya laciniosa	6310, 6318, 6332, 6414, 6416	5
Neosartorya pseudofischeri	6422	1
Neosartorya siamensis sp. nov.	6303, 6307, 6308, 6309, 6312, 6322, 6324, 6327, 6330, 6331, 6349, 6365, 6402, 6404, 6405, 6412, 6423, 6424, 6425, 6426, 6429, 6431, 6442, 6443, 6465, 6480, 6481, 6482, 6492, 6495, 6498, 6520, 6523, 6524, 6530, 6531, 6532, 6534, 6535, 6537, 6547, 6548, 6559, 6570, 6571,	45
Neosartorya spinosa	6301, 6302, 6304, 6305, 6306, 6319, 6321, 6323, 6325, 6329, 6333, 6334, 6336, 6337, 6338, 6339, 6340, 6342, 6345, 6347, 6348, 6350, 6352, 6354, 6356, 6362, 6366, 6369, 6389, 6397,	

Table 5 (Continued)

Fungal species	Accession number (KUFC)	Total isolates
Neosartorya spinosa	6401, 6411, 6418, 6427, 6428, 6432, 6437, 6438, 6439, 6440, 6446, 6449, 6450, 6451, 6452, 6453, 6454, 6460, 6470, 6472, 6473, 6478, 6479, 6484, 6487, 6489, 6490, 6496, 6497, 6500, 6502, 6504, 6507, 6510, 6515, 6517, 6526, 6529, 6540, 6550, 6554, 6555, 6563, 6564, 6565, 6567, 6573, 6576, 6577	79
Neosartorya takakii	6355, 6503, 6560	3
Neosartorya tatenoi	6315, 6316, 6320, 6326, 6347, 6363, 6364, 6377, 6403, 6415, 6417, 6419, 6421, 6433, 6436, 6437, 6444, 6457, 6458, 6462 6543, 6556, 6557, 6572, 6574, 6575	26
Neosartorya sp.1	6341	1
Neosartorya sp.2	6513	1
Neosartorya sp.3	6579	1
	total	248

KUFC = Kasetsart University Fungal Collection



AT = Angthong
CB = Chon Buri
CM = Chiang Mai
KK = Khon Kaen
KP = Kamphaeng Phet
LE = Loei
NP = Nakhon Pathom
NR = Nakhon Ratchasima
NS = Nakhon Sawan
PB = Phetchabun
PL = Phitsanulok
PT = Pathum Thani
RY = Rayong
ST = Surat Thani
UR = Ubon Ratchathani
UT = Uttaradit

Figure 9 Map of Thailand indicating the soil collection sites from 16 provinces

Table 6 Distribution of *Neosartorya* spp. from soil samples at different locations using alcohol and heat treatment methods

KUFC	Isolation method	Soil type	Location
			Neosartorya fischeri
6317	alc	A	basil plantation, Amphoe Khlong Luang, Pathum Thani
6346	alc	CF	Samaesarn Isand, Chonburi
6357	alc	NA	termite mound, Samaesarn Isand, Chonburi
6358	alc	A	physic nut plantation, Nakhon Pathom
6359	alc	A	physic nut plantation, Nakhon Pathom
6371	alc	A	banana plantation, Amphoe Wisetchaichan, Angthong
6372	alc	A	banana plantation, Amphoe Wisetchaichan, Angthong
6382	alc	A	banana plantation, Amphoe Wisetchaichan, Angthong
6386	ht	A	forage grass field, Amphoe Wisetchaichan, Angthong
6406	ht	NA	Doi Suthep temple, Chiang Mai
6407	alc	F	Khlong Lan waterfall, Kamphaeng Phet
6445	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6447	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6448	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6455	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6456	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6459	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6461	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6463	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6464	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6466	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6468	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6471	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6475	ht	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6476	ht	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6483	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6485	ht	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6486	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6501	alc	F	Ko Wau Talab, Mu Ko Angthong National Park, Surat Thani
6508	ht	F	Ko Wau Talab, Mu Ko Angthong National Park, Surat Thani
6522	ht	F	Mu Ko Angthong National Park, Surat Thani
6544	alc	F	Phu Luang wildlife Sanctuary, Loei
6545	ht	F	Phu Luang wildlife Sanctuary, Loei

Table 6 (Continued)

KUFC	Isolation method	Soil type	Location
			Neosartorya fischeri
6546	ht	F	Phu Hor, Phu Luang wildlife Sanctuary, Loei
6549	ht	F	Phu Hor, Phu Luang wildlife Sanctuary, Loei
6552	alc	F	Phu Luang wildlife Sanctuary, Loei
6553	ht	F	Phu Luang wildlife Sanctuary, Loei
6558	alc	F	Phu Luang wildlife Sanctuary, Loei
6561	alc	F	Phu Luang wildlife Sanctuary, Loei
6568	alc	F	Phu Luang wildlife Sanctuary, Loei
6569	ht	F	Phu Hor, Phu Luang wildlife Sanctuary, Loei
			Neosartorya glabra
6311	alc	NA	Amphoe Mueang, Uttaradit
6360	ht	A	Amphoe Kamphaeng Saen, Nakhon Pathom
6361	ht	A	Amphoe Kamphaeng Saen, Nakhon Pathom
6367	ht	A	banana plantation, Amphoe Wisetchaichan, Angthong
6368	alc	A	banana plantation, Amphoe Wisetchaichan, Angthong
6370	alc	A	banana plantation, Amphoe Wisetchaichan, Angthong
6373	ht	A	banana plantation, Amphoe Wisetchaichan, Angthong
6374	ht	A	bamboo plantation, Amphoe Wisetchaichan, Angthong
6375	ht	A	bamboo plantation, Amphoe Wisetchaichan, Angthong
6376	ht	A	mango plantation, Amphoe Wisetchaichan, Angthong
6378	alc	A	banana plantation, Amphoe Wisetchaichan, Angthong
6379	alc	A	forage grass field, Amphoe Wisetchaichan, Angthong
6380	ht	A	banana plantation, Amphoe Wisetchaichan, Angthong
6381	ht	A	banana plantation, Amphoe Wisetchaichan, Angthong
6383	alc	A	banana plantation, Amphoe Wisetchaichan, Angthong
6384	alc	A	banana plantation, Amphoe Wisetchaichan, Angthong
6385	ht	A	banana plantation, Amphoe Wisetchaichan, Angthong
6387	ht	A	eggplant plantation, Amphoe Wisetchaichan, Angthong
6390	ht	A	Amphoe Kamphaeng Saen, Nakhon Pathom
6391	ht	A	banana plantation, Amphoe Wisetchaichan, Angthong
6392	ht	A	physic nut plantation, Nakhon Pathom
6394	ht	A	physic nut plantation, Nakhon Pathom
6395	ht	A	banana plantation, Amphoe Wisetchaichan, Angthong
6398	ht	A	physic nut plantation, Nakhon Pathom

Table 6 (Continued)

KUFC	Isolation method	Soil type	Location
			Neosartorya glabra
6399	ht	A	banana plantation, Amphoe Wisetchaichan, Angthong
6400	ht	A	physic nut plantation, Nakhon Pathom
6410	ht	NA	Maesa Elephant Camp, Chiang Mai
6413	alc	NA	termite mound, Maesa Elephant Camp, Chiang Mai
6420	ht	A	Amphoe Kamphaeng Saen, Nakhon Pathom
6430	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6435	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6441	ht	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6467	ht	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6474	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6477	ht	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6514	ht	A	Field Crops Research Institute, Rayong
6518	alc	F	cassava plantation, Nakhon Ratchasima
6519	alc	F	Khao Yai National Park, Nakhon Ratchasima
6521	ht	A	cassava plantation, Field Crops Research Institute, Rayong
6525	ht	A	physic nut plantation, Nakhon Ratchasima
6527	ht	A	physic nut plantation, Khon Kaen
6528	ht	A	rambutan plantation, Rayong
6533	ht	A	peanut plantation, Khon Kaen
6536	ht	A	physic nut plantation, Khon Kaen
6538	ht	A	sugarcane plantation, Nakhon Ratchasima
			Neosartorya laciniosa
6310	alc	A	mangosteen plantation, Amphoe Ban Khai, Rayong
6318	ht	F	Khao Yai National Park, Nakhon Ratchasima
6332	alc	F	Phu Luang wildlife Sanctuary, Loei
6414	ht	F	Khlong Lan waterfall, Kamphaeng Phet
6416	ht	NA	Maesa Elephant Camp, Chiang Mai
			Neosartorya pseudofischeri
6422	ht	A	rose apple plantation, Amphoe Wisetchaichan, Angthong
			***Neosartorya siamensis* sp. nov.**
6303	alc	A	pineapple plantation, Amphoe Ban Khai, Rayong

Table 6 (Continued)

KUFC	Isolation method	Soil type	Location
			***Neosartorya siamensis* sp. nov.**
6307	ht	A	para rubber plantation, Amphoe Ban Khai, Rayong
6308	alc	A	para rubber plantation, Amphoe Ban Khai, Rayong
6309	alc	A	para rubber plantation, Amphoe Ban Khai, Rayong
6327	ht	A	para rubber plantation, Amphoe Ban Khai, Rayong
6330	ht	A	pineapple plantation, Amphoe Ban Khai, Rayong
6331	ht	A	cassava plantation, Amphoe Ban Khai, Rayong
6349	alc	CF	Samaesarn Island, Chonburi
6365	alc	A	physic nut plantation, Nakhon Pathom
6402	ht	F	Khlong Lan waterfall, Kamphaeng Phet
6404	ht	NA	hot spring, Amphoe San Kamphaeng, Chiang Mai
6405	ht	F	Khlong Lan waterfall, Kamphaeng Phet
6412	ht	NA	termite mound, Maesa Elephant Camp, Chiang Mai
6423	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6424	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6425	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6426	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6429	ht	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6431	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6442	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6443	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6465	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6480	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6481	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6482	ht	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6492	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6495	alc	F	Ko Wau Talab, Mu Ko Angthong National Park, Surat Thani
6498	alc	F	Ko Wau Talab, Mu Ko Angthong National Park, Surat Thani
6520	ht	A	Field Crops Research Institute, Rayong
6523	ht	A	physic nut plantation, Khon Kaen
6524	ht	A	physic nut plantation, Khon Kaen
6530	alc	A	physic nut plantation, Khon Kaen
6531	alc	A	bamboo plantation, Khon Kaen
6532	ht	A	cassava plantation, Nakhon Ratchasima
6534	alc	A	physic nut plantation, Rayong

Table 6 (Continued)

KUFC	Isolation method	Soil type	Location
			***Neosartorya siamensis* sp. nov.**
6535	alc	A	bamboo plantation, Khon Kaen
6537	ht	A	sugarcane plantation, Khon Kaen
6547	ht	F	Phu Luang wildlife Sanctuary, Loei
6548	ht	NA	Khon Kaen University, Khon Kaen
6559	ht	F	Phu Luang wildlife Sanctuary, Loei
6570	alc	F	Phu Luang wildlife Sanctuary, Loei
6571	alc	F	Phu Luang wildlife Sanctuary, Loei
			Neosartorya spinosa
6301	alc	A	pineapple plantation, Amphoe Ban Khai, Rayong
6302	ht	A	pineapple plantation, Amphoe Ban Khai, Rayong
6304	alc	A	mangosteen plantation, Amphoe Ban Khai, Rayong
6305	ht	A	mangosteen plantation, Amphoe Ban Khai, Rayong
6306	ht	A	mangosteen plantation, Amphoe Ban Khai, Rayong
6319	alc	F	Khao Yai National Park, Nakhon Ratchasima
6321	alc	F	Khao Yai National Park, Nakhon Ratchasima
6323	alc	A	para rubber plantation, Amphoe Ban Khai, Rayong
6325	ht	A	mangosteen plantation, Amphoe Ban Khai, Rayong
6329	ht	A	mangosteen plantation, Amphoe Ban Khai, Rayong
6333	ht	F	Phu Luang wildlife Sanctuary, Loei
6334	ht	F	Phu Luang wildlife Sanctuary, Loei
6336	ht	F	Phu Luang wildlife Sanctuary, Loei
6337	alc	F	Phu Luang wildlife Sanctuary, Loei
6338	ht	F	Phu Luang wildlife Sanctuary, Loei
6339	alc	CF	Samaesarn Island, Chonburi
6340	alc	CF	Samaesarn Island, Chonburi
6342	alc	CF	Samaesarn Island, Chonburi
6345	alc	CF	Samaesarn Island, Chonburi
6347	alc	A	bamboo plantation, Phetchabun
6348	alc	CF	Samaesarn Island, Chonburi
6350	ht	F	Phu Luang wildlife Sanctuary, Loei
6352	ht	F	Phu Luang wildlife Sanctuary, Loei
6354	alc	A	strawberry plantation, Phetchabun
6356	alc	F	Phu Luang wildlife Sanctuary, Loei

Table 6 (Continued)

KUFC	Isolation method	Soil type	Location
			Neosartorya spinosa
6362	alc	NA	Maesa Elephant Camp, Chiang Mai
6366	alc	A	mango plantation, Amphoe Wisetchaichan, Angthong
6369	alc	A	banana plantation, Amphoe Wisetchaichan, Angthong
6389	alc	A	banana plantation, Amphoe Wisetchaichan, Angthong
6397	ht	NA	termite mound, Maesa Elephant Camp, Chiang Mai
6401	alc	A	chili plantation, Ubon Ratchathani
6411	alc	F	Khlong Lan waterfall, Kamphaeng Phet
6418	alc	F	Khlong Lan waterfall, Kamphaeng Phet
6427	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6428	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6432	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6437	ht	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6438	ht	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6439	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6440	ht	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6446	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6449	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6450	ht	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6451	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6452	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6453	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6454	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6460	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6470	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6472	ht	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6473	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6478	ht	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6479	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6484	ht	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6487	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6489	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6490	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6496	alc	F	Ko Wau Talab, Mu Ko Angthong National Park, Surat Thani
6497	alc	F	Ko Wau Talab, Mu Ko Angthong National Park, Surat Thani

Table 6 (Continued)

KUFC	Isolation method	Soil type	Location
			Neosartorya spinosa
6500	ht	F	Ko Wau Talab, Mu Ko Angthong National Park, Surat Thani
6502	ht	F	Ko Wau Talab, Mu Ko Angthong National Park, Surat Thani
6504	alc	F	Ko Wau Talab, Mu Ko Angthong National Park, Surat Thani
6507	ht	F	Ko Wau Talab, Mu Ko Angthong National Park, Surat Thani
6510	alc	F	Ko Wau Talab, Mu Ko Angthong National Park, Surat Thani
6515	alc	NA	bamboo plantation, Khon Kaen
6517	ht	A	rambutan plantation, Rayong
6526	ht	F	Ko Wau Talab, Mu Ko Angthong National Park, Surat Thani
6529	alc	F	Khao Yai National Park, Nakhon Ratchasima
6540	ht	F	Khao Yai National Park, Nakhon Ratchasima
6550	alc	F	Phu Luang wildlife Sanctuary, Loei
6554	ht	F	Phu Luang wildlife Sanctuary, Loei
6555	ht	F	Phu Luang wildlife Sanctuary, Loei
6563	alc	F	Phu Luang wildlife Sanctuary, Loei
6564	ht	F	Phu Luang wildlife Sanctuary, Loei
6565	alc	F	Phu Luang wildlife Sanctuary, Loei
6567	ht	F	Phu Luang wildlife Sanctuary, Loei
6573	ht	F	Phu Luang wildlife Sanctuary, Loei
6576	alc	F	Phu Luang wildlife Sanctuary, Loei
6577	ht	F	Phu Luang wildlife Sanctuary, Loei
			Neosartorya takakii
6355	ht	F	Phu Luang wildlife Sanctuary, Loei
6503	alc	F	Ko Wau Talab, Mu Ko Angthong National Park, Surat Thani
6560	alc	F	Phu Luang wildlife Sanctuary, Loei
			Neosartorya tatenoi
6315	alc	NA	Amphoe Mueang, Uttaradit
6316	alc	A	basil plantation, Amphoe Khlong Luang, Pathum Thani
6320	alc	F	Khao Yai National Park, Nakhon Ratchasima
6326	alc	A	mangosteen plantation, Amphoe Ban Khai, Rayong
6347	alc	NA	bamboo plantation, Phetchabun
6363	ht	F	Khlong Lan waterfall, Kamphaeng Phet
6364	ht	A	physic nut plantation, Nakhon Pathom
6377	ht	A	banana plantation, Amphoe Wisetchaichan, Angthong

Table 6 (Continued)

KUFC	Isolation method	Soil type	Location
			Neosartorya tatenoi
6403	ht	F	Khlong Lan waterfall, Kamphaeng Phet
6415	ht	NA	termite mound, Maesa Elephant Camp, Chiang Mai
6417	alc	NA	Maesa Elephant Camp, Chiang Mai
6419	ht	F	Khlong Lan waterfall, Kamphaeng Phet
6421	ht	NA	Maesa Elephant Camp, Chiang Mai
6433	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6436	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6437	ht	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6444	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6457	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6458	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6543	ht	F	Phu Hor, Phu Luang wildlife Sanctuary, Loei
6556	ht	F	Phu Luang wildlife Sanctuary, Loei
6557	alc	F	Phu Luang wildlife Sanctuary, Loei
6462	alc	F	Ko Paluai, Mu Ko Angthong National Park, Surat Thani
6572	ht	F	Phu Luang wildlife Sanctuary, Loei
6574	ht	F	Phu Luang wildlife Sanctuary, Loei
6575	alc	F	Phu Luang wildlife Sanctuary, Loei
			***Neosartorya* sp.1**
6341	ht	F	Phu Luang wildlife Sanctuary, Loei
			***Neosartorya* sp.2**
6513	alc	F	Samaesarn Island, Chonburi
			***Neosartorya* sp.3**
6579	ht	A	rice field, Amphoe Wisetchaichan, Angthong

KUFC = Kasetsart University Fungal Collection

alc = alcohol treatment method, ht = heat treatment method

A = agricultural soil, CF = coastal forest soil, NA = non-agricultural soil,

F = forest soil

2. Morphological study of *Neosartorya* spp.

1. *Neosartorya fischeri* (Wehmer) Malloch & Cain (Figures 10-12, Tables 5-7)
Strains examined: KUFC 6338 agricultural soil, Phu Luang Wildlife Sanctuary, Loei province; KUFC 6447 coastal forest soil, Mu Koh Ang Thong, Surat Thani province
Reference : Raper & Fennell, 1965

Anamorphic state: *Aspergillus fischeri* Wehmer

Colonies on Czapek yeast autolysate agar (CYA) spreading broadly, attaining a diameter of 38-65 and 41-71 mm in 7 and 14 days, respectively, incubation at 28˚C (Figures 10 A, a, D, d), white to yellowish white, consisting of a thin mycelial felt and very abundant cleistothecia; conidiogenesis few in number; exudates absent; reverse yellowish brown to pale luteous (Rayner 11).

Colonies on Czapek agar (CZA) growing rapidly, attaining a diameter of 37-63 and 40-71 mm in 7 and 14 days, respectively, incubation at 28˚C (Figures 10 B, b, E, e), at first white, later becoming pale cream, consisting of a thin mycelial felt; abundant cleistothecia, conidiogenesis few in number in pale blue green shades; white exudates, reverse pale cream to flesh (R 37).

Colonies on malt extract agar (MEA) growing rapidly, attaining a diameter of 38-63 and 40-69 mm in 7 and 14 days respectively, incubation at 28˚C (Figures 10 C, c, F, f), white to creamish white, consisting of a thin mycelial felt and abundant cleistothecia, conidiogenesis few in number; colorless exudates; reverse white to creamish white.

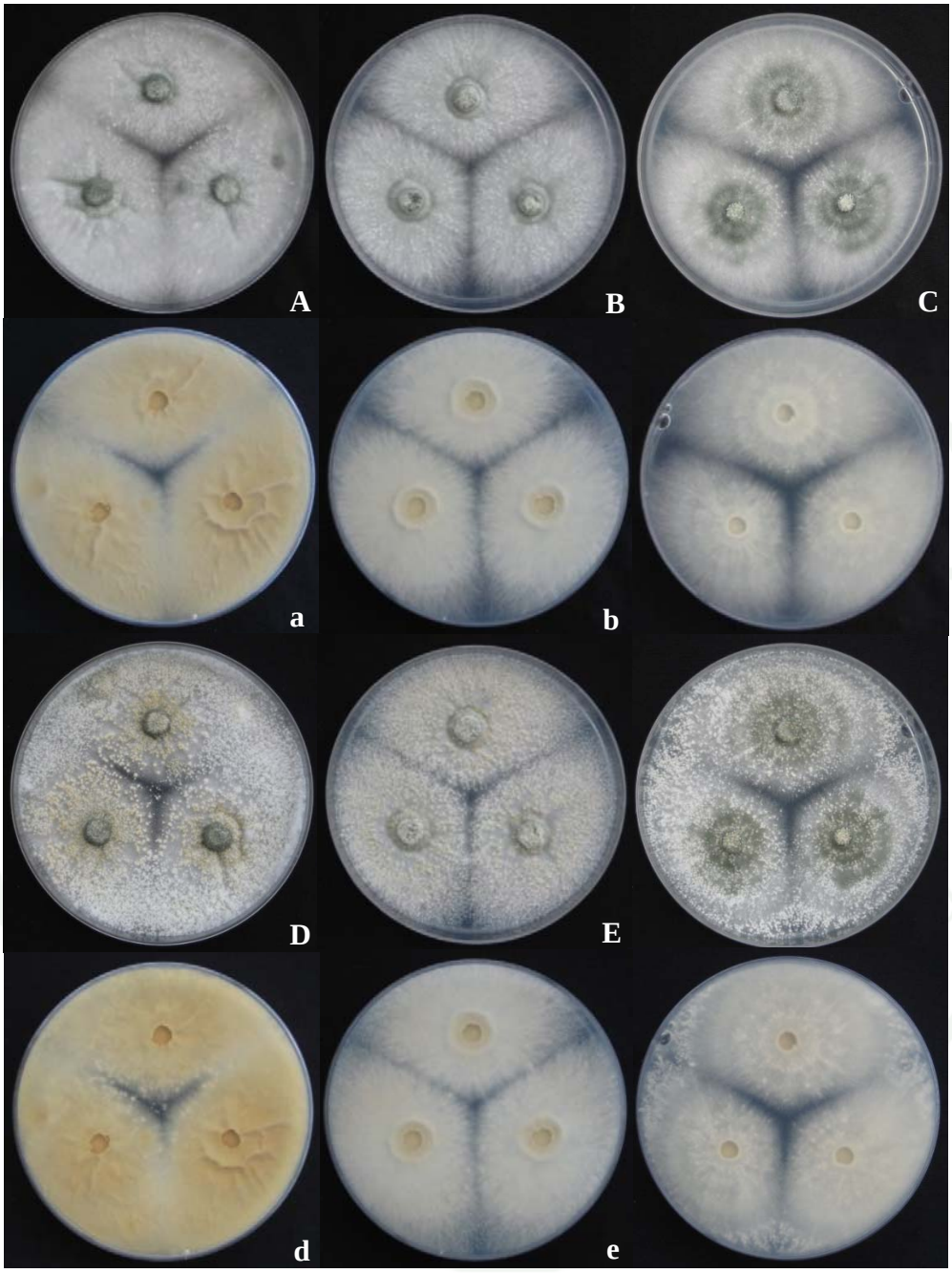


Figure 10 *Neosartorya fischeri* (Wehmer) Malloch & Cain (KUFC 6338)
Obverse and reverse views of colonies incubated at 28˚C on CYA for 7 days (A, a,) 14 days (D, d); CZA 7 days (B, b) 14 days (E, e); MEA 7 days (C, c) 14 days (F, f).

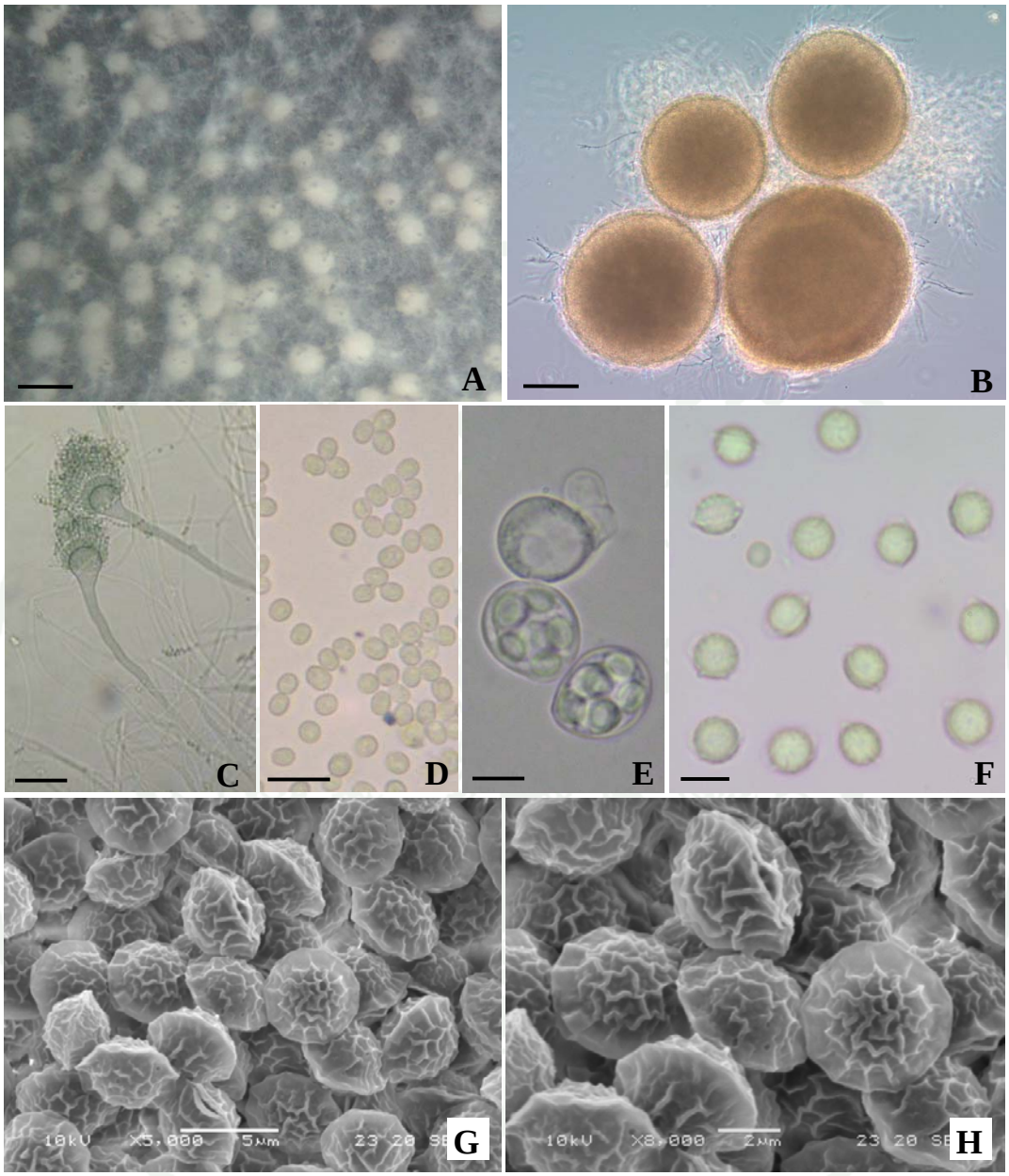


Figure 11 *Neosartorya fischeri* (Wehmer) Malloch & Cain (KUFC 6338)

A. Stereo photomicrograph of Ascomata and Conidial heads;

B-F. Light photomicrographs of Ascomata (B), Conidial heads (C), Conidia (D), Asci and ascospores (E), Ascospores (F);

G, H. SEM photomicrographs of ascospores

(Bars: A = 1,000 µm; B = 100 µm; C = 40 µm; D, E = 10 µm; F, G = 5 µm; H = 2 µm)

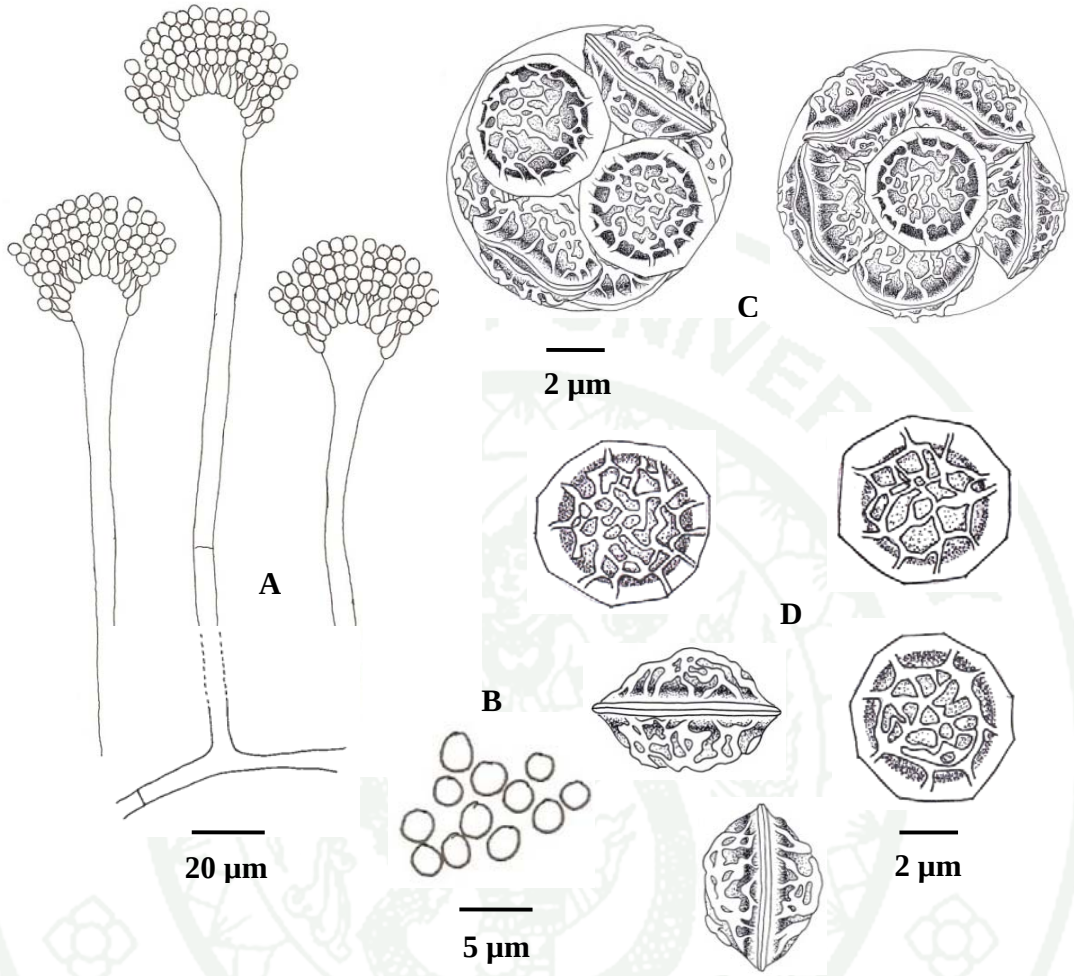


Figure 12 *Neosartorya fischeri* (Wehmer) Malloch & Cain (KUFC 6338) Camera lucida drawings of conidial heads (A), conidia (B), asci and ascospores (C), ascospores (D).

Homothallic, ascomata superficial, borne singly or in small clusters within a loose hyphal envelope, pale yellow to brownish yellow, typically globose to subglobose, 250 to 400 µm (Figures 11 A, B), consisting of irregularly flattened cell; asci maturing rapidly, globose to subglobose, 8-spored, 9.5-12 x 7.5-10.3 µm (Figures 11 E; 12 C); ascospores lenticular, hyaline, usually 3.8-5.0 x 4.0-5.5 µm, with two distinctly convex surfaces bearing anastomosing ridges about 1 µm wide (Figures 11 F-H; 12 D).

Short to loosely columnar conidial head, conidiophores mostly 170-450 µm length by 3.0-5.5 µm (Figures 11 C; 12 A) wide in diameter, smooth; uniseriate; vesicles usually flask-shaped, mostly 12- 18 µm in diameter, faintly to definitely colored in grayish green shades, bearing phialides over the upper one-haft to two-third; phialides in a single, crowded, usually in pale to dull greenish shades, 5.2-6.7 x 1.9-2.5 µm (Figure 12 A); conidia globose to subglobose, delicately roughened, faintly pigmented, 2.0-2.5 µm in diameter (Figures 11 D; 12 B).

Neosartorya fischeri was commonly found in most soil samples. Samson *et al.* (2007) reported this species from rice, cotton, potato, groundnut, leather, paper products, canned products. Guarro *et al.* (2012) recorded this fungus from soil in Australia, Japan, Nigeria, the Natherlands, Spain and England.

In Thailand, *Neosartorya fischeri* was widely distributed in soil and termite mound in Chiang Rai, Chon Buri, Mae Hong Son, Ratchaburi, Sakon Nakhon Si and Sa Ket provinces (Manoch *et al.*, 2004, 2007; Manoch and Chana, 1995). Busarakam (2002) reported *N. fischeri* from rhizosphere soil of terrestrial orchid, *Goodyera procera* collected from Queen Sirikit Botanic Garden, Chiang Mai province. Moreover, twenty-seven isolates of these fungi were found from buffalo, cow, deer, eld's deer, elephant, gaur, goat, rat and toad dung in Bangkok, Suphanburi, Surat Thani and Surin provinces (Jeamjitt, 2007; Sudsanguan, 2012). In this study, 41 isolates of *N. fischeri* were found mainly from forest soil (Tables 5, 6).

Proksa *et al.* (1998) reported a new metabolite, neosartorin from the mycelium of *Neosartorya fischeri* isolated from sediment in the river Vah, Slovakia. Fujimoto *et al.* (1993) found a new toxic metabolite named fischerin (1,4–dihydroxy-3,5-disubstitute-2(1H)-pyridone) from an ascomycete. *N. fischeri* var. *fischeri*. This toxic compound caused lethal peritonitis in mice. Wong *et al.* (1993) reported three new compounds, fiscalins A, B and C produced in culture broth of *N. fischeri*. These compounds inhibited the binding of radiolabeled substance P ligand to the human neurokinin (NK-1) receptor, with Ki values of 57, 174, and 68 μM, respectively.

Littera *et al.* (2011) reported arsenic removal from aqueous solutions by biomass of fungal strain *Neosartorya fischeri*. The biosorption capacity of fungal biomass was studied within the As (V) concentration range of approximately 0.2 to 5.0 mgL^{-1} at two different pH values. Igbinigie *et al.* (2008) recorded the first report of *N. fischeri*-mediated coal biodegradation and possible application in coal biotechnology, the finding may enable development of sustainable technologies in coal mine rehabilitation. Shen *et al.,* (2009) found that a marine fungus, *N. fischeri* could inhibit *Tobacco mosaic virus* and two tumor cell lines. Phattanawasin *et al*. (2007) reported the activity of chloroform and ethyl acetate extracts of *Aspergillus fischeri* TISTR to control seed germination of *Mimosa pigra* (giant sensitive tree) and *Echinochloa crus-galli* (barnyard grass). Bioassay-directed fractionation of the active extracts led to the isolation of five known compounds, (+)-terrein, (-)-6-hydroxymellein, two diketopiperazines (*cyclo*-(*S*-Pro-*S*-Leu), *cyclo*-(*S*-Pro-*S*-Val) and butyrolactone I. Samson *et al*. (2007) found 13 known compounds including terrein, fumitremorgins A, C, tryptoquivaline A, trypacidin, TR-2, verruculogen, sarcin, aszonalenins, fischerin, neosartorin, fiscalins and helvolic acid from cultures of *N. fischeri* grown on Czapex yeast autolysate agar (CYA), oat meal agar (OA) and yeast extract sucrose agar (YES agar).

2. *Neosartorya glabra* (Fennell & Raper) Kozakiewicz (Figures 13-15, Tables 5-7)
Strains examined: KUFC 6311 non-agricultural soil, Uttaradit province; KUFC 6413 non-agricultural soil, Maesa Elephant Camp, Amphoe Mae Rim, Chiang Mai province
Reference : Raper and Fennell, 1965

Anamorphic state: *Aspergillus fischeri* var. *glaber* (1955)

Synonym Anamorphic state: *Aspergillus neoglaber* (1989)

Colonies on Czapek yeast autolysate agar (CYA) growing rapidly, attaining a diameter of 40-57 and 43-71 mm in 7 and 14 days, respectively, incubation at 28˚C (Figures 13 A, a, D, d), white to yellowish white, consisting of a thin mycelial felt, cleistothecia very abundantly produced; velvety to floccose, conidiogenesis few in number; exudates absent; reverse yellowish brown to pale luteous (R 11).

Colonies on Czapek agar (CZA) spreading broadly, attaining a diameter of 35-50 and 55-71 mm in 7 and 14 days, respectively, incubation at 28˚C (Figures 13 B, b, E, e), white to pale cream, velvet, consisting of a thin mycelial felt and loose aerial hyphae; abundant cleistothecia, granular in conidiogenesis few in number in pale blue green shades; exudates absent, reverse pale cream to flesh (R 37).

Colonies on malt extract agar (MEA) growing rapidly, attaining a diameter of 32-53 and 40-67 mm in 7 and 14 days, respectively, incubation at 28˚C (Figures 13 C, c, F, f), white, consisting of a thin mycelial felt and abundant cleistothecia, conidiogenesis absent; exudates absent; reverse white to creamish white.

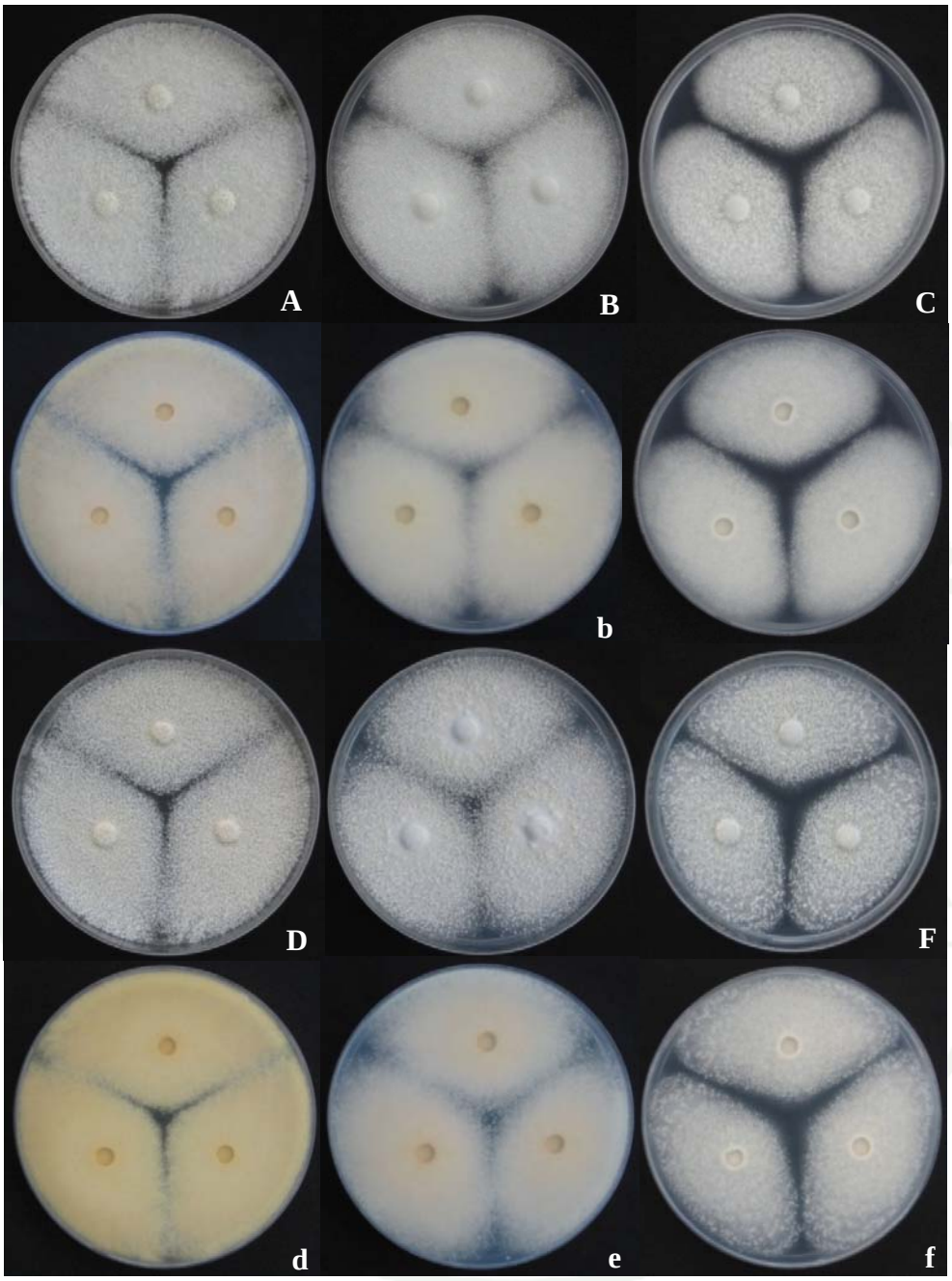


Figure 13 *Neosartorya glabra* (Fennell & Raper) Kozakiewicz (KUFC 6311) Obverse and reverse views of colonies incubated at 28˚C on CYA for 7 days (A, a,) 14 days (D, d); CZA 7 days (B, b) 14 days (E, e); MEA 7 days (C, c) 14 days (F, f).

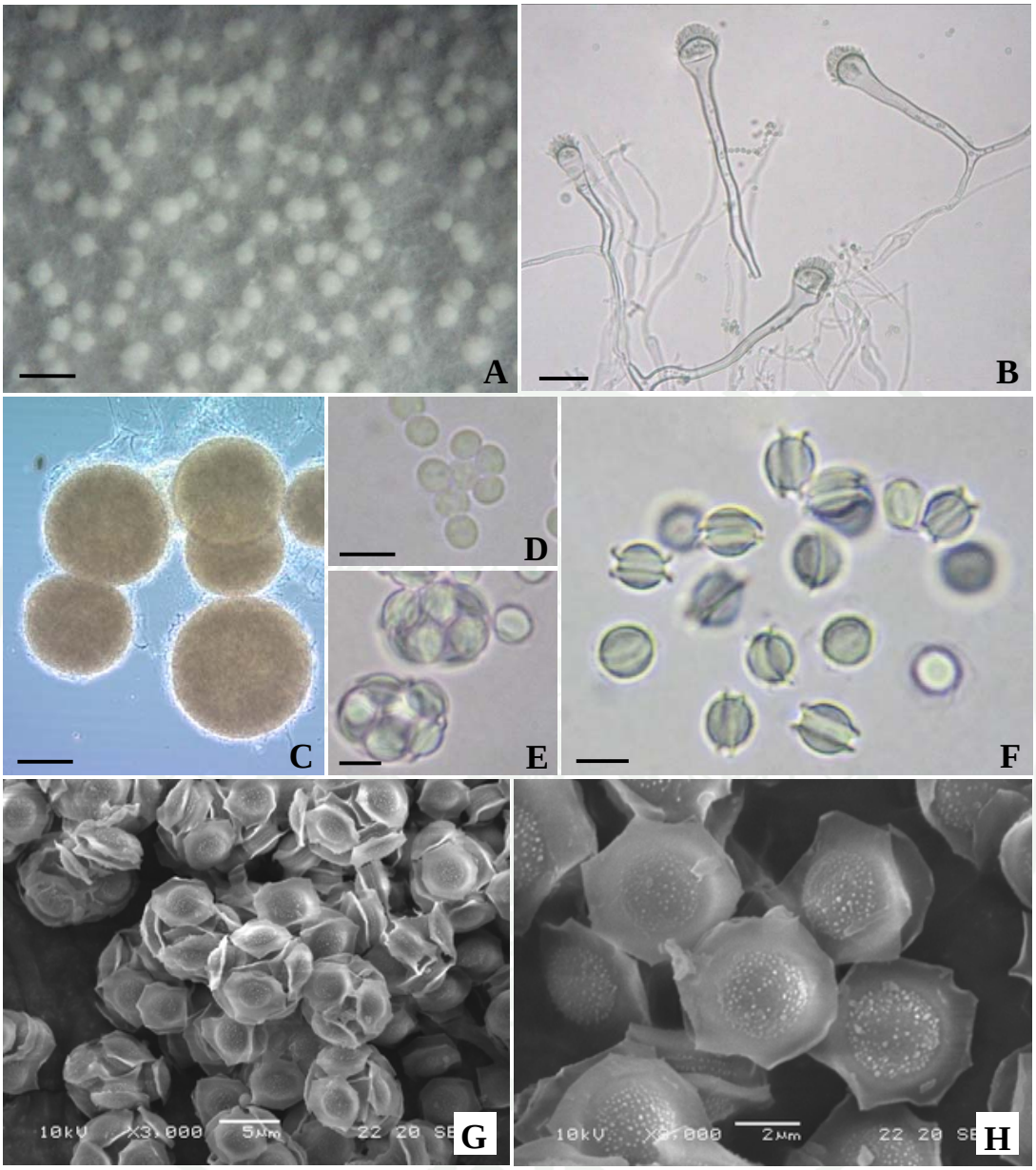


Figure 14 ***Neosartorya glabra*** (Fennell & Raper) Kozakiewicz (KUFC 6311)
A. Stereo photomicrograph of Ascomata and Conidial heads;
B-F. Light photomicrographs of Conidial heads (B), Ascomata (C), Conidia (D), Asci and ascospores (E), Ascospores (F);
G, H. SEM photomicrographs of ascospores
(Bars: A = 1,000 µm; B = 40 µm; C = 100; D – G = 5; H = 2 µm)

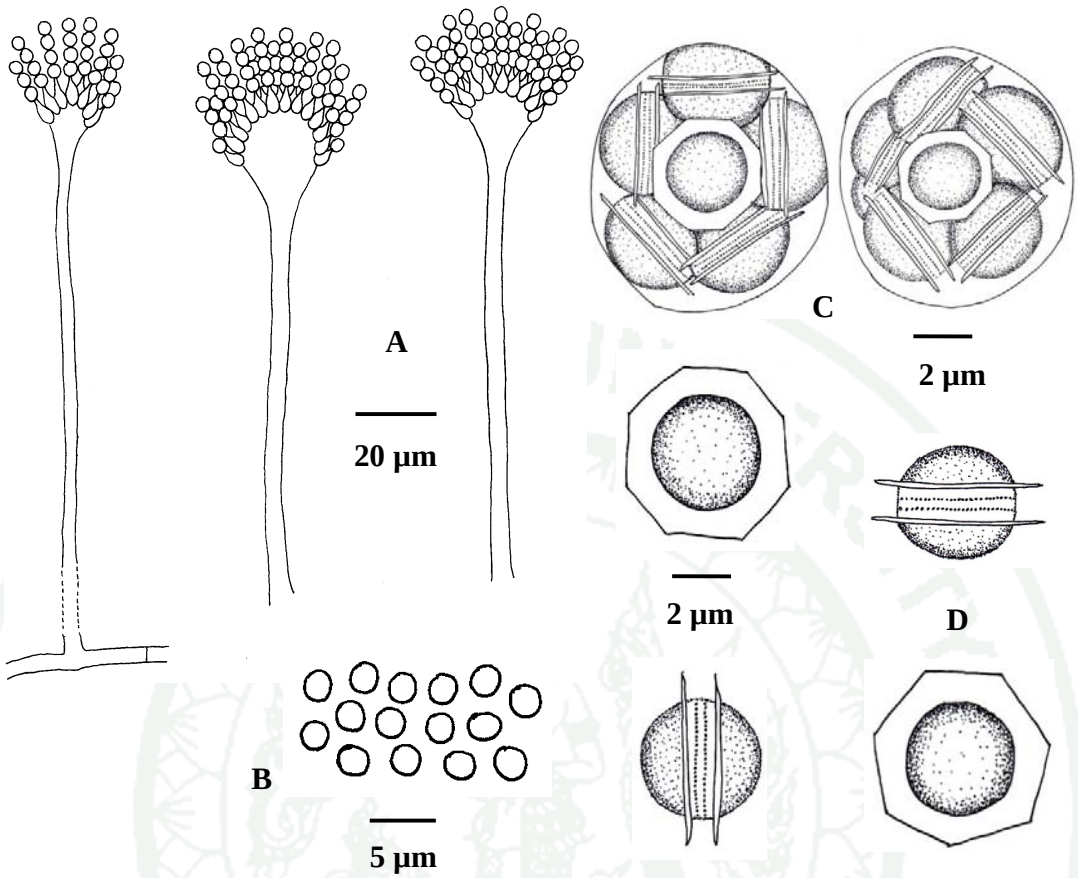


Figure 15 *Neosartorya glabra* (Fennell & Raper) Kozakiewicz (KUFC 6311) Camera lucida drawings of conidial heads (A), conidia (B), asci and ascospores (C), ascospores (D).

Homothallic, ascomata superficial, white to pale yellow, globose to subglobose, mostly 165- 220 µm in diameter (Figures 14 A, C); asci 8-spored, globose to subglobose, 12-14 x 11-13 µm (Figures 14 E; 15 C); ascospores lenticular, hyaline, with two prominent equatorial crests and with convex surfaces smooth, mostly 4.5-5.0 x 5.5-6.5 µm, including crests about 1.0-1.5 µm wide (Figures 14 F-H; 15 D).

Conidiophores arising from aerial hyphae, smooth, 250- 485 µm long, 3-6.5 µm wide at the middle (Figures 14 B; 15 A); vesicles flask-shaped, 10-18 µm in diameter; conidial heads short columnar; uniseriate, phialides 6-8 x 2-4 µm (Figure 15 A), covering the upper half of vesicle; conidia, globose to subglobose, microtubulate, 2.0-3.5 µm in diameter (Figure 15 B).

Neosartorya glabra has been reported from canned apples, desert sand, maize field, sesame field and peper field soils in Japan, Namibia, Nepal, Dominican Replublic, Korea, Australia, Ireland, England and U.S.A (Hong *et al.*, 2006; Guarro *et al*., 2012). In Thailand, this species has been reported from burned soil from Bang Pakong district, Chachoengsao province using alcohol treatment method (Manoch and Chana, 1995).

Samson *et al.* (2007) stated that *Neosartorya glabra* was similar to *N. papuensis* and *N. australensis* by its ascospore ornamentation but *N. glabra* grows slower than the other species and can be distinguished from those species using β-tubulin, calmodulin and actin genes sequences data and secondary metabolite profiles. Jayasuriya *et al*. (2009) discovered antibacterial activity of glabramycin A-C from *N. glabra* for the treatment of drug-resistant bacteria. Glabramycin C showed strong antibiotic activity against *Streptococcus pneumoniae* and modest antibiotic activity against *Staphylococcus aureus*. Kijjoa *et al.* (2011) reported sartoryglabins A-C, which were evaluated for their *in vitro* growth inhibitory activity on three human cell lines; breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and melanoma (A375-C5). All compounds exhibited strong to moderate activity against the breast adenocarcinoma.

3. *Neosartorya laciniosa* Hong, Frisvad & Samson (Figures 16-18, Tables 5-7)
Strains examined: KUFC 6318 forest soil, Khao Yai National Park, Nakhon Ratchasima province; KUFC 6414 forest soil, Khlong Lan Waterfall, Kamphaeng Phet province
Reference : Hong *et al.,* 2006

Anamorphic state: *Aspergillus laciniosus* Hong, Frisvad & Samson (2006)

Colonies on CYA growing rapidly, attaining a diameter of 39-58 and 43-72 mm in 7 and 14 days, respectively, incubation at 28˚C (Figures 16 A, a, D, d), white to pale yellow, granular due to the abundant production of cleistothecia, loosely overgrown by aerial hyphae; conidiogenesis few in number, scattered, pale blue green colored; colorless exudates; reverse yellowish orange to luteous (R 12).

Colonies on CZA growing rapidly, attaining a diameter of 36-53 and 50-69 mm in 7 and 14 days, respectively, incubation at 28˚C (Figures 16 B, b, E, e), cream to pale yellow, consisting of a thin mycelial felt; abundant cleistothecia; velvety; exudates absent; conidiogenesis few in number; reverse slightly orange to salmon (R 41).

Colonies on MEA spreading broadly, attaining a diameter of 37-50 and 40-65 mm in 7 and 14 days, respectively, incubation at 28˚C (Figures 16 C, c, F, f), white, plane, loosely overgrown by aerial hyphae, cleistothecia abundantly produced; conidiogenesis absent; reverse white to creamish white.

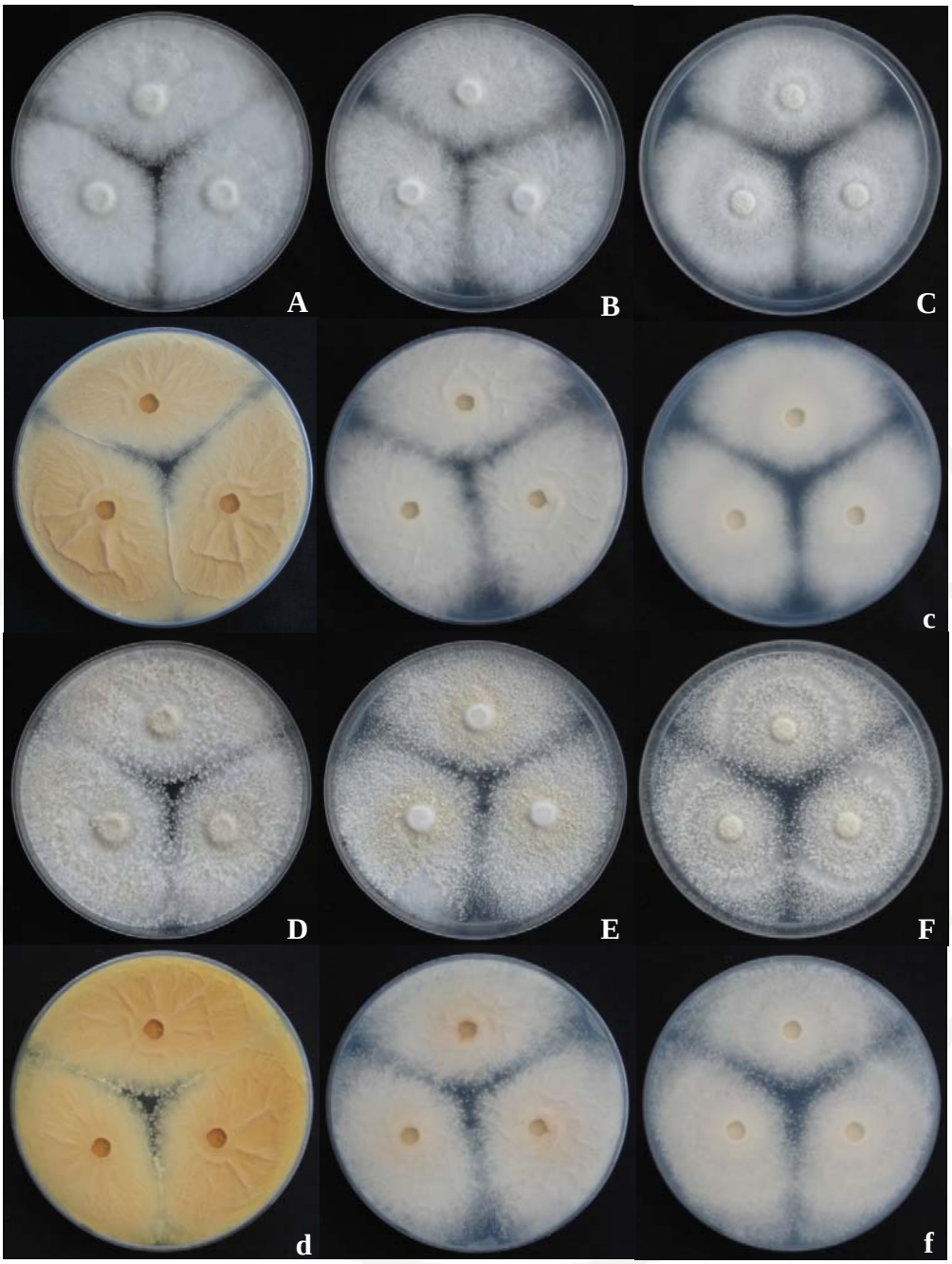


Figure 16 *Neosartorya laciniosa* Hong, Frisvad & Samson (KUFC 6318)
Obverse and reverse views of colonies incubated at 28˚C on CYA for 7 days (A, a,) 14 days (D, d); CZA 7 days (B, b) 14 days (E, e); MEA 7 days (C, c) 14 days (F, f).

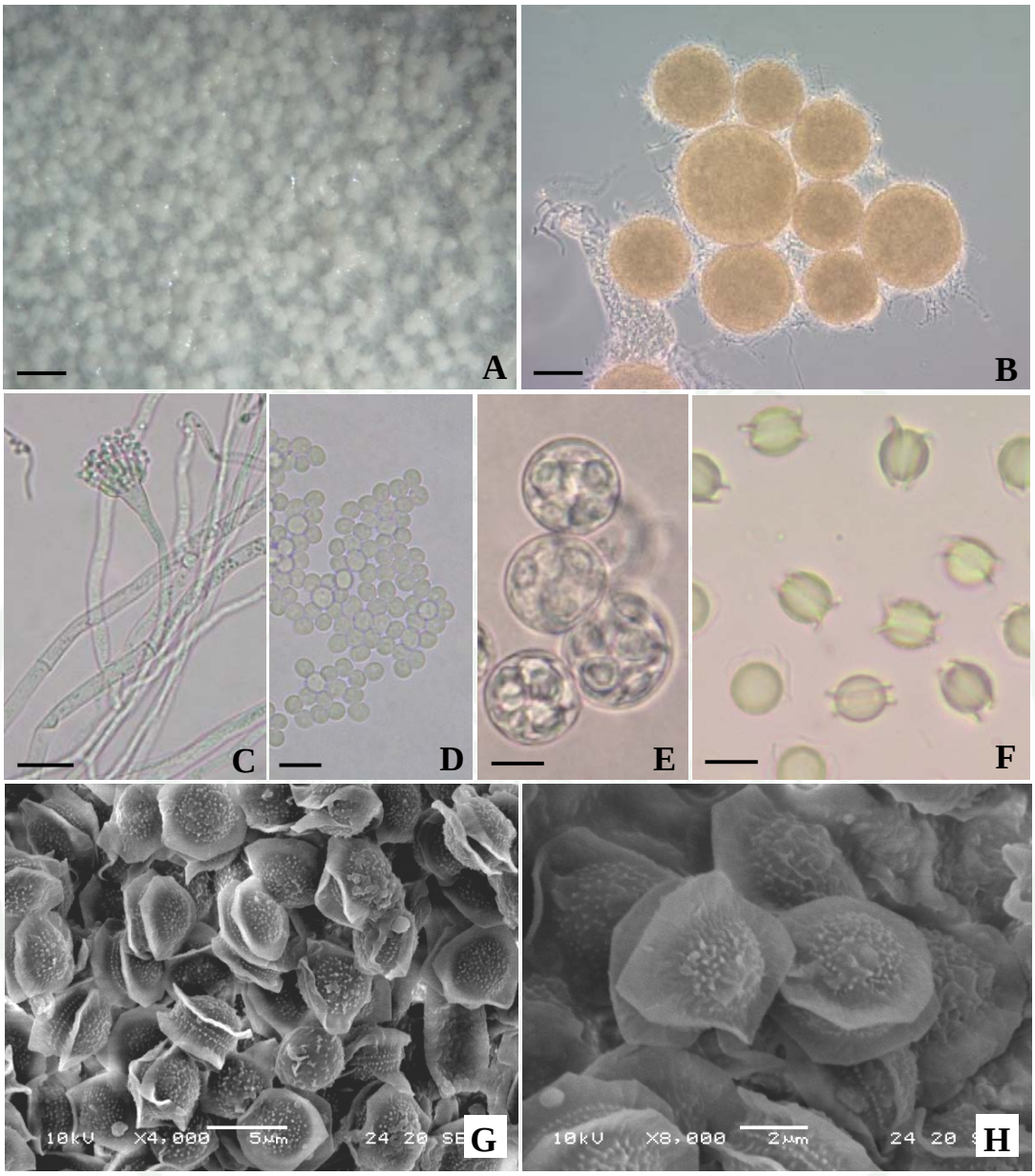


Figure 17 *Neosartorya laciniosa* Hong, Frisvad & Samson (KUFC 6318)

A. Stereo photomicrograph of Ascomata and Conidial heads;

B-F. Light photomicrographs of Ascomata (B), Conidial head (C), Conidia (D), Asci and ascospores (E), Ascospores (F);

G, H. SEM photomicrographs of ascospores

(Bars: A = 1,000 µm; B = 100 µm; C = 40 µm; D – G = 5 µm; H = 2 µm)

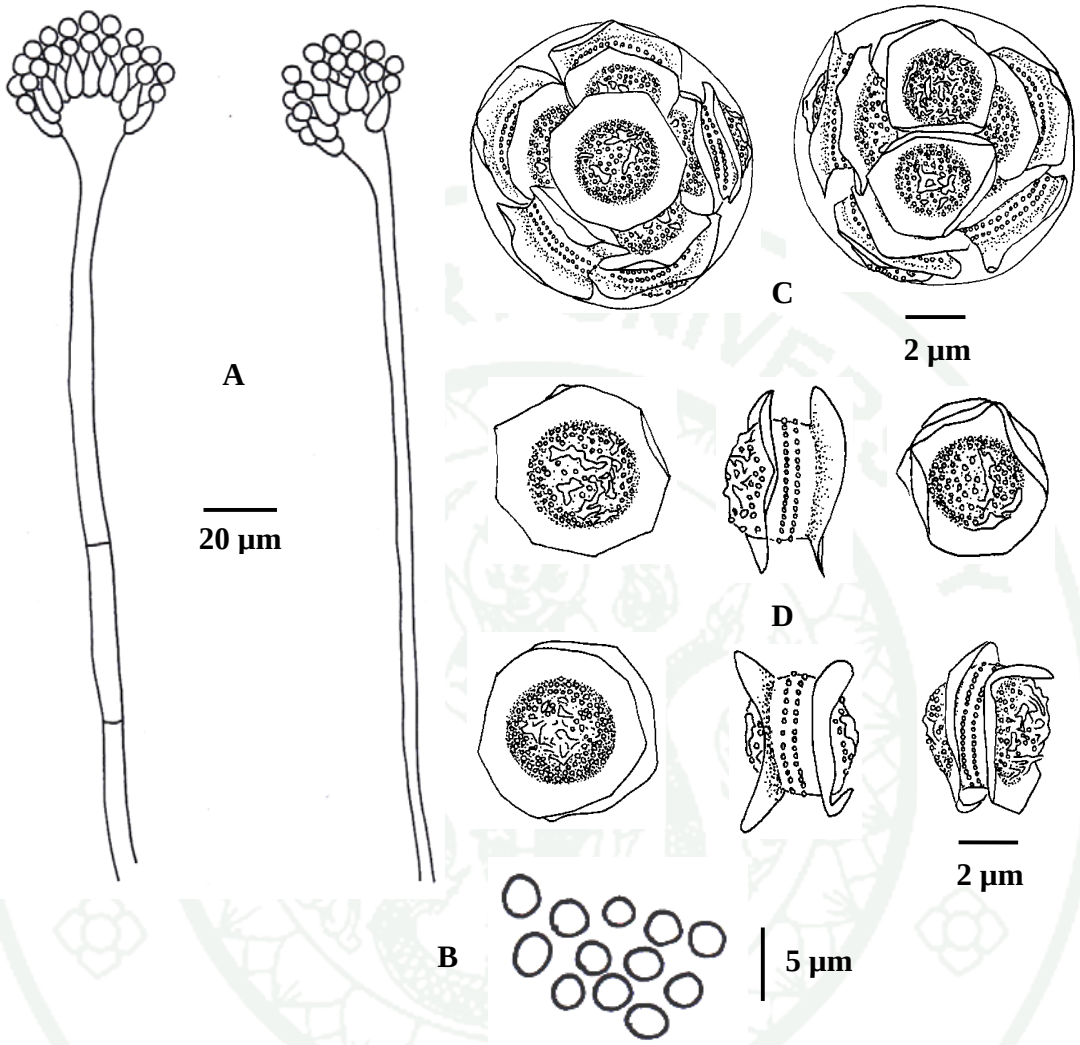


Figure 18 *Neosartorya laciniosa* (KUFC6318)
Camera lucida drawings of conidial heads (A), conidia (B), asci and ascospores (C), ascospores (D).

Homothallic, ascomata white to light yellow, cleistothecia superficial, globose to subglobose, 270-420 µm in diameter (Figures 17 A, B), surrounded by a loose covering of hyaline to yellowish white, 2-4 µm wide hyphae, cleistothecial peridium hyaline to light yellow, consisting of angular; asci globose to subglobose, 8-spored, 10-12 x 11-13 µm diameter, evanescent at maturity (Figures 17 E; 18 C); ascospores broadly lenticular, spore body 3.7-4.5 x 4-5 µm diameter, provided with two distinct equatorial crests which are up to 2 µm wide with distinct equatorial rings of small projections in the ascospore furrow; convex surfaces finely rough or smooth in light microscopy, appearing rugose to tuberculate in SEM (Figures 17 F-H; 18 D).

Conidiophores arising from aerial hyphae, smooth, 155-230 µm long, 3-4 µm wide at the middle (Figure 17 C); vesicles subclavate, 13-18 µm in diameter; conidial heads columnar; uniseriate, phialides 7-8 x 2-3 µm, covering the upper half of vesicle (Figure 18 A); conidia, globose to subglobose, sometimes broadly elliptical, smooth, 2.0-3.5 µm diameter (Figures 17 D; 18 B).

Hong *et al.* (2006) recorded *Neosartorya laciniosa* as a new species from soil in Korea planted with perilla, tomato, peper, vineyard and strawberry pulp. Phylogenetic analyses of *N. laciniosa* based on β-tubulin and calmodulin gene sequences, *N. laciniosa* was closer to *N. spinosa* but differs by its microtuberculate ascospores with two bent crests and two distinct equatorial rings of small projections (SEM). They also reported that this fungus produced three compounds including aszonalenins, tryptoquivalins and tryptoquivalons. Guarro *et al.* (2012) reported this fungus from soil in Surinam, Dominican Replublic, Kenya, Pakistan, the Netherland, Brazil and U.S.A. *N. laciniosa* is a new record for Thailand. Three isolates were found from forest soil, whereas two isolates were recorded from agricultural and non-agricultural soil (Tables 5, 6)

4. *Neosartorya pseudofischeri* Peterson (Figures 19-21, Tables 5-7)

Strain examined: KUFC 6422 agricultural soil, Amphoe Wisetchaichan, Angthong province

Reference: Peterson, 1992

Anamorphic state: *Aspergillus thermomutatus* (Paden) Peterson (1992)

Synonym anamorpic state: *A. fischeri* Wehmer var. *thermomutatus* Paden (1968)

Colonies on Czapex yeast autolysate agar (CYA) growing rapidly, attaining a diameter of 39-69 and 41-70 mm at 7 and 14 days, respectively, incubation at 28˚C (Figures 19 A, a, D, d), white to creamish white, consisting of a thin mycelial felt and very abundant cleistothecia; conidiogenesis few in number; exudates absent; reverse yellowish brown to pale luteous (R 11).

Colonies on CZA growing rapidly, , attaining a diameter of 37-45 and 47-64 mm at 7 and 14 days, respectively, incubation at 28˚C (Figures 19 B, b, E, e), at first white to creamish white, consisting of a thin mycelial felt, velvety to floccose; abundantly cleistothecia produced, conidiogenesis few in number; exudates absent; reverse cream.

Colonies on malt extract agar (MEA) growing restrictedly, attaining a diameter of 25-31 and 34-50 mm at 7 and 14 days, respectively, incubation at 28˚C (Figures 19 C, c, F, f), white, consisting of a thin mycelial felt; granular cleistothecia at center, pale yellow; conidiogenesis absent; exudates absent; reverse white to creamish white.

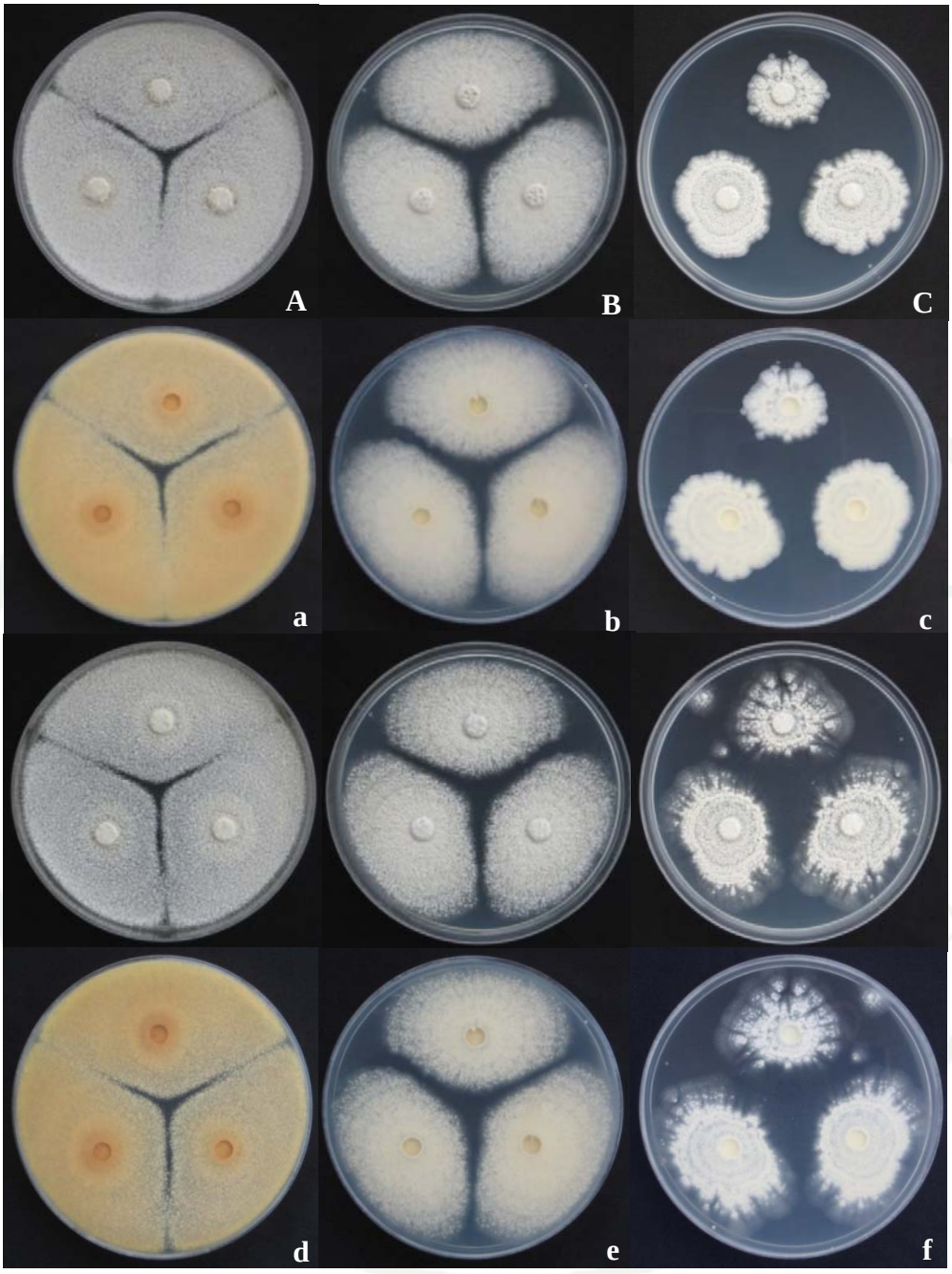


Figure 19 *Neosartorya pseudofischeri* Peterson (KUFC 6422)
Obverse and reverse views of colonies incubated at 28˚C on CYA for 7 days (A, a,) 14 days (D, d); CZA 7 days (B, b) 14 days (E, e); MEA 7 days (C, c) 14 days (F, f).

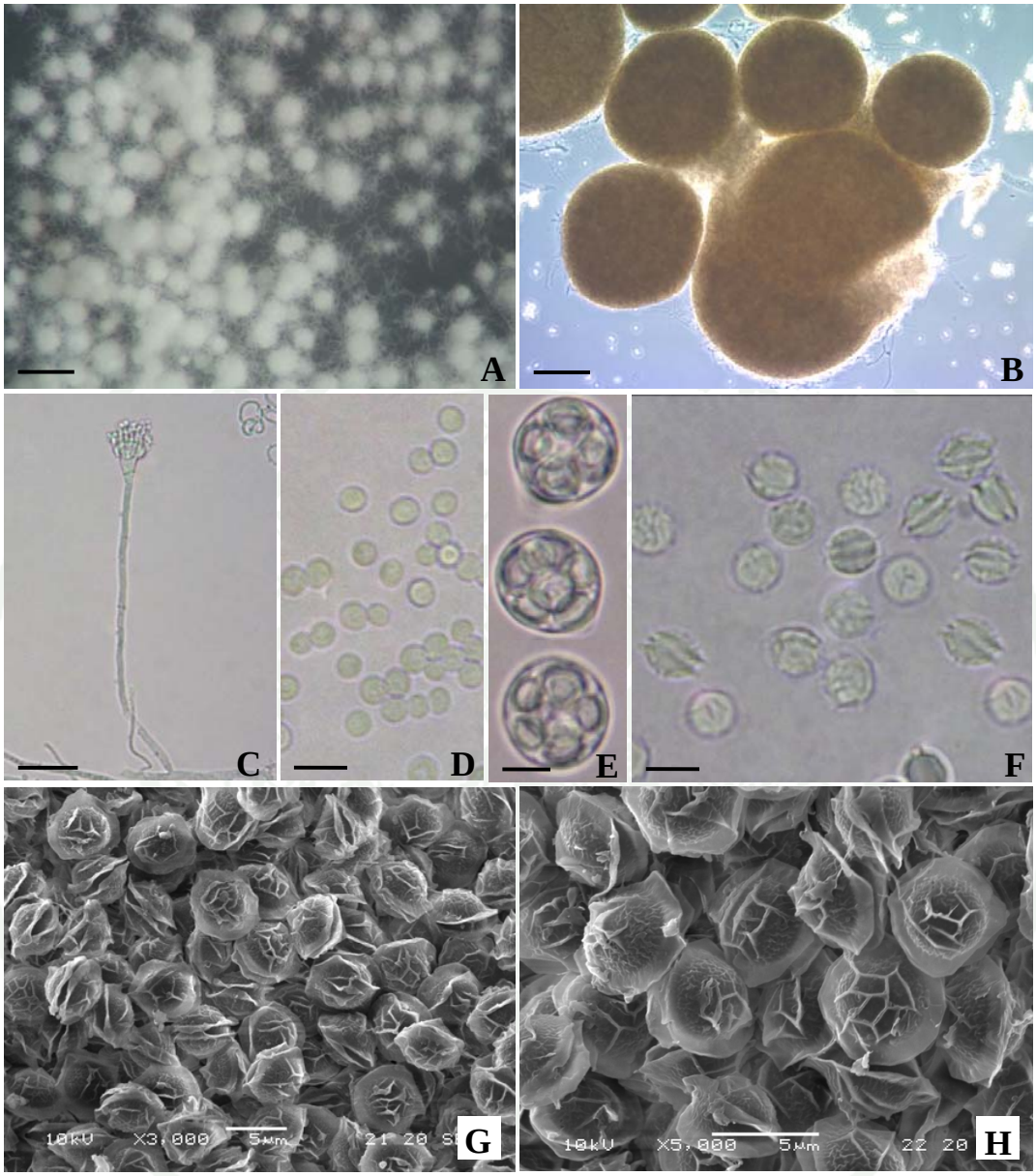


Figure 20 *Neosartorya pseudofischeri* Peterson **(**KUFC 6422)

A. Stereo photomicrograph of Ascomata and Conidial heads;

B-F. Light photomicrographs of Ascomata (B), Conidial head (C), Conidia (D), Asci and ascospores (E), Ascospores (F);

G, H. SEM photomicrographs of ascospores

(Bars: A = 1,000 μm; B = 100 μm; C = 40 μm; D - H = 5 μm)

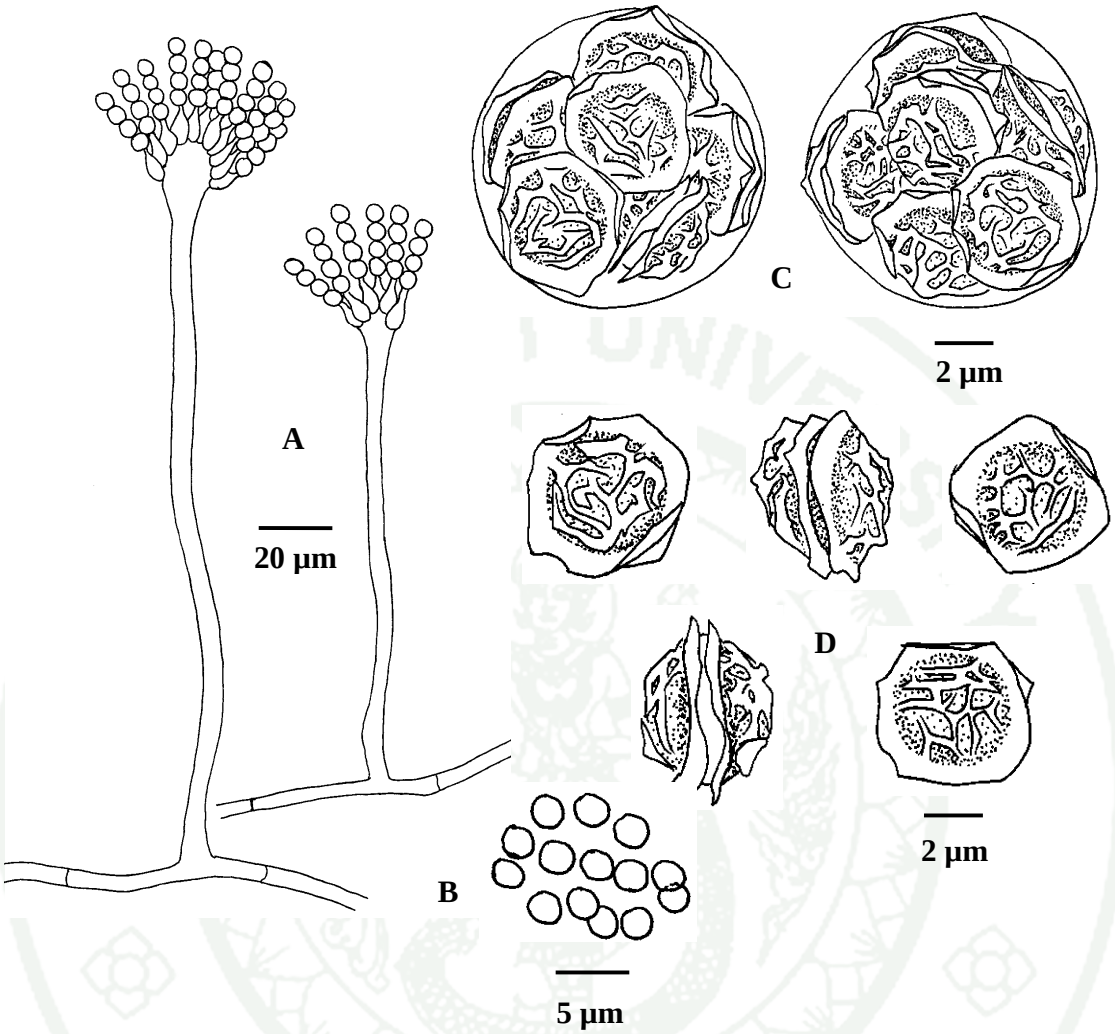


Figure 21 *Neosartorya pseudofischeri* Peterson (KUFC6422)
Camera lucida drawings of conidial heads (A**)**, conidia (B), asci and ascospores (C), ascospores (D).

Homothallic, ascomata superficial, scattered in small cluster, non-ostiolate, white to slightly cream colored, globose to subglobose, 200-315 µm in diameter (Figures 20 A, B), covered loosely with hyaline, branched, smooth, septate; asci 8 ascospored, subglobose, 11-13 x 9-11 µm (Figures 20 E, 21 C); ascospores, subglobose, 4.5-5 x 5-6 µm, with two equatorial rings, 1 µm, convex surfaces with raised flaps resembling triangular projections (Figures 20 F-H, 21 D).

Conidiophores arising from the basal mycelium or aerial hyphae, smooth, hyaline, 200-300 x 4-6 µm in diameter (Figure 20 C); conidial heads loosely columnar; vesicles hyaline, subglobose, 11-16 µm; uniseriate; phialides hyaline, ampulliform, 7.5-11 x 3.5-5 µm in diameter (figure 21 A); conidia, globose to subglobose, green colored, smooth, 3-4 µm in diameter (Figures 20 D; 21 B).

Peterson (1992) first isolated *Neosartorya pseudofischeri* from human dead body at autopsy from a fungal lesion occurring in human neck vertebrae whereas the fungal lesion was not the proximal cause of death. Järv *et al.* (2004) reported a case of invasive pulmonary aspergillosis caused by *N. pseudofischeri* from a neutropenic patient blood. Ghebremedhin *et al.* (2009) recorded the peritonitis caused by this fungus in an elderly patient undergoing peritoneal dialysis. Guarro *et al.* (2012) recorded *N. pseudofischeri* from soil planted with tomato and perilla, cherry filling in Korea, USA, Denmark, Canada and Japan. In this study, *N. pseudofischeri* is a new record for Thailand in agricultural soil planted with rose apple at Amphoe Wisetchaichan, Angthong province.

Eamvijarn *et al.* (2012) reported three new compounds, 1,4-diacetyl-2,5-dibenzylpiperazine 3,7´´-oxide, pseudofischerine and 2,4-dihydroxy-6-methylbenzoic acid and four known compounds including cadinene, eurochevalierine, brasiliamide B and pyripyropene A from a culture of fungus *N. pseudofischeri*. Eurochevalierine displayed *in vitro* growth inhibitory activity with six human cancer cell lines. Samson *et al.* (2007) recorded five known compounds; asperfuran, cytochalasin-like compound, fiscalin-like compound, pyripyropens and gliotoxin from this fungus.

5. *Neosartorya siamensis* Manoch & Eamvijarn sp. nov. (Figures 22-24, Table 5-7)
Strains examined: KUFC 6349 (MycoBank 561946) coastal forest soil, Samaesarn Island, Chonburi province; KUFC 6412 nonagricultural soil, Maesa Elephant Camp, Amphoe Mae Rim, Chiang Mai province
Reference: Eamvijarn *et al.*, 2013

Anamorphic state: *Aspergillus siamensis* Manoch & Eamvijarn (2013)

Colonies on CYA growing rapidly, attaining a diameter of 45-55 and 57-67 mm at 7 and 14 days, respectively, incubation at 28°C (Figures 22 A, a, D, d), velvety to granulose, white to faintly yellow with finely pink exudates; consisting of thick mycelial layer; cleistothecia abundantly produced; conidiogenesis few in number; reverse yellowish brown to amber (R 47).

Colonies on CZA growing rapidly, attaining a diameter of 35-55 and 45-60 mm at 7 and 14 days, respectively, incubation at 28°C (Figures 22 B, b, E, e), velvety to granulose, white with a tint, turned to pinkish with finely pink exudates; cleistothecia abundantly produced; conidiogeneses inconspicuous, reverse bright orange pink to coral (R 38).

Colonies on MEA attaining a diameter of 45-53 and 55-61 mm at 7 and 14 days, respectively, incubation at 28°C (Figures 22 C, c, F, f), white to pale yellow consisting of thin mycelial felt with concentric rings; granular cleistothecia abundantly produced, no conidiogenesis; exudates absent; reverse straw (R 46) to yellowish green.

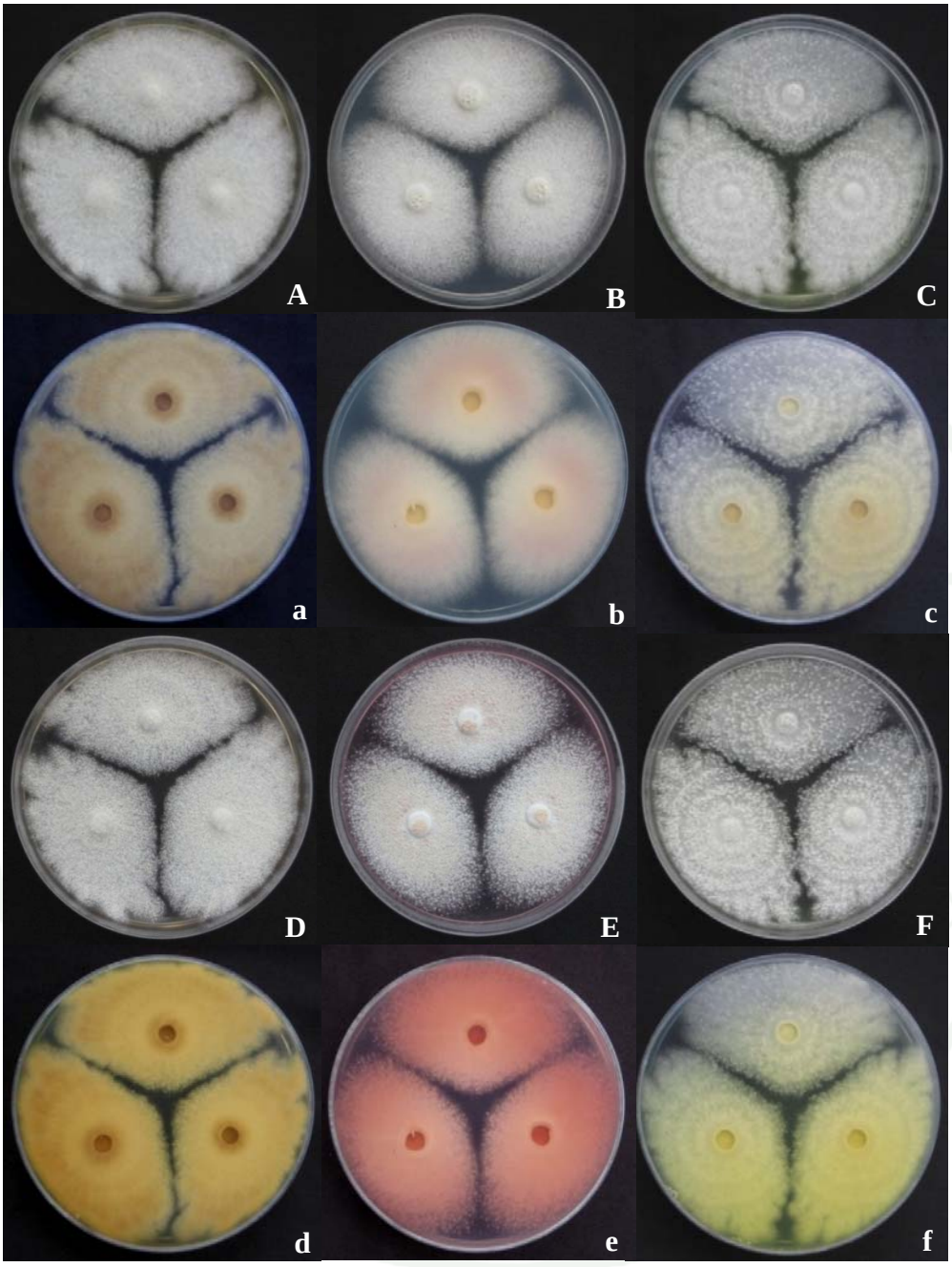


Figure 22 *Neosartorya siamensis* Manoch & Eamvijarn sp. nov. (KUFC 6349) Obverse and reverse views of colonies incubated at 28˚C on CYA for 7 days (A, a,) 14 days (D, d); CZA 7 days (B, b) 14 days (E, e); MEA 7 days (C, c) 14 days (F, f).

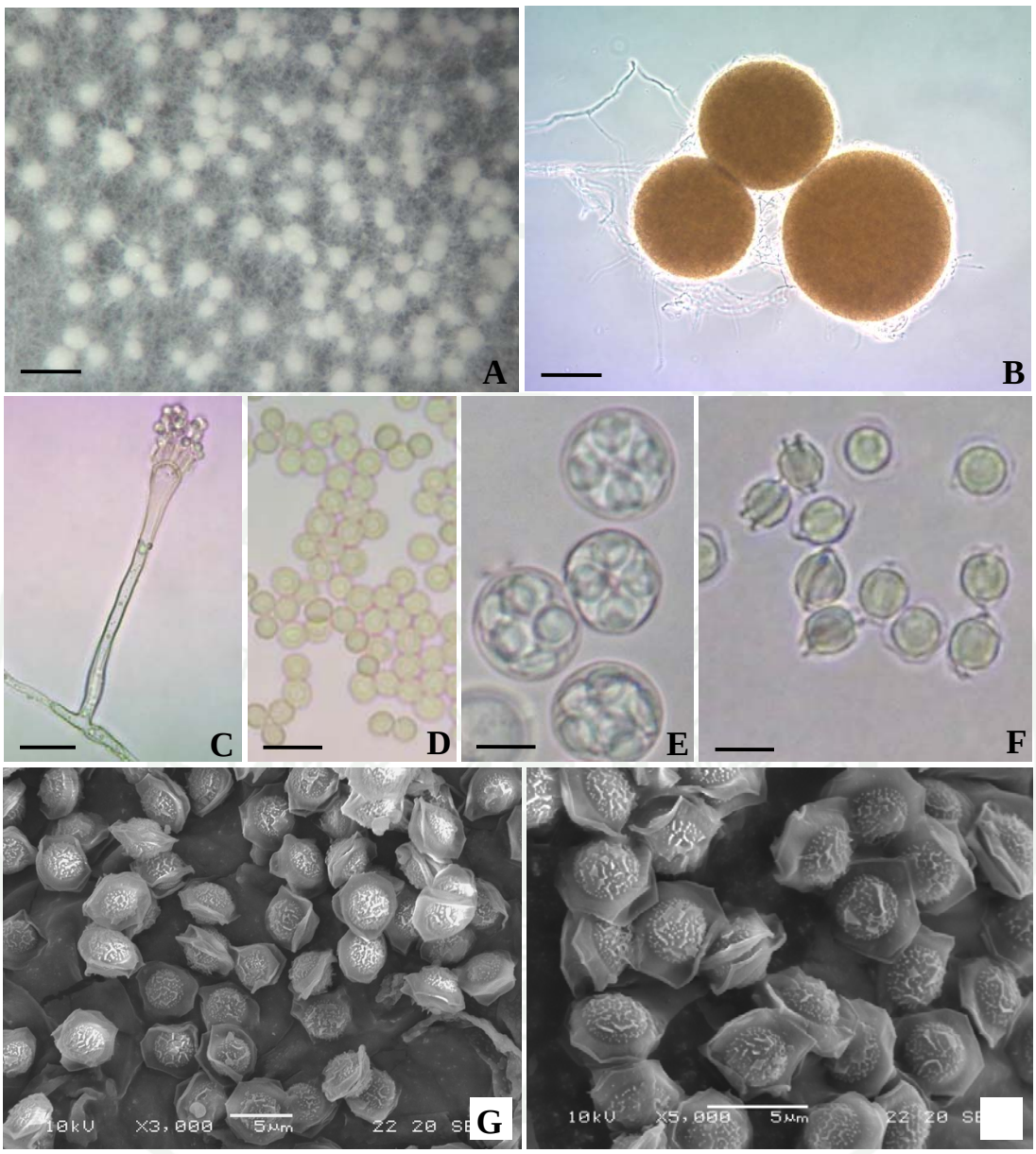


Figure 23 *Neosartorya siamensis* Manoch and Eamvijarn sp. nov. **(**KUFC 6349)
A. Stereo photomicrograph of Ascomata and Conidial heads;
B-F. Light photomicrographs of Ascomata (B), Conidial head (C), Conidia (D), Asci and ascospores (E), Ascospores (F);
G, H. SEM photomicrographs of ascospores
(Bars: A = 1,000 µm; B = 100 µm; C = 20 µm; D – H = 5 µm)

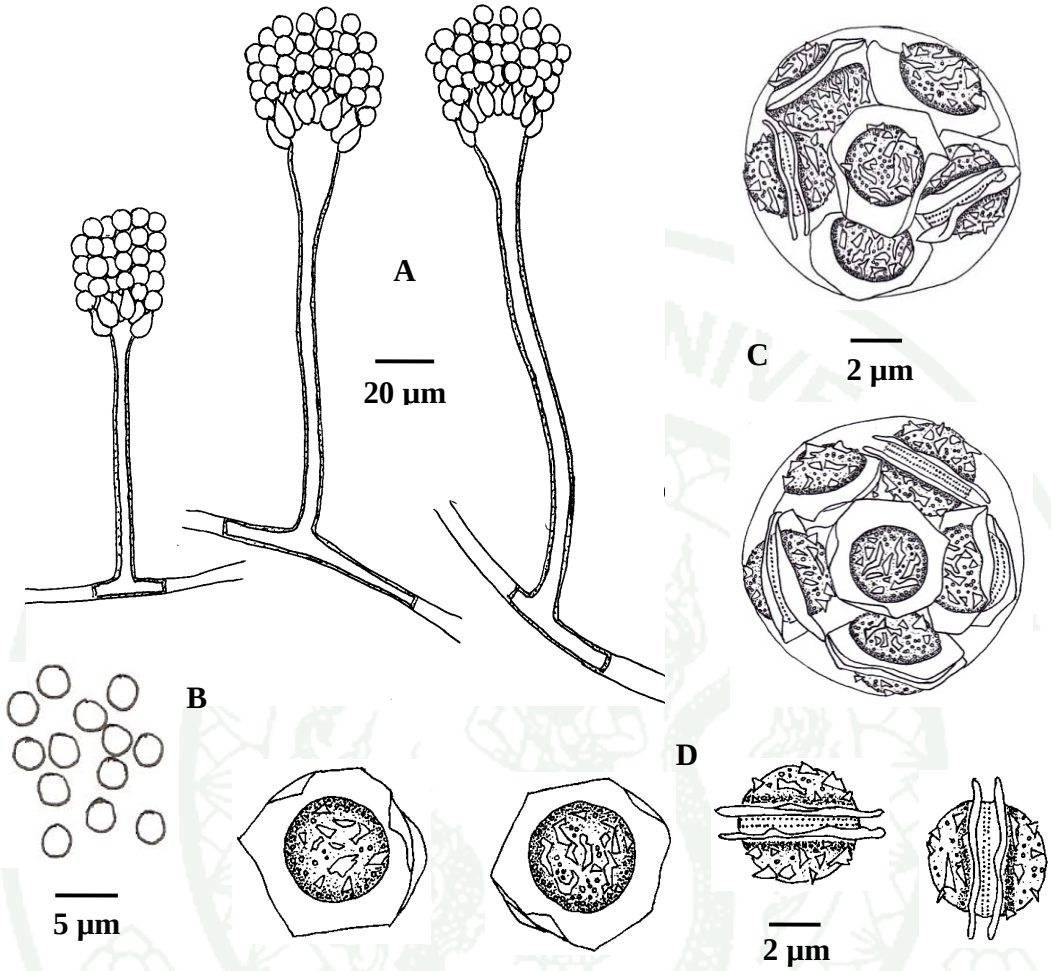


Figure 24 *Neosartorya siamensis* Manoch and Eamvijarn sp. nov. (KUFC 6349) Camera lucida drawings of conidial heads (A), conidia (B), asci and ascospores (C), ascospores (D).

Homothallic, ascomata superficial, 250-470 µm in diameter (Figures 23 A, B), white to creamish white, containing numerous asci and ascospores; asci 8-spored, globose to subglobose, 10-12.5 x 9.5-11 µm (Figures 23 E; 24 C); ascospores globose to subglobose, 4.7-5 x 5.5-6 µm, with broadly lenticular ascospores with two wide equatorial crests and finely spinulose and rugose convex surfaces (Figures 23 F-H; 24 D).

Loosely columnar conidial head; conidiophores arising from surface and aerial mycelium, hyaline, smooth, 65-110 µm long and 3.5-4 µm wide (Figure 23 C), uniseriate, pale grayish green; hyaline subglobose apical vesicle, 10-13 µm in diam., metulae lacking, phialides hyaline, ampulliform 5-6 x 3-4 µm (Figure 24 A), conidia globose to subglobose, 2-2.5 µm in diameter, smooth-walled (Figures 23 D; 24 B).

Eamvijarn et al. (2013) reported that KUFC 6349 (MycoBank 561946), *N. aureola*, *N. indohii*, *N. udagawae* and *A. viridinutans* formed a monophyletic clade. However, those KUFC strains and the others were clearly separated and had similarities of 96.0-96.9% at the β-tubulin gene sequence. On comparison with the species forming a monophyletic clade on morphology, *N. aureola* and *N. indohii* have ascospores with echinulate or tuberculate convex surfaces and *N. udagawae* is heterothallic species and have broadly lenticular, with two equatorial, convex surfaces tuberculate and *A. viridinutans* is anamorph species not found sexual state. Moreover, the distinctive features of the anamorphic states of *A. viridinutans*, *N. aureola* and *N. indohii* is in the presence of shorter conidiophores 20-35, 50 and 85-130 µm, respectively (Samson *et al.*, 2007) while *N. udagawae* has conidiophores of up to 530 µm long (Horie *et al.*, 1995a). With the evidence from morphology and this phylogenetic analysis, these strains should be considered a distinct new species.

Therefore, *N. siamensis* Manoch & Eamvijarn sp. nov. (*A. siamensis* Manoch & Eamvijarn sp. nov.) is a new species reported from soil in Thailand. It can be distinguished from other known species of *Neosartorya* by producing pinkish with pale pink exudates on CZA medium. Forty-five isolates of *N. siamensis* sp.nov. were widely distributed in forest and agricultural soil in various parts of Thailand.

6. *Neosartorya spinosa* (Raper & Fennell) Kozakiewicz (Figures 25-27, Tables 5-7)

Strains examined: KUFC 6325 agricultural soil, Amphoe Ban Khai, Rayong province; KUFC 6339 forest soil, Samaesarn Island, Chonburi province

Reference: Raper & Fennell, 1965

Anamorphic state: *Aspergillus fischeri* var. *spinosus* (1965)

Synonym anamorphic state: *Aspergillus spinosus* (1989)

Colonies on CYA growing rapidly, attaining a diameter of 37-53 and 54-69 mm at 7 and 14 days, respectively, incubation at 28°C (Figures 25 A, a, D, d), white to faintly yellow, consisting of thick mycelial felt, velvety to floccose, irregular margin; very abundant cleistothecia produced, conidiogenesis few in number, loose columnar, colorless exudates, reverse luteous (R 12) to orange (R 7).

Colonies on CZA growing rapidly, attaining a diameter of 38-50 and 55-69 mm at 7 and 14 days, respectively, incubation at 28°C (Figures 25 B, b, E, e), white to creamish white, consisting of thick mycelial layer, velvety to floccose; cleistothecia abundantly produced; conidiogenesis absent; colorless exudates; reverse yellowish white to pale luteous (R 11).

Colonies on MEA spreading broadly, attaining a diameter of 36-52 and 53-70 mm at 7 and 14 days, respectively, incubation at 28°C (Figures 25 C, c, F, f), white, consisting of thin mycelial felt, plane; less cleistothecia produced, granular in appearance; conidiogenesis absent; colorless exudates, reverse hyaline.

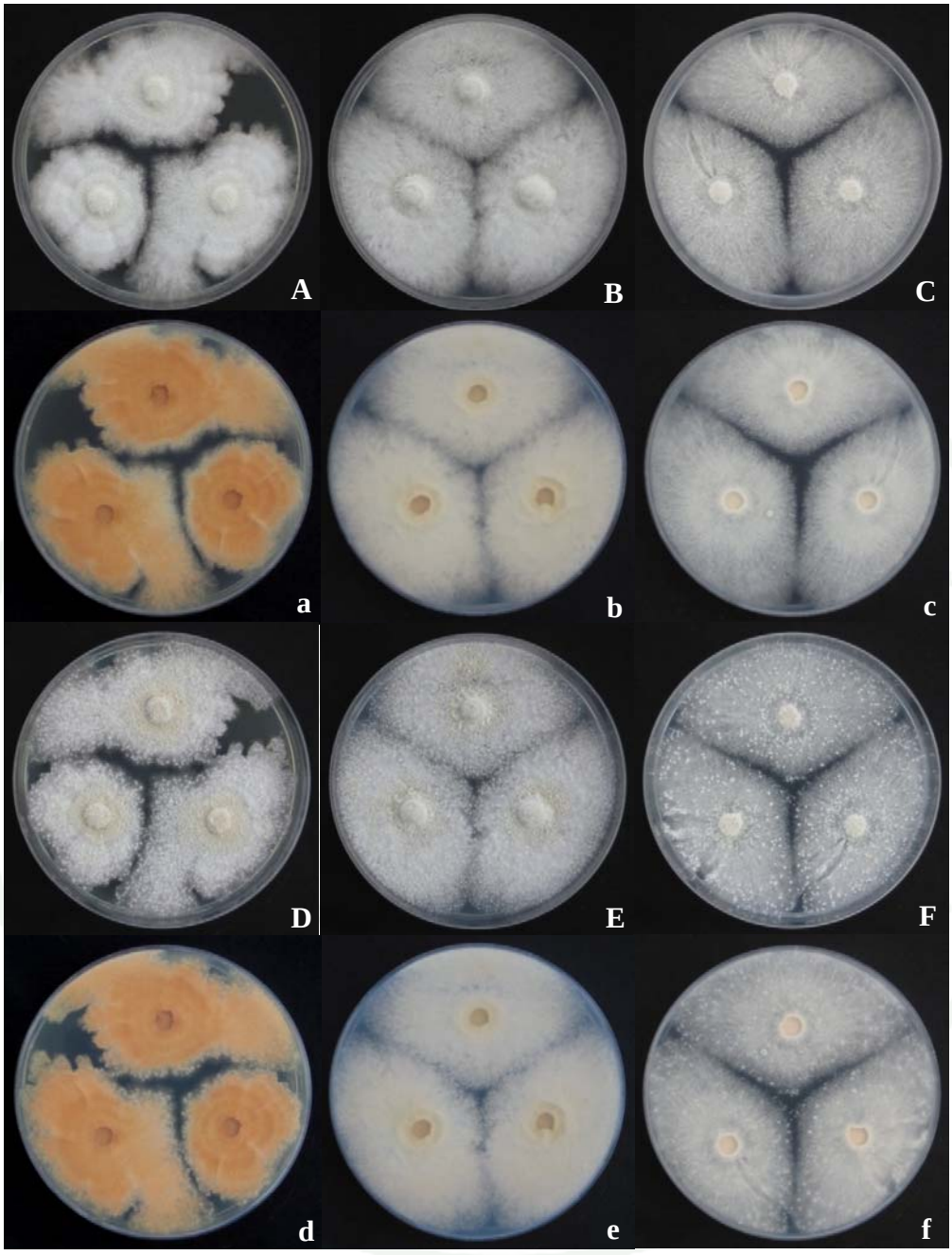


Figure 25 *Neosartorya spinosa* (Raper & Fennell) Kozakiewicz (KUFC 6325) Obverse and reverse views of colonies incubated at 28˚C on CYA for 7 days (A, a,) 14 days (D, d); CZA 7 days (B, b) 14 days (E, e); MEA 7 days (C, c) 14 days (F, f).

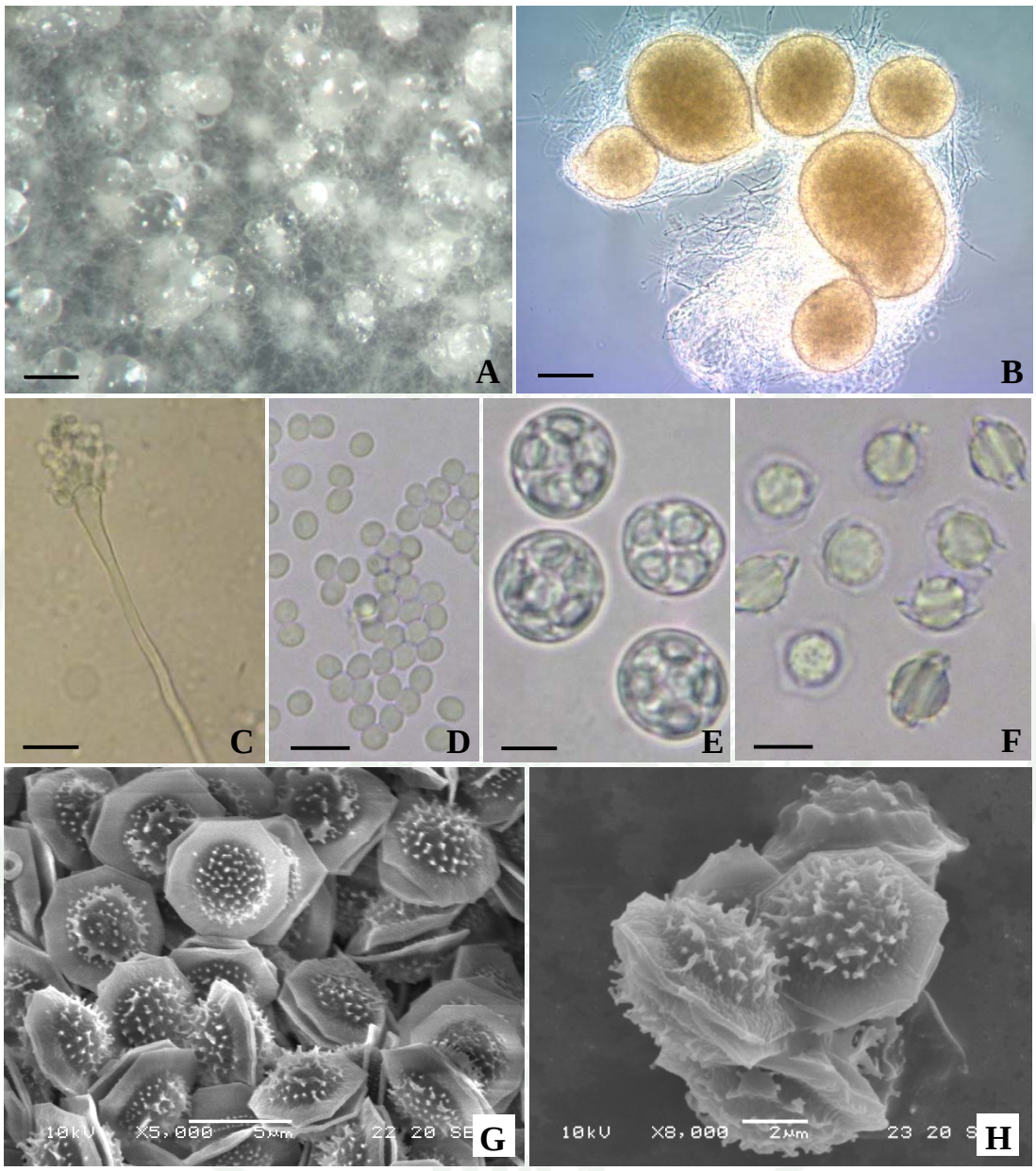


Figure 26 *Neosartorya spinosa* (Raper & Fennell) Kozakiewicz (KUFC 6325)
A. Stereo photomicrograph of Ascomata and Conidial heads;
B-F. Light photomicrographs of Ascomata (B), Conidial head (C), Conidia (D), Asci and ascospores (E), Ascospores (F);
G, H. SEM photomicrographs of ascospores
(Bars: A = 1,000 µm; B = 100 µm; C = 20 µm; D - G = 5 µm; H = 2 µm)

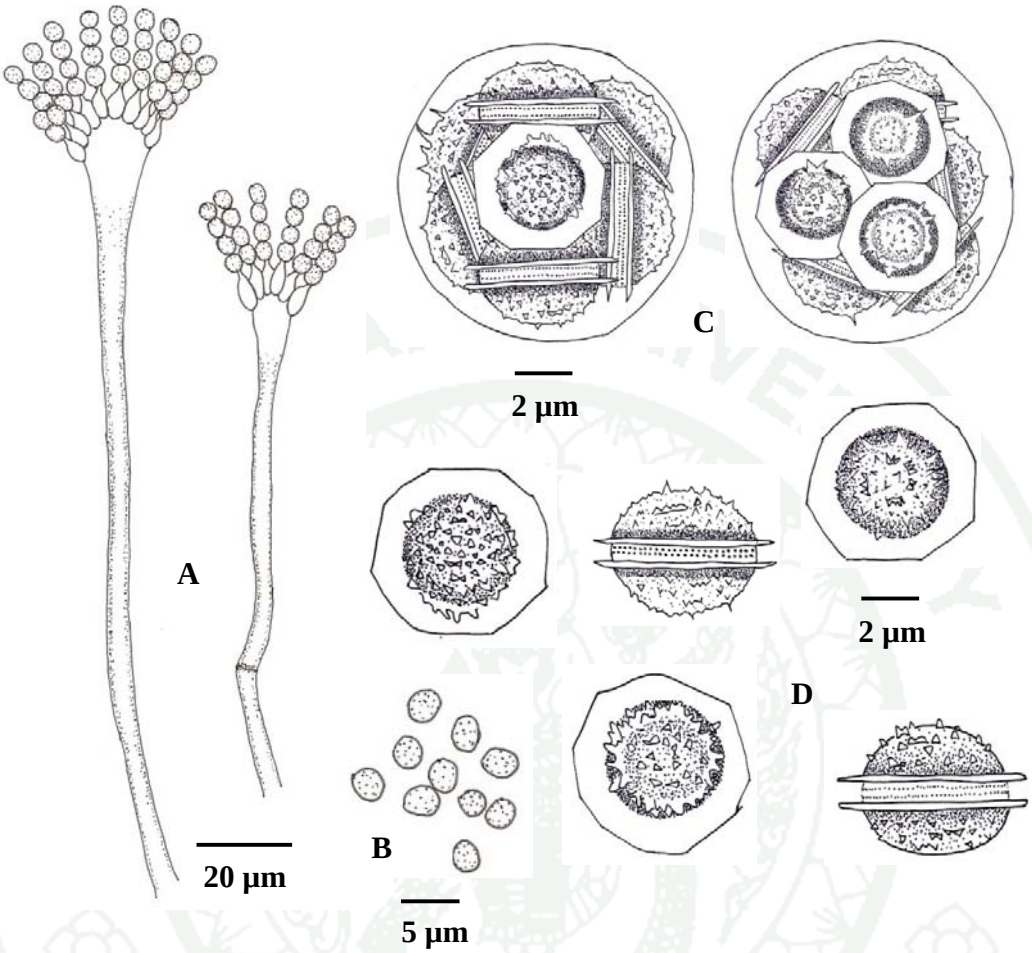


Figure 27 *Neosartorya spinosa* (Raper & Fennell) Kozakiewicz (KUFC 6325) Camera lucida drawings of conidial heads (A), conidia (B), asci and ascospores (C), ascospores (D).

Homothallic, ascomata superficial, borne singly or in small clusters within a loose hyphal envelope, spherical, white to pale yellow, 190-300 µm in diameter (Figures 26 A, B); asci, globose to subglobose, 8-spored, 10-12 x 8-10 µm diam. (Figures 26 E; 27 C); ascospores spherical to subspherical, 4-5 µm in diam., hyaline, with two prominent, flexuose and widely separated equatorial crests, with convex surfaces bearing spine-like projections (Figures 26 F-H; 27 D).

Conidiophores arising from surface and aerial mycelium, hyaline to pale green, smooth-walled, 300-500 µm in diameter (Figure 26 C), conidial heads columnar, pale blue-grey, anamorph uniseriate; vesicles flask-shaped, up to 18 µm, phialides hyaline to pale greyish-green, 5-7 x 3-4.5 µm in diameter (Figure 27 A); conidia spherical to subspherical, smooth-walled, 2.5-4.5 µm, pale greyish-green (Figures 26 D; 27 B).

In Thailand, Manoch and Chana (1995) reported *Neosartorya spinosa* on cow dung from Kanchanaburi province. Manoch *et al*. (2007) found *N. spinosa* from soil and termite mound from Ratchaburi province. Kokaew (2011) recorded *N. spinosa* as an endophytic fungus in the healthy plant tissue of *Dracaena conferta* (Dracaenaceae) from Mu Ko Similan National Park, Phangnga province.

Guarro *et al.* (2012) reported *N. spinosa* from soil in Nicaragua, Kenya, Denmark, Dominican Republic, U.S.A, Belgium, Sudan, India, Pakistan and South Korea, from fruit juice in Japan. This fungus produces aszonalenins, 2-pyrovoylaminobenzamide and pseurotin (Samson *et al.,* 2007). Ferulic acid producing fungus *N. spinosa* NRRL 185 releases a full complement of enzymes from corn bran and corn fibers (Shin *et al.,* 2006). On the other hand, *N. spinosa* was reported as pathogenic to human. Summerbell *et al.* (1992) described the first case of endocarditis caused by *N. fischeri* var. *spinosa* in child who received a calf pericardium graft.

In this study, *N. spinosa* was the most frequently found and 79 isolates were recorded mainly from forest soil.

7. *Neosartorya takakii* Horie, Abliz & Fukushima (Figures 28-30, Table 5-7)
Strain examined: KUFC 6355 forest soil, Phu Luang Wildlife Sanctuary, Loei province
Reference: Horie *et al.,* 2001

Anamorphic state: *Aspergillus takakii* Horie, Abliz & Fukushima (2001)

Colonies on CYA growing rapidly, attaining a diameter of 38-53 and 55-68 mm at 7 and 14 days, respectively, incubation at 28°C (Figures 28 A, a, D, d), white to faintly yellow, consisting of thick mycelial felt, velvety to floccose; very abundant production of cleistothecia, conidiogenesis few in number, loose columnar, colorless exudates, reverse pale orange (R 7) to saffron (R 10).

Colonies on CZA spreading broadly, attaining a diameter of 36-49 and 52-65 mm at 7 and 14 days, respectively, incubation at 28°C (Figures 28 B, b, E, e), white to yellowish white, consisting of thin mycelial layer, floccose in appearance; producing abuntdant cleistothecia on mycelial felts; conidiogenesis few in number; colorless exudates; reverse creamish white to pale luteous (R 11).

Colonies on MEA spreading broadly, attaining a diameter of 36-51 and 52-68 mm at 7 and 14 days, respectively, incubation at 28°C (Figures 28 C, c, F, f), white, consisting of thin mycelial felt, plane; less production of cleistothecia, velvet to floccose in appearance due to less production of cleistothecia, loosely overgrown by aerial hyphae; conidiogenesis absent; colorless exudates, reverse hyaline to pale yellow.

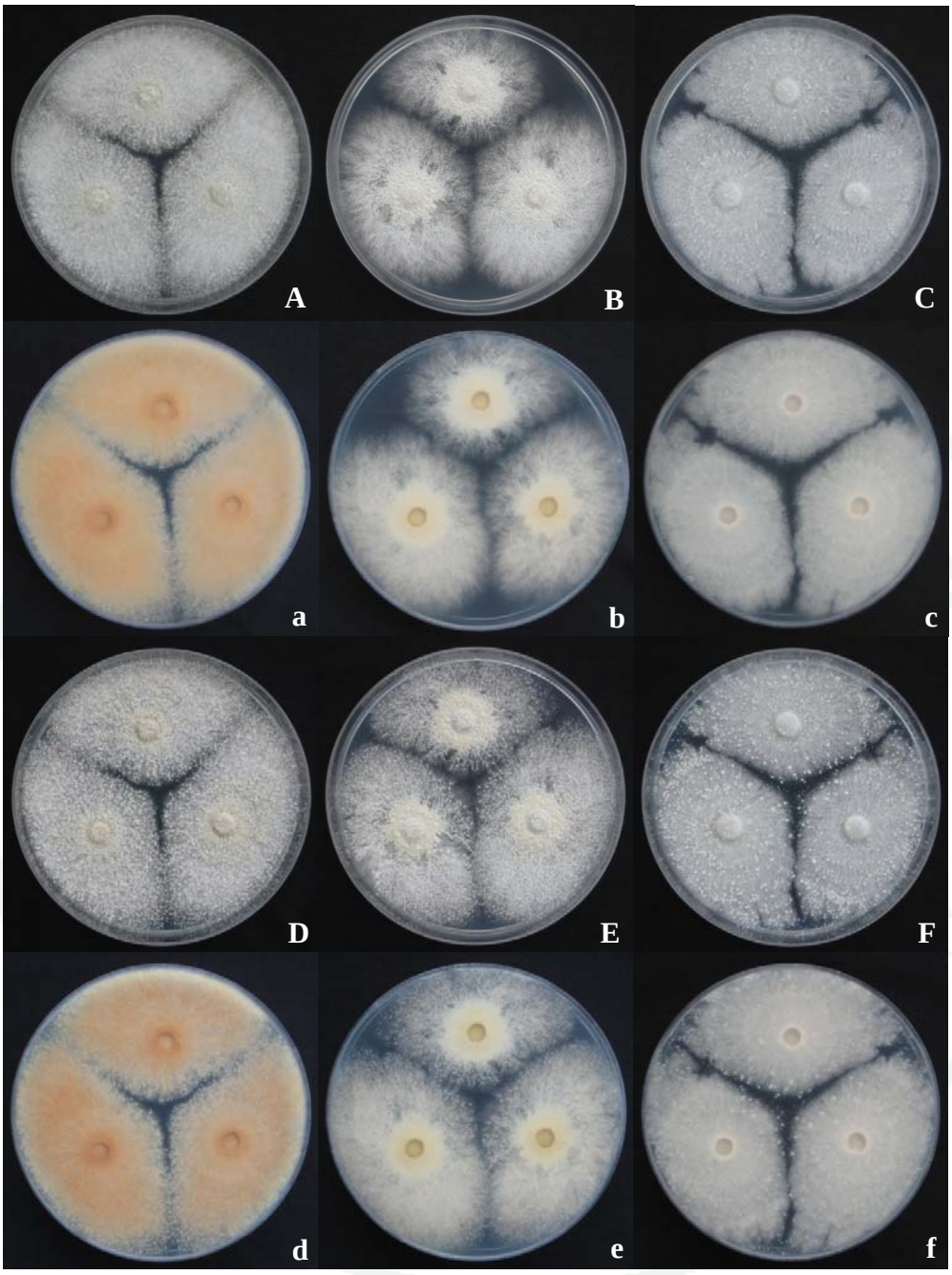


Figure 28 *Neosartorya takakii* Horie, Abliz & Fukushima (KUFC 6355)
Obverse and reverse views of colonies incubated at 28˚C on CYA for 7 days (A, a,) 14 days (D, d); CZA 7 days (B, b) 14 days (E, e); MEA 7 days (C, c) 14 days (F, f).

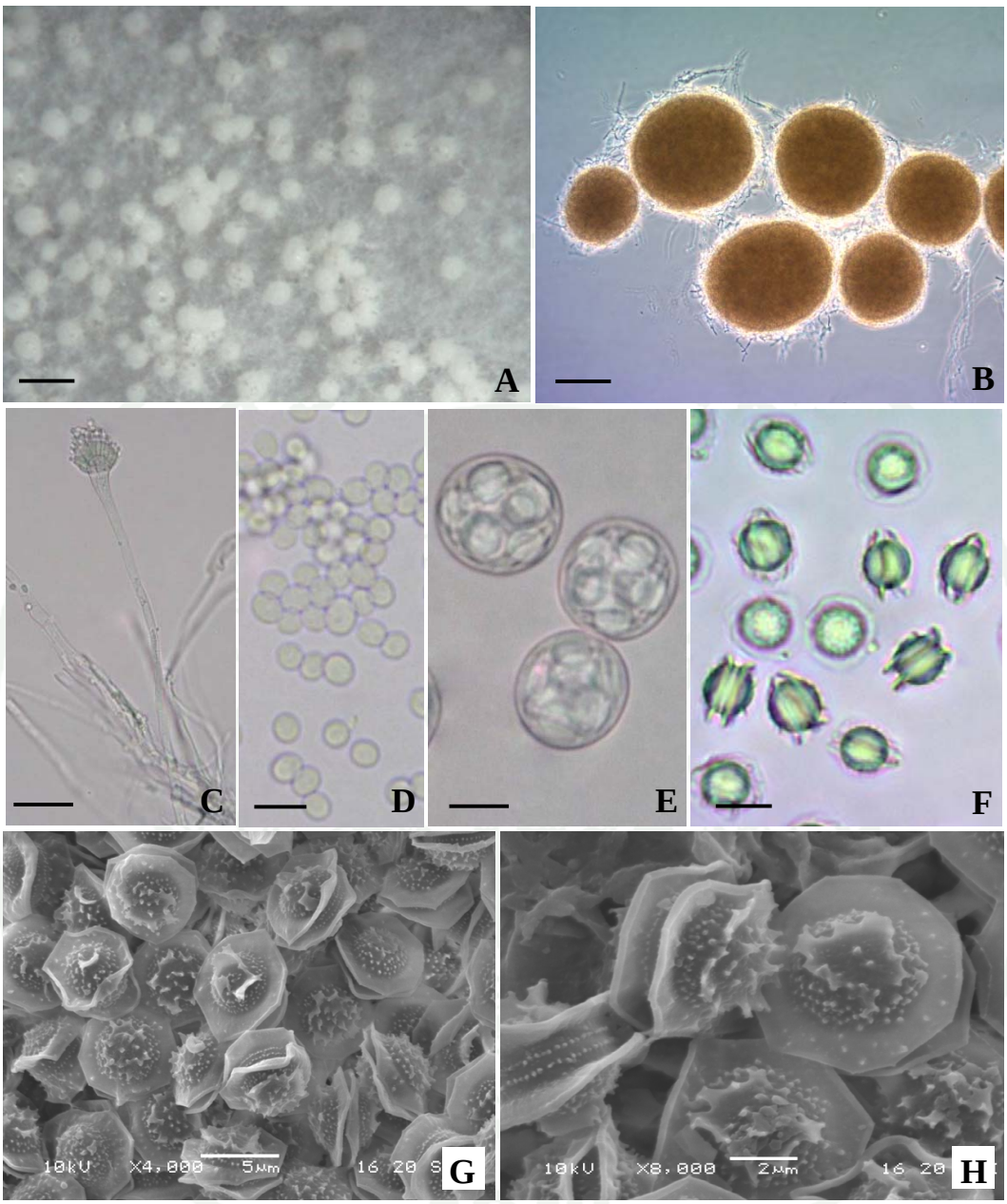


Figure 29 *Neosartorya takakii* Horie, Abliz & Fukushima (KUFC 6355)

A. Stereo photomicrograph of Ascomata and Conidial heads;

B-F. Light photomicrographs of Ascomata (B), Conidial head (C), Conidia (D), Asci and ascospores (E), Ascospores (F);

G, H. SEM photomicrographs of ascospores

(Bars: A = 1,000 µm; B = 100 µm; C = 20 µm; D – G = 5 µm, H = 2 µm)

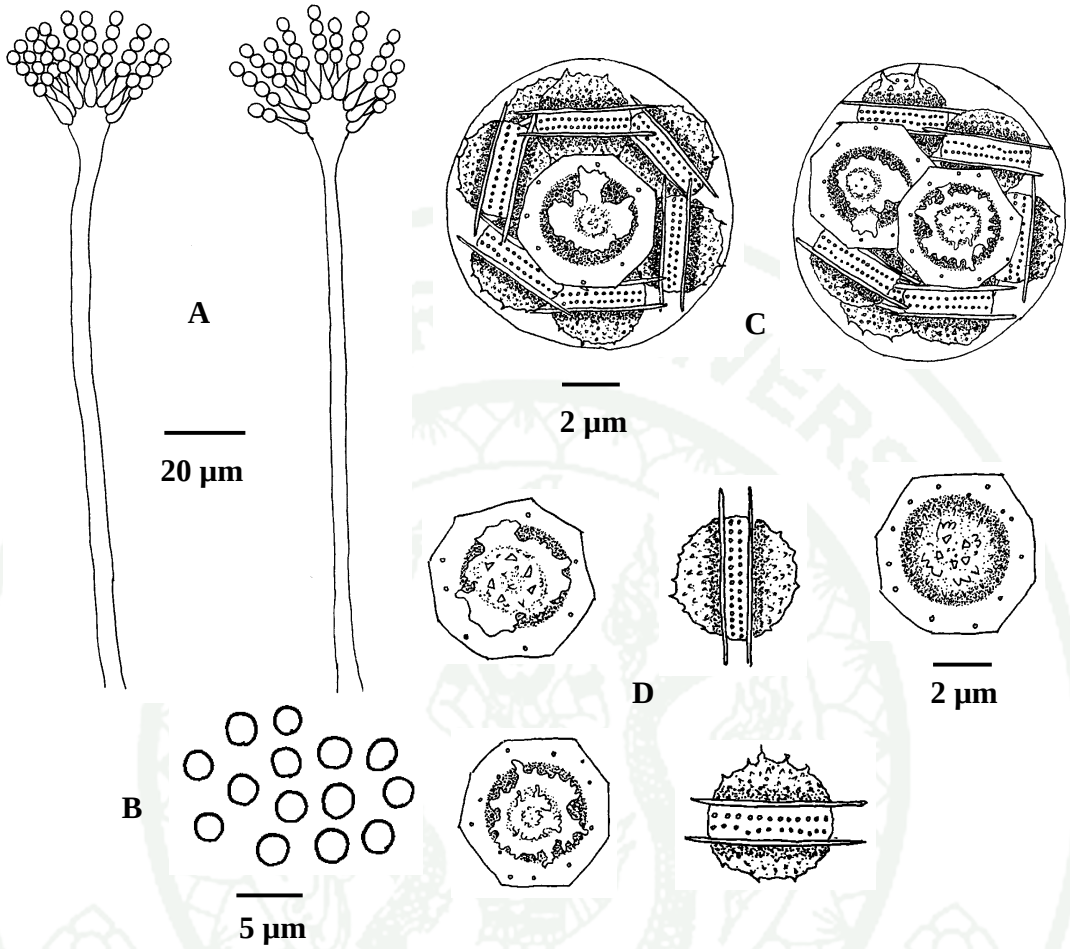


Figure 30 *Neosartorya takakii* (KUFC 6355)

Camera lucida drawings of conidial heads (A), conidia (B), asci and ascospores (C), ascospores (D).

Homothallic, ascotama superficial, scattered, white to yellowish white, globose to subglobose, 220-490 µm in diameter (Figures 29 A, B), surrounded by a loose covering of aerial hyphae, cleistothecial wall hyaline to pale yellowish brown, thin, membranaceous, consisting of angular; asci 8-ascospored, globose to subglobose, 10.5-13 x 10-12 µm, evanescent at maturity (Figures 29 E, 30 C); ascospores hyaline to pale yellow, broadly lenticular, spore body 4.5-5 x 4-4.5 µm, provided with two widely separated equatorial crests which are up to 1.5 µm wide with convex surfaces bearing roughly circularly arranged projections and microtuberculate on the convex surfaces (Figures 29 F-H; 30 D).

Mycelium composed of hyaline, branched, septate, smooth-walled hyphae; conidial heads short columnar, grayish green; conidiophores arising from aerial hyphae or the basal mycelium, hyaline to pale yellowish white, smooth, 287-395 µm long, 4-5 µm wide at the middle (Figure 29 C); vesicles hyaline, hemispherical to flask-shaped, 10-15 µm in diameter; uniseriate; phialides hyaline, 5.5-7 x 1.5-3 µm, covering the upper half of the vesicle (Figure 30 A); conidia hyaline to pale grayish green, globose to subglobose, smooth, 2.2-3.1 µm in diameter (Figures 29 D; 30 B).

In Thailand, *Neosartorya takakii* was isolated from termite mound in Sakon Nakhon province using alcohol treatment method (Manoch *et al.,* 2004). In this study, 3 isolates of this fungus were found from forest soil in Loei and Surat Thani provinces (Tables 5, 6).

Horie *et al.* (2001) reported *Neosartorya takakii* isolated from soil during a survey of pathogenic and mycotoxin-producing fungi from glassland in Roraima State, Brazil. *N. takakii* differs from the other known species of the genus *Neosartorya* by having lenticular ascospores with two distinct equatorial crests and with roughly circularly arranged projections on convex surfaces.

8. *Neosartorya tatenoi* Horie, Miyaji, Yokoyama, Udagawa and Takagi (Figures 31-33, Tables 5-7)

Strains examined: KUFC 6377 forest soil, Khao Yai National Park, Nakhon Ratchasima province; KUFC 6403 forest soil, Khlong Lan Waterfall, Kamphaeng Phet province

Reference: Horie *et al.,* 1992

Anamorphic state: *Aspergillus tatenoi* Horie, Miyaji, Yokoyama, Udagawa and Takagi (1992)

Colonies on CYA, attaining a diameter of 38-67 and 40-68 mm at 7 and 14 days, respectively, incubation at 28°C (Figures 31 A, a, D, d), creamy to faintly yellow, consisting of thick mycelial layer, velvety to floccose; abundantly cleistothecia produced, conidiogenesis few in number; colorless exudates; reverse brownish orange to ochreous (R 44).

Colonies on CZA growing rapidly, attaining a diameter of 37-64 and 40-69 mm at 7 and 14 days, respectively, incubation at 28°C (Figures 31 B, b, E, e), cream to pale yellow, consisting of thick mycelial layer, floccose, sulcate; abundantly cleistothecia produced; conidiogenesis absent; colorless exudates; reverse pale yellow to light buff (R 45).

Colonies on MEA, attaining a diameter of 37-64 and 38-67 mm after 7 and 14 days, respectively, incubation at 28°C (Figures 31 C, c, F, f), white to pale yellow, consisting of thin mycelial layer, plane; less cleistothecia produced, granular in appearance; conidiogenesis absent; exudates absent, reverse hyaline.

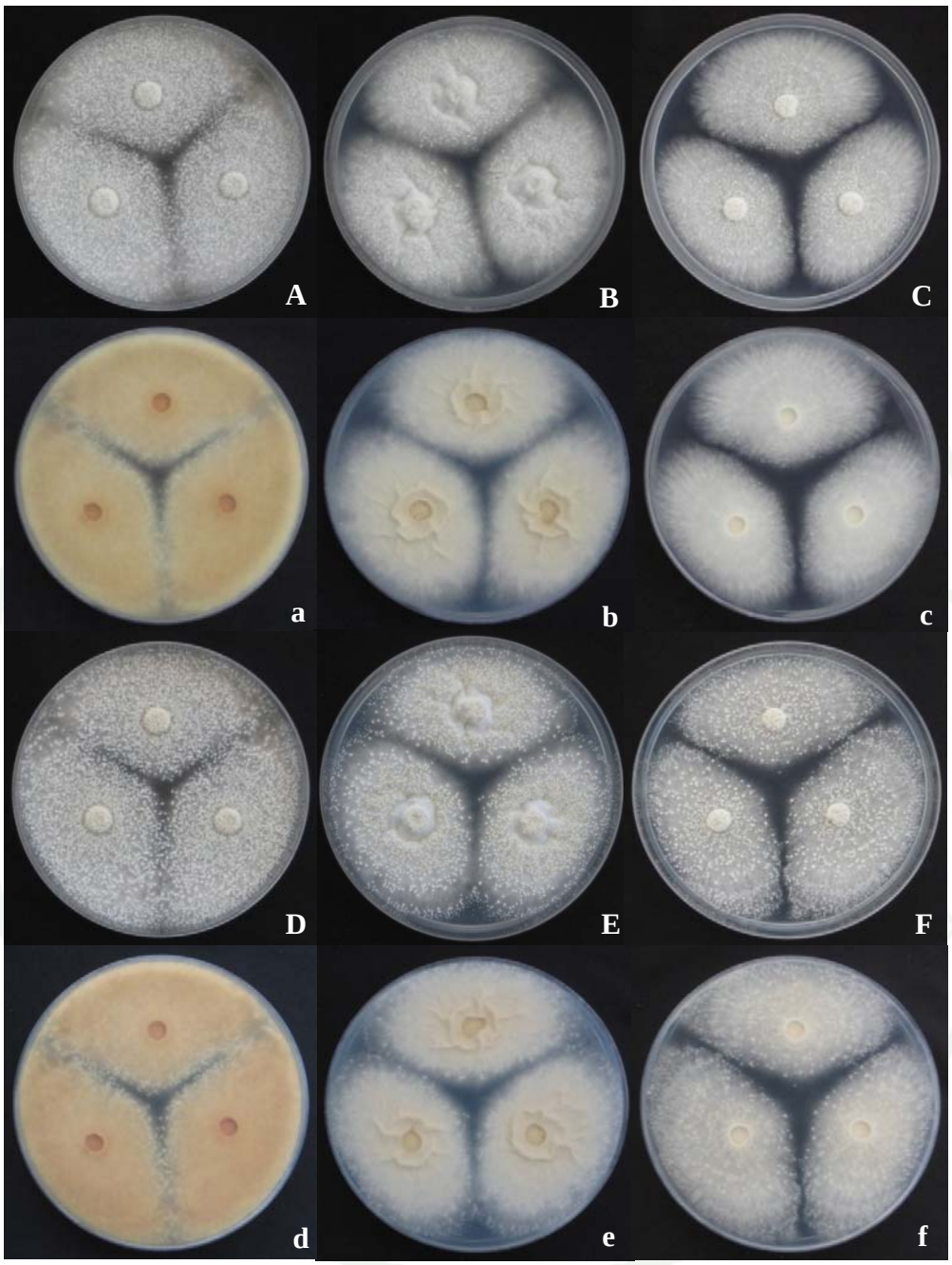


Figure 31 *Neosartorya tatenoi* Horie, Miyaji, Yokoyama, Udagawa and Takagi (KUFC 6377) Obverse and reverse views of colonies incubated at 28˚C on CYA for 7 days (A, a,) 14 days (D, d); CZA 7 days (B, b) 14 days (E, e); MEA 7 days (C, c) 14 days (F, f).

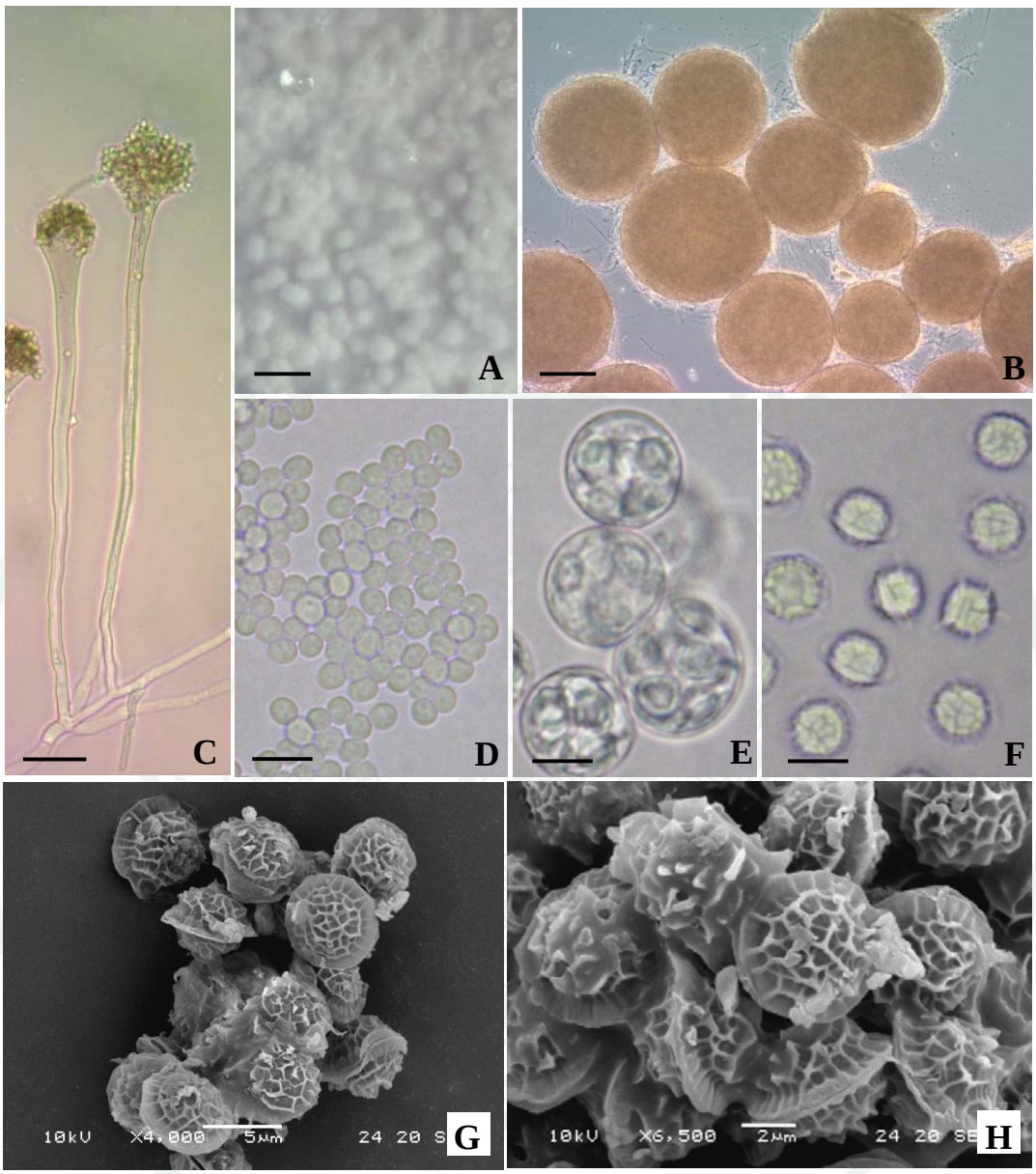


Figure 32 *Neosartorya tatenoi* Horie, Miyaji, Yokoyama, Udagawa and Takagi (KUFC 6377)
A. Stereo photomicrograph of Ascomata and Conidial heads;
B-F. Light photomicrographs of Ascomata (B), Conidial heads (C), Conidia (D), Asci and ascospores (E), Ascospores (F);
G, H. SEM photomicrographs of ascospores
(Bars: A = 1,000 µm; B = 100 µm; C = 40 µm; D – G = 5 µm; H = 2 µm)

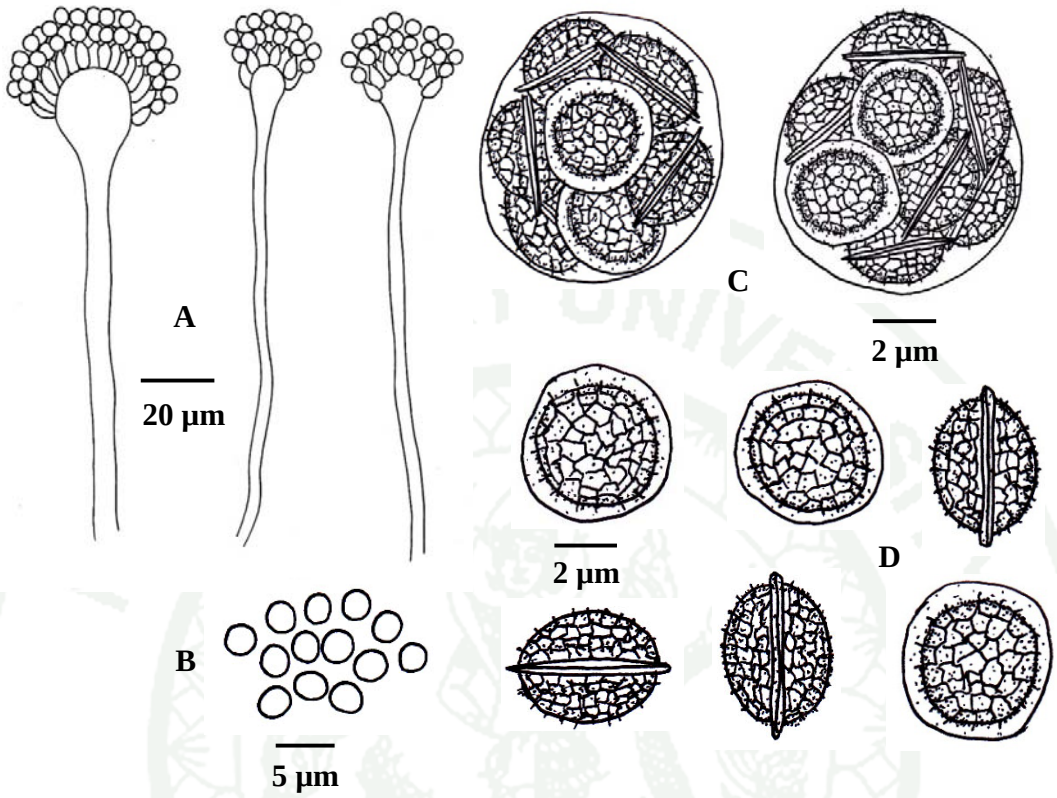


Figure 33 *Neosartorya tetanoi* Horie, Miyaji, Yokoyama, Udagawa and Takagi (KUFC 6377) Camera lucida drawings of conidial heads (A), conidia (B), asci and ascospores (C), ascospores (D).

Homothallic, ascomata superficial, white to slightly yellow, globose to subglobose, 100-220 µm in diameter (Figures 32 A, B), surrounded by a loose covering which is hyaline to pale yellowish brown; asci 8-spored, globose to subglobose, 12.5-15 x 12-14 µm in diameter, evanescent at maturity (Figures 32 E; 33 C); ascospores hyaline, lenticular, spore body 5-6 x 5-5.5 µm, provided with two narrow equatorial crests measuring 0.5-1 µm wide, with convex surfaces ornamented by distinctly and narrowly reticulate ridges (Figures 32 F-G; 33 D).

Conidiophores arising from surface and aerial mycelium, hyaline, 150- 270 µm long, 4-7.5 µm wide in diameter (Figure 32 C), smooth, thin-walled; conidial heads grayish green, short columnar; vesicles hyaline, hemispherical to flask-shaped, 10-20 µm in diam.; uniseriate; phialides hyaline, 7-7.5 x 2.5-3 µm, covering the upper half to two-third of the vesicle (Figure 33 A); conidia pale grayish green, globose to ovoid, 2-3 µm in diameter, smooth (Figures 32 D; 33 B).

Twenty-six isolates of *Neosartorya tatenoi* were recorded from various soil samples from nine provines using alcohol and heat treatment methods (Tables 5, 6). In this study, *N. tatenoi* is a new record for Thailand.

Horie *et al*. (1992) reported *Neosartorya tatenoi* from soil in a sugarcane plantation in Brazil. It differs from other known species of the genus *Neosartorya* by having convex surfaces of ascospores, ornamented with distinctly reticulate ridges. Aszonalenins was analyzed from cultures grown on CYA, oat meal agar and yeast extract sucrose agar (Samson *et al*., 2007)

9. *Neosartorya* sp.1 (Figures 34-36, Tables 5-7)

Strains examined: KUFC 6341 forest soil, Phu Luang Wildlife Sanctuary, Loei province

Anamorphic state: absent

Colonies on CYA, attaining a diameter of 27-36 and 34-45 mm after 7 and 14 days, respectively, incubation at 28°C (Figures 34 A, a, D, d), yellow with a wink at center, consisting of thick mycelial layer; pinkish cleistothecia abundantly produced; conidiogenesis absent; wrinkle, zonate, pink exudates, reverse red pink (R 2).

Colonies on CZA, attaining a diameter of 24-29 and 30-32 mm after 7 and 14 days, respectively, incubation at 28°C (Figures 34 B, b, E, e), pale yellow with a pink at center, consisting of thin mycelial layer; pinkish cleistothecia abundantly produced; conidiogenesis absent; zonate, irregular margin, exudates absent; reverse yellowish orange to luteous (R 12).

Colonies on MEA, attaining a diameter of 21-22 and 27-32 mm after 7 and 14 days, respectively, incubation at 28°C (Figures 34 C, c, F, f), pale yellow, consisting of thin mycelial layer; cleistothecia less produced, granular in appearance, conidiogenesis absent; wrinkle, exudates absent, irregular margin, reverse orange yellow (R 7) and terned to pinkish red (R 2) at 14 days.

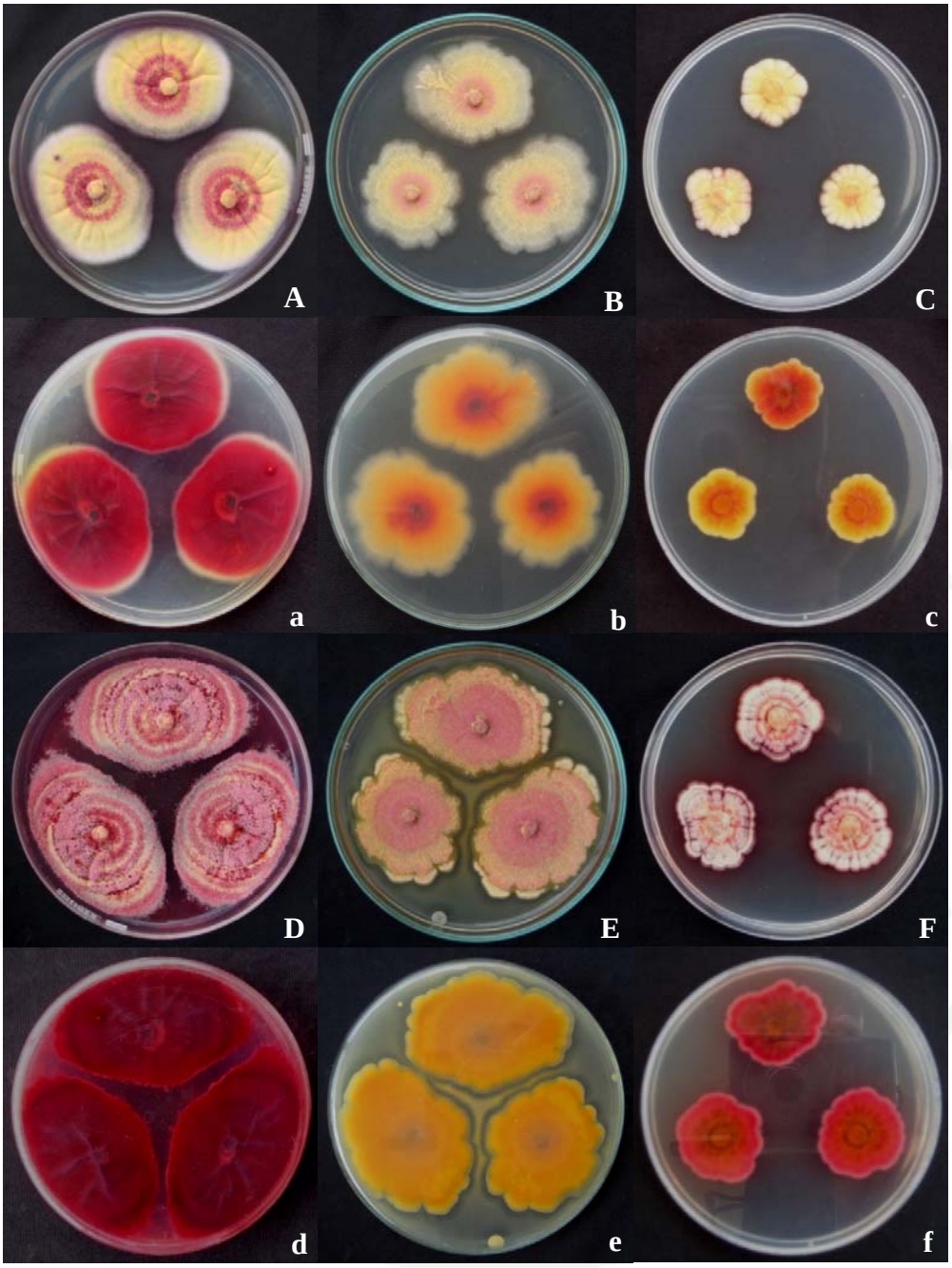


Figure 34 *Neosartorya* sp.1 (KUFC 6341)
Obverse and reverse views of colonies incubated at 28˚C on CYA for 7 days (A, a,) 14 days (D, d); CZA 7 days (B, b) 14 days (E, e); MEA 7 days (C, c) 14 days (F, f).

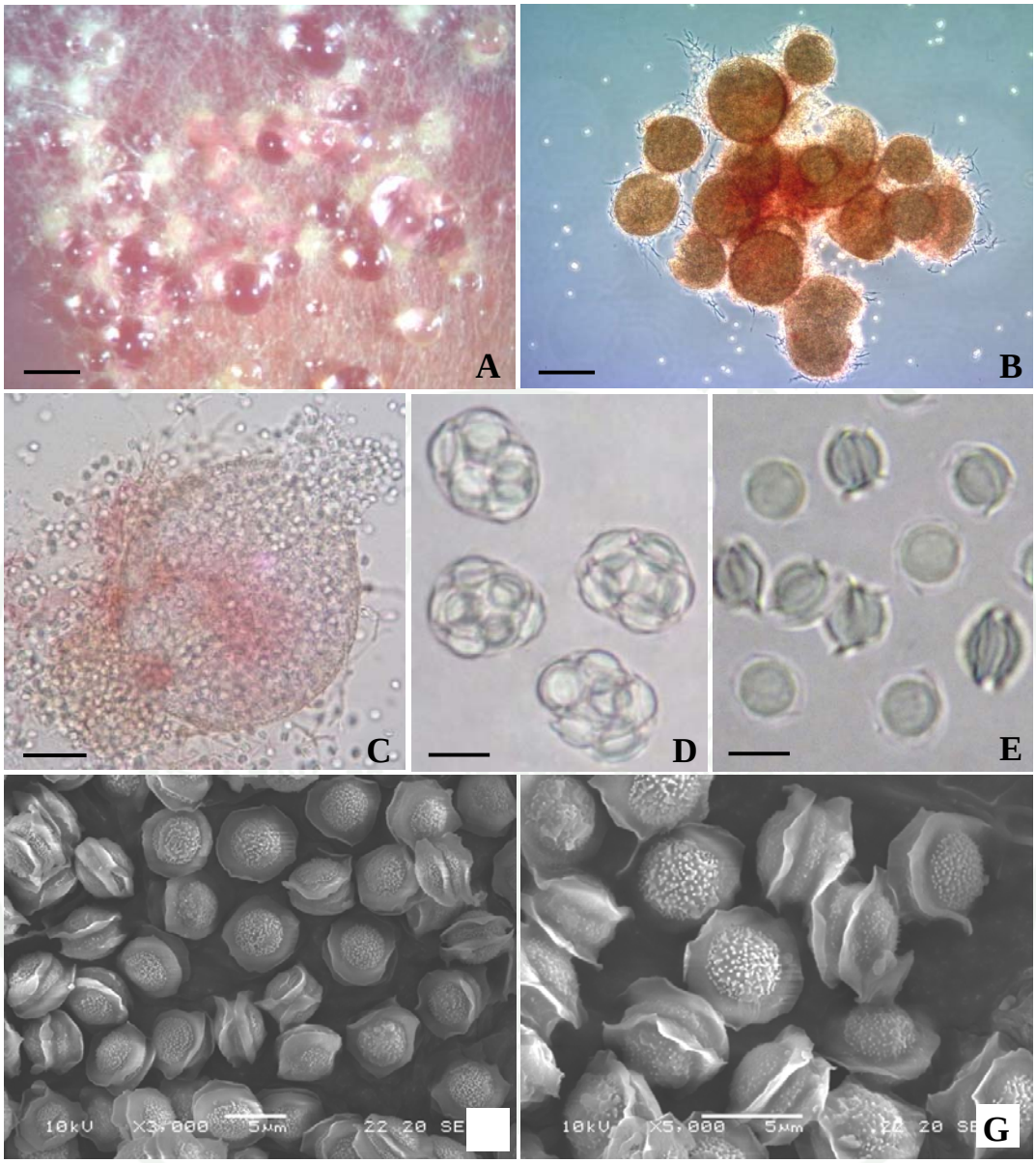


Figure 35 *Neosartorya* sp.1 **(**KUFC 6341)

A. Stereo photomicrograph of Ascomata;

B-E. Light photomicrographs of Ascomata (B, C), Asci and ascospores (D), Ascospores (E);

F, G. SEM photomicrographs of ascospores

(Bars: A = 1,000 μm; B = 100 μm; C = 40 μm; D – G = 5 μm)

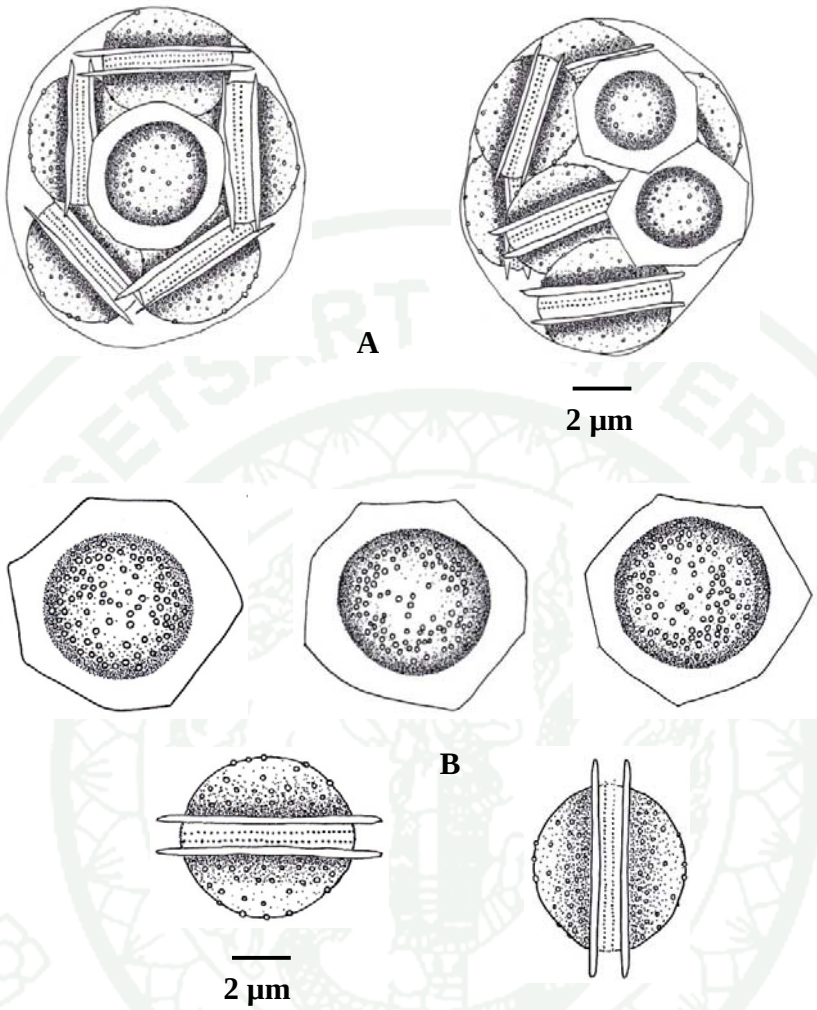


Figure 36 *Neosartorya* sp.1 **(**KUFC 6341)
Camera lucida drawings of asci and ascospores (A**)**, ascospores (B).

Homothallic, ascomata superficial, yellowish pink to pinkish red, globose to subglobose, 111-193 µm in diameter (Figures 35 A-C), surrounded by a loose covering of pale yellowish brown aerial hyphae, peridium hyaline to pinkish yellow, thin; asci 8-spored, globose to subglobose, 11.5-13 x 12-14 µm in diameter (Figures 35 D; 36 A), evanescent at maturity; ascospores hyaline, lenticular, spore body 5-6 x 5-5.5 µm, provided with two wide equatorial crests measuring 0.5-1 µm wide, with convex surfaces microtuberculate ornamentation (Figures 35 E-F; 36 B); mycelium composed of hyaline, branched, septate, smooth-walled hyphae; conidiogenesis absent.

Neosartorya sp.1 (KUFC 6341) was a single isolate found from forest soil at Phu Luang Wildlife Sanctuary, Loei province using the heat treatment medthod. However, Kamnerdngam (2013) reported the similar fungus as an endophytic ascomycete (KUFC 7494) from twig of *Mussaenda* sp. (Rubiaceae) from Mu Ko Surin National Park, Phangnga province. The ornamentation of ascospores suggested a relationship with *N. galapagensis* Frisvad, Hong & Samson*, N. glabra* (Fennell & Raper) Kozakiewicz*, N. laciniosa* Hong, Frisvad & Samson*, N. papuensis* Samson, Hong & Varga*,* and *N. warcupii* Peterson, Varga & Samson. In addition, the distinctive difference in producing of red soluble exudates on CYA and absent of anamorphic state production. This isolate was distinguished from *N. glabra*, *N. papuensis* and *N. warcupii* in having ascospores smoother convex surfaces; from *N. laciniosa* in having rougher convex surfaces; from *N. galapagensis* in having smaller cleistothecia.

The sequence data of the strain 6341 was not identical to those of known *Neosartorya* spp. The morphological characteristics were not also identical to any of those known *Neosartorya* spp. The strain KUFC 6341, *N. australensis* CBS 112.55^{T}, and *N. hiratsukae* NHL 3008^{T} formed a monophyletic clade 38%. However, the strain *Neosartorya* sp.1 (KUFC 6341) and the others were clearly separated and had similarities of 93.7 and 94.5 % at the β-tubulin gene sequence (Figure 43). In addition, *Neosartorya* (KUFC 6341) produced unique reddish colonies (Figure 34). Morphological characteristic and molecular analyses of *Neosartorya* sp.1 (KUFC 6341) did not fit with any described *Neosartorya* spp.

10. *Neosartorya* sp.2 (Figures 37-39, Tables 5-7)

Strain examined: KUFC 6513 coastal forest soil, Samaesarn Island, Chonburi province

Anamorphic state: absent

Colonies on CYA growing rapidly, attaining a diameter of 36-62 and 41-65 mm at 7 and 14 days, respectively, incubation at 28°C (Figures 37 A, a, D, d), white to creamish white, consisting of thin mycelial felt, velvety to floccose; cleistothecia very abundantly produced, conidiogenesis absent; exudates absent, reverse brownish green to citrine green (R 67).

Colonies on CZA growing restrictedly, attaining a diameter of 26-30 and 28-32 mm at 7 and 14 days, respectively, incubation at 28°C (Figures 37 B, b, E, e), white to yellowish white, consisting of thin mycelial layer, irregular colony margin, floccose in appearance; cleistothecium absent; conidiogenesis absent; exudates absent; odor production; reverse creamish white to pale luteous (R 11).

Colonies on MEA spreading broadly, attaining a diameter of 33-41 and 36-50 mm at 7 and 14 days, respectively incubation at 28°C (Figures 37 C, c, F, f), white, consisting of thin mycelial felt, plane; less cleistothecia produced, zonate and irregular colony margin, velvety to floccose in appearance; conidiogenesis absent; exudates absent; reverse faintly brownish green to pale buff (R 45).

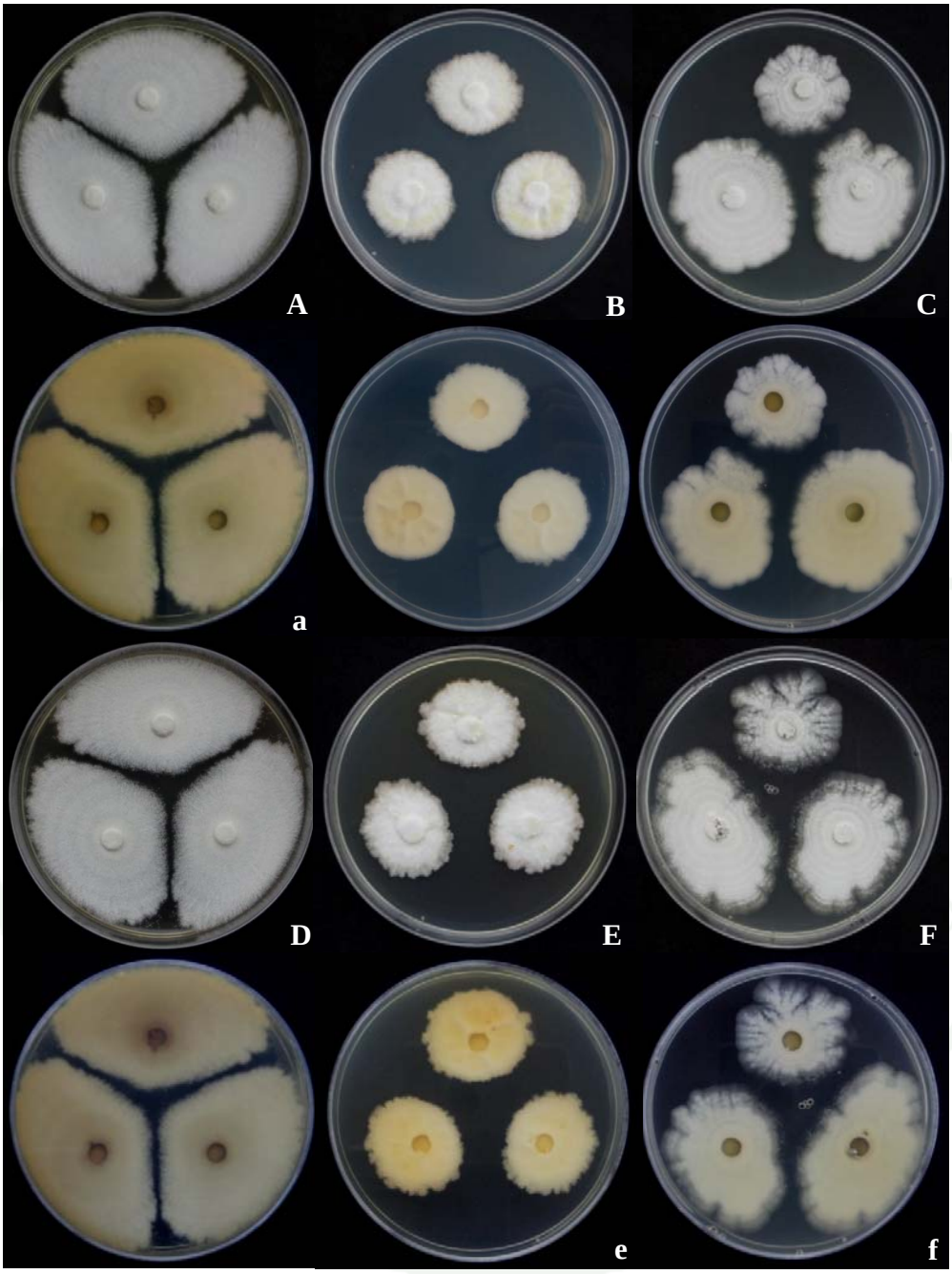


Figure 37 *Neosartorya* sp.2 (KUFC 6513)
Obverse and reverse views of colonies incubated at 28˚C on CYA for 7 days (A, a,) 14 days (D, d); CZA 7 days (B, b) 14 days (E, e); MEA 7 days (C, c) 14 days (F, f).

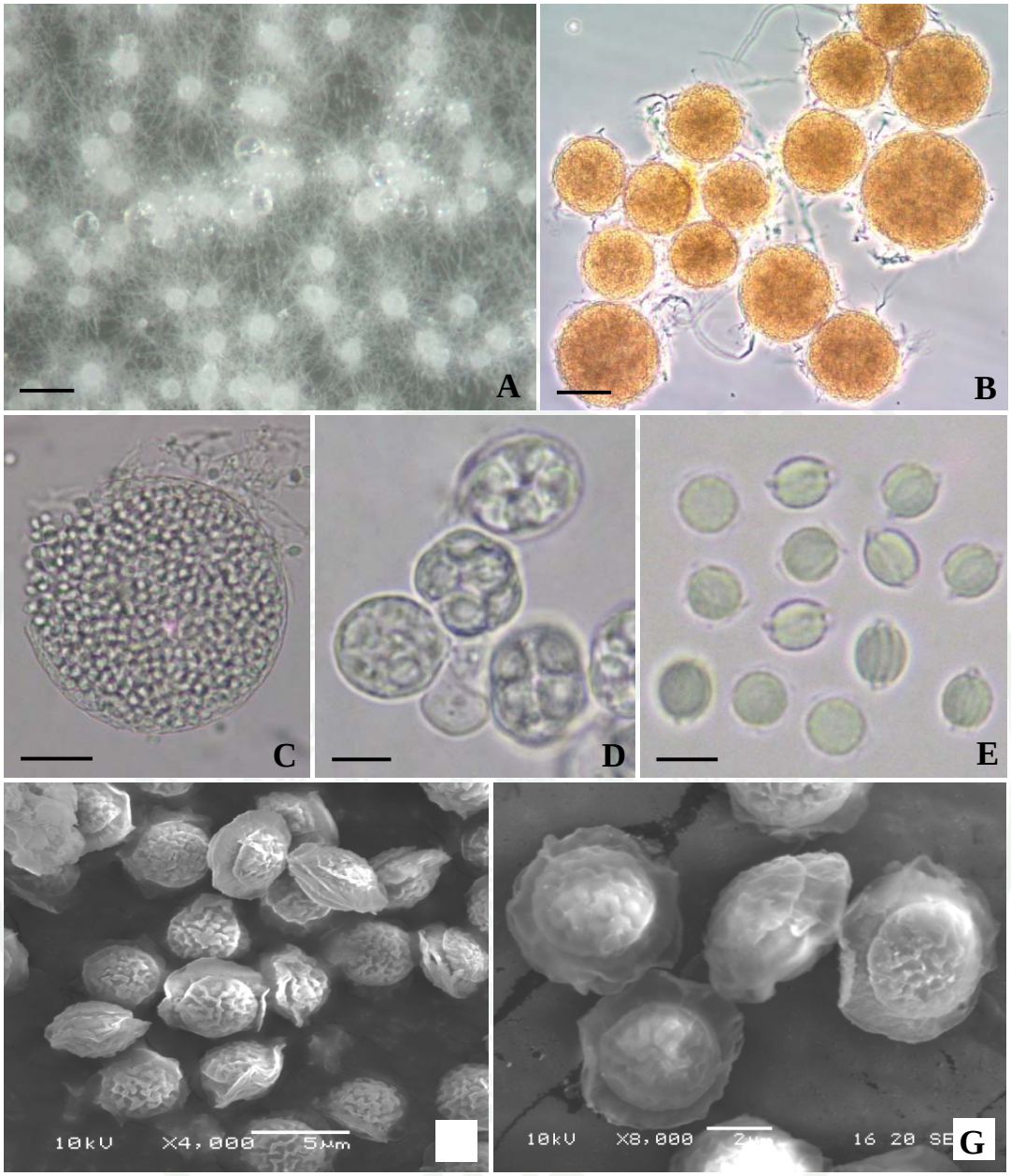


Figure 38 *Neosartorya* sp.2 **(**KUFC 6513)

A. Stereo photomicrograph of Ascomata;

B-E. Light photomicrographs of Ascomata (B, C), Asci and ascospores (D), Ascospores (E);

F, G. SEM photomicrographs of ascospores

(Bars: A = 1,000 µm; B = 100 µm; C = 40 µm; D – F = 5 µm; G =2 µm)

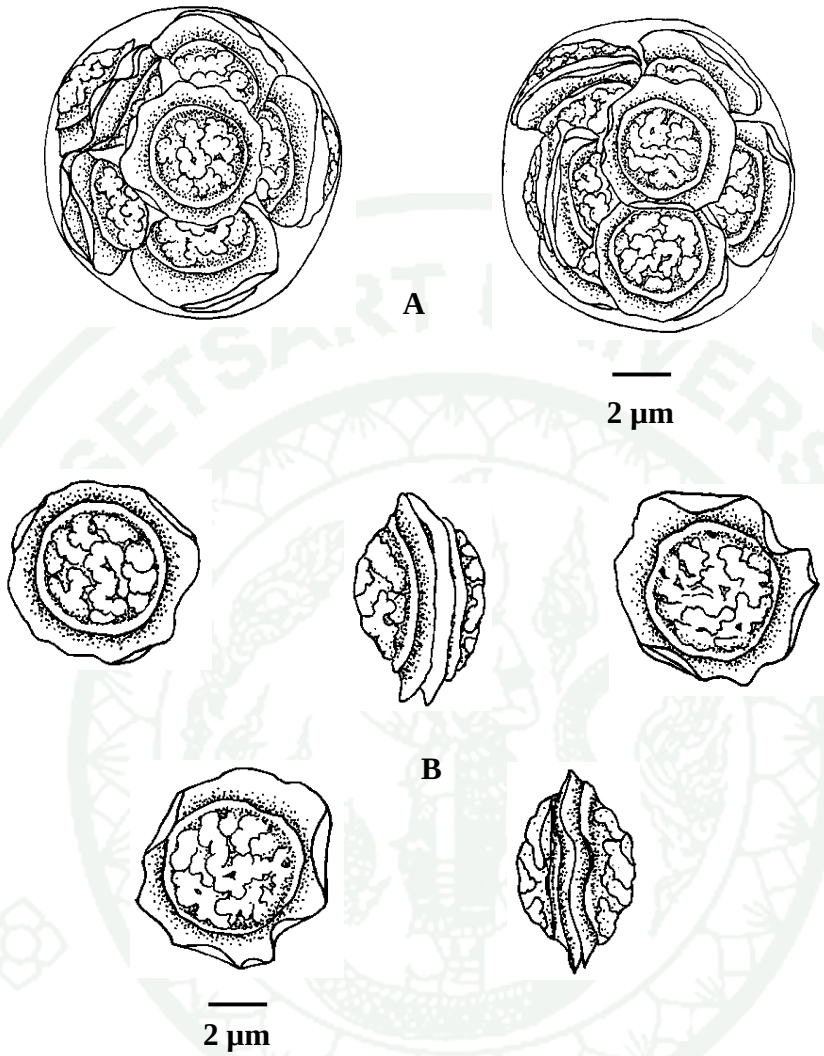


Figure 39 *Neosartorya* sp.2 **(**KUFC 6513)

Camera lucida drawings of asci and ascospores (A), ascospores (B).

Homothallic, cleistothecia superficial, scattered, white to yellowish white, globose to subglobose, 86-152 (117) µm in diameter (Figures 38 A-C), surrounded by a loose covering of hyaline aerial hyphae; peridium yellow to yellowish brown, thin, membranaceous, consisting of angular; asci 8-ascospored, globose to subglobose to ovoid, 10-12 x 10.5-12.5 µm, evanescent at maturity (Figures 38 D; 39 A); ascospores hyaline to pale yellow, broadly lenticular, spore body 4.5-5 x 4.2-4.5 µm, provided with two widely separated equatorial crests which are 1-1.5 µm wide with convex surfaces delicately roughened (Figures 38 E-G; 39 B).

Neosartorya sp.2 (KUFC 6513) was the only one isolate found from forest soil in Samaesarn Island, Chonburi province. This strain produced broadly lenticular with two widely separated equatorial crests and convex surfaces delicately roughened. The ascospores ornamentations were somewhat similar to those of *N. aurata* (Warcup) Malloch & Cain, *N. fennelliae* Kwon-Chung & Kim. In addition to common difference in smaller ascospores and absent anamophic state production, this isolate was distinguished from *N. aurata* in producing of faintly brownish green to pale buff in MEA. *N. fennelliae* is a heterothallic species. Moreover, *Neosartorya* sp.2 (KUFC 6513) grew faster than *N. aurata* on CZA.

Although the strain KUFC 6513, and *N. pseudofischeri* CBS 208.92^{T} formed a monophyletic clade 100%, its morphological characteristics were not similar to that of *N. pseudofischeri* (Figure 43). On comparison with ascospore ornamentation, *N. pseudofischeri* had convex surfaces with raised flaps resembling triangular projections, whereas the strain KUFC 6513 had widely separated equatorial crests with convex surfaces delicately roughened. Morphological characteristic of *Neosartorya* sp.2 (KUFC 6513) did not fit with any described *Neosartorya* spp.

11. *Neosartorya* sp.3 (Figures 40-42, Tables 5-7)

Strain examined: KUFC 6579 agricultural soil, Amphoe Wisetchaichan, Angthong province

Anamorphic state: *Aspergillus* sp.3

Colonies on CYA growing rapidly, attaining a diameter of 33-37 and 42-51 mm in 7 and 14 days, respectively, incubation at 28˚C (Figures 40 A, a, D, d), velvety, sulcate, irregular margin, consisting of a rather compact basal felt; slowly developed sacomata, white in color; margins broad and submerged; conidiogenesis absent, not sufficiently produced to influence the colony appearance; exudates absent; reverse redish orange to bay (R 6).

Colonies on CZA growing rather restrictedly, attaining a diameter of 16-20 and 19-25 mm in 7 and 14 days, respectively, incubation at 28˚C (Figures 40 B, b, E, e), plane, velvety, consisting of a thin basal felt, white in color; ascomata slowly developed throughout the colony; margins thin and irregular; conidiogenesis absent; exudates absent; reverse yellowish orange to pale luteous (R 11).

Colonies on MEA growing very restrictedly, attaining a diameter of 0.5-0.6 mm in 14 days incubation at 28˚C (Figures 40 C, c, F, f), floccose, plane, consisting of a very thin basal, white in color; conidiogenesis absent; exudates absent; reverse yellowish brown to amber (R 47).

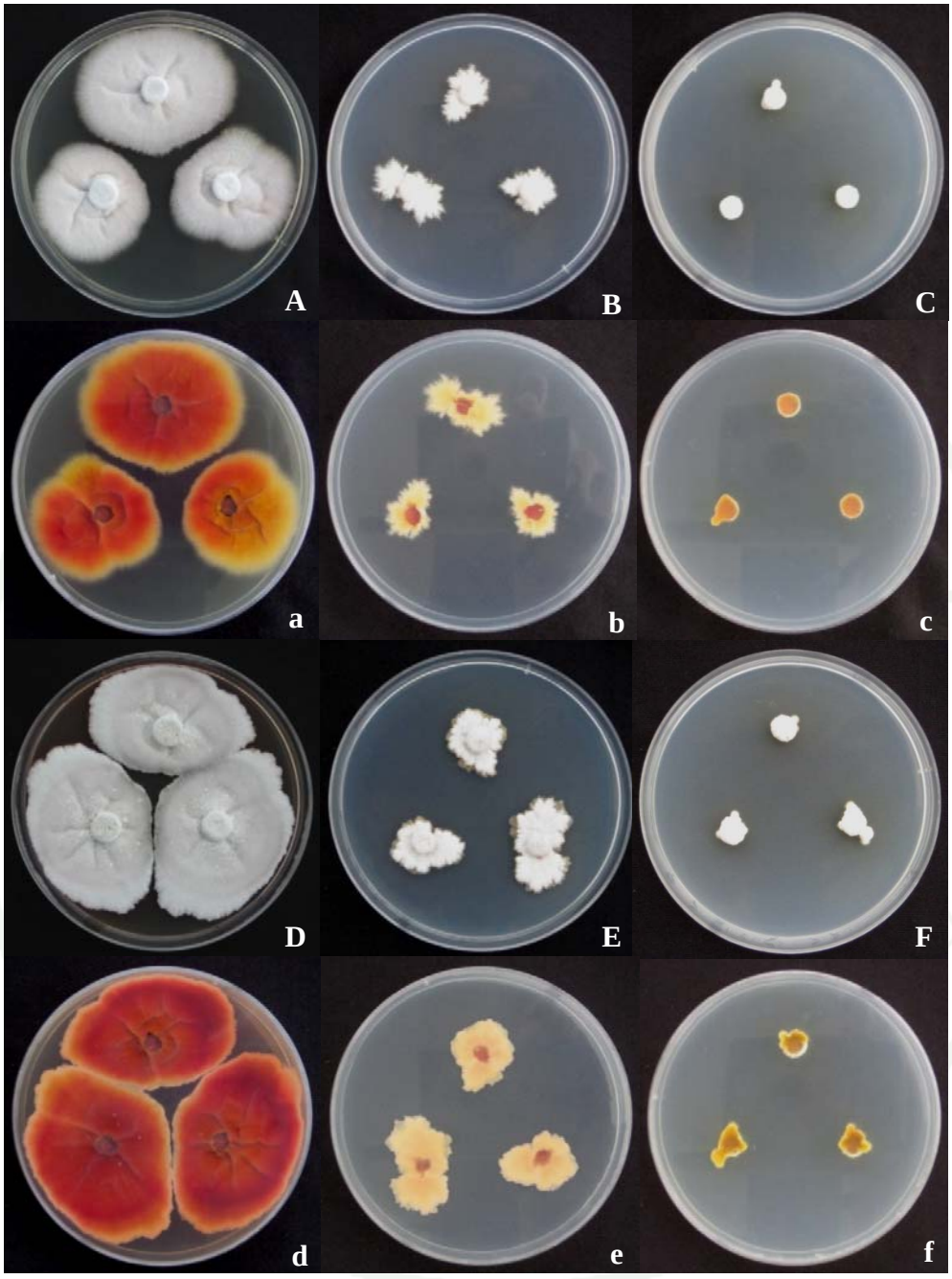


Figure 40 *Neosartorya* sp.3 (KUFC 6579)
Obverse and reverse views of colonies incubated at 28˚C on CYA for 7 days (A, a,) 14 days (D, d); CZA 7 days (B, b) 14 days (E, e); MEA 7 days (C, c) 14 days (F, f).

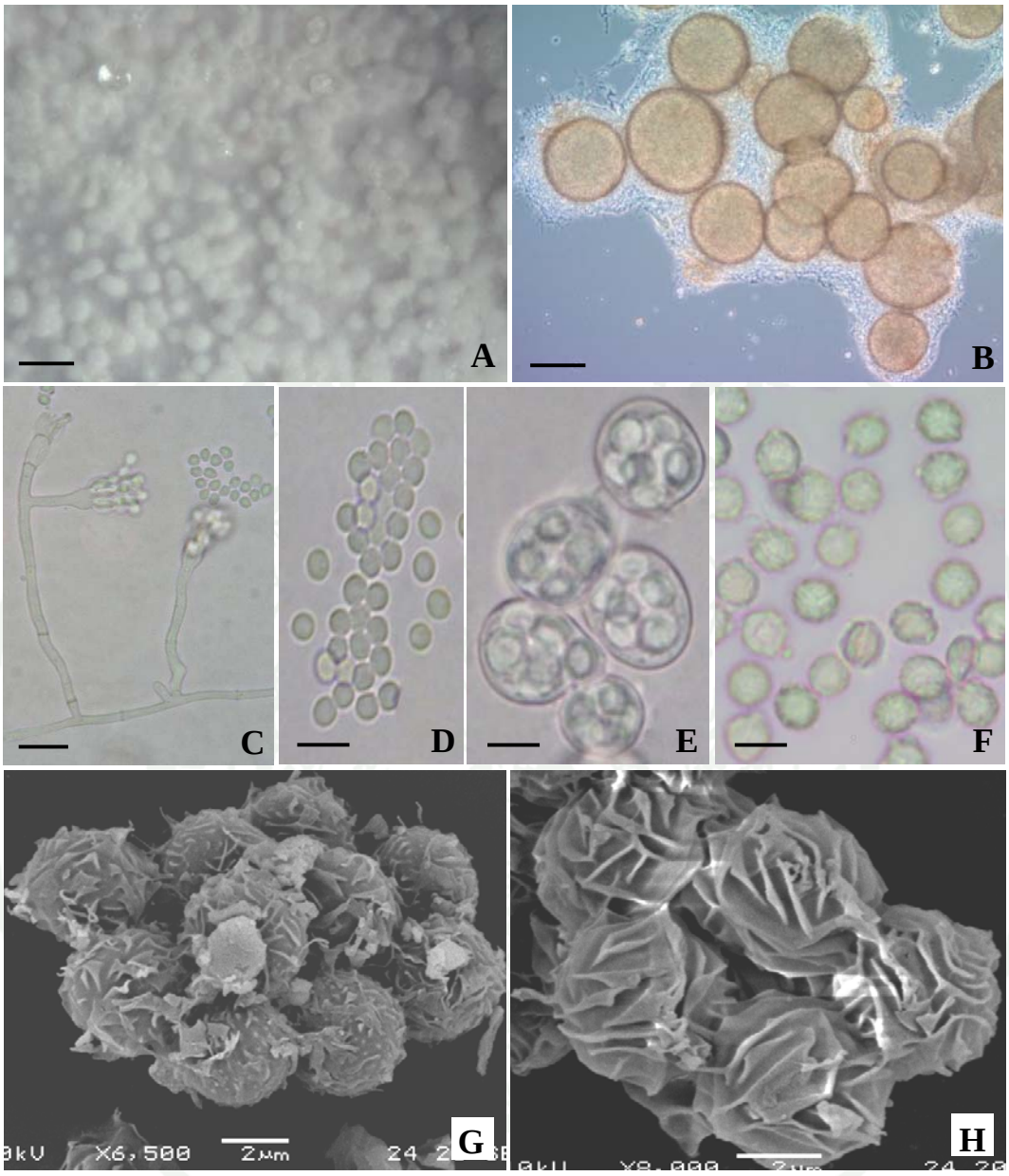


Figure 41 *Neosartorya* sp.3 **(**KUFC 6579)

A. Stereo photomicrograph of Ascomata and Conidial heads;

B-F. Light photomicrographs of Ascomata (B), Conidial heads (C), Conidia (D), Asci and ascospores (E), Ascospores (F);

G, H. SEM photomicrographs of ascospores

(Bars: A = 1,000 µm; B = 100 µm; C = 20 µm; D – F = 5 µm; G – H = 2 µm)

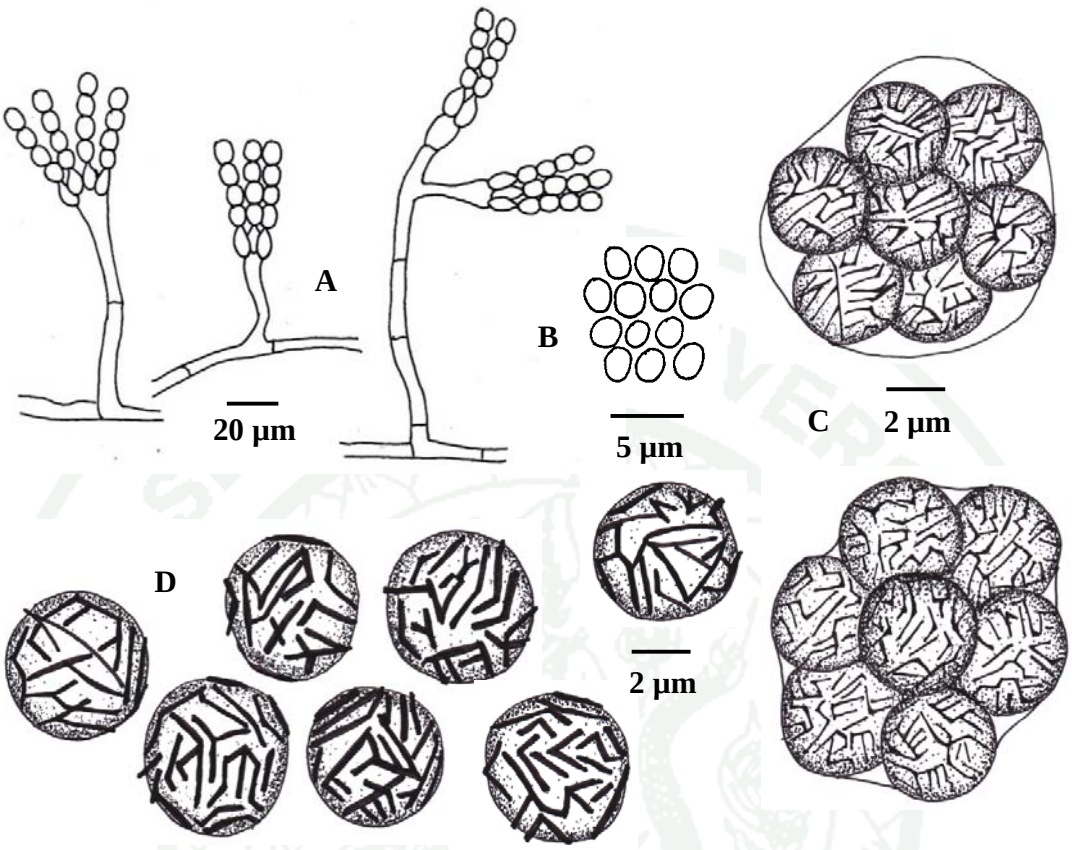


Figure 42 *Neosartorya* sp.3 (KUFC6579)
Camera lucida drawings of conidial heads (A), conidia (B), asci and ascospores (C), ascospores (D).

Ascomata superficial, scattered or confluent in small cluster, non-ostiolate, white to slightly cream colored, globose to subglobose, 100-280 µm in diameter (Figures 41 A, B), covered loosely with hyaline, branched, smooth, septate, 1.5-3.6 µm wide hyphae; asci 8 ascospored, borne singly, globose to ovoid, 10-13 x 10-12 µm, evanescent at maturiry (Figures 41 E; 42 C); ascospores hyaline, globose to subglobose, 4-5 µm in diameter, with a shallow furrow in the lateral view but without distinct equatorial crests, ornamented on surfaces by several linear ridges about 0.5 µm high, presenting ribbed or somewhat reticulate pattern (Figures 41 F-H; 42 D).

Conidiophores arising from the basal mycelium or aerial hyphae, hyaline, 40-120 x 2.5-3.0 µm in diameter (Figures 41 C; 42 A), smooth, often septate near the base; vesicles hyaline, clavate to flask-shaped, 3-6 µm in diameter; uniseriate conidial heads; phialides hyaline, ampuliform or cylindrical, 4.3-7.0 x 2.5-4.0 µm, covering the upper half of the vesicle; conidia hyaline, globose to subglobose, 2.0-3.5 µm in diameter, smooth-walled (Figures 41 D; 42 B).

Neosartorya sp.3 (KUFC 6579) was an only one isolate found from paddy field soil in Amphoe Wisetchaichan, Angthong province. The ascospore ornamentation was similar to *N. multiplicata* Yaguchi, Someya & Udagawa. Based on morphogical characteristics, the stipe of this strain is longer than *N. multiplicata. Neosartorya* sp.3 (KUFC 6579) grew slowly on MEA. Morphological characteristic and molecular analyses of *Neosartorya* sp.3 (KUFC 6579) did not fit with any described *Neosartorya* spp.

Table 7 Morphological characteristics of eleven *Neosartorya* species found in this study

Species	Conidial heads	Phialides and vesicles	Conidiophores	Conidia	Asci	Cleistothecia and ascospores
Neosartorya fischeri (KUFC 6338)	short to loosely columnar	phialide; flask shaped, 5.2-6.7 x 1.9-2.5 µm. vesicle flask shaped, mostly 12- 18 µm	smooth, 170-450 x 3.0-5.5 µm	globose to subglobose, delicately roughened, faintly pigmented, 2.0-2.5 µm	globose to subglobose, 9.5-12 x 7.5-10.3 µm	cleistothecia globose to subglobose, 250 to 400 µm; ascospore lenticular, 3.8-5.0 x 4.0-5.5 µm, with two distinctly convex surfaces bearing anastomosing ridges 1 µm wide
N. glabra (KUFC 6311)	short columnar	phialides 6-8 x 2-4 µm; vesicles flask-shaped, 10-18 µm	smooth, 250-485 x 3.0-6.5 µm	globose to subglobose, microtubulate, 2.0-3.5 µm	globose to subglobose, 12-14 x 11-13 µm	cleistothecia globose to subglobose, mostly 165- 220 µm; ascospore lenticular, 4.5-5.0 x 5.5-6.5 µm, with two prominent equatorial crests and with convex surfaces smooth, crests about 1.0-1.5 µm.
N. laciniosa (KUFC 6318)	columnar	phialides 7-8 x 2-3 µm; vesicles subclavate, 13-18 µm	smooth, 155-230 x 3-4 µm	globose to subglobose, sometimes broadly elliptical, smooth, 2.0-3.5 µm	asci globose to subglobose, 8-spored, 10-12 x 11-13 µm	cleistothecia globose to subglobose, 270-420 µm; ascospore lenticular, 3.7-4.5 x 4-5 µm, with two distinct equatorial crests wide with distinct equatorial rings, convex surfaces rugose to tuberculate

Table 7 (Continued)

Species	Conidial heads	Phialides and vesicles	Conidiophores	Conidia	Asci	Cleistothecia and ascospores
N. pseudofischeri (KUFC 6422)	loosely columnar	phialide ampulliform, 7.5-11 x 3.5-5 µm; vesicles subglobose, 11-16 µm	smooth, 200-300 x 4-6 µm	globose to subglobose, smooth, 3-4 µm	subglobose, 11-13 x 9-11 µm	cleistothecia globose to subglobose, 200-315 µm; ascospore subglobose, 4.5-5 x 5-6 µm, with two equatorial rings
N. siamensis sp. nov (KUFC 6349)	loosely columnar	phialides ampulliform 5-6 x 3-4 µm; vesicle subglobose apical, 10-13 µm	smooth, 65-110 x 3.5-4 µm	globose to subglobose, smooth, 2-2.5 µm	globose to subglobose, 10-12.5 x 9.5-11 µm	cleistothecia globose to subglobose, 250-470 µm; ascospore globose to subglobose, 4.7-5 x 5.5-6 µm, with broadly lenticular ascospores with two wide equatorial crests and finely spinulose and rugose convex surfaces
N. spinosa (KUFC 6325)	columnar	phialide 5-7 x 3-4.5 µm; vesicle flask-shaped, up to 18 µm	smooth, 300-500 µm	spherical to subspherical, smooth, 2.5-4.5 µm	globose to subglobose, 10-12 x 8-10 µm	cleistothecia spherical, 190-300 µm; ascospores spherical to subspherical, 4-5 µm, with convex surfaces bearing spine-like projections

Table 7 (Continued)

Species	Conidial heads	Phialides and vesicles	Conidiophores	Conidia	Asci	Cleistothecia and ascospores
N. takakii (KUFC 6355)	short columnar	phialides 5.5-7 x 1.5-3 µm; vesicle hemispherical to flask-shaped, 10-15 µm	smooth, 287-395 x 4-5 µm	globose to subglobose, smooth, 2.2-3.1 µm	globose to subglobose, 10.5-13 x 10-12 µm	cleistothecia globose to subglobose, 220-490 µm; broadly lenticular, 4.5-5 x 4-4.5 µm, with two widely separated equatorial crests and convex surfaces bearing roughly, circularly arranged projections ornamentation
N. tatenoi (KUFC 6377)	short columnar	phialides 7-7.5 x 2.5-3 µm; vesicle hemispherical to flask-shaped, 10-20 µm	smooth, 150-270 x 4-7.5 µm	globose to ovoid, 2-3 µm	globose to subglobose, 12.5-15 x 12-14 µm	cleistothecia globose to subglobose, 100-220 µm; lenticular, spore body 5-6 x 5-5.5 µm, with two narrow equatorial crests and convex surfaces ornamented by distinctly and narrowly reticulate ridges
Neosartorya sp.1 (KUFC 6341)	none				globose to subglobose, 11.5-13 x 12-14 µm	cleistothecia globose to subglobose, 111-193 µm; lenticular, 5-6 x 5-5.5 µm, with two wide equatorial crests measuring 0.5-1 µm wide and convex surfaces microtuberculate

Table 7 (Continued)

Species	Conidial heads	Phialides and vesicles	Conidiophores	Conidia	Asci	Cleistothecia and ascospores
Neosartorya sp.2 (KUFC 6513)	none				globose to subglobose to ovoid, 10-12 x 10.5-12.5 µm	cleistothecia globose to subglobose, 86-152 µm; broadly lenticular, 4.5-5 x 4.2-4.5 µm, with two widely equatorial crests which are 1-1.5 µm wide, convex surfaces delicately roughened
Neosartorya sp.3 (KUFC 6579)	loosely columnar	phialide ampuliform or cylindrical, 4.3-7.0 x 2.5-4.0 µm; vesicles clavate to flask-shaped, 3-6 µm	smooth, 40-120 x 2.5-3.0 µm	smooth, globose to subglobose, 2.0-3.5 µm	globose to ovoid, 10-13 x 10-12 µm	cleistothecia globose to subglobose, 100-280 µm; ascospore globose to subglobose, 4-5 µm, ornamented on surfaces by several linear ridges about 0.5 µm high, presenting ribbed or somewhat reticulate pattern

2. The molecular study of *Neosartorya* spp.

DNA sequences of the β-tubulin gene of the strains KUFC 6331, 6341, 6342, 6344, 6349, 6377, 6397, 6412, 6422, and 6513 were determined and new sequences have been deposited in the DNA Data Bank of Japan (DDBJ). The identity of the KUFC strains were analyzed based on the sequence similarities of the β-tubulin gene using BLAST search (http://www.ncbi. nlm.nih.gov). As a result, these strains were found to be related with the species of *Neosartorya*. The phylogenic relationships based on the β-tubulin gene for the KUFC strains and related species of *Neosartorya* and *Aspergillus* section *Fumigati* were analyzed by using a Neighbor Joining method (Figure 43). A bootstrap analysis was performed with 1000 replications to determine the support for each clade. Sequences from an *A. clavatus* strain was used as an outgroup in this experiment.

The strains KUFC6331, 6349 (MycoBank 561946) and 6412, *N. aureola* CBS 105.55^{T}, *N. indohii* CBM-FA-934^{T}, *N. udagawae* CBM.FA.702^{T} and *A. viridinutans* IMI 133982 formed a monophyletic clade. However, those KUFC strains and the others were clearly separated and had similarities of 96.0-96.9% at the β-tubulin gene sequence (Figure 43). Furthermore, the phylogenic positions of the KUFC strains clearly differed from that of *N. paulistensis* having similar ascospores. On comparison with the species forming a monophyletic clade on morphology, *N. aureola* and *N. indohii* have ascospores with echinulate or tuberculate convex surfaces and *N. udagawae* is heterothallic species and have broadly lenticular, with two equatorial or often irregular crests, convex surfaces tuberculate and *A. viridinutans* is anamorph species not found sexual state. Moreover, the distinctive features of the anamorphic states of *A. viridinutans*, *N. aureola* and *N. indohii* is in the presence of shorter conidiophores 20-35, 50 and 85-130 µm in diameter, respectively (Samson *et al.*, 2007) while *N. udagawae* has conidiophores of up to 530 µm long (Horie *et al.*, 1995a). With the evidence from morphology and this phylogenetic analysis, these strains should be considered a distinct new species.

The sequence data of the strain 6341 was not identical to those of known *Neosartorya* spp. The morphological characteristics were not also identical to any of those known *Neosartorya* spp. The strain KUFC 6341, *N. australensis* CBS 112.55^{T}, and *N. hiratsukae* NHL 3008^{T} formed a monophyletic clade 38%. However, the strain *Neosartorya* sp.2 KUFC 6341 and the others were clearly separated and had similarities of 93.7 and 94.5 % at the β-tubulin gene sequence (Figure 43). On morphology, *N. hiratsukae* had ascospores with two closely appressed equatorial crests and convex surfaces finely reticulate, and *N. australensis* had ascospores with narrower equatorial crests. Furthermore, the strain 6341 had unique reddish colonies. Therefore, the strain 6341 was considered a new species.

Although the strain KUFC 6513, and *N. pseudofischeri* CBS 208.92^{T} formed a monophyletic clade 100%, its morphological characteristics were not similar to that of *N. pseudofischeri* (Figure 43). On comparison with ascospore ornamentation, *N. pseudofischeri* had convex surfaces with raised flaps resembling triangular projections, whereas the strain KUFC 6513 had widely separated equatorial crests with convex surfaces delicately roughened. Based on these finding, the strain KUFC 6513 was considered a new species.

The sequence data of the strain KUFC 6344 was identical to that of *N. fischeri* CBS 544.65^{T} and the morphological characteristics was also identical to that of *N. fischeri* (Figure 43) 95%. Therefore, the strain 6344 was identified as *N. fischeri*.

The sequence data of the strain KUFC 6342 was almost identical to that of *N. spinosa* CBS 483.65^{T} and the similarity was 98.7% at the β-tubulin gene sequence (Figure 43) 90%. The morphological characteristics were also identical to that of *N. spinosa*. Therefore, the strain KUFC 6342 was identified as *N. spinosa*. The sequence data of the strain KUFC 6422 was identical to that of *N. pseudofischeri* CBS 208.92^{T} and the similarity was 98.6% at the β-tubulin gene sequence (Figure 43) 100%. The morphological characteristics were also identical to that of *N. pseudofischeri*. Therefore, the strain KUFC 6422 was identified as *N. pseudofischeri*.

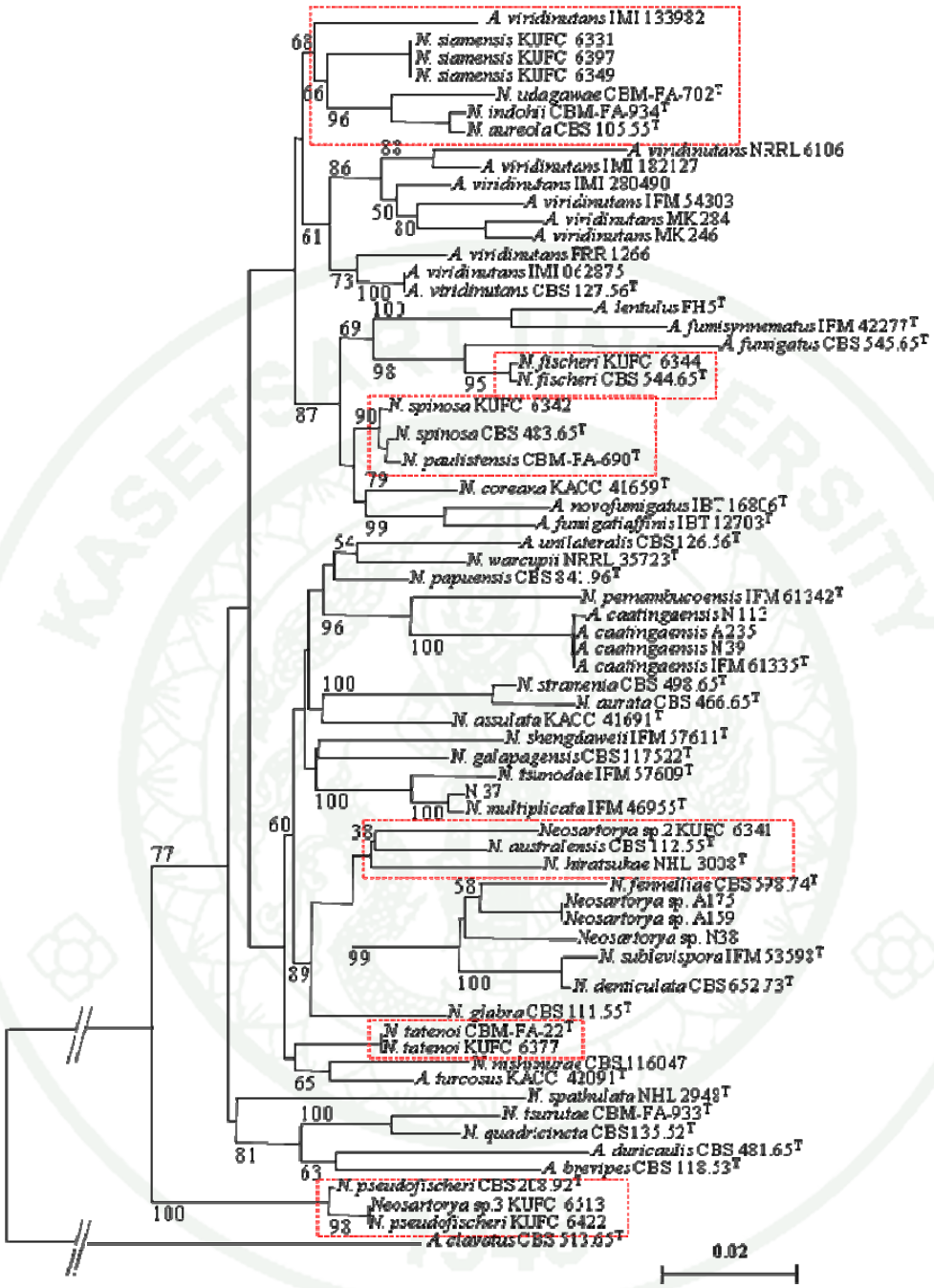


Fig 43 Relationships of *Neosartorya* species based on the partial sequences of the β-tubulin gene using Neighbor-joining method. Each number indicates the percentage of bootstrap samplings, derived from 1000 samples, supporting the internal branches of 50% or higher.

3. Antagonistic effect of *Neosartorya* against plant pathogenic fungi

3.1 Mycelial growth inhibition of plant pathogenic fungi

Nine strains of *Neosartorya* including *N. fischeri* (KUFC 6344), *N. pseudofischeri* (KUFC 6422), *N. siamensis* sp. nov. (KUFC 6349), *N. spinosa* (KUFC 6325), *N. tatenoi* (KUFC 6377), *Neosartorya tatenoi* (KUFC 6315), *Neosartorya* sp.1 (KUFC 6341), *Neosartorya* sp.2 (KUFC 6513) and *Neosartorya* sp.3 (KUFC 6579) were selected for antagonistic activity test against nine isolates of plant pathogenic fungi (Figure 53).

The test for inhibition of mycelial growth of three Hyphomycetes (Table 8, Figures 44-46) *Alternaria brassicicola*, *Neosartorya fischeri* inhibited 56.1%, *N. tatenoi* (KUFC 6315) (54.4%), *N. tatenoi* (KUFC 6377) (55.6%), *Neosartorya* sp.1 (53.9%), *N. spinosa* (53.3%), *N. siamensis* sp. nov. (52.2%), *N. pseudofischeri* (50%), *Neosartorya* sp.2 (48.3%) and *Neosartorya* sp.3 (36.1%). *Curvularia oryzae*, *Neosartorya* sp.1 provided 55% of inhibition, followed by *N. fischeri* (53.9%), *N. spinosa* and *N. tatenoi* (KUFC 6377) (52.8%), *N. tatenoi* (KUFC 6315) (52.2%), *N. siamensis* sp. nov. (51.7%), *Neosartorya* sp.2 (46.1%), *N. pseudofischeri* (44.4%) and *Neosartorya* sp.3 (42.8%). *Fusarium oxysporum* f. sp. *cubense*, *N. fischeri* provided 58.3% of inhibition, followed by *Neosartorya* sp.1 (54.4%), *N. tatenoi* (KUFC 6377) and *N. tatenoi* (KUFC 6315) (53.9%), *N. pseudofischeri* (52.8%), *N. siamensis* sp. nov. and *N. spinosa* (51.1%), *Neosartorya* sp.2 (43.3%) and *Neosartorya* sp.3 (40.6%).

For the inhibition of mycelial growth of *Colletotrichum capsici* (Table 8, Figures 47, 48) *Neosartorya fischeri* provided 55.1% of inhibition, followed by *N. tatenoi* (KUFC 6315) (54.0%), *N. spinosa* (52.3%), *N. pseudofischeri*, *N. siamensis* sp. nov., *N. tatenoi* (KUFC 6377) and *Neosartorya* sp.1 (50%), *Neosartorya* sp.2 (47.2%) and *Neosartorya* sp.3 (46.6%). *Lasiodiplodia theobromae*, *N. fischeri* provided 48.9% of inhibition, followed by *N. tatenoi* KUFC 6315 (46.1%), *N. siamensis* sp. nov. (45.0%) and *Neosartorya* sp.1 (32.2%), whereas *N. pseudofischeri*, *N. spinosa, N. tatenoi* (KUFC 6377), *Neosartorya* sp.2 and *Neosartorya* sp.3 could not inhibit *L. theobromae.*

Table 8 Percent inhibition on mycelial growth of three Hyphomycetes (*Alternaria brassicicola*, *Curvularia oryzae* and *Fusarium oxysporum*) and two Coelomycetes (*Colletotrichum capsici* and *Lasiodiplodia theobromae*) by nine isolates of *Neosartorya* on PDA as dual culture at 28˚C for 14 days

Fungal isolate	Mycelial growth inhibition (%)				
	Alternaria brassicicola	*Curvularia oryzae*	*Fusarium oxysporum*	*Colletotrichum capsici*	*Lasiodiplodia theobromae*
N. fischeri	56.1	53.9	58.3	55.1	48.9
N.pseudofischeri	50.0	44.4	52.8	50.6	0*
N. siamensis sp. nov.	52.2	51.7	51.1	50.0	45.0
N. spinosa	53.3	52.8	51.1	52.3	0*
N. tatenoi	54.4	52.2	53.9	54.0	46.1
N. tatenoi	55.6	52.8	53.9	50.0	0*
Neosartorya sp.1	53.9	55.0	54.4	50.0	32.2
Neosartorya sp.2	48.3	46.1	43.3	47.2	0*
Neosartorya sp.3	36.1	42.8	40.6	47.2	0*

0* = plant pathogenic fungus overgrew colonies of five *Neosartorya* spp.

The growth of two Oomycetes (Table 9, Figure 49, 50), *Pythium aphanidermatum*, *Neosartorya tatenoi* KUFC 6377 provided 51.1% of inhibition, followed by *N. siamensis* sp. nov. (50.6%), *N. fischeri* (49.4%) and *Neosartorya tatenoi* KUFC 6315 (41.1%) whereas, *N. pseudofischeri*, *N. spinosa, Neosartorya* sp.1, *Neosartorya* sp.2 and *Neosartorya* sp.3 could not inhibit *P. aphanidermatum. Phytophthora palmivora* showed that *Neosartorya* sp.1 and *N. tatenoi* KUFC 6377 provided 62.8 and 62.2% of inhibition, followed by *N. siamensis* sp. nov. (61.7%), *N. pseudofischeri* and *N. fischeri* (61.1%), *Neosartorya tatenoi* KUFC 6315 (59.4%), *Neosartorya* sp.2 (58.3%), *N. spinosa* (56.1%) and *Neosartorya* sp.3 (54.4%).

Nine isolates of *Neosartorya* spp. could not inhibit mycelial growth of two Basidiomycetes (Table 9, Figures 51, 52), *Rhizoctonia oryzae* and *Sclerotium rolfsii*.

Table 9 Percent inhibition on mycelial growth of two Oomycetes (*Pythium aphanidermatum* and *Phytophthora palmivora*) and two Basidiomycetes (*Rhizoctonia oryzae* and *Sclerotium rolfsii*) by nine isolates of *Neosartorya* on PDA as dual culture at 28˚C for 14 days

Fungal isolate	Mycelial growth inhibition (%)			
	Pythium aphanidermatum	*Phytophthora palmivora*	*Rhizoctonia oryzae*	*Sclerotium rolfsii*
N. fischeri	49.4	61.1	0*	0*
N. pseudofischeri	0*	61.1	0*	0*
N. siamensis sp. nov.	50.6	61.7	0*	0*
N. spinosa	0*	56.1	0*	0*
N. tatenoi	41.1	59.4	0*	0*
N. tatenoi	51.1	62.2	0*	0*
Neosartorya sp.1	0*	62.8	0*	0*
Neosartorya sp.2	0*	58.3	0*	0*
Neosartorya sp.3	0*	54.4	0*	0*

0* = plant pathogenic fungi overgrew colonies of nine *Neosartorya* spp.

Shen *et al.* (2009) reported a marine derived fungus *Neosartorya fischeri* and the inhibitory effects of their crude extract on *Tobacco mosaic virus* and two tumor cell lines. Crude extracts was obtained by extracting with MeOH. The extract was dissolved in water and water insoluble. The inhibitory effects on *Tobacco mosaic virus* was evaluated by indirect enzyme linked immunosorbant assay, and the antitumor activity was tested by methyl thaialyl tetrazolium method. The active fraction inhibited *Tobacco mosaic virus* and was insoluble in water, whereas the fraction inhibited tumor cell lines was water-soluble. Furthermore, the inhibitory activity of water-soluble fraction against BEL7404 human hepatoma cell line was much higher than SGC-7901 human gastric cancer cell line.

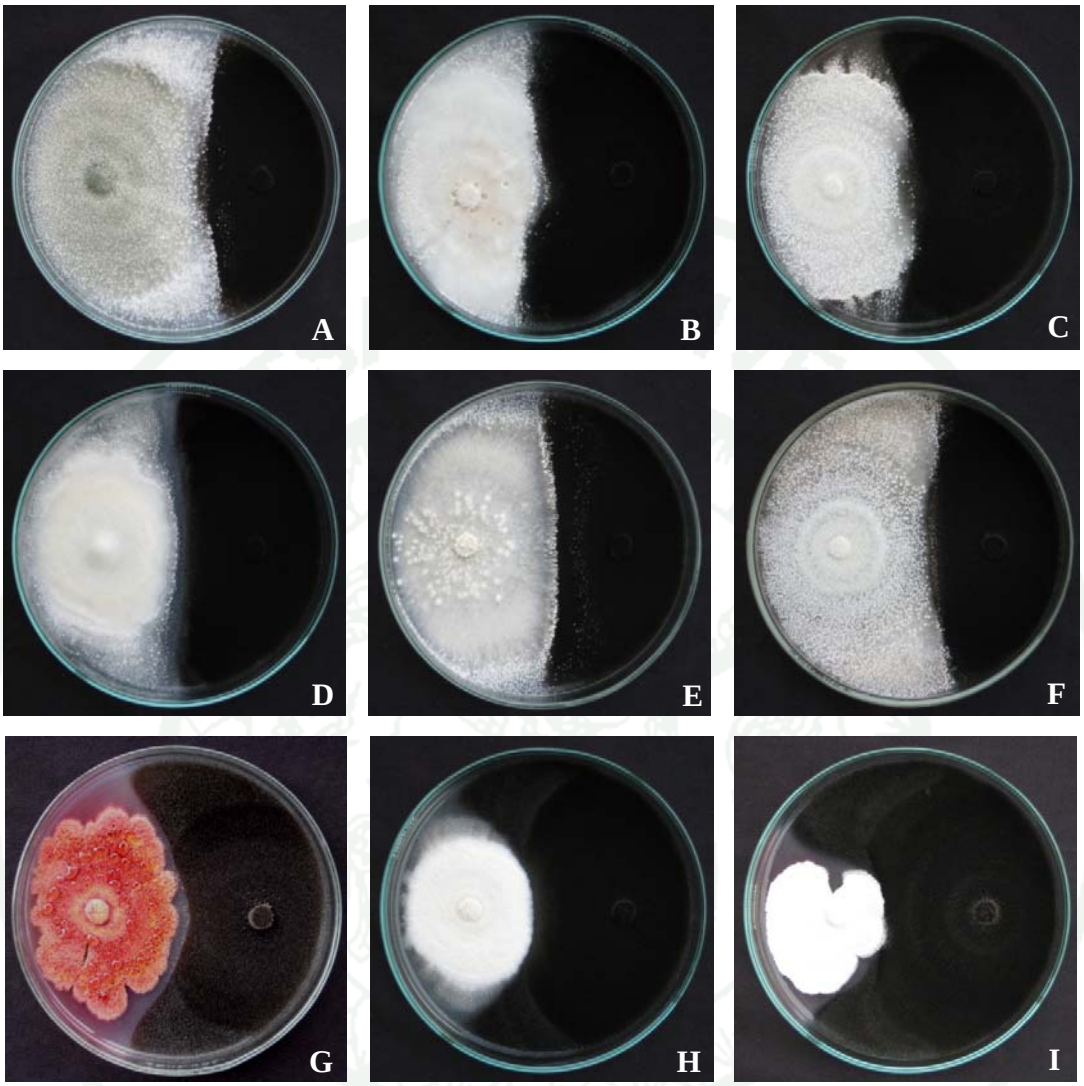


Figure 44 *In vitro* antagonistic test of *Neosartorya* spp. against ***Alternaria brassicicola*** as dual culture on PDA incubated at 28˚C for 14 days:

A. *Neosartorya fischeri* (KUFC 6344) B. *N. pseudofischeri* (KUFC 6422)
C. *N. siamensis* sp. nov. (KUFC 6349) D. *N. spinosa* (KUFC 6325)
E. *N. tatenoi* (KUFC 6315) F. *N. tatenoi* (KUFC 6377)
G. *Neosartorya* sp.1 (KUFC 6341) H. *Neosartorya* sp.2 (KUFC 6513)
I. *Neosartorya* sp.3 (KUFC 6579).

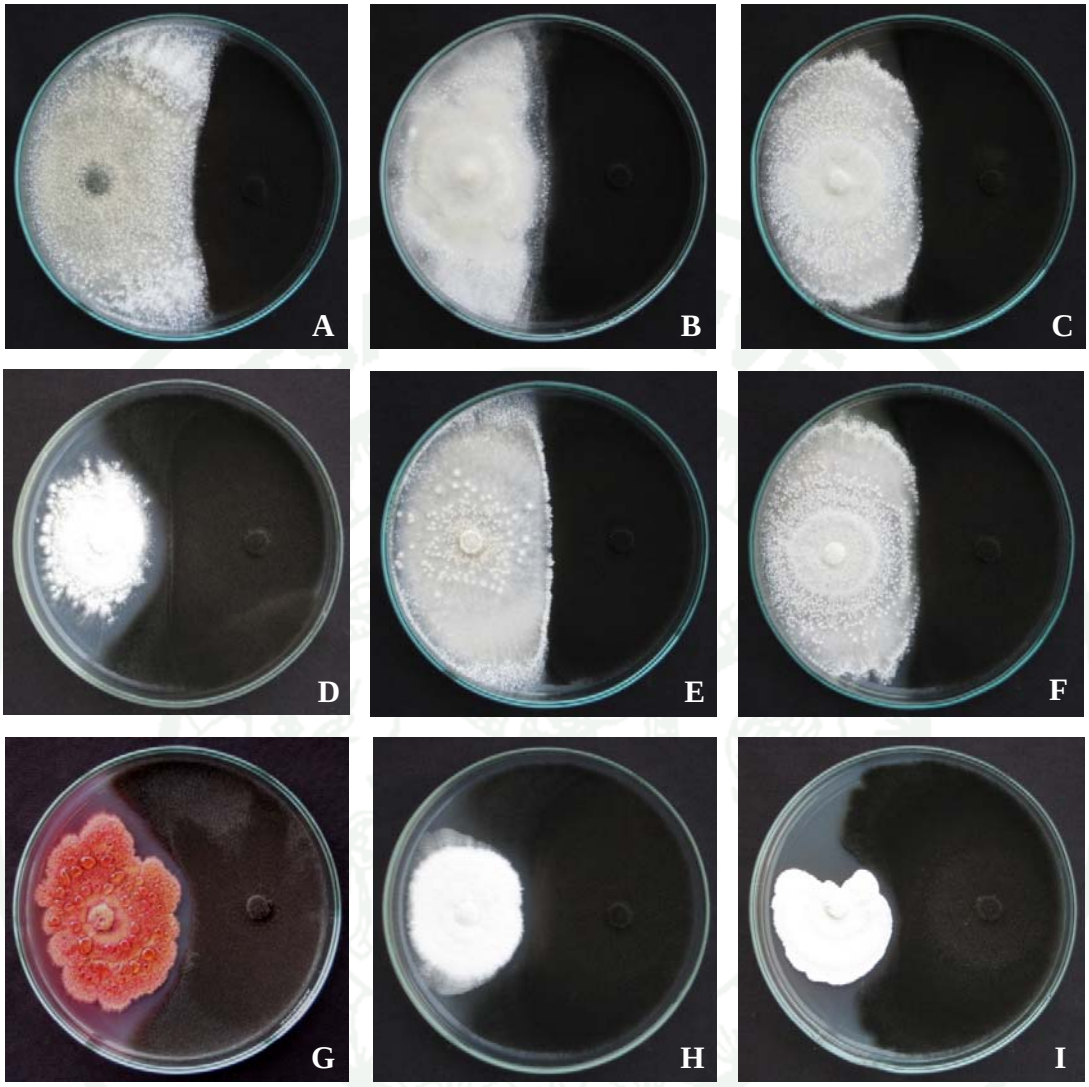


Figure 45 *In vitro* antagonistic test of *Neosartorya* spp. against ***Curvularia oryzae*** as dual culture on PDA incubated at 28˚C for 14 days:

A. *Neosartorya fischeri* (KUFC 6344) B. *N. pseudofischeri* (KUFC 6422)
C. *N. siamensis* sp. nov. (KUFC 6349) D. *N. spinosa* (KUFC 6325)
E. *N. tatenoi* (KUFC 6315) F. *N. tatenoi* (KUFC 6377)
G. *Neosartorya* sp.1 (KUFC 6341) H. *Neosartorya* sp.2 (KUFC 6513)
I. *Neosartorya* sp.3 (KUFC 6579).

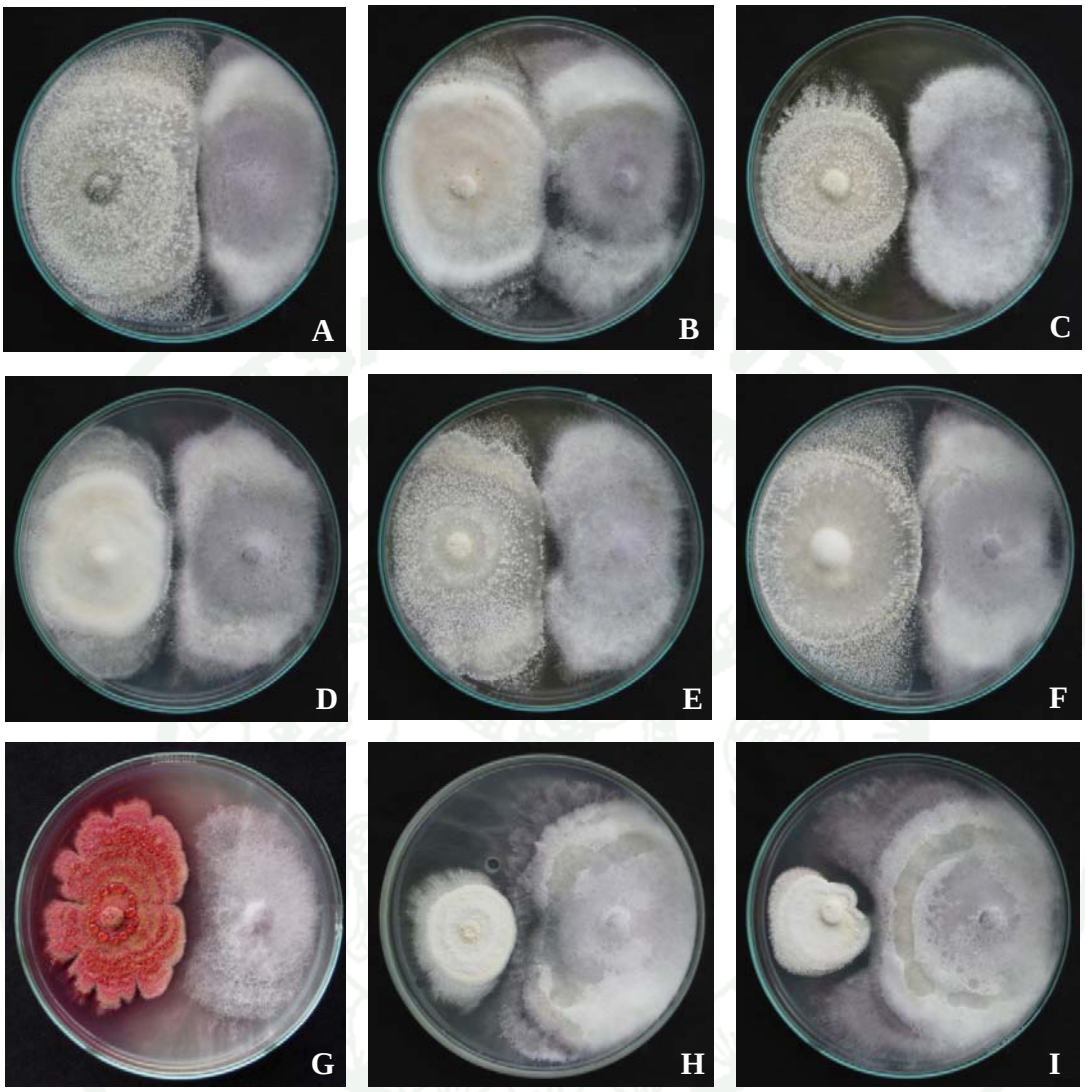


Figure 46 *In vitro* antagonistic test of *Neosartorya* spp. against ***Fusarium oxysporum* f. sp. *cubense*** as dual culture on PDA incubated at 28˚C for 14 days:
A. *Neosartorya fischeri* (KUFC 6344) B. *N. pseudofischeri* (KUFC 6422)
C. *N. siamensis* sp. nov. (KUFC 6349) D. *N. spinosa* (KUFC 6325)
E. *N. tatenoi* (KUFC 6315) F. *N. tatenoi* (KUFC 6377)
G. *Neosartorya* sp.1 (KUFC 6341) H. *Neosartorya* sp.2 (KUFC 6513)
I. *Neosartorya* sp.3 (KUFC 6579).

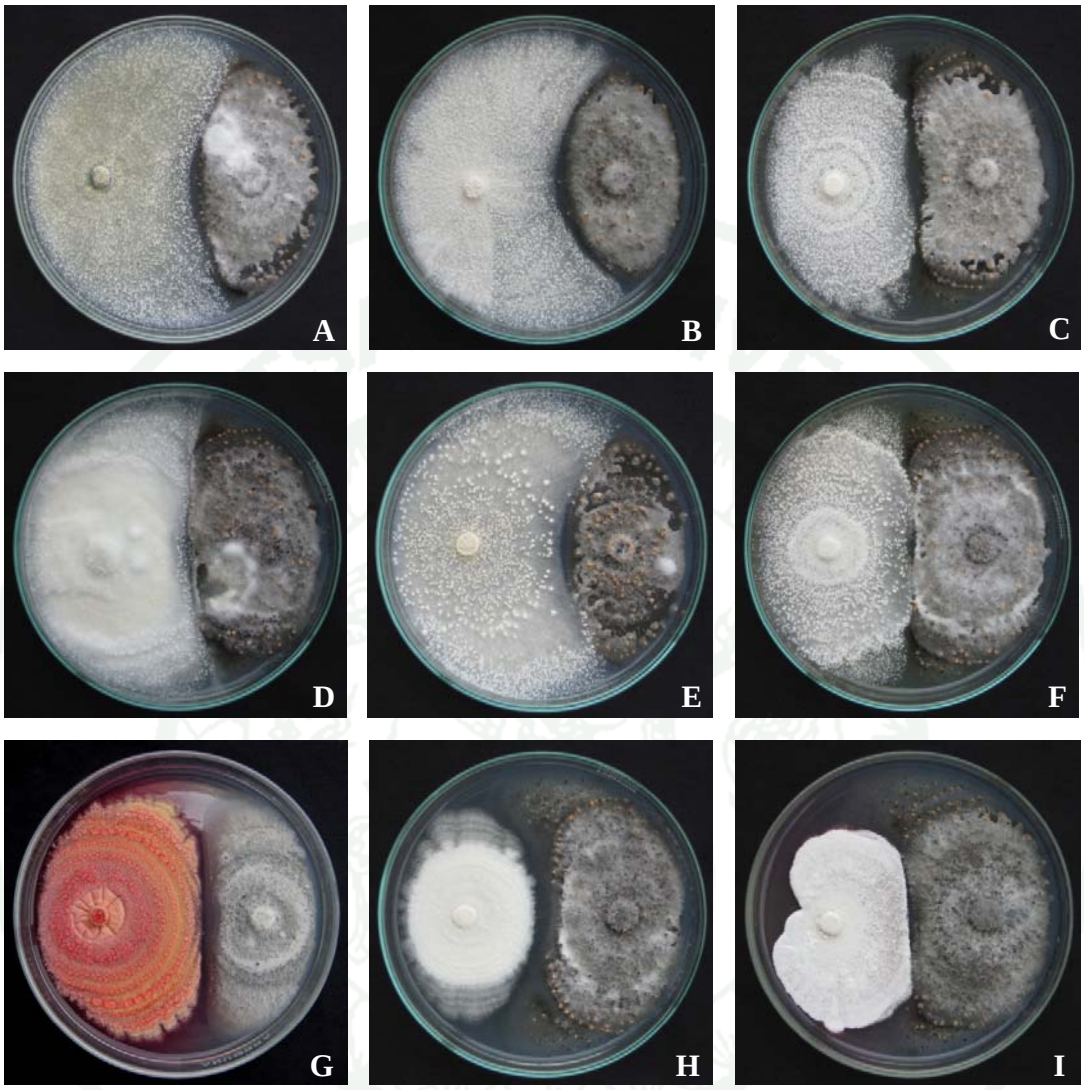


Figure 47 *In vitro* antagonistic test of *Neosartorya* spp. against ***Colletotrichum capsici*** as dual culture on PDA incubated at 28˚C for 14 days:

A. *Neosartorya fischeri* (KUFC 6344) B. *N. pseudofischeri* (KUFC 6422)
C. *N. siamensis* sp. nov. (KUFC 6349) D. *N. spinosa* (KUFC 6325)
E. *N. tatenoi* (KUFC 6315) F. *N. tatenoi* (KUFC 6377)
G. *Neosartorya* sp.1 (KUFC 6341) H. *Neosartorya* sp.2 (KUFC 6513)
I. *Neosartorya* sp.3 (KUFC 6579).

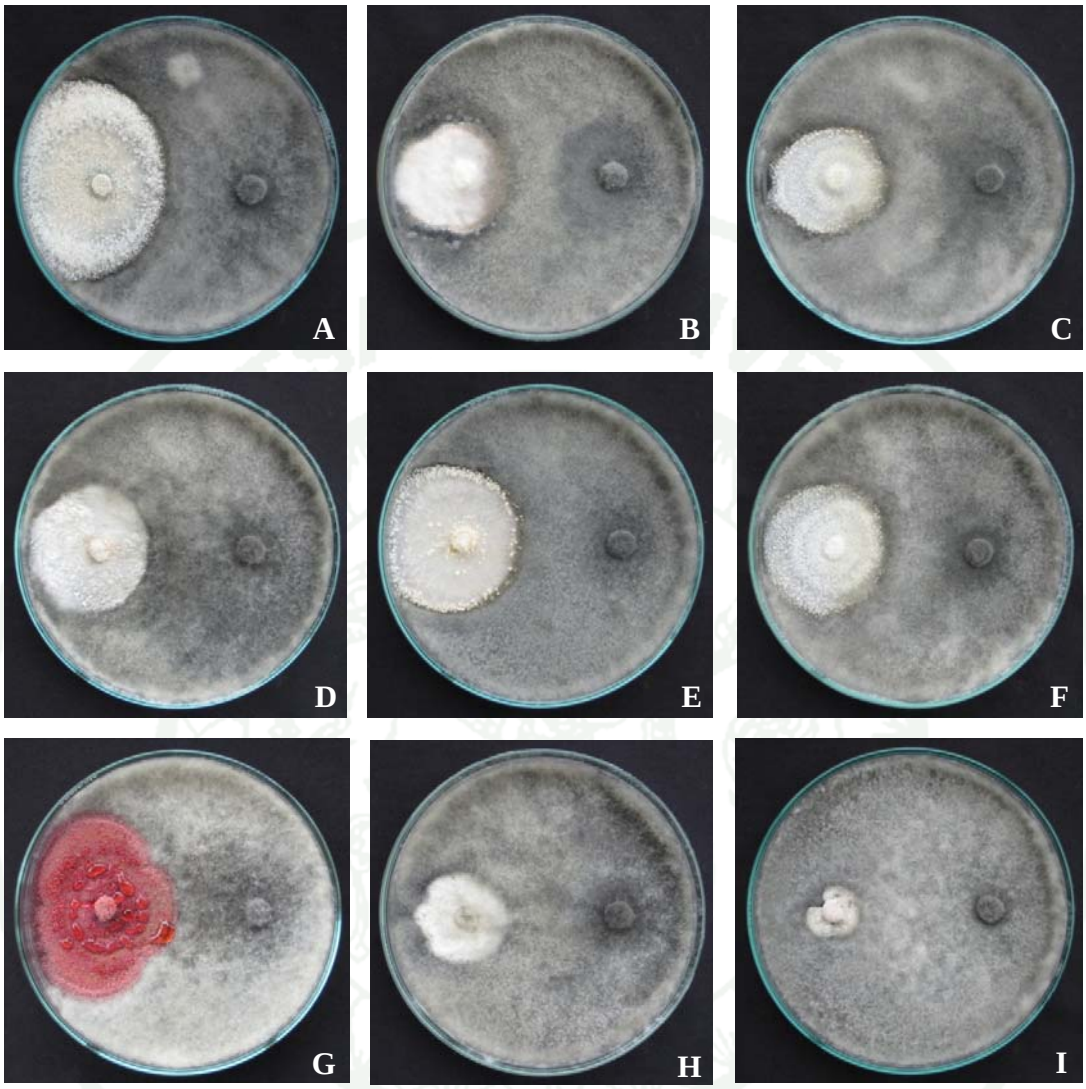


Figure 48 *In vitro* antagonistic test of *Neosartorya* spp. against ***Lasiodiplodia theobromae*** as dual culture on PDA incubated at 28˚C for 14 days:
A. *Neosartorya fischeri* (KUFC 6344) B. *N. pseudofischeri* (KUFC 6422)
C. *N. siamensis* sp. nov. (KUFC 6349) D. *N. spinosa* (KUFC 6325)
E. *N. tatenoi* (KUFC 6315) F. *N. tatenoi* (KUFC 6377)
G. *Neosartorya* sp.1 (KUFC 6341) H. *Neosartorya* sp.2 (KUFC 6513)
I. *Neosartorya* sp.3 (KUFC 6579).

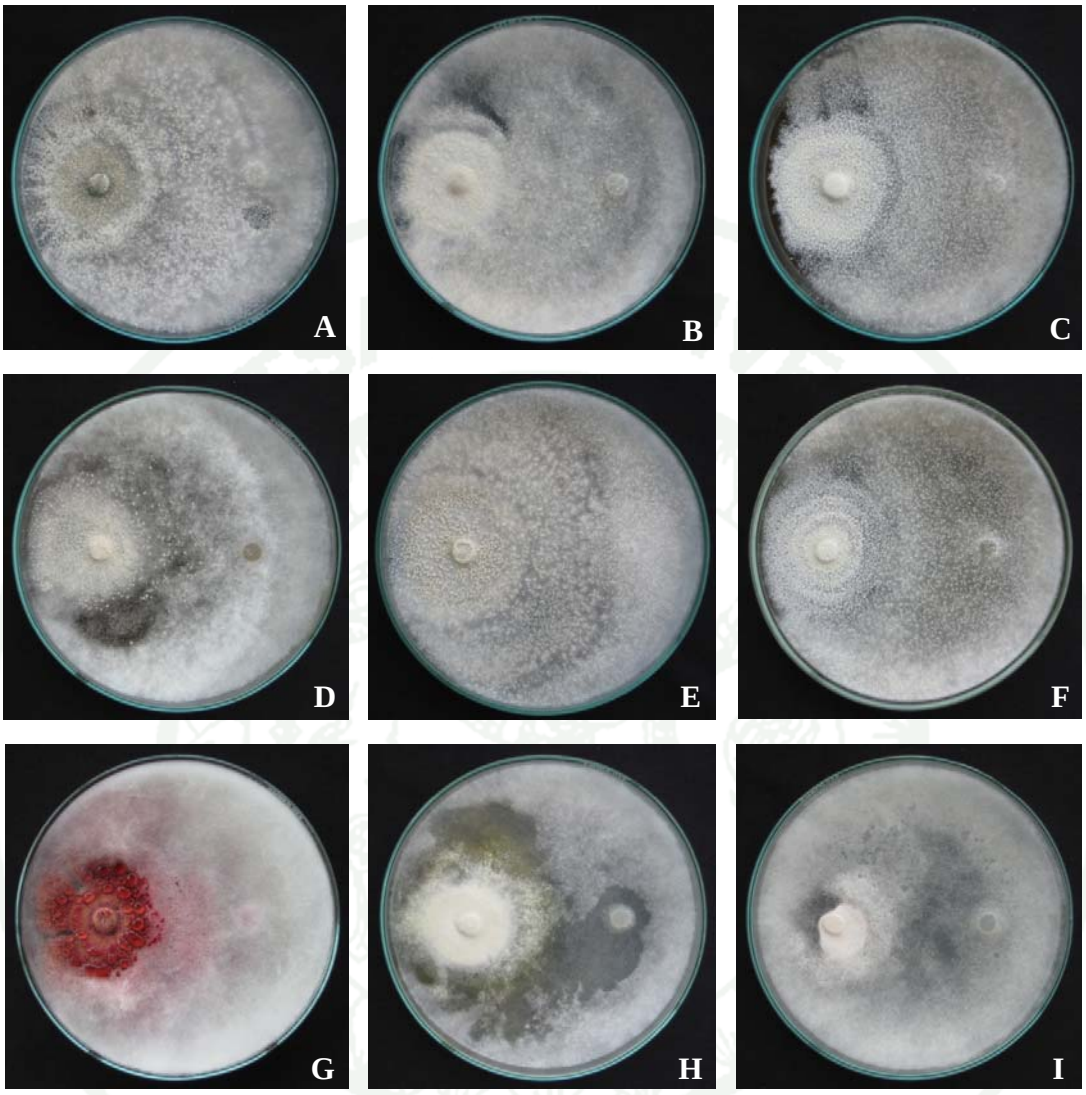


Figure 49 *In vitro* antagonistic test of *Neosartorya* spp. against ***Pythium aphanidermatum*** as dual culture on PDA incubated at 28˚C for 14 days:
A. *Neosartorya fischeri* (KUFC 6344) B. *N. pseudofischeri* (KUFC 6422)
C. *N. siamensis* sp. nov. (KUFC 6349) D. *N. spinosa* (KUFC 6325)
E. *N. tatenoi* (KUFC 6315) F. *N. tatenoi* (KUFC 6377)
G. *Neosartorya* sp.1 (KUFC 6341) H. *Neosartorya* sp.2 (KUFC 6513)
I. *Neosartorya* sp.3 (KUFC 6579).

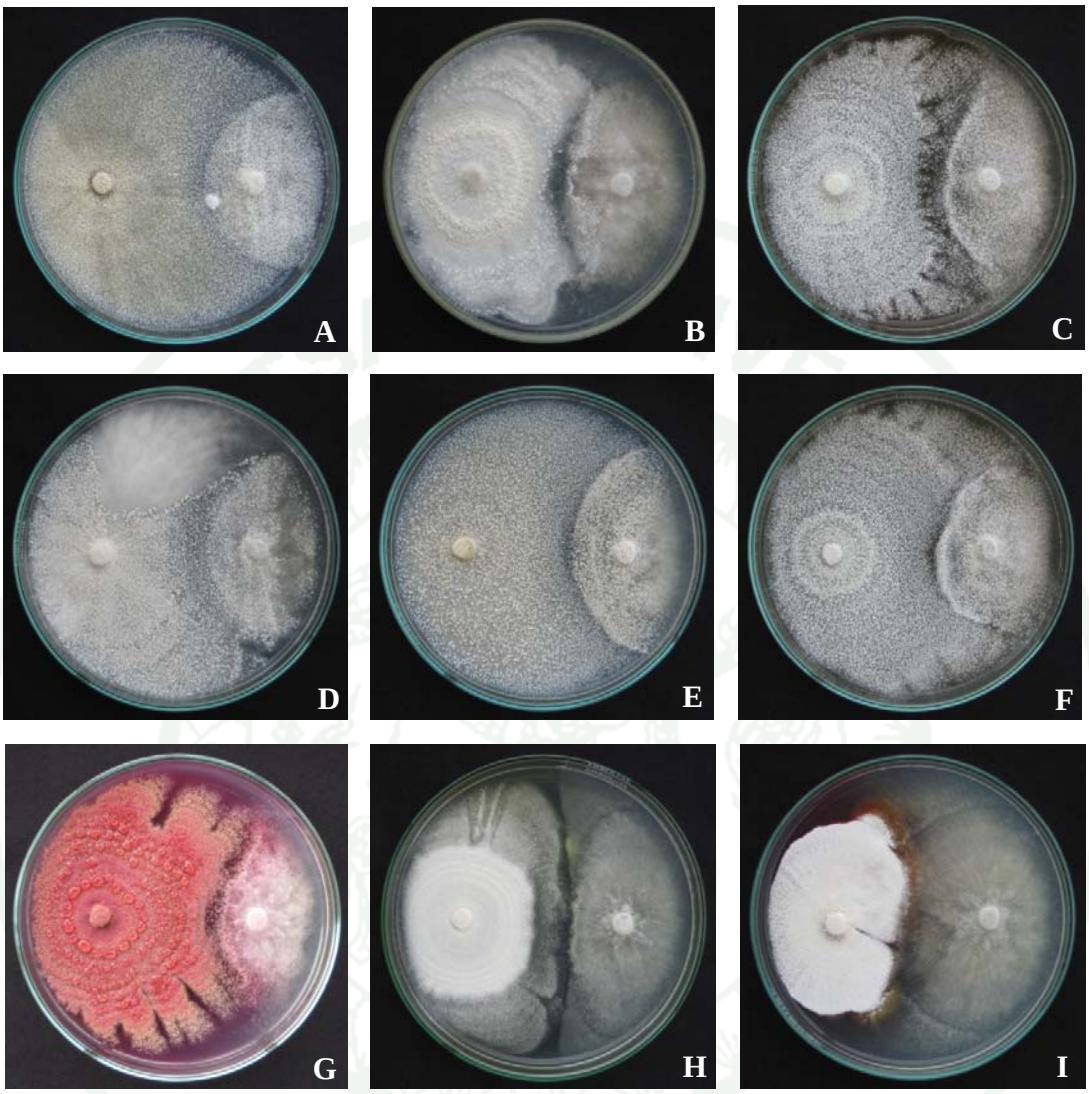


Figure 50 *In vitro* antagonistic test of *Neosartorya* spp. against ***Phytophthora palmivora*** as dual culture on PDA incubated at 28˚C for 14 days:
A. *Neosartorya fischeri* (KUFC 6344) B. *N. pseudofischeri* (KUFC 6422)
C. *N. siamensis* sp. nov. (KUFC 6349) D. *N. spinosa* (KUFC 6325)
E. *N. tatenoi* (KUFC 6315) F. *N. tatenoi* (KUFC 6377)
G. *Neosartorya* sp.1 (KUFC 6341) H. *Neosartorya* sp.2 (KUFC 6513)
I. *Neosartorya* sp.3 (KUFC 6579).

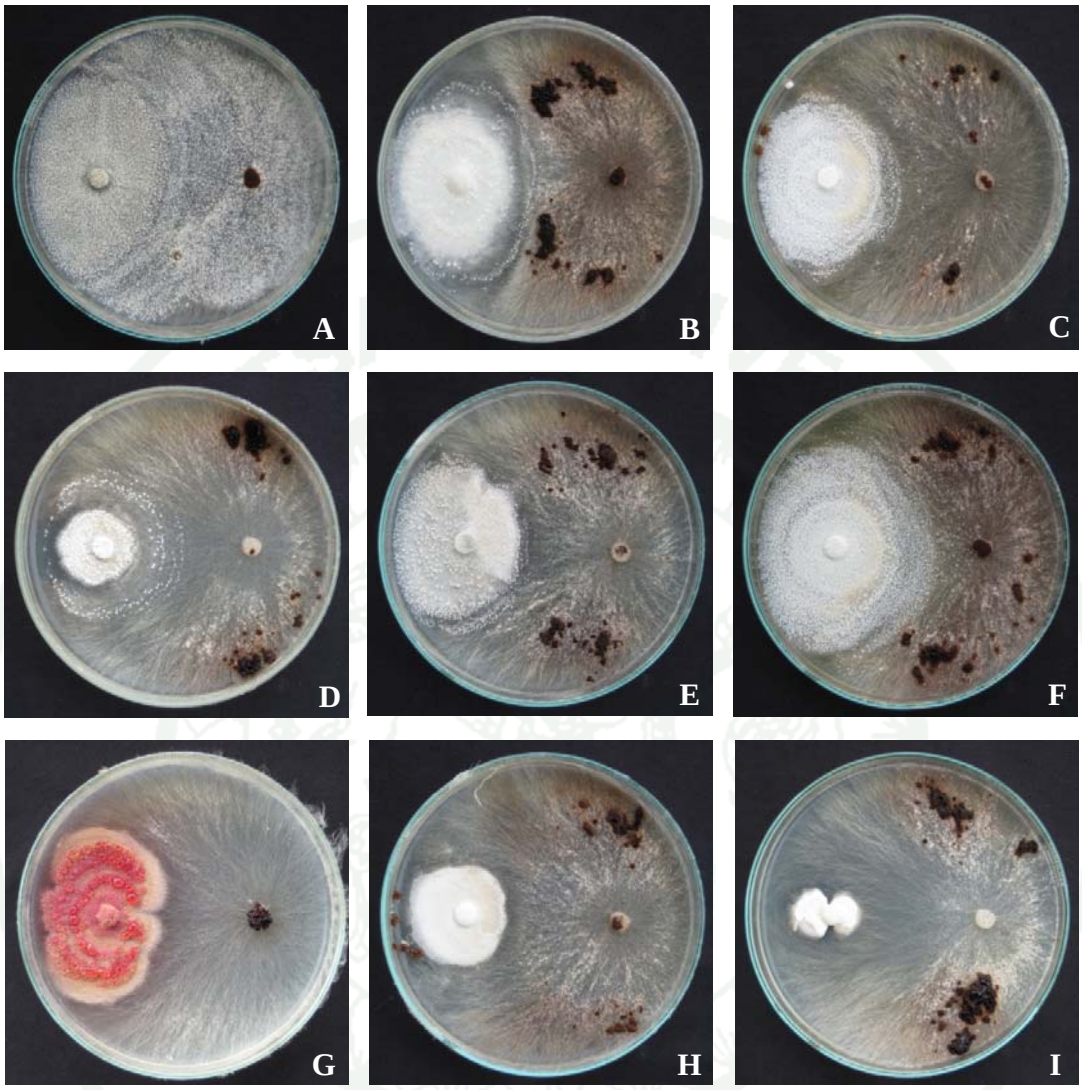


Figure 51 *In vitro* antagonistic test of *Neosartorya* spp. against ***Rhizoctonia oryzae*** as dual culture on PDA incubated at 28˚C for 14 days:
A. *Neosartorya fischeri* (KUFC 6344) B. *N. pseudofischeri* (KUFC 6422)
C. *N. siamensis* sp. nov. (KUFC 6349) D. *N. spinosa* (KUFC 6325)
E. *N. tatenoi* (KUFC 6315) F. *N. tatenoi* (KUFC 6377)
G. *Neosartorya* sp.1 (KUFC 6341) H. *Neosartorya* sp.2 (KUFC 6513)
I. *Neosartorya* sp.3 (KUFC 6579).

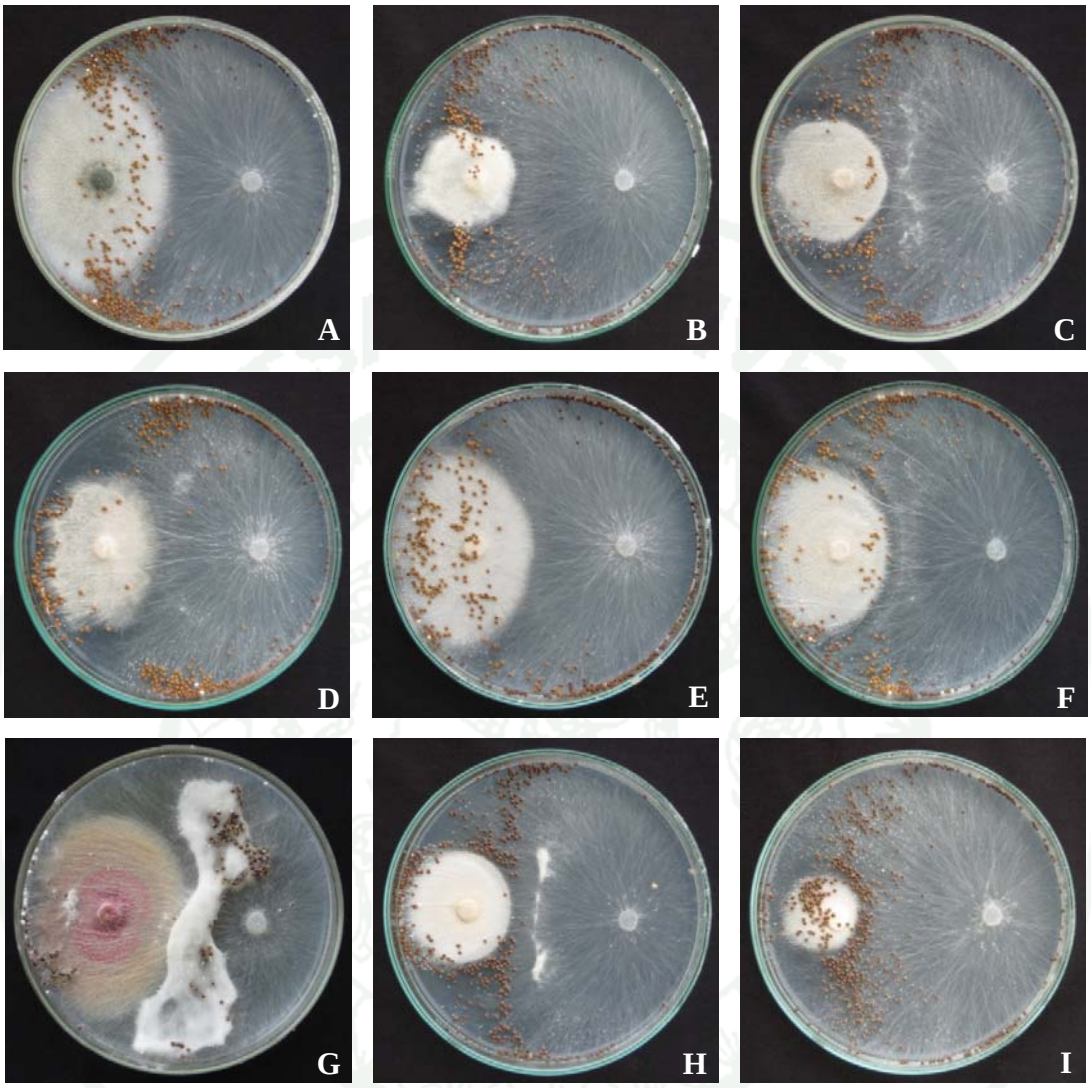


Figure 52 *In vitro* antagonistic test of *Neosartorya* spp. against ***Sclerotium rolfsii*** as dual culture on PDA incubated at 28˚C for 14 days:
A. *Neosartorya fischeri* (KUFC 6344) B. *N. pseudofischeri* (KUFC 6422)
C. *N. siamensis* sp. nov. (KUFC 6349) D. *N. spinosa* (KUFC 6325)
E. *N. tatenoi* (KUFC 6315) F. *N. tatenoi* (KUFC 6377)
G. *Neosartorya* sp.1 (KUFC 6341) H. *Neosartorya* sp.2 (KUFC 6513)
I. *Neosartorya* sp.3 (KUFC 6579).

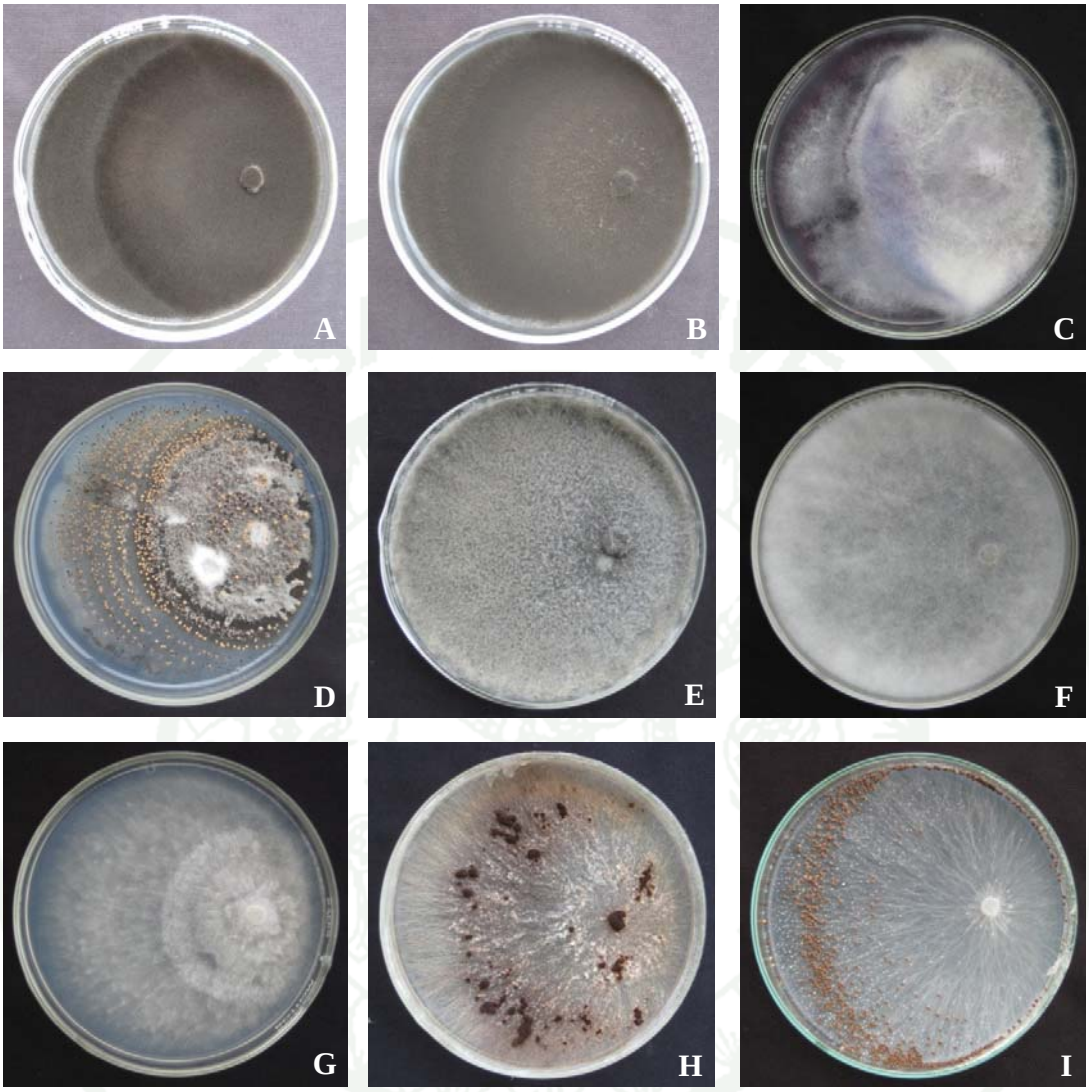


Figure 53 Nine isolates of plant pathogenic fungi (control), *Alternaria brassicicola* (A), *Curvularia oryzae* (B), *Fusarium oxysporum* f.sp. *cubense* (C), *Colletotrichum capsici* (D), *Lasiodiplodia theobromae* (E), *Pythium aphanidermatum* (F), *Phytophthora palmivora* (G), *Rhizoctonia oryzae* (H) and *Sclerotium rolfsii* (I) on PDA, incubated at 28˚C for 14 days.

3.2 *In vitro* antagonistic activity test of crude extracts against plant pathogenic fungi *in vitro*

The efficacy of crude extracts derived from six *Neosartorya* species including, *Neosartorya fischeri* (KUFC 6344), *N. pseudofischeri* (KUFC 6422), *N. siamensis* (KUFC 6349), *N. spinosa* (KUFC 6325), *Neosartoya* sp.1 (KUFC 6341) and *Neosartorya* sp.2 (KUFC 6513) were selected to test with plant pathogenic fungi on PDA.

The result showed that three plant pathogenic hyphomycete, *Alternaria brassicicola* was completely inhibited by *N. pseudofischeri, N. fischeri* and *Neosartorya* sp.2 (100%), 45-65% inhibition by *N. siamensis* sp. nov., *N. spinosa* and *Neosartoya* sp.1 at 10,000 ppm, six *Neosartorya* crude extracts at 10,000 ppm inhibited *Curvularia oryzae* over 80% and at 1,000 ppm more than 31-80%. All *Neosartorya* crude extracts at 10,000 ppm provided *Fusarium oxysporum* f. sp. *cubense* over 88% mycelial growth inhibition exception for *N. spinosa* with only 60% inhibition (Table 10, Figures 54-56).

For the inhibition of mycelial growth of two coelomycete, *Neosartorya pseudofischeri, N. fischeri, Neosartorya* sp.1 and *Neosartorya* sp.2 completely inhibited (100%) *Colletotrichum capsici* at 10,000 ppm, but they could not inhibit *Lasiodiplodia theobromae* (Table 11, Figures 57, 58).

The test for inhibition of mycelial growth of two oomycete, six *Neosartorya* crude extracts inhibited almost 100% of *Pythium aphanidermatum. Phytophthora palmivora* was inhibited mostly 100 % by five *Neosartorya* crude extracts at 10,000 ppm but *N. siamensis* sp. nov. provided only 64 % (Table 12, Figures 59, 60). However, *N. fischeri* crude extract at 1,000 ppm strongly inhibited for both oomycete.

The test for inhibition of mycelial growth of two basidiomycete, six *Neosartorya* crude extracts at 10,000 ppm completely inhibited 100% *Rhizoctonia oryzae* and *Sclerotium rolfsii*. Moreover, *N. fischeri* showed very good results at 1,000 ppm (Table 13, 61, 62).

Table 10 Percent inhibition on mycelial growth of three Hyphomycetes (*Alternaria brassicicola*, *Curvularia oryzae* and *Fusarium oxysporum* f. sp. *cubense*) using six crude extractions (ppm) of *Neosartorya* spp. on PDA at 28˚C for 7 days

Fungal isolate	Mycelial growth inhibition (%)											
	Alternaria brassicicola				***Curvularia oryzae***				***Fusarium oxysporum* f.sp. *cubense***			
	10	100	1000	10000	10	100	1000	10000	10	100	1000	10000
N. fischeri	2.1	14.6	72.9	100	12.8	24.4	41.1	100	0	22.8	55.6	100
N. pseudofischeri	4.2	66.7	100	100	31.7	65.7	71.1	100	41.1	63.9	77.8	100
N. siamensis sp. nov.	14.9	16.7	28.1	45.8	0	11.1	31.7	91.1	20.0	27.8	59.4	91.1
N. spinosa	5.2	26.0	35.4	64.6	0	65.0	80.6	84.4	0	9.4	30.6	60.0
Neosartorya sp.1	0	0	47.9	60.4	0	35.6	52.2	100	0	23.9	36.6	100
Neosartorya sp.2	8.3	32.3	56.3	100	10.6	17.2	51.1	84.4	0	0	18.9	88.9

Table 11 Percent inhibition on mycelial growth of two Coelomycetes (*Colletotrichum capsici* and *Lasiodiplodia theobromae*) using six crude extractions (ppm) of *Neosartorya* spp. on PDA at 28˚C for 7 days

Fungal isolate	Mycelial growth inhibition (%)							
	Colletotrichum capsici				***Lasiodiplodia theobromae***			
	10	100	1000	10000	10	100	1000	10000
N. fischeri	0	0	46.2	100	0	0	0	0
N. pseudofischeri	13.2	40.6	70.6	100	0	0	0	0
N. siamensis sp. nov.	5.7	5.7	14.2	23.6	0	0	0	19.4
N. spinosa	0	0	0	5.7	0	0	0	0
Neosartorya sp.1	0	8.5	26.4	100	0	0	0	0
Neosartorya sp.2	0	0	2.0	100	0	0	0	0

Table 12 Percent inhibition on mycelial growth of two Oomycetes (*Pythium aphanidermatum* and *Phytophthora palmivora*) using six crude extractions (ppm) of *Neosartorya* spp. on PDA at 28˚C for 7 days

Fungal isolate	Mycelial growth inhibition (%)							
	Pythium aphanidermatum				***Phytophthora palmivora***			
	10	100	1000	10000	10	100	1000	10000
N. fischeri	0	0	100	100	0	11.1	100	100
N. pseudofischeri	0	0	15.0	100	40.0	40.0	78.3	100
N. siamensis sp. nov.	0	0	0	100	0	0	29.4	64.44
N. spinosa	0	0	0	100	0	4.4	11.1	100
Neosartorya sp.1	0	0	0	100	0	16.7	63.3	100
Neosartorya sp.2	0	0	0	100	0	0	27.8	100

Table 13 Percent inhibition on mycelial growth of two Basidiomycetes (*Rhizoctonia oryzae* and *Sclerotium rolfsii*) using six crude extractions (ppm) of *Neosartorya* spp. on PDA at 28˚C for 7 days

Fungal isolate	Mycelial growth inhibition (%)							
	Rhizoctonia oryzae				***Sclerotium rolfsii***			
	10	100	1000	10000	10	100	1000	10000
N. pseudofischeri	0	0	28.89	100	0	0	47.78	100
N. fischeri	0	0	59.44	100	0	0	100	100
N. siamensis sp. nov.	0	0	0	100	0	0	0	100
N. spinosa	0	37.78	68.89	100	0	0	0	100
Neosartorya sp.1	0	0	0	100	0	0	0	100
Neosartorya sp.2	0	0	0	100	0	0	0	100

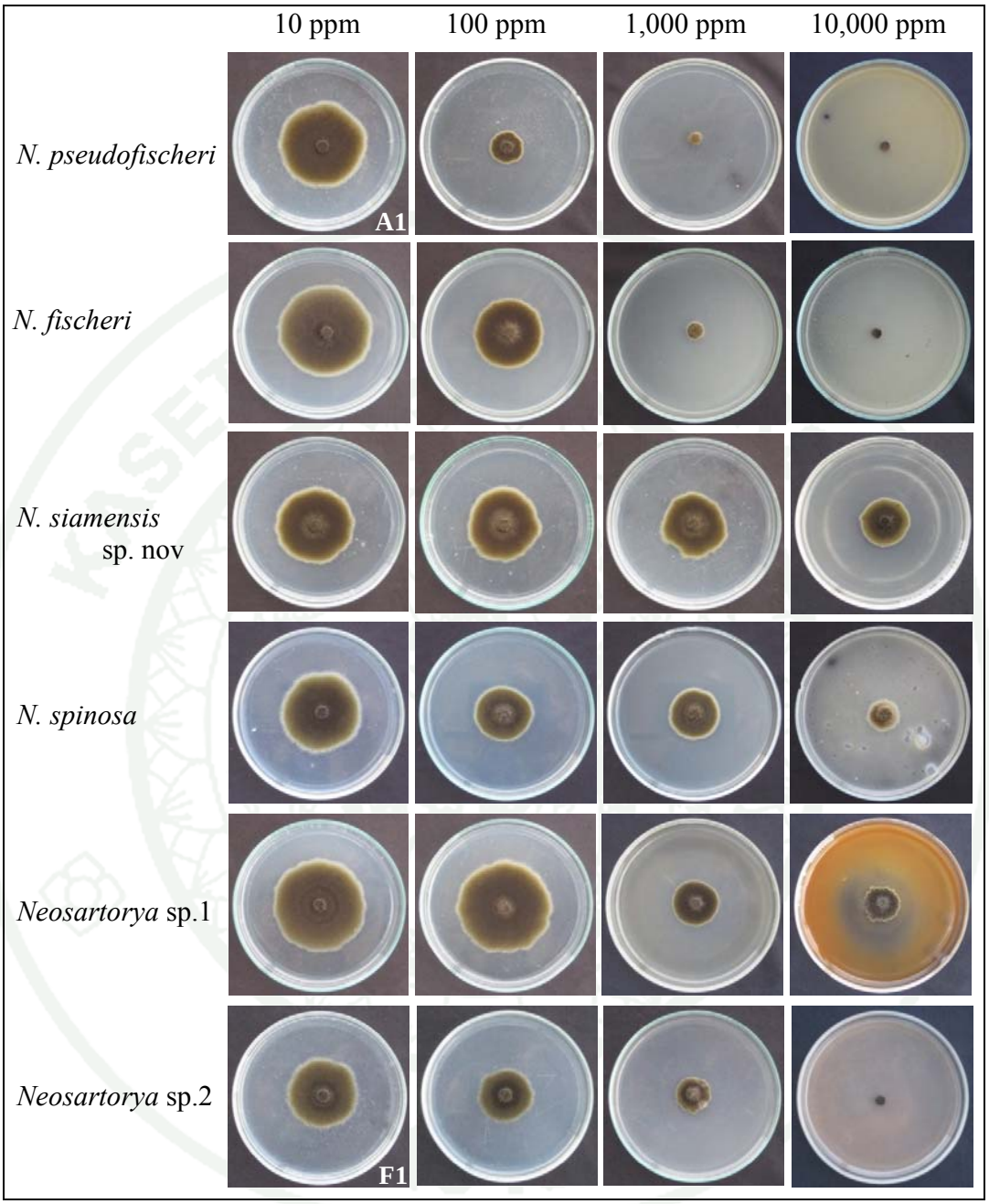


Figure 54 Antagonistic effects of various crude extracts of *Neosartorya* spp. at different concentrations against ***Alternaria brassicicola*** on PDA at 28˚C for 7 days:

A1 - A4. *Neosartorya pseudofischeri* B1 - B4. *N. fischeri*

C1 - C4. *N. siamensis* sp. nov. D1 - D4. *N. spinosa*

E1 - E4. *Neosartorya* sp.1 F1 - F4. *Neosartorya* sp.2

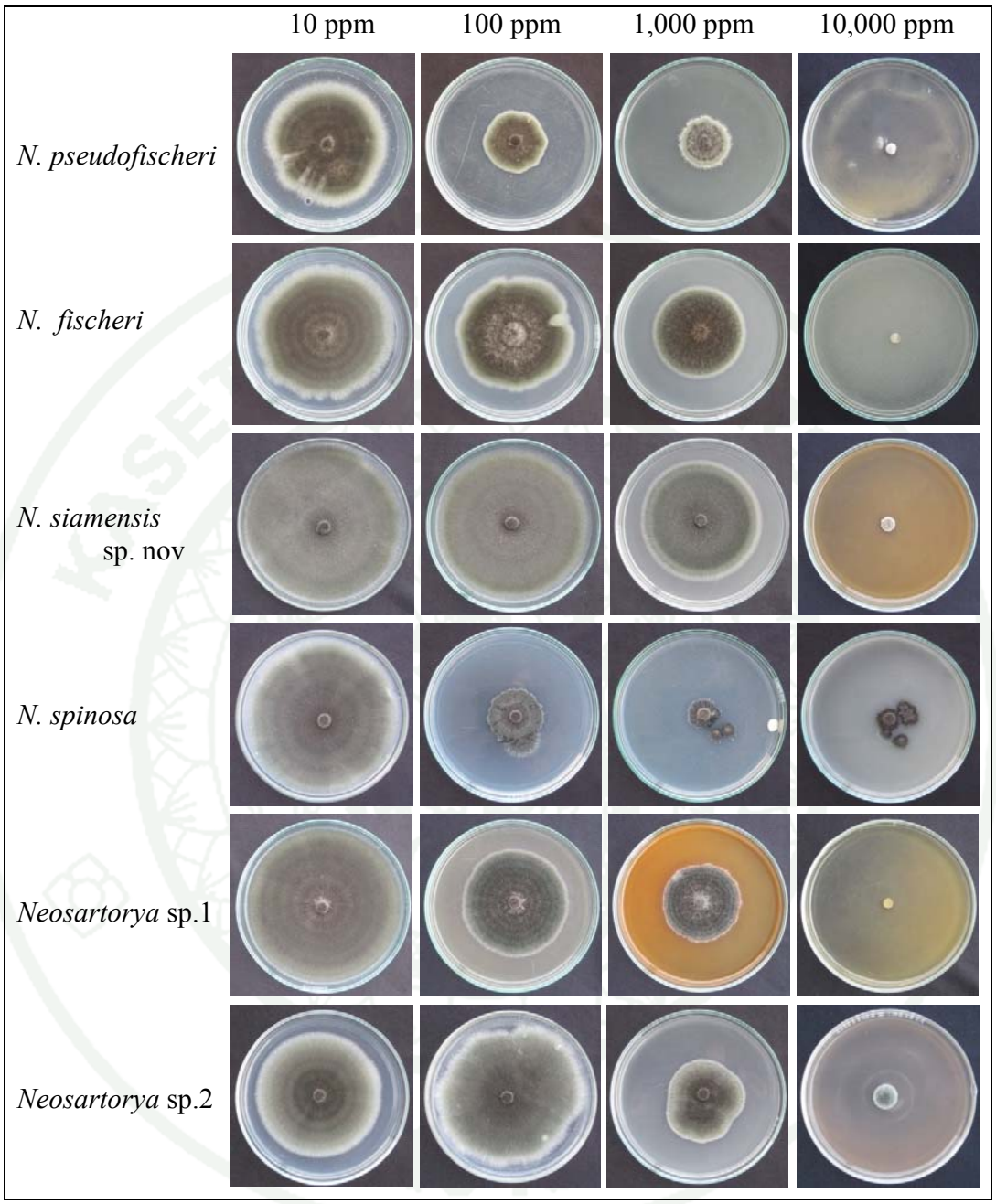


Figure 55 Antagonistic effects of various crude extracts of *Neosartorya* spp. at different concentrations against ***Curvularia oryzae*** on PDA at 28˚C for 7 days:

A1 - A4. *Neosartorya pseudofischeri* B1 - B4. *N. fischeri*

C1 - C4. *N. siamensis* sp. nov. D1 - D4. *N. spinosa*

E1 - E4. *Neosartorya* sp.1 F1 - F4. *Neosartorya* sp.2

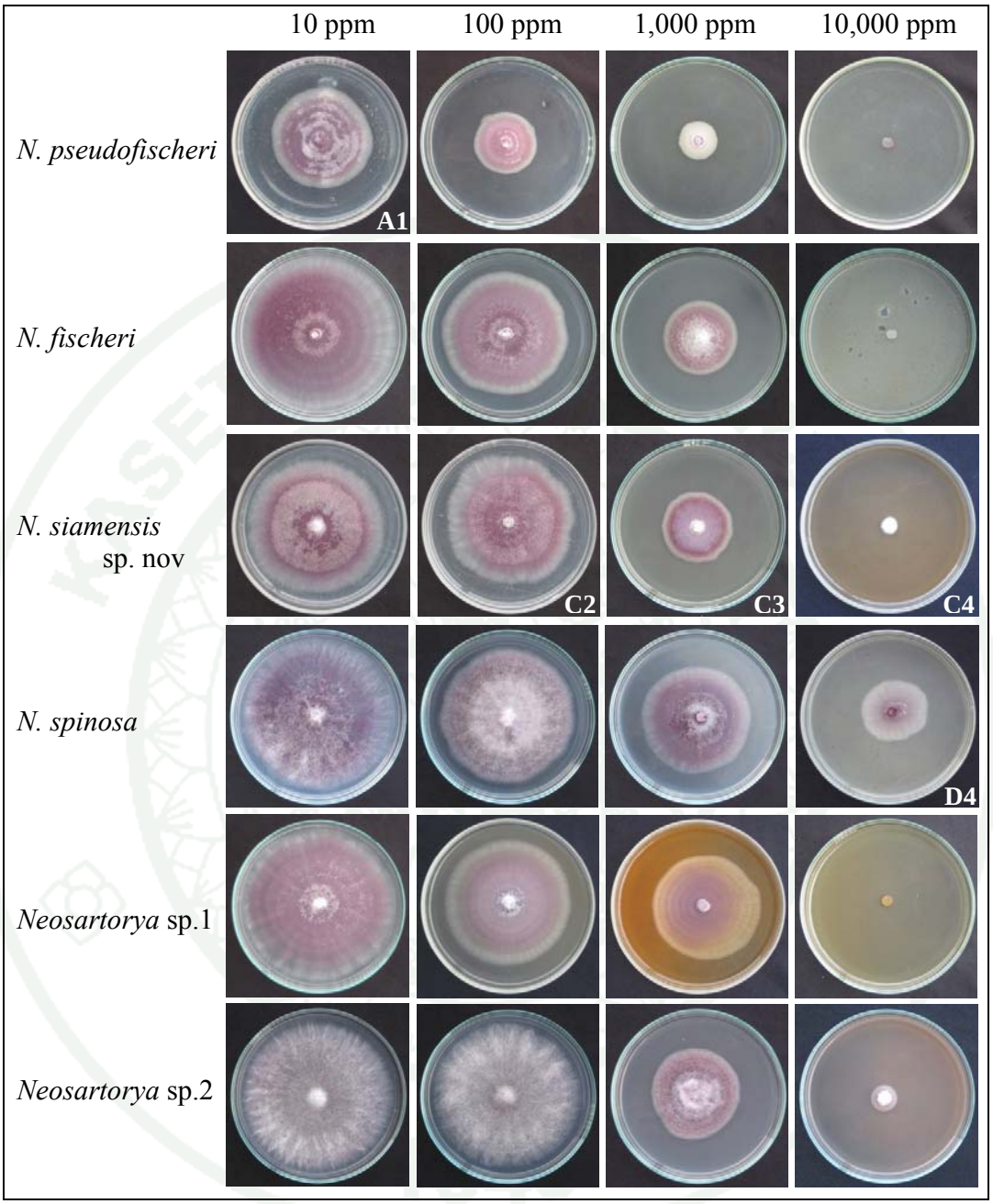


Figure 56 Antagonistic effects of various crude extracts of *Neosartorya* spp. at different concentrations against ***F. oxysporum* f.sp. *cubense*** on PDA at 28˚C for 7 days:

A1 - A4. *Neosartorya pseudofischeri* B1 - B4. *N. fischeri*

C1 - C4. *N. siamensis* sp. nov. D1 - D4. *N. spinosa*

E1 - E4. *Neosartorya* sp.1 F1 - F4. *Neosartorya* sp.2

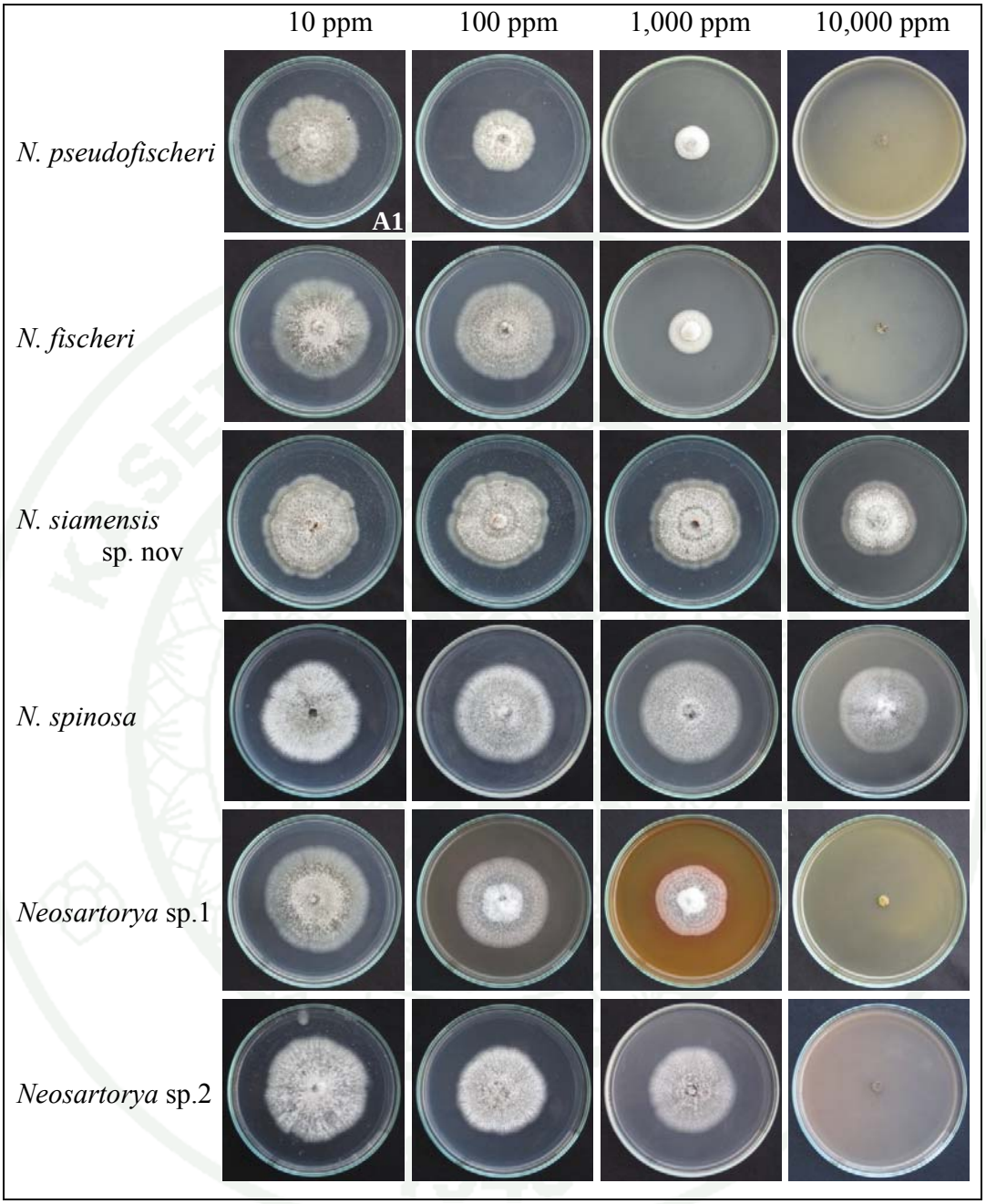


Figure 57 Antagonistic effects of various crude extracts of *Neosartorya* spp. at different concentrations against ***Colletotrichum capsici*** on PDA at 28˚C for 7 days:

A1 - A4. *Neosartorya pseudofischeri* B1 - B4. *N. fischeri*

C1 - C4. *N. siamensis* sp. nov. D1 - D4. *N. spinosa*

E1 - E4. *Neosartorya* sp.1 F1 - F4. *Neosartorya* sp.2

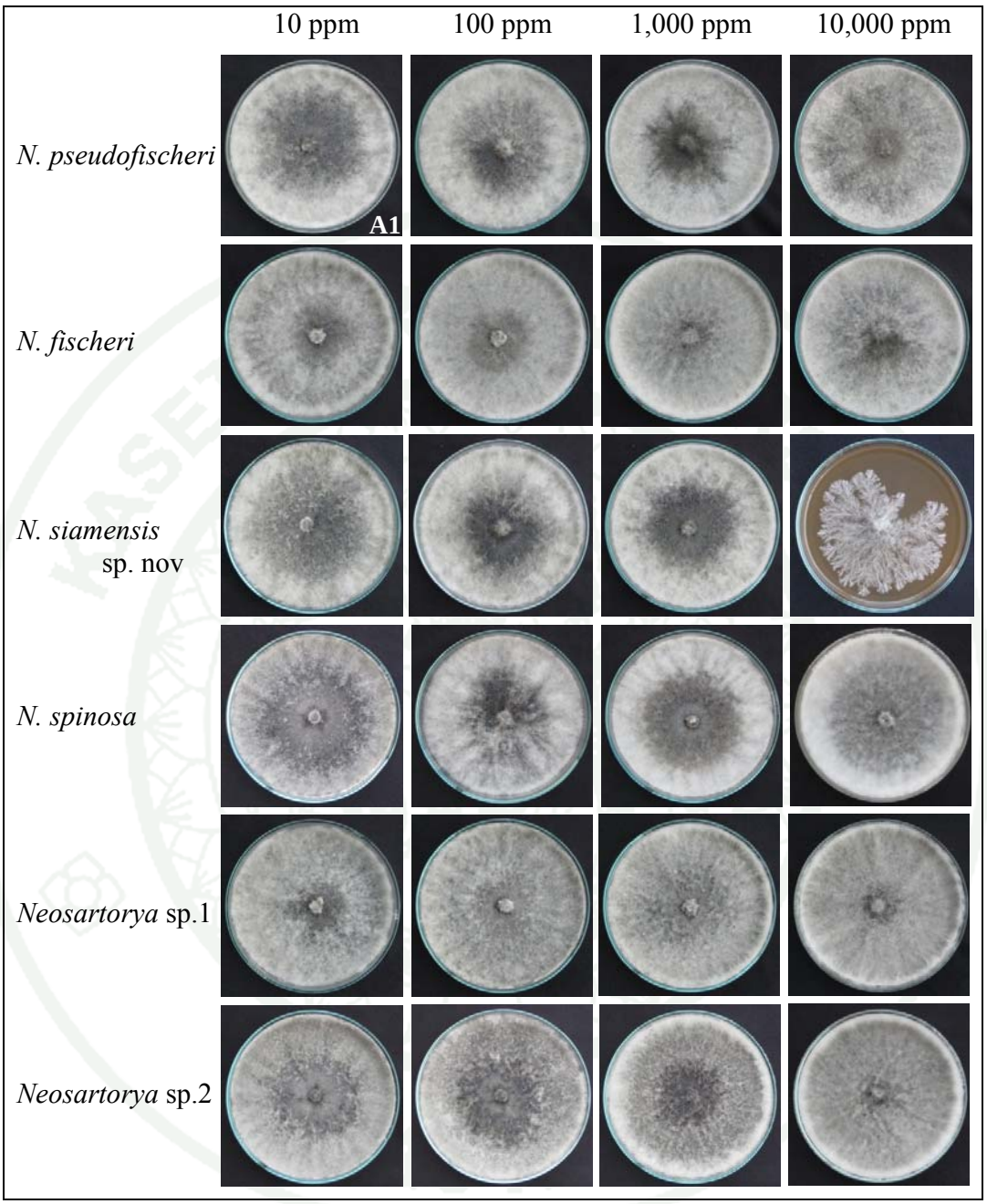


Figure 58 Antagonistic effects of various crude extracts of *Neosartorya* spp. at different concentrations against ***Lasiodiplodia theobromae*** on PDA at 28˚C for 7 days:

A1 - A4. *Neosartorya pseudofischeri* B1 - B4. *N. fischeri*

C1 - C4. *N. siamensis* sp. nov. D1 - D4. *N. spinosa*

E1 - E4. *Neosartorya* sp.1 F1 - F4. *Neosartorya* sp.2

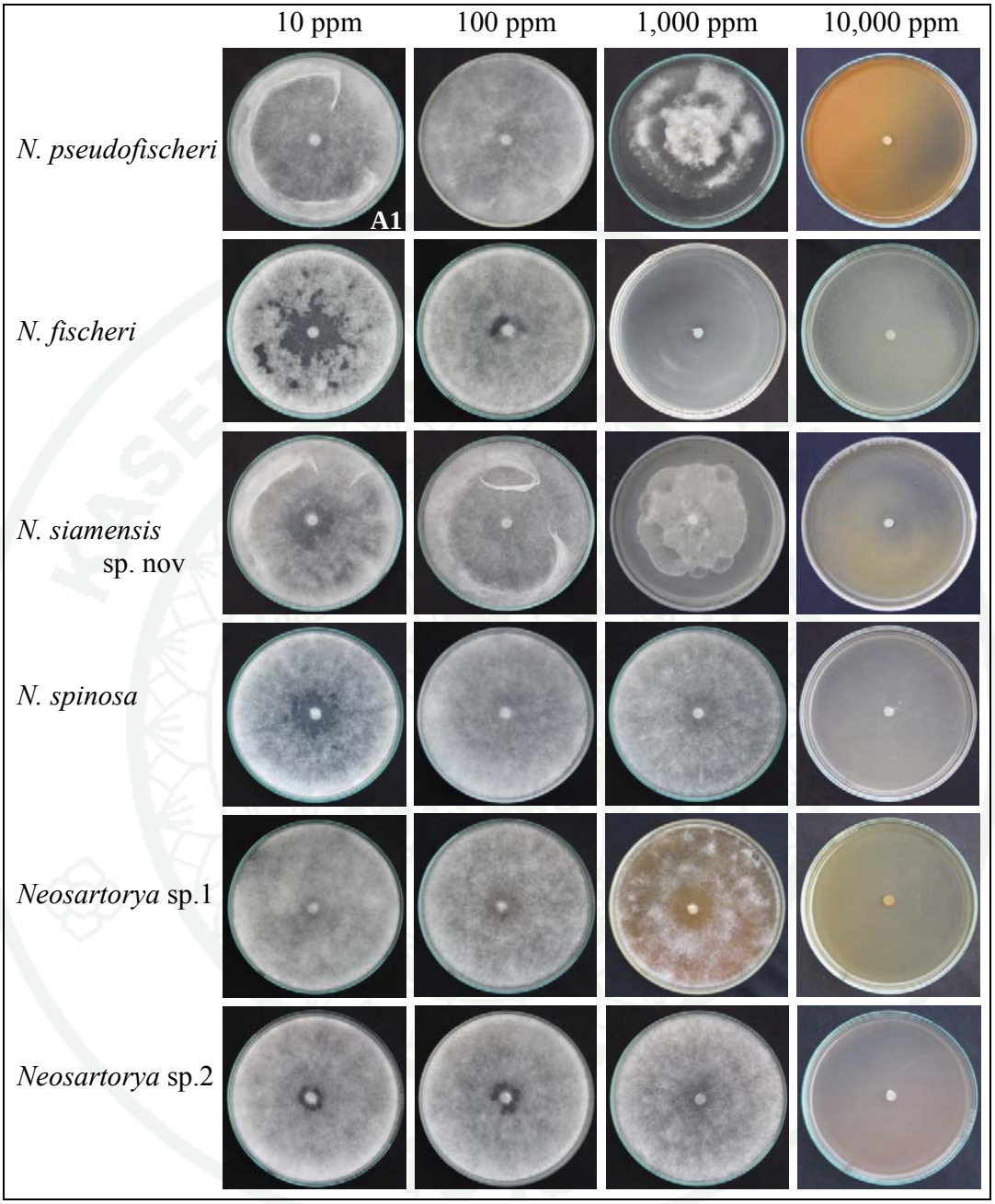


Figure 59 Antagonistic effects of various crude extracts of *Neosartorya* spp. at different concentrations against ***Pythium aphanidermatum*** on PDA at 28˚C for 7 days:

A1 - A4. *Neosartorya pseudofischeri* B1 - B4. *N. fischeri*

C1 - C4. *N. siamensis* sp. nov. D1 - D4. *N. spinosa*

E1 - E4. *Neosartorya* sp.1 F1 - F4. *Neosartorya* sp.2

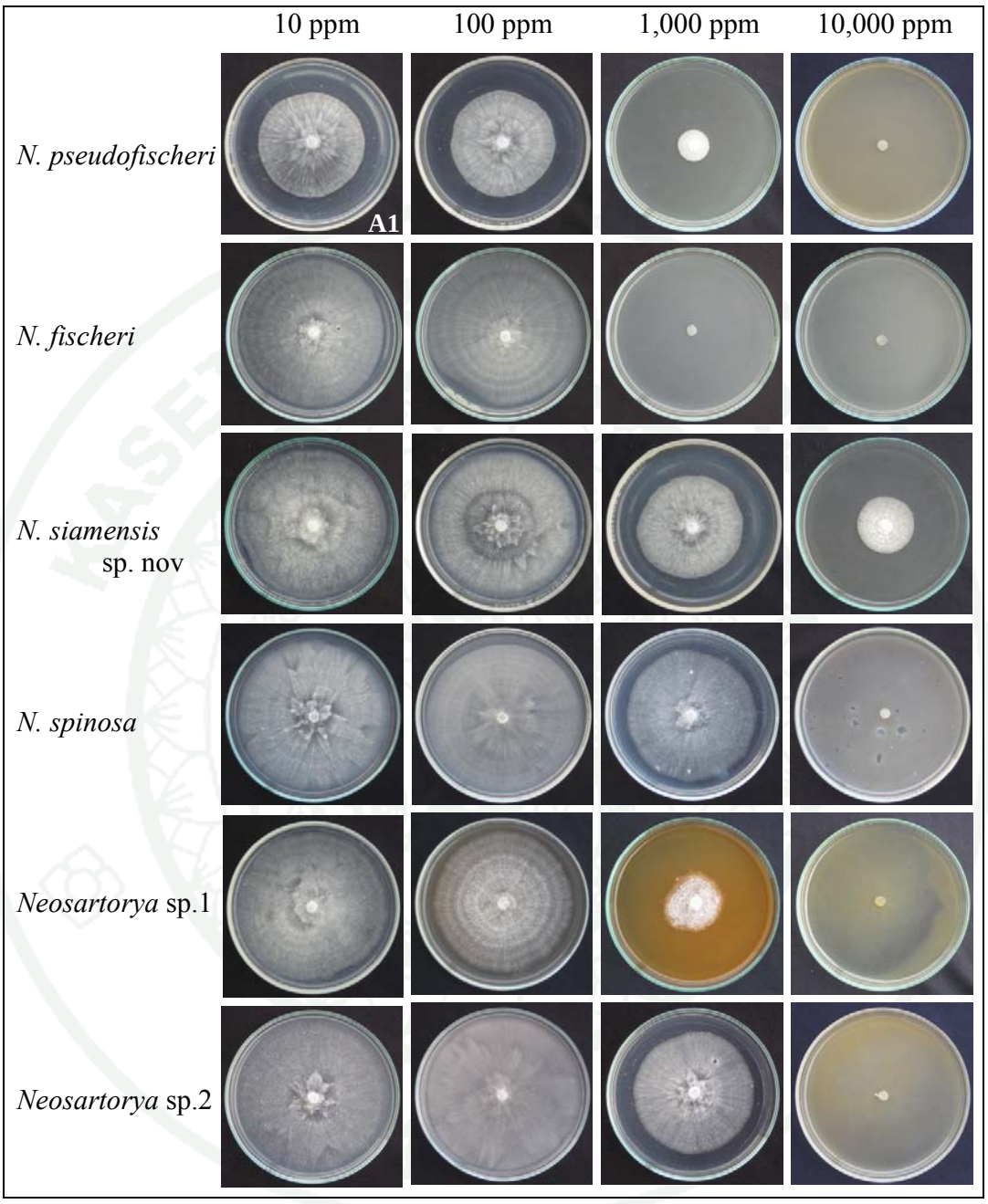


Figure 60 Antagonistic effects of various crude extracts of *Neosartorya* spp. at different concentrations against ***Phytophthora palmivora*** on PDA at 28˚C for 7 days:

A1 - A4. *Neosartorya pseudofischeri* B1 - B4. *N. fischeri*

C1 - C4. *N. siamensis* sp. nov. D1 - D4. *N. spinosa*

E1 - E4. *Neosartorya* sp.1 F1 - F4. *Neosartorya* sp.2

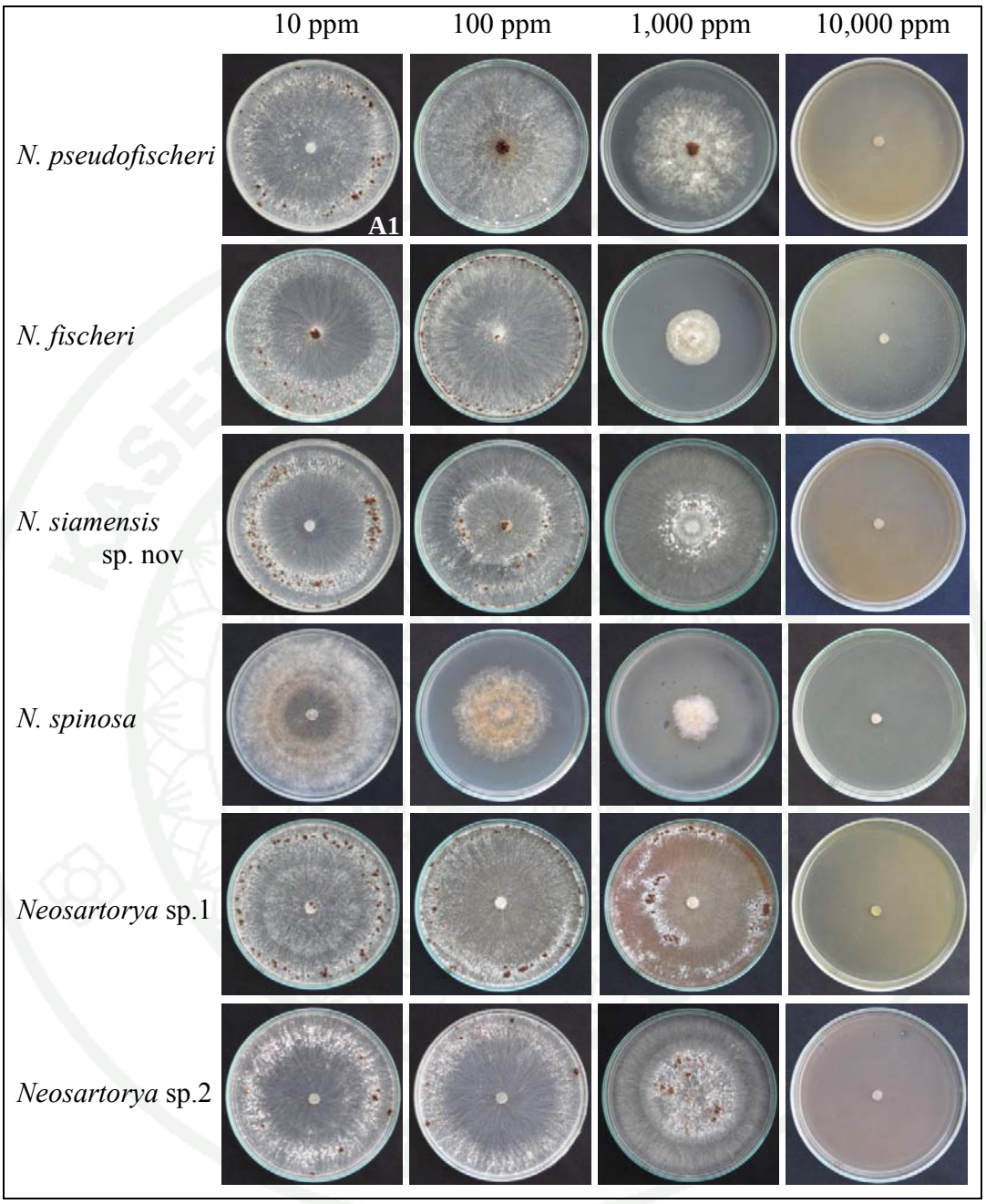


Figure 61 Antagonistic effects of various crude extracts of *Neosartorya* spp. at different concentrations against ***Rhizoctonia oryzae*** on PDA at 28˚C for 7 days:

A1 - A4. *Neosartorya pseudofischeri* B1 - B4. *N. fischeri*

C1 - C4. *N. siamensis* sp. nov. D1 - D4. *N. spinosa*

E1 - E4. *Neosartorya* sp.1 F1 - F4. *Neosartorya* sp.2

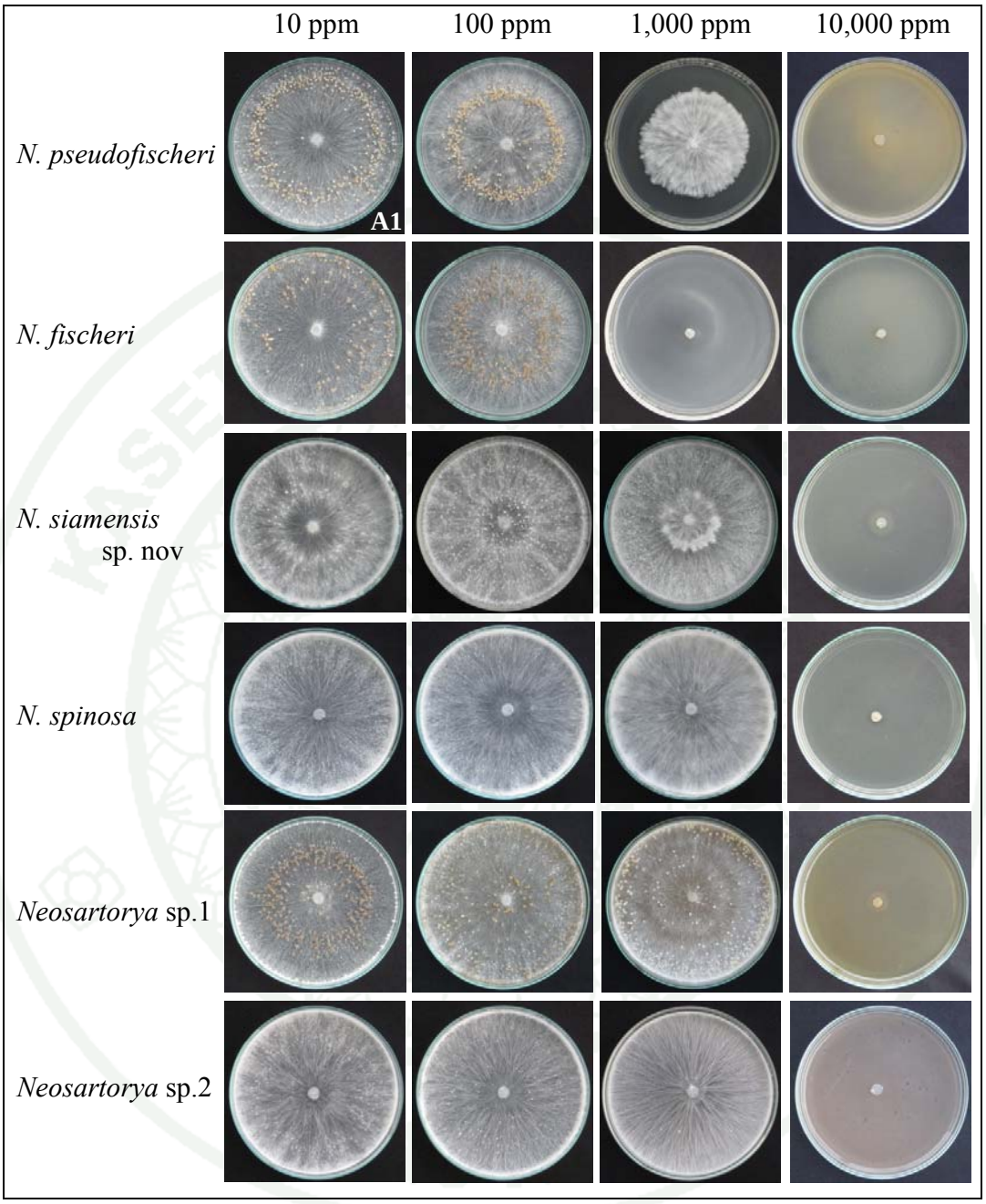


Figure 62 Antagonistic effects of various crude extracts of *Neosartorya* spp. at different concentrations against ***Sclerotium rolfsii*** on PDA at 28˚C for 7 days:

A1 - A4. *Neosartorya pseudofischeri* B1 - B4. *N. fischeri*

C1 - C4. *N. siamensis* sp. nov. D1 - D4. *N. spinosa*

E1 - E4. *Neosartorya* sp.1 F1 - F4. *Neosartorya* sp.2

4. Inhibitory activity of *Neosartorya* crude extracts for weed germination and growth

The efficacy of crude extracts derived from five *Neosartorya* including, *Neosartorya pseudofischeri* (KUFC 6422), *N. siamensis* sp. nov. (KUFC 6349), *N. spinosa* (KUFC 6325), *Neosartoya* sp.1 (KUFC 6341) and *Neosartorya* sp.2 (KUFC 6513) were selected to test with three kinds of weeds comprising *Phaseolus lathyroides* L., *Mimosa pigra* L. and *Corchorus* sp.

Results in Table 14 showed that among the extracts from five *Neosartorya* species at 10,000 ppm, *N. pseudofischeri* (KUFC 6422) extract showed the strongest germination and growth inhibition (91.6 and 81.0%) on *Phaseolus lathyroides* while *N. spinosa* (KUFC 6325) extract was the second most effective (81.4 and 45.9%), followed by *N. siamensis* sp. nov. (KUFC 6349) 81.4 and 47.4%, *Neosartorya* sp.2 (KUFC 6513) 20.3 and 13.4%, whereas *Neosartorya* sp.1 (KUFC 6341) could not inhibit germination and growth of this weed (Figure 63).

Table 14 Percent inhibition of five *Neosartorya* crude extracts at different concentrations on growth germination of *Phaseolus lathyroides* L. seeds

Isolate	Concentration of crude extracts (%)									
	10 ppm		100 ppm		1,000 ppm		5,000 ppm		10,000 ppm	
	shoot	root	shoot	root	shoot	root	shoot	root	shoot	root
N. pseudofischeri	0.2	-13.1	-7.4	-11.0	19.0	5.9	27.1	13.9	81.0	91.6
N. siamensis	-11.4	40.1	-5.6	48.1	15.7	21.5	29.3	35.4	47.4	81.4
N.spinosa	1.8	0	-0.7	41.8	0.9	55.3	14.3	60.8	45.9	85.7
Nesartorya sp.1	5.4	26.2	-1.1	-1.3	1.6	5.1	14.5	-0.4	16.1	-8.0
Nesartorya sp.2	-16.1	8.9	-7.4	-5.9	-5.8	-7.6	19.9	10.6	13.4	20.3
Control	0	0	-	-	-	-	-	-	-	-

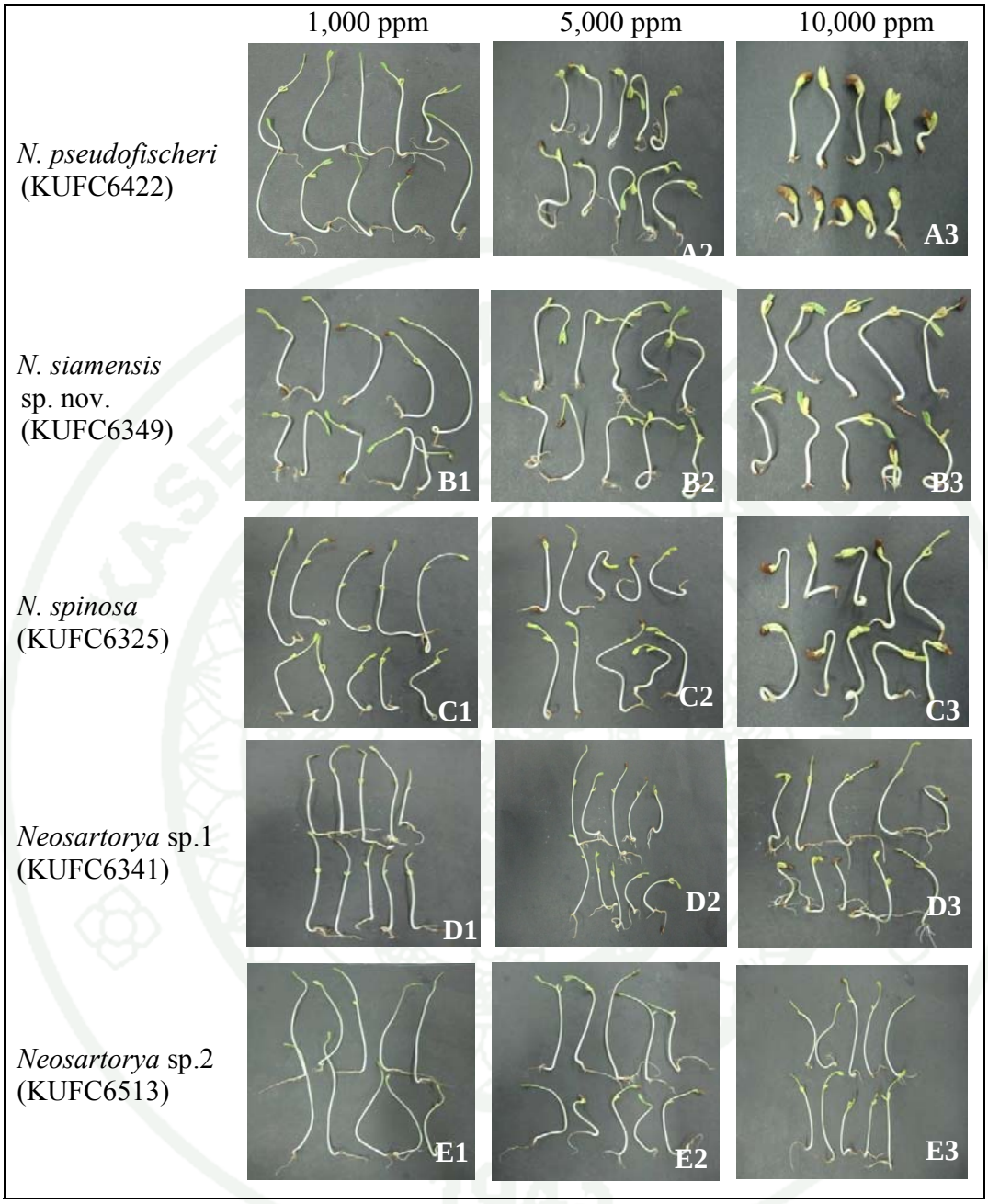


Figure 63 Growth inhibition of shoot and root of ***Phaseolus lathyroides* L.** using various concentrations of crude extracts of *Neosartorya* spp. incubated at 28˚C for 7 days:

A1 - A3. *Neosartorya pseudofischeri* B1 - B3. *N. siamensis* sp. nov.

C1 - C3. *N. spinosa* D1 - D3. *Neosartorya* sp.1

E1 - E3. *Neosartorya* sp.2

Neosartorya siamensis sp. nov. (KUFC6349) crude extract showed strong seed germination and growth inhibition (98.7 and 86.3%) on *Mimosa pigra* followed by *N. spinosa* (KUFC6325) 66.4 and 62.4%, *N. pseudofischeri* (KUFC 6422) 65 and 58.8%, *Neosartorya* sp.2 (KUFC 6513) 50.7 and 60.1% and *Neosartorya* sp.1 (KUFC 6341) 31.4 and 52.9% at 10,000 ppm, respectively (Table 15, Figure 64).

Table 15 Percent inhibition of five *Neosartorya* crude extracts at different concentrations on growth germination of *Mimosa pigra* L. seeds

Isolate	Concentration of crude extracts (%)									
	10 ppm		100 ppm		1,000 ppm		5,000 ppm		10,000 ppm	
	shoot	root	shoot	root	shoot	root	shoot	root	shoot	root
N. pseudofischeri	21.0	7.4	14.4	-1.8	17.0	-17.0	63.1	84.3	58.8	65.0
N. siamensis	30.7	-5.7	12.1	9.2	14.7	-8.3	52.6	34.9	86.3	98.7
N.spinosa	19.6	3.1	27.8	15.7	31.0	43.2	69.9	59.0	62.4	66.4
Nesartorya sp.1	0.6	-11.8	22.2	-4.8	13.1	-15.7	26.1	-7.0	52.9	31.4
Nesartorya sp.2	-5.9	-2.2	26.5	-14.0	37.9	0.4	*	*	60.1	50.7
Control	0	0	-	-	-	-	-	-	-	-

* = contamination

Phattanawasin *et al.* (2007) studied (+)-terrein, (-)-6-hydroxymellein, *cyclo*-(*S-Pro-S-Leu*), *cyclo*-(*S-Pro-S-Val*), and butyrolactone I from a culture broth of *Aspergillus fischeri* TISTR 3272 isolated from soil in Thailand They reported that (+)-terrein showed strongest inhibition, especially on root growth of *M. pigra* L. and *E. crus-galli* [L.] Beauv.

Eamvijarn *et al.* (2012) reported that *N. pseudofischeri* isolated from soil in Angthong province, Thailand produced four known compounds and three new compounds namely a 1, 4-diacetyl-2, 5-dibenzylpiperazine derivative, pseudofischerine and 2,4-dihydroxy-6-methylbenzoic acid from the culture. In this study, *N. pseudofischeri* crude extract was tested on three seeds and these compounds were effective on seed germination of these weeds.

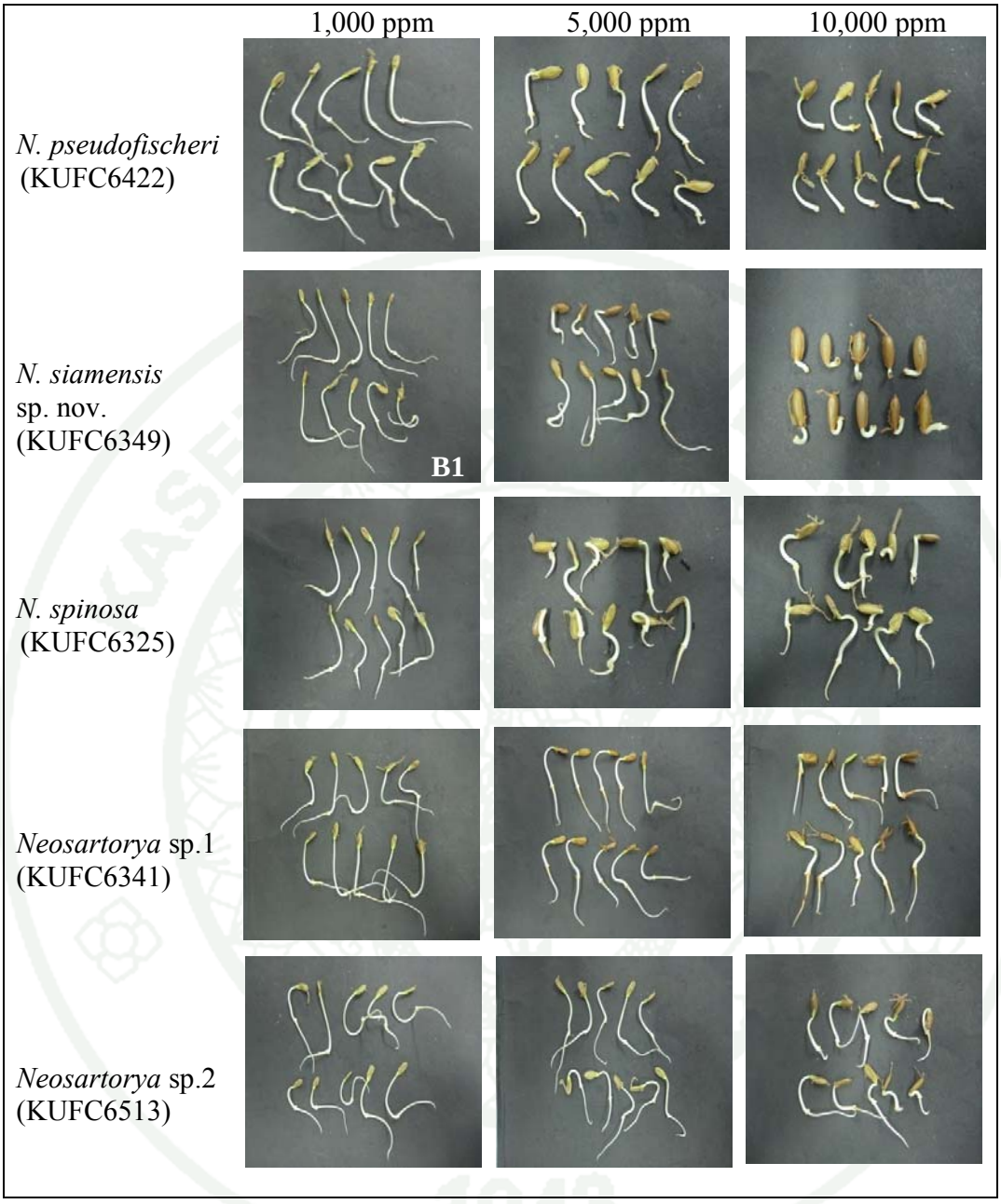


Figure 64 Growth inhibition of shoot and root of ***Mimosa pigra* L.**using various concentrations of crude extracts of *Neosartorya* spp. incubated at 28˚C for 7 days:

A1 - A3. *Neosartorya pseudofischeri* B1 - B3. *N. siamensis* sp. nov.

C1 - C3. *N. spinosa* D1 - D3. *Neosartorya* sp.1

E1 - E3. *Neosartorya* sp.2

Neosartorya siamensis sp. nov. (KUFC 6349) crude extract completely inhibited seed germination and growth (99.3 and 100%) on *Corchorus* sp. followed by *N. spinosa* (KUFC 6325) 95.8 and 91.2%, *N. pseudofischeri* (KUFC 6422) 93.7 and 48.3%, *Neosartorya* sp.2 (KUFC 6513) 63.9 and 70.6% and *Neosartorya* sp.1 (KUFC 6341) 35.7 and 45.8% at 10,000 ppm, respectively (Table 16, Figure 65).

Table 16 Percent inhibition of five *Neosartorya* crude extracts at different concentrations on growth germination of *Corchorus* sp. seeds

Isolate	Concentration of crude extracts (%)									
	10 ppm		100 ppm		1,000 ppm		5,000 ppm		10,000 ppm	
	shoot	root	shoot	root	shoot	root	shoot	root	shoot	root
N.pseudofischeri	15.8	27.1	24.2	30.5	26.7	64.3	40.4	89.6	48.3	93.7
N. siamensis	7.5	-23.8	25.0	6.3	33.3	43.5	59.2	81.4	100	99.3
N.spinosa	27.5	13.8	25.0	11.2	32.5	23.2	64.2	86.2	91.2	95.8
Nesartorya sp.1	20.8	20.4	21.7	13.0	28.3	-6.3	23.8	27.1	45.8	35.7
Nesartorya sp.2	39.6	-0.4	39.2	5.2	28.8	4.1	30.8	16.7	70.6	63.9
Control	0	0	-	-	-	-	-	-	-	-

Buttachon *et al.* (2012) reported seven known indole alkaloids including 2,4-dihydroxy-3-methylacetophenone, tryptoquivaline, tryptoquivalines L, H, F, and fiscalins A and C in addition to seven new metabolites including sartorymensin, 3′-(4-oxoquinazolin-3-yl)spiro[1H-indole-3,5′-oxolane]-2,2′-dione, epi-fiscalin C, epi-fiscalin A, neofiscaline A, and epi-neofiscalin A. Some of these compounds were effective on seed germination of *M. pigra* and *Corchorus* sp (Table 14, 15). Phattanawasin *et al*. (2006) studied the screening of effective crude extract of twenty fungal species for seed germination and growth inhibitory activity at 35 mg/ml of monocotyledonous and dicotyledonous weed, *Echinochloa crus-galli* [L.] Beauv. and *Mimosa pigra* L. The crude extracts of *Aspergillus fischeri* TISTR 3272 and *Aspergillus usamii* TISTR 3258 showed remarkable germination and growth inhibition on both weeds.

Figure 65 Growth inhibition of shoot and root of ***Corchorus* sp.**using various concentrations of crude extracts of *Neosartorya* spp. incubated at 28˚C for 7 days:

A1 - A3. *Neosartorya pseudofischeri* B1 - B3. *N. siamensis* sp. nov.

C1 - C3. *N. spinosa* D1 - D3. *Neosartorya* sp.1

E1 - E3. *Neosartorya* sp.2

5. Secondary metabolites isolated from *Neosartorya pseudofischeri* (KUFC 6422) and their *in vitro* cytostatic activity in human cancer cells

5.1 Secondary metabolites isolated from *Neosartorya pseudofischeri* (KUFC 6422)

The ethyl acetate extract of the culture of *Neosartorya pseudofischeri* furnished (Figure 66), besides cadinene (1), eurochevalierine (2), brasiliamide B (3) and pyripyropene A (6), were reported from the fungus *Eurotuim chevalieri* (Kanokmedhakul *et al*., 2011), and three new compounds including a 1,4-diacetyl-2,5-dibenzylpiperazine derivative (4), a quinazolinone-containing indole derivative (pseudofischerine, 5), and a new ester of 2,4-dihydroxy-6-methylbenzoic acid (7). The structures of these new compounds have been established by spectroscopic methods (^{1}H, ^{13}C NMR, COSY, HSQC and HMBC).

Cadinene (1): White crystals, (*1R, 2S, 3R, 6S, 7R, 10R*)-3-Acetoxy-1,2-dihydroxycadinan-4, 11-diene (1): White crystals with an mp of 173-174ºC, $[\alpha]^{20}_{D}$ -53º (*c* 0.15, $CHCl_3$), IR(KBr) υ_{max} 3440, 3320, 2930, 1730, 1640, 1240 cm^{-1},^{1}H and ^{13}C NMR (Table 17), HRESIMS *m/z* 294. 18252 (M^{+}) (calculated for $C_{17}H_{26}O_4$, 294.18246).

Eurochevalierine (2): Yellow gum, $[\alpha]^{20}_{D}$ − 159 (c 0.1, $CHCl_3$), UV ($CHCl_3$) λ_{max} (log ε): 215 (3.30), 246 (3.75), 260 (3.60), 398 (3.47) nm; IR (KBr) ν_{max} 3440, 2930, 1740, 1630, 2928, 1520, 1230, 1020 cm^{-1}; ^{1}H and ^{13}C NMR (Table 18) ASAP-HRMS, m/z 527.2737 $(M + H)^{+}$ (calcd. for $C_{29}H_{38}N_2O_7$, 527.2757).

Brasiliamide B (3): Yellow gum, $[\alpha]^{20}_{D}$ +142.0 (c 0.24, $CHCl_3$), UV ($CHCl_3$) λ_{max} (log ε): 240 (3.7), 278 (3.6), IR (KBr) ν_{max} 2956, 2926, 1633, 1506, 1428, 1396, 1193, 1089 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.33 (2H, ddd, *J* = 7.3, 7.3, 1.5 Hz, H-3″,5″), 7.25 (1H, m, H-4″), 7.13 (2H, dd, *J* = 7.3, 1.5 Hz, H-2″,6″), 6.49 (2H, s, H-2′, 6′), 6.36 (1H, brs, H-3), 6.00 (2H, s, OCH2O), 5.50 (1H, d, *J* = 15.0 Hz, H-7′b), 4.37 (1H, d, *J* = 12.9 Hz, H-6*β*), 4.06 (1H, m, H-5), 3.94 (3H, s, OMe-3′), 3.69 (1H, d, *J* = 15.0 Hz, H-

7′a), 3.43 (1H, dd, *J* = 12.9, 4.8, H-6α), 2.96 (1H, dd, *J* = 13.7, 8.4 Hz, H-7″b), 2.87 (1H, dd, *J* = 13.7, 6.1 Hz, H-7″a), 2.29 (3H, s, N1-Ac), 1.67 (3H, s, N4-Ac). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.8 (CO, N1-Ac), 169.6 (CO, N4-Ac), 149.2 (C, C-5′), 143.7 (C, C-3′), 137.1 (C, C-1″), 135.3 (C, C-4′), 134.0 (C, C-1′), 129.2 (2CH, C-2″,6″), 128.9 (2CH, C-3″,5″), 127.1 (CH, C-4″), 126.5 (CH, C-3), 108.8 (CH, C-2′), 102.9 (CH, C-6′), 101.7 (CH_2, OCH*2*O), 56.8 (CH_3, OMe-3′), 55.8 (CH, C-5), 46.5 (CH_2, C-6), 38.2 (CH_2,C-7′), 37.2 (CH_2,C-7″), 21.8 (CH_3, N1-Ac), 20.9 (CH_3, N4-Ac). ASAP-HRMS, m/z 423.1918 $(M + H)^+$ (calcd. for $C_{24}H_{27}N_2O_5$, 423.1935).

3,8-Diacetyl-4-(3-methoxy-4,5-methylenedioxy)benzyl-7-phenyl6-oxa-3,8-diazabicyclo[3.2.1]octane (4): Yellow gum, $[\alpha]_D^{20}$ +62.9 (c 0.18, $CHCl_3$), UV ($CHCl_3$) λ_{max} (log ε): 204 (3.3), 243 (3.7), 276 (3.1), IR (KBr) ν_{max} 3082, 2929, 1650, 1633, 1507, 1447, 1428, 1130, 1088, 1038 cm^{-1}; ^{1}H and ^{13}C NMR (Table 19) ,ASAPHRMS, m/z 439.1860 $(M + H)^+$ (calcd. for $C_{24}H_{27}N_2O_6$, 439.1869).

Pseudofischerine (5): Yellow gum, $[\alpha]_D^{20}$ − 16.9 (c 0.18, $CHCl_3$), UV ($CHCl_3$) λ_{max} (log ε): 251 (3.5), 303 (3.2), 314 (3.1), IR (KBr) ν_{max} 3402, 2961, 2930, 1725, 1677, 1607, 1476, 1378, 1325, 1266, 1173 cm^{-1}; ^{1}H and ^{13}C NMR (Table 20) ASAP-HRMS, m/z 431.1721 $(M + H)^+$ (calcd. for $C_{24}H_{23}N_4O_4$, 431.1719).

3-Hydroxy-5-methylphenyl 2,4-dihydroxy-6-methylbenzoate (7): Pale yellow solid, mp 232°C. UV ($CHCl_3$) λ_{max} (log ε): 218 (4.6), 260 (3.7), 300 (3.5), IR (KBr) ν_{max} 3446, 2958, 1619, 1601, 1456, 1230, 1263, 1159 cm^{-1}; ^{1}H NMR (DMSO, 300 MHz) δ 9.58 (brs, OH), 6.43 (1H, brs, H-4′), 6.34 (1H, d, *J* = 2.2 Hz, H-5), 6.32 (1H, brs, H-6′), 6.24 (1H, dd, *J* = 2.2, 2.2 Hz, H-2′), 6.21 (1H, d, *J* = 2.2 Hz, H-3), 2.38 (3H, s, Me-6), 2.21 (3H, s, Me-5′); ^{13}C NMR (DMSO, 75 MHz) δ 170.8 (CO, C-7), 160.8 (C, C-2), 160.1 (C, C-4), 158.6 (C, C-1′), 156.0 (C, C-3′), 141.7 (C, C-6), 140.5 (C, C-5′), 112.3 (CH, C-4′), 111.5 (CH, C-5), 111.3 (C, C-1), 111.0 (CH, C-6′), 104.0 (CH, C-2′), 102.6 (CH, C-3), 22.2 (CH_3, Me-6), 21.1 (CH_3, Me-5′), ASAP-HRMS, m/z = 275.0916 $(M + H)^+$ (calcd. for $C_{15}H_{15}O_5$, 275.0919).

Table 17 NMR data (300.13 MHz, $CDCl_3$) for cadinene (1)

position	δ_C, type	δ_H (*J* in Hz)	COSY	HMBC
1	73.2, C			
2	71.2, CH	3.93, brd (2.0)	H-3, OH-2	1, 4
3	75.8, CH	5.11, brs	H-2	1, 2, 4, 5, CO (Ac)
4	128.9, C			
5	127.5, CH	5.42, s	H-15	1, 3, 7, 15
6	38.2, CH	2.70, brs	H-15	
7	41.1, CH	2.46, brd (12.3)		
8	25.9, CH_2	1.57, m		
9	30.4, CH_2	1.47, m		1, 7, 10
10	30.5, CH	1.92, m	H-14	
11	147.3, C			
12	110.8, CH_2	4.94, dd (2.7, 1.3)	H-13	7, 13
		4.71, brs		7, 13
13	22.4, CH_3	1.74, s	H-12	7, 11, 12
14	14.8, CH_3	0.84d, (6.7)	H-10	1, 9, 10
15	19.9, CH_3	1.67, brs	H-5, 6	3, 4, 5
CO (Ac)	171.2, C			
Me (Ac)	21.0, CH_3	2.11, s		CO (Ac)
OH-2		3.63, d (3.8)	H-2	

Table 18 NMR data for eurochevalierine (2) ($CDCl_3$, 75.47 and 300.13 MHz)

position	δ_C, type	δ_H (*J* in Hz)	COSY	HMBC
1	170.2, C	----		
2	47.6, CH	5.03, ddd (7.6, 4.1, 3.8)	NHCHO, H-3	1, 4
3	40.5, CH_2	3.83, dd (17.8, 4.1)	H-2	1, 2, 4
		3.63, dd (17.8, 3.8)		
4	199.9, C			
1’	116.2, C			
2’	152.5, C			
3’	111.9, CH	6.69, d (7.8)	H-4’	1’, 5’
4’	136.4, CH	7.40, ddd (7.8, 7.8, 1.1)	H-3’, 5’	C-2’, 6’
5’	114.6, CH	6.58, dd (7.8, 7.8)	H-4’, 6’	1’, 3’
6’	131.9, CH	7.68, dd (7.8, 1.1)	H-5’	4, 2’, 4’
1”	72.4, C			
2”	74.9, CH	5.43, d (1.5)	H-3”	1, 1”, 4”, 6”
3”	72.7, CH	5.15, s	H-2”	3”, 4”,CO (Ac)
4”	129.4, C			
5”	127.5, CH	5.33, brs	H-6”	1”, 4”
6”	39.4, CH	2.25, m	H-5”	
7”	40.7, CH	2.40, m	H-6”, 8”	
8”	25.8, CH_2	1.50, m	H-7”, 9”	
9”	30.1, CH_2	1.50, m	H-8”, 10”	
10”	30.7, CH	1.97, m	H-9”, 14”	
11”	146.9, C			
12”	111.0, CH_2	4.83, bs	H-13”	7”, 13”
		4.61, brs		
13”	21.5, CH_3	1.24, s	H-12”	7”, 11”, 12”
14”	14.9, CH_3	0.95, d (6.7)	H-10”	1”, 9”
15”	19.8, CH_3	1.64, s	H-5”	3”, 4”
CHO	160.8, CH	8.19, s		
CO (Ac)	169.9, CO			
Me (Ac)	20.8, CH_3	2.11, s		CO (Ac)
NHMe	29.5, CH_3	2.92, d (5.0)	NHMe	2’
NHMe		8.67, brd (5.0)	NHMe	
NHCHO		6.7d, (7.6)	H-2	

Table 19 NMR data of the major rotamer of 3,8-Diacetyl-4-(3-methoxy-4,5-methylenedioxy)benzyl-7-phenyl6-oxa-3,8-diazabicyclo[3.2.1]octane (4a) in $CDCl_3$ (^{1}H300.13MHz, ^{13}C 75.47 MHz).

position	δ_{C9} type	δ_H (/ in Hz)	COSY	HMBC	NOESY
1	-	-			
2	6.10,CH	4.19, br	H-3		H-3,6′,7′
3	84.1,CH	5.42, br (2.5)	H-2		H-2, CH_3(N_4-Ac)
4	-	-			
5	53.3,CH	5.10, br	H-6α,7″		H-6*α*,6*β*,2″,6″
6β	43.5CH2	3.59, br	H-5, 6α		H-5, 6 *α*
6α		3.16, d (11.3)	H-6*β*		H-6 *β*
1′	131.8,C	-			
2′	108.6,CH	6.65, s		C-4′,6′,7′	H-2,7′a,7′b,OMe
3′	143.7, C	-			
4′	134.0, C	-			
5′	148.9, C	-			
6′	103.7, CH	6.69, s		C-2′,4′	H-7′a,7′b,
7′a	35.0, CH_2	3.37, br	H-2, 7′b		H-2,6′
7′b		3.00, t (11.2)	H-2, 7′a		H-6′
1″	136.2, C	-			
2″	125.9, CH	7.46, m		C-4″,6″,7″	H-7″
3″	129.0, CH	7.46, m		C-1″,5″	
4″	128.2, CH	7.37, m		C-2″,6″	
5″	129.0, CH	7.46, m	H-4″, 6″	C-1″,3″	
6″	125.9, CH	7.46, m	H-5″	C-2″,4″,7″	
7″	80.7, CH	5.02, brd (3.5)	H-5	C-5,6,2″,6″	H-2″
OMe-3′	56.6, CH_3	3.92, s		C-3′	H-2′
OCH_2O	101.4, CH_2	5.97, s		C-4′,5′	
N-1 Ac	172.0, CO	-			
	22.6, CH3	1.96, 2		N-1CO	H-6*α*,6*β*
N-4 Ac	167.6, CO	-			
	21.1. CH3	2.02, 2		N-4C0	H-3

Table 20 NMR data of pseudofischerine (5) in DMSO-d_6 (^{1}H300.13MHz,^{13}C 75.47 MHz).

position	δ_{C9} type	δ_H (/ in Hz)	COSY	HMBC	NOESY
1	124.7, CH	7.55, d(7.8)	H-2	C-3,4a,9a	H-2,9*β*,OH-9a
2	125.3, CH	7.26, ddd(7.8, 7.8, 1.5)	H-1,3	C-4,9b	H-1,H-3
3	130.2, CH	7.44,dd (7.8,7.8)	H-2,4	C-1,4a	H-2,H-4
4	114.3, CH	7.47,dd(7.8, 1.5)	H-3	C-2,9b	H-3
4a	140.3, C	-			
5	-	-			
5a	83.8, CH	5.82, s		C-4a,7,9a,9b	H-12, 13, 14,6′, OH-9a
6	-	-			
7	164.9, C	-			
8	55.7, CH	4.86,dd (8.2,5.3)	H-9*α*,6*β*	C-7,9,9a,6′,8′	H-9*β*(str),6′
9*α*	34.1, CH_2	3.01, dd(13.9,8.2)	H-8,9*β*	C-5a,7,8,9a,9b	H-9*á*(str), OH-9a
B		2.77, dd(13.9,5.3)	H-8,9*α*	C-5a,7,8,9a,9b	H-1, 8(str), 9*á*(str)
9a	73.9, C	-			
9b	134.9, C	-			
10	173.9, C	-			
11	69.4, CH_3	4.21, d(9.3)	H-12	C-5a, 7, 10, 13, 14	H-12,13,14
12	28.2, CH	2.45, m	H-11, 13, 14	C-11,13,14	H-5a,11,13,14
13	20.2, CH_3	1.12, d(6.7)	H-12	C-11,12,14	H-5a,11,12
14	18.7, CH_3	1.16, d (6.7)	H-12	C-11,12,13	H-5a,11,12
1′	126.2, CH	8.19, dd(7.9,1.5)	H-2′	C-3′,4′a,8′	H-2′
2′	127.4, CH	7.59,dd(7.9,7.9)	H-1′,3′	C-4′,8′a	H-1′,3 ′
3′	134.7, CH	7.88, ddd(7.9, 7.9, 1.5)	H-2′,4′	C-1′,4′a	H-2′,4′
4′	127.2, CH	7.71, d(7.9)	H-3	C-2′,8′a	H-3′
4′a	147.5, C	-			
5′	-	-			
6′	146.9, CH	8.31, s		C-8,4′a,8′	H-5a,8
8′	159.8, C	-			
8′a	121.5, C	-			
OH-9a	-	6.30, s		5a,9,9a,9b	H-1,5a,9α

Cadinene (1) was obtained as white crystals, and its molecular formula was determined by HREIMS to be $C_{17}H_{26}O_4$, indicating five degrees of saturation for the molecule. The following 17 carbons were categorized based on ^{13}C NMR and HSQC data (Table 17): one ester carbonyl (δ_C 171.2), two quaternary sp^2 carbons (δ_C 147.3 and 128.9), one sp^2 methine (δ_C 128.9), one sp^2 methylene (110.8), one oxygenated quaternary carbon (δ_C 73.2), two oxygenated methines (δ_C 75.8, 71.2), three methines (δ_C 41.1, 38.2, 30.5), two methylenes (δ_C 30.4, 25.9) and four methyl carbons (δ_C 22.4, 21.0, 19.9, 14.8). The COSY and HMBC correlations (Table 16) indicated the presence of a substituted 3, 4, 5, 6, 7, 8, 9, 10-octahydronaphthalene system. The one isopropenyl (δ_C 22.4 CH_3, 147.34C, 110.8CH_2) and two methyl (δ_C 19.9 and 14.8) substituents at the 8^{th}, 2^{nd} and 5^{th} positions, respectively, of the octahydronaphthalene ring indicated that cadinene was a cadinane-type sesquiterpene. Consequently, we have adopted the numbering system used for cadinane sesquiterpenes (Kuo *et al.*, 2002) for cadinene. The HMBC correlations (Table 17) between the oxygenated methine proton signal at δ_H 5.11, the oxygenated quaternary carbon (δ_C 73.2) and the carbonyl carbon (δ_C 171.2) indicated that the acetoxy and hydroxyl groups were on C-3 and C-1, respectively. In turn, the correlations between the oxygenated methine proton signal at δ_H 3.93 and the quaternary sp^2 carbon (δ_C 128.9) placed another hydroxyl group on C-2, which was confirmed by the correlation between this proton signal (δ_H 3.93 brd) and the hydroxyl proton doublet at δ_H 3.63 (J = 3.8 Hz) with the COSY spectrum. Although cadinene has been previously obtained from the selective degradation of a natural anthelmintic pyrrolobenzoxazine terpenoid CJ-12662, which was isolated from the fermentation broth of *Aspergillus fischeri* var. *thermomutatus* (Didier *et al.*, 2004).

The molecular formula $C_{29}H_{38}N_2O_7$ for eurochevalierine (2) was deduced on the basis of HRFABMS m/z 527.2722 [M+H^+]. The ^{13}C NMR, DEPTs and HSQC spectra (Table 18) revealed the presence of one conjugated ketone (δ_C 199.9), two ester carbonyls (δ_C 170.2, 169.9), one aldehyde (δ_C 160.8), four quaternary sp^2 carbons, five methine sp^2 carbons, one methylene sp^2, one oxygenated quaternary sp^3, two oxygenated sp^3 carbons, four methines, three methylene and four methyl carbons. The low frequency of the aldehyde carbonyl was indicative of a highly electronegative nature of its substituent.

Analysis of the correlations from the COSY and HMBC spectra (Table 18) revealed the presence of a cadinane sesquiterpene moiety in its structure. Another portion of the molecule, an *ortho*-methylamino benzoyl moiety ($C_{12}H_{13}N_2O_3$), was substantiated by the correlations with the H-6 signal (δ_H 7.68 dd, *J*= 7.8, 1.3 Hz) and C-2 (δ_C 152.5), C-4 (δ_C 136.4) and the carbonyl carbon (C-4) at δ_C 199.9 as well as the correlation between a methyl group signal at δ_H 2.92 d (*J*= 5.0) and at C-2 (δ_C 152.5). The correlation between the aldehyde proton signal at δ_H 8.19 s and the methylene carbon at δ_C 47.6, as well as the correlation between the methine proton at δ_H 5.03 (*J* = 7.6, 4.1, 3.8) with the carbonyl carbons at δ_C 199.9 (C-4) and δ_C 170.2 (C-1), suggested that the formamido substituent was on the C-2 of the 4-oxobutanoate moiety. This was confirmed by the coupling of the methine proton (H-2) at δ_H 5.03 to the NH at δ_H 6.7 d, *J* = 7.6 and the methylene protons at δ_H 3.83 dd, *J* =17.8, 4.1 and 3.63 dd, J = 17.8, 3.8 in the COSY spectrum. Correlations between H-2 at δ_H 5.43 d, *J*= 1.5 and C-1 at δ_C 170.2, which were observed in the HMBC spectrum (Table 18), indicated that the hydroxyl group on the C-2 carbon of the cadinane sesquiterpene and the C-1 carbon of the 4-oxobutanoate moiety form an ester linkage. Although eurochevalierine has been previously reported from the bioactive meroterpenoids and alkaloids, which was isolated from the fungus *Eurotium chevalieri* (Kanokmedhakul *et al.*, 2011).

The structure of eurochevalierine may have originated from the pyrrolobenzoxazine terpenoid, namely CJ-12663 (brasiliamide B), a minor metabolite that was also obtained from the fermentation broth of *Aspergillus fischeri* var. *thermomutatus* ATCC 18618 (Didier *et al.*, 2004).

Taken together, the pyrrolobenzoxazine terpenoid CJ-12663 could be a biosynthetic precursor of pseudofischerine. Schwaebisch *et al*. (2004) have proposed that an *N*-methylated L-tryptophan is a precursor for the biomimetic biosynthesis of the pyrrolobenzoxazine core unit of paecilozaxine. Consequently, we anticipate that the configuration of C-2 in pseudofischerine is the same as that of the corresponding carbon in CJ-12663.

The molecular formula $C_{24}H_{26}N_2O_5$, of brasiliamide B (3), was established on the basis of ASAP-HRMS m/z 423.1918 [M + H^+]. Analysis of the ^1H, ^{13}C, DEPTs, COSY, HSQC, and HMBC spectra revealed that the structure of compound 3 was compatible with that of brasiliamide B (Fujita *et al.*, 2002). However, the chemical shift values of the protons and carbons of the tetrahydropyrazine ring and the acetamide groups of compound 3 were slightly different from those reported for the major rotamer of brasiliamide B (Fujita *et al.*, 2002), suggesting that compound 3 was another rotamer of brasiliamide B. That the carbonyl group of the N4-acetamide was directed to the C-3 side in compound 3 was evidenced by the shielding of H-5 (δ_H 4.06 m) when compared to the corresponding proton in the major rotamer of brasiliamide B (δ_H 4.93 br) reported by Fujita *et al.* (2002). This was supported by the deshielding of H-3 (δ_H 6.36 s) as well as the correlation between the signal of the methyl protons (δ_H 1.67 s) of the N4-acetamide and H-5 signal (δ_H 4.06 m) in the NOESY spectrum. On the other hand, the equatorial proton on C-6 (δ_H 4.37 d, J = 12.9 Hz) of compound 3 was deshielded when compared to the corresponding proton of the previously reported major rotamer of brasiliamide B (δ_H 3.59 br), which suggested that the carbonyl group of the N1-acetamide was directed to the C-6 side. This orientation was also supported by the absence of the correlations between the signal of the methyl protons (δ_H 2.29 s) of the N1-acetamide and the signals of the methylene protons on C-6 (δ_H 4.37 d, J = 12.9 Hz; 3.43 dd, J = 12.9, 4.8 Hz). Thus, compound 3 is an enantiomeric rotamer of the brasiliamide B major rotamer, reported by Fujita *et al.* (2002).

3,8-Diacetyl-4-(3-methoxy-4,5-methylenedioxy)benzyl-7-phenyl6-oxa-3,8-diazabicyclo[3.2.1]octane (4a and 4b) displayed an ion peak at m/z 439.1860 (ASAP-HRMS) corresponding to $C_{24}H_{27}N_2O_6$ and was consistent with $[M + H]^+$. The IR spectrum showed absorption bands for aromatic (3082 cm^{-1}) and carbonyl (1650 and 1633 cm^{-1}) groups. The ^{13}C NMR, DEPT, and HSQC spectra (Table 19) revealed the presence of twenty-two carbon signals which can be categorized as two carbonyls (δ_C 172.0, 167.6), five quaternary sp^2 (δ_C 148.9, 143.7, 136.2, 134.0, 131.8), seven methine sp^2 (two at δ_C 129.0, two at δ_C 125.9, one at δ_C 128.2, 108.6, 103.7), one methylenedioxy (δ_C 101.4), one methoxy (δ_C 56.6), four methine sp^3 (δ_C 84.1, 80.7, 61.0, 52.3), two

methylene sp^3 (δ_C 43.5, 35.0), and two methyl carbons (δ_C 21.1, 22.6). However, the intensity of the signal of the methyl carbon at δ_C 22.6 was less than half of that of the methyl carbon at δ_C 21.1, while the intensities of the signals of the methylene sp^3 carbon at δ_C 43.5 and the methine sp^3 carbon at δ_C 61.0 were also less than half of those of the methylene sp^3 carbon at δ_C 35.0 and the methine sp^3 carbon at δ_C 52.3, respectively. On the other hand, the ^{1}HNMR spectrum exhibited apparently two sets of closely related proton signals, most of which were broad, similar to those of the rotamers of brasiliamide B (Fujita *et al.*, 2002). The two methyl carbons were associated with two *N*-acetyl groups whose carbonyls were those referred to earlier, as shown by cross-peaks in the HMBC spectrum. Analysis of the HSQC, COSY, and HMBC spectra (Table 19) of the major component (4a) revealed the presence of the phenyl and 3-methoxy-4,5-methylenedioxyphenyl portions of the molecule. The former was substantiated by the existence of a coupled system consisting of two pairs of chemically equivalent methine sp^2 carbon at δ_C 129.0 (C-3″,C-5″) and δ_C 125.9 (C-2″,C-6″) as well as another methine sp^2 carbon at δ_C 128.2 (C-4″) and the quaternary sp^2 carbon at δ_C 136.2 (C-1″), while the latter was evidenced by the correlations of the signals of the aromatic protons at δ_H 6.65 s (H-2′) and δ_H 6.69 s (H-6′) with those of C-4′ (δ_C 134.0), C-6′ (δ_C 103.7) and C-2′ (δ_C 108.6), C-4′ (δ_C 134.0), respectively, as well as of the methylenedioxy protons signal (δ_H 5.97 s) with those of C-4′ (δ_C 134.0) and C-5′ (δ_C 148.9) in the HMBC spectrum (Table 19). The COSY spectrum also showed the coupling system of H-2 (δ_H 4.19 br) and H-3 (δ_H 5.42 brd, J = 2.5 Hz) as well as the two methylene protons (H2-7′) at δ_H 3.37 br and 3.00 t, J = 11.2 Hz, while the HMBC spectrum showed the correlations between the signal of C-7′ (δ_C 35.0) and H-2′ (δ_H 6.65 s), which suggested that the three-carbon unit was connected to the 3-methoxy-4,5-methylenedioxyphenyl ring through the methylene carbon at δ_C 35.0. The COSY spectrum also showed correlations of the methine proton signal at δ_H 5.10 br (δ_C 52.3) to the signals of the oxymethine proton at δ_H 5.02 brd, J =3.5 Hz (δ_C 80.7) and the methylene protons at δ_H 3.16 brd (J = 11.2 Hz) and 3.59 br (δ_C 43.5), while the HMBC spectrum exhibited correlations of the oxymethine proton signal at δ_H 5.02 brd, J =3.5 Hz (δ_C 80.7) to the carbon signals at δ_C 125.8 (C-2″,C-6″), 52.3 (C-5) and C-6 (δ_C 43.5), suggesting that the second three-carbon unit was connected to the phenyl ring through the oxymethine carbon at δ_C 80.7. Thus, it was now obvious that the

benzylic carbon C-7″ (δ_C 80.7) was linked to C-3 (δ_C 84.1) by an oxygen bridge. Since the signals of H-6*β* and H-6α appeared, respectively, as a broad signal at δ_H 3.59 and a broad doublet at δ_H 3.16 (J = 11.3 Hz), the piperazine ring conformation of 4a must be in a chair form, similar to that of the major rotamer of the dihydro-derivative of brasiliamide D, in which H-5 and H6*β* were diequatorial (Fujita *et al.*, 2004). Since the signal of H-6α appeared as a broad doublet, the average value of $J_{6\alpha}$, 6*β* (11.2 Hz) was smaller than the typical value for the germinal coupling. The chair conformation of the piperazine ring was also supported by a strong correlation between the signals of H-5 (δ_H 5.10 br) and H-6*β* (δ_H 3.59 br) as well as a weaker correlation between the signals of H5 (δ_H 5.10 br) and H-6α (δ_H 3.16 brd, J = 11.3 Hz). On the other hand, the signal of H-3 appeared as a broad doublet with $J_{2,3}$ = 2.5 Hz, suggesting the diequatorial relationship between H2 and H-3. Therefore, the relative configuration of H-2 and H-5 was trans. That H-7″ was directed to the N4-acetamide side was evidenced by the fact that the NOESY spectrum did not show a cross-peak between the signal of H-7″ (δ_H 5.02 brd, J = 3.5 Hz) and H-6*β* (δ_H 3.59 br). Since the chemical shift values of H-7″ (δ_H 5.02 brd, J = 3.5 Hz) and H-5 (δ_H 5.10 br) were very similar, it was not possible to discern their correlation in the NOESY spectrum; however, it was possible to observe a weak cross-peak between the signals of H-7″ (δ_H 5.02 brd, J = 3.5 Hz) and H-3 (δ_H 5.42 brd, J = 2.5 Hz). Furthermore, the COSY spectrum revealed that the signal of H-2 (δ_H 4.19 br) also gave a cross-peak to a broad signal at δ_H 5.90 of the minor component, suggesting that the latter was due to H-3 of another rotamer (4b). As this proton was de-shielded when compared to H-3 (δ_H 5.42 brd, J = 2.5 Hz) of the major rotamer (4a), the carbonyl group of the N-4 acetamide was directed to the C-3 side in the minor rotamer (4b). This was supported by the fact that H-5 in this rotamer (4b) was shielded, appearing at δ_H 4.50 br (δ_C 56.2) when compared to H-5 (δ_H 5.10 br; δ_C 52.3) of the major rotamer (4a). That the carbonyl of the N1 acetamide was directed to the C-2 side of both rotamers (4a and 4b) was supported by the deshielded H-2 (δ_H 4.19 br) as well as the shielded H-6 (δ_H 3.16 brd, J = 11.2 Hz and 3.59 br). The directions of the carbonyl groups of the N-1 and N-4 acetamides in the major (4a) and minor (4b) rotamers (Figure 66) were supported by the observations in the NOESY spectrum (Table 19) which showed strong correlations of H-3 (δ_H 5.42 brd, J = 2.5 Hz) to H-2 (δ_H 4.19 br) and the methyl protons at δ_H 2.02 s, as well as correlations of

H-6α (δ_H 3.16 brd, J = 11.3 Hz) and H-6β (δ_H 3.59 br) to the methyl protons at δ_H 1.96 s. On the other hand, the signal of H-2 (δ_H 4.19 br) gave cross-peaks to the signals of H-7′ (δ_H 3.37 br), H-3 of 4a (δ_H 5.42 brd, J = 2.5 Hz), and H-3 of 4b (δ_H 5.90 br).

Pseudofischerine (5) exhibited an ion peak at *m/z* 431.1721 (ASAPHRMS) corresponding to $C_{24}H_{23}N_4O_4$, consistent with the $[M + H]^+$. The IR spectrum showed absorption bands for hydroxyl (3402 cm^{-1}), aromatic (1607 cm^{-1}), and carbonyl (1725 and 1677 cm^{-1}) groups. The ^{13}C NMR and DEPT spectra run in $DMSOd_6$ (Table 20) displayed signals of three carbonyls at δ_C 173.9, 164.9, and 159.8, four quaternary sp^2 carbons (δ_C 147.5, 140.3, 134.9, 121.5), nine methine sp^2 carbons (δ_C 146.9, 134.7, 130.2, 127.4, 127.2, 126.2, 125.3, 124.7, 114.3), one quaternary sp^3 carbon (δ_C 73.9), four methine sp^3 carbons (δ_C 83.8, 69.4, 55.7, 28.2), one methylene (δ_C 34.1), and two methyl carbons (δ_C 20.2, 18.7). The HSQC, COSY, and HMBC spectra permitted identification of two 1, 2-disubstituted benzene rings. The HMBC spectrum (Table 20) revealed that the proton signal at δ_H 8.19 dd (J = 7.9, 1.5 Hz), assignable to H-1′ of one of these rings, exhibited cross-peaks to those of the carbonyl at δ_C 159.8 (C-8′) and the quaternary sp^2 carbon at δ_C 147.5 (C-4′a). In turn, the latter exhibited a cross-peak to the proton signal at δ_H 8.31 s (δ_C 146.9). The chemical shift of the carbonyl carbon at δ_C 159.8 suggested its amide functionality while the shifts of the proton at δ_H 8.31 s and a methine sp^2 carbon at δ_C 146.9 suggested that they were part of an imine. All this indicated that 5 contained a 1H-quinazoline-4-one moiety. The HSQC, COSY, and HMBC spectra further suggested that a second 1, 2-disubstituted benzene ring was part of an indole. As shown in Table 20, HMBC cross-peaks between the H-1 signal at δ_C 7.55 d (J = 7.8 Hz) and the quaternary sp^2 carbon signal at δ_C 140.3 led to identification of the latter as that of C-4a while the H-1 signal also gave a cross-peak with the signal of a quaternary carbon at δ_C 73.9 (C-9a), indicating that the latter carried an oxygen atom. As the proton signal at δ_H5.82 s (δ_C 83.8) exhibited one cross-peak with the signal of C-4a at δ_C 140.3, it was assigned to H-5a. The latter also showed cross-peaks with the signals of an oxoquaternary carbon at δ_C 73.9 (C-9a), the methine sp^2 carbons at δ_C 134.9 (C-9b) and δ_C 140.3 (C-4a), and the carbonyl at δ_C 164.9, which suggested that the indole was fused onto a piperidone ring. This was confirmed by cross-peaks between H-8 at δ_H 4.86 dd (J = 8.2, 5.3 Hz) and C-9a

at δ_C 73.9 and between H-9α (δ_H 3.01 dd, J = 13.9, 8.2 Hz), H-9*β* (δ_H 2.77 dd, J = 13.9, 5.3 Hz) and C-8 (δ_C 55.7), C-9a (δ_C 73.9), C-5a (δ_C 83.8), CO-7 (δ_C 164.9), C-9b (δ_C 134.9). That the 1H-quinazoline-4-one portion and the pyridone ring were linked through N-7′ of the former and C-8 of the latter was supported by the cross-peaks in the HMBC spectrum between H-8 (δ_H 4.86 dd, J = 8.2, 5.3 Hz) and C-8′ (δ_C 159.8), as well as C-6′ (δ_C 146.9). Further expansion of the structure was made possible by noting that H-12 at δ_H 2.45 m (δ_C 28.2) was coupled to H-11 at δ_H 4.21 d, J =9.3 Hz (δ_C 69.4) and to two methyl doublets at δ_H 1.16 d, J =6.7 Hz (δ_C 18.7) and δ_H 1.12 d, J =6.7 Hz (δ_C 20.2). The HMBC spectrum (Table 20) showed cross-peaks between the H-11 signal (δ_H 4.21 d, J = 9.3 Hz) and the signals of the carbonyls at δ_C 164.9 (C-7), 173.9 (C-10) and the methine carbon at δ_C 83.8 (C-5a). These correlations suggested that C-11 was connected to a nitrogen atom of the pyridone ring as well as to the carbonyl at δ_C 173.9. In turn, the carbonyl at δ_C 173.9 (C-10) should be on N-5 of the indole ring, thus leading to structure 5. The coupling constants between H-8 and H-9*α* ($J_{8,9\alpha}$ = 8.2) and between H-8 and H-9*β* ($J_{8,9\beta}$ = 5.3 Hz) suggested that the piperidone ring was in a twist-boat conformation. The NOESY spectrum (Table 20) showed that H-5a (δ_H 5.82 s) exhibited strong correlations with H-12 (δ_H 2.45 m), CH_3-13 (δ_H 1.12 d, J = 6.7 Hz), CH_3-14 (δ_H 1.16 d, J = 6.7 Hz), H-6′ (δ_H 8.31 s), and OH-9a (δ_H 6.30 s) but not with H-8 (δ_H 4.86 dd, J = 8.2, 5.3 Hz). In turn, the signal of OH-9a (δ_H 6.30 s) gave cross-peaks to the signals of H-5a (δ_H 5.82 s), H-1 (δ_H 7.55 d, J = 7.8 Hz), and H-9*α* (δ_H 3.01 dd, J = 13.9, 8.2 Hz). These correlations placed H-5a in the *α* orientation and H-8, H-11 in the *β* position, and since H-8 (δ_H 4.86 dd, J = 8.2, 5.3 Hz) showed correlation with only the proton signal at δ_H 2.77 dd (J = 13.9, 5.3 Hz) but not with that at δ_H 3.01 dd, J = 13.9, 8.2 Hz), the former was assigned to H-9*β*. Therefore, the stereochemistry of 5 was the one shown. The structure of 5 resembles that of chaetominine, a cytotoxic alkaloid produced by endophytic Chaetomium sp. IFBE015 (Jiao *et al.*, 2006). However, the configuration of C-8 and C-11 of 5 is opposite to that of the corresponding carbons of chaetominine. This was also supported by the fact that, contrary to chaetominine, the chemical shift value of H-9*α* (δ_H 3.01 dd, J = 13.9, 8.2 Hz) is higher than that of H-9*β* (2.77dd, J = 13.9, 5.3 Hz), suggesting that the former was deshielded by the carbonyl group (C-8′) of the quinazolinone system. Thus, 5 is a new compound which we have named

pseudofischerine and was presumably biosynthesized from L-tryptophan, anthranilic acid, and D-valine.

The remaining new constituent, mp 232°C, displayed an ion peak at *m/z* 275.0919 (ASAP-HRMS) corresponding to $C_{15}H_{15}O_5$, consistent with the $[M + H]^+$. A positive $FeCl_3$ test showed that it was a phenol. The IR spectrum showed absorption bands for hydroxyl (3446 cm^{-1}), aromatic (1601 cm^{-1}), and carbonyl (1619 cm^{-1}) groups. The ^1H NMR spectrum exhibited signals of a phenolic hydroxyl group at δ_H 9.58 brs, five aromatic protons at δ_H 6.43 brs, 6.34 d (J = 2.2 Hz), 6.32 brs, 6.24 dd (J = 2.2, 2.2 Hz), 6.21d (J = 2.2 Hz), and two methyls at δ_H 2.21 s and 2.38 s. The ^{13}C NMR, DEPT, and HSQC spectra exhibited, in addition to an ester carbonyl carbon at δ_C 177.8, the signals of seven quaternary sp^2 carbons (δ_C 161.8, 160.1, 158.6, 156.0, 141.7, 140.5, 111.3), five methine sp^2 carbons (δ_C 112.3, 111.5, 111.0, 104.0, 102.6), and two methyls at δ_C 22.2 and 21.1. The COSY spectrum showed cross-peaks between the broad singlet of the aromatic proton at δ_H 6.43 and the aromatic double doublet at δ_H 6.24 (J = 2.2, 2.2 Hz) as well as with the singlet of the methyl group at δ_H 2.21. In turn, the proton associated with the signal at δ_H 6.24 dd (J = 2.2, 2.2 Hz) showed a cross-peak with a broad singlet at δ_H 6.32. This system suggested the presence of a 5-methylresorcinol unit. Similarly, the doublet at δ_H 6.34 (J = 2.2 Hz) was correlated to another doublet at δ_H 6.21 (J = 2.2 Hz) as well as to the methyl singlet at δ_H 2.38. Also the HMBC spectrum displayed correlations between the signals of the methyl protons at δ_H 2.38 s and of the carbons at δ_C 141.7, 111.3, and 111.5, which confirmed that the carbonyl and this methyl group were vicinal and suggested that the second half of the substance was a 2, 4-dihydroxy-6-methylbenzoyl moiety. Combination of the two partial structures then led to structure 7 or 3′-hydroxy-5′-methylphenyl-2, 4-dihydroxy-6-methylbenzoate.

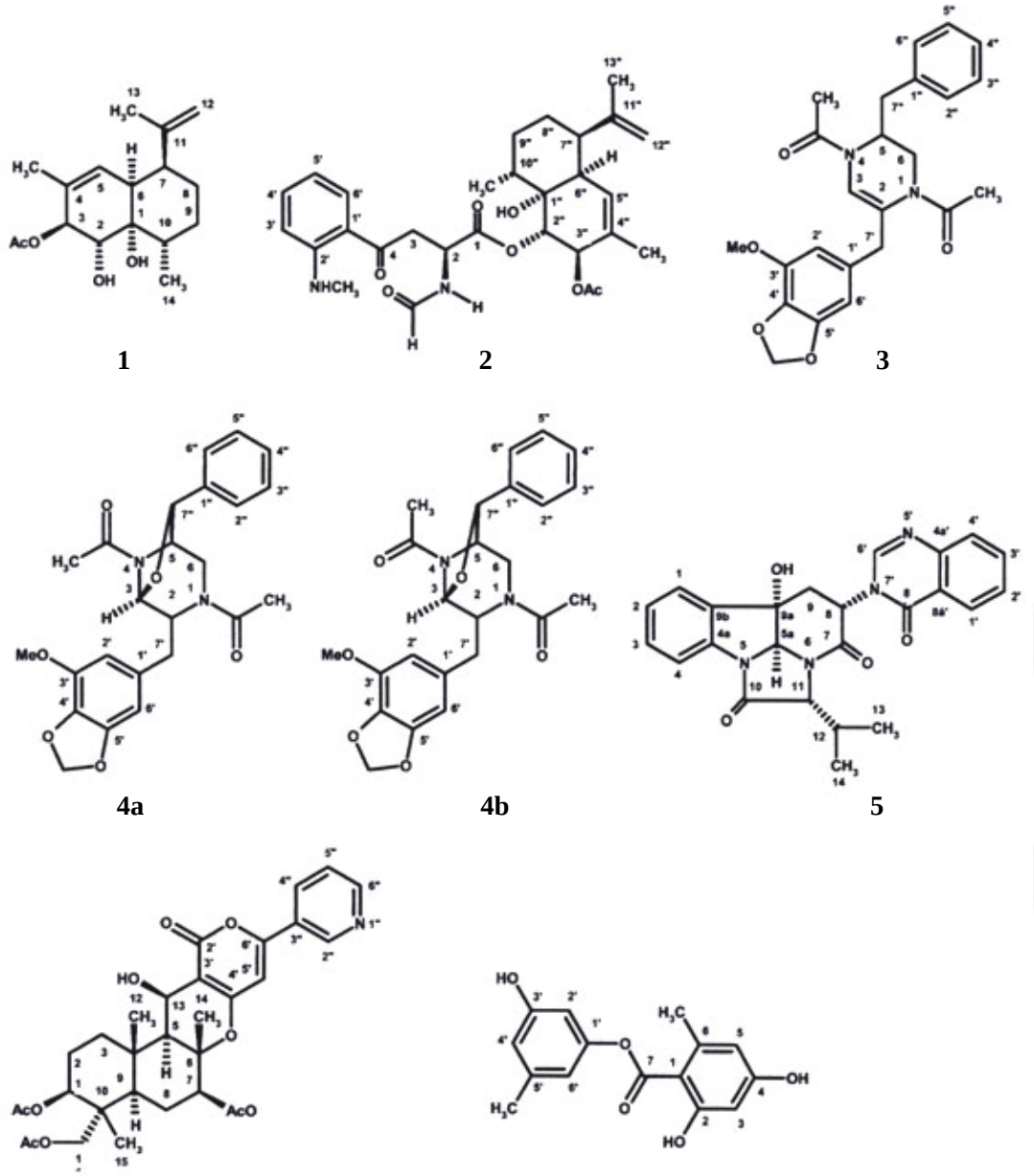


Figure 66 Constituents of *Neosartorya pseudofischeri* (KUFC6422)
1 = cadinene, 2 = eurochevalierine, 3 = brasiliamide B, 4a, 4b = 3,8-diacetyl-4-(3-methoxy-4,5-methylenedioxy)benzyl-7-phenyl6-oxa-3,8-diazabicyclo[3.2.1]octane, 5 = pseudofischerine, 6 = pyripyropene A, and 2,4-dihydroxy-6-methylbenzoic acid

5.2 *In vitro* cytostatic activity in human cancer cells by secondary metabolites of *Neosartorya pseufischeri* KUFC 6422

5.2.1 Determination of *in vitro* growth-inhibitory activity by the MTT colorimetric assay

The *in vitro* growth inhibitory activities of cadinene**,** eurochevalierine, 1,4-diacetyl-2,5-dibenzylpiperazine, pseudofischerine , pyripyropene A, and 2,4-dihydroxy-6-methylbenzoic acid (purity >95%) have been compared to two reference compounds, etoposide (Pommier *et al.*, 2010) and carboplatin (Stinchcombe *et al.*, 2010) that are cytotoxic pro-apoptotic compounds and widely used to treat cancer patients. The data in Table 21 illustrate that eurochevalierine displayed *in vitro* anticancer activity in the range displayed by etoposide and carboplatin, whereas cadinene exhibited less activity than eurochevalierine and similar activity to that of carboplatin. Compounds 1,4-diacetyl-2,5-dibenzylpiperazine, pseudofischerine , pyripyropene A, and 2,4-dihydroxy-6-methylbenzoic acid were found to be inactive in all cell lines at the highest concentration tested.

5.2.2 Computer-assisted phase contrast videomicroscopy (quantitative videomicroscopy)

Computer-assisted phase-contrast microscopy demonstrated that 2 displayed cytostatic, not cytotoxic, effects in human U373 glioblastoma (GBM) and A549 non-small cell lung cancer (NSCLC) cells (Figure 67). Both U373 GBM (Lefranc *et al.*, 2005b) and A549 NSCLC (Mijatovic *et al.*, 2006) cells are resistant to pro-apoptotic stimuli, and thus to conventional chemotherapies and radiotherapies. U373 GBM cells are sensitive to sustained pro-autophagic stimuli (Lefranc *et al.*, 2008), whereas A549 NSCLC cells are not (Mijatovic *et al.*, 2006). We have chosen these two models because U373 GBM (Lefranc *et al.*, 2005a) and A549 NSCLC (Mathieu *et al.*, 2004) best mimic the biological aggressiveness and resistance to conventional chemotherapeutics of cancers seen in the clinic.

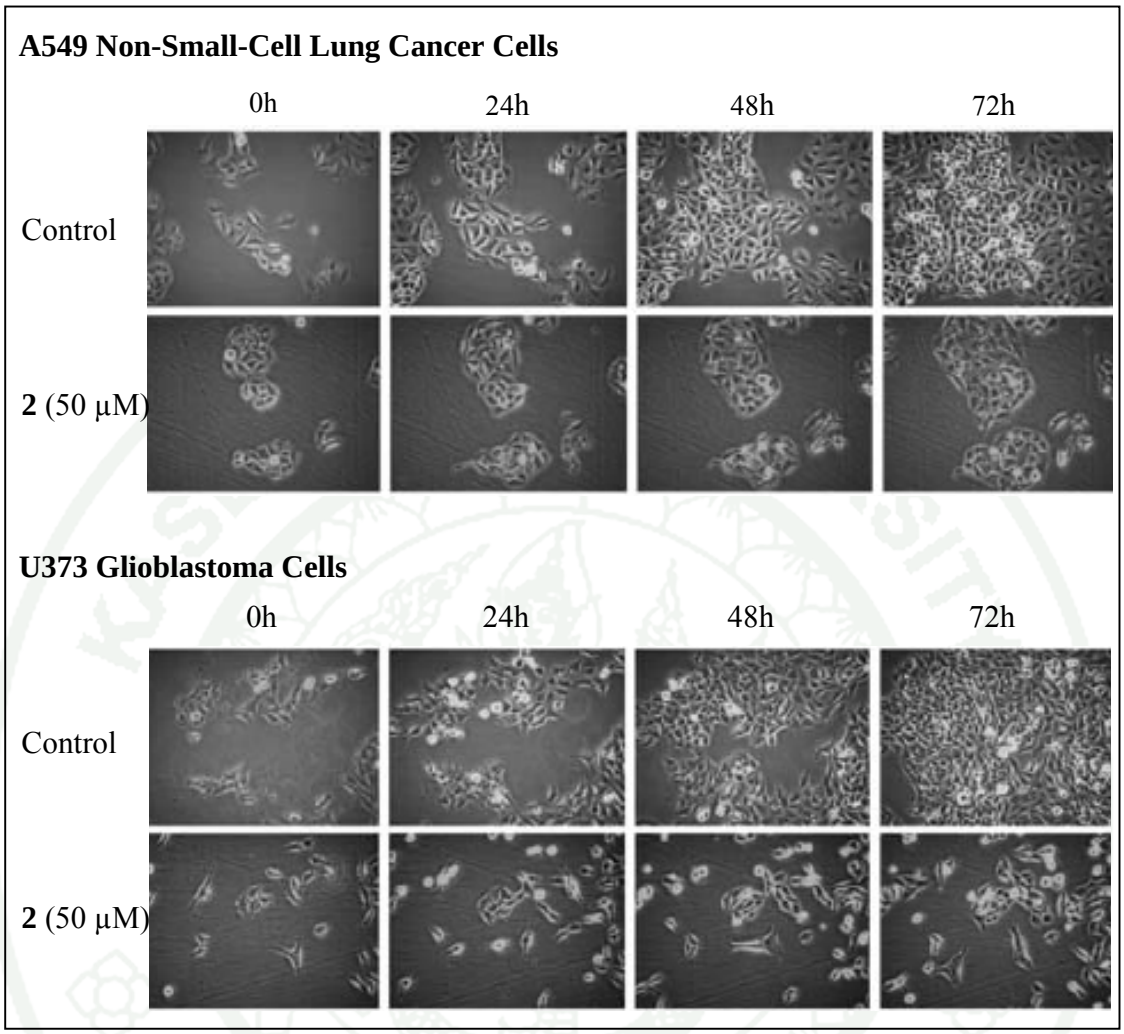


Figure 67 Morphological illustrations (x100) of A549 NSCLC (upper panel) and U373 GBM (lower panel) cell growth when untreated (control) or treated with 50 μM of chevalierine (**2**). Digitized images have been recorded for 24, 48, and 72 h post-treatment with computer-assisted phase-contrast microscopy (quantitative videomicroscopy).

The global growth analysis in control and 2-treated conditions, which showed that 50 μM of 2 reduced the growth of U373 GBM cells by 65% and reduced the growth of A549 NSCLC cells by 50% over a 72-h period of observation (Figure 67), confirmed the MTT colorimetric assay-related data (Table 20). The MTT colorimetric

assay indirectly determines the growth rate of a given cell population, whereas quantitative videomicroscopy analysis directly determines the growth rate.

5.2.3 Flow cytometry determination of apoptosis and cell cycle kinetics

Our data demonstrate that chevalierine (2) is cytostatic (Figure 67) and is able to overcome the intrinsic resistance of human GBM (U373) and NSCLC (A549) cells to pro-apoptotic stimuli. This intrinsic resistance to apoptosis, which gives rise to an unresponsiveness to a large majority of currently available anticancer apoptosis-inducing drugs, results in tumor metastases that account for more than 90% of cancer patient deaths (Simpson *et al.*, 2008). Many cancer types such as NSCLCs (Denlinger *et al.*, 2004), gliomas (Lefranc *et al.*, 2005a), pancreatic cancers (El Maalouf *et al.*, 2009), melanomas (Soengas and Lowe, 2003), and esophageal cancers display intrinsic resistance to proapoptotic stimuli before metastasizing (D'Amico and Harpole, 2000).

Table 21 Determination of the *in vitro* growth inhibitory activity of compound 1, 2, 4-8 and of two reference compounds, i.e. etoposide and carboplatin in six human cancer cell lines.

Compounds	IC50 in vitro growth inhibitory concentrations (μM)			
	Hs683	U373	A549	MCF-7
Etoposide	4 ± 2	0.4 ± 0.1	4.2 ± 0.7	-
Carboplatin	46 ± 6	28 ± 3	54 ± 4	-
1*	52 ±2	82 ± 2	44 ± 1	68 ± 2
2*	33 ± 1	31 ± 1	28 ± 1	25 ± 1
4*	> 100	> 100	> 100	> 100
5*	> 100	> 100	> 100	> 100
6*	> 100	> 100	> 100	91
7*	> 100	> 100	> 100	> 100

Table 21 (Continued)

Compounds	IC50 in vitro growth inhibitory concentrations (μM)				
	OE21	SKMEL28	Min-max	Median	Mean ± SEM
Etoposide	2.6 ± 0.4	1.8 ± 0.1	0.4-4.2	2.6	2.6 ± 0.7
Carboplatin	31 ± 4	69 ± 6	28-69	46	45.6 ± 7.7
1	-	56 ± 2	44-82	56	60.4 ± 1.6
2	24 ± 1	24 ± 1	24-33	28	27.5 ± 1.6
4	-	> 100	> 100	> 100	> 100
5	-	> 100	> 100	> 100	> 100
6	> 100	> 100	91-> 100	> 100	> 99
7	> 100	> 100	> 100	> 100	> 100

1* = cadinene, 2* = chevalierine, 4* = 3,8-Diacetyl-4-(3-methoxy-4,5-methylenedioxy) benzyl-7-phenyl6-oxa-3,8-diazabicyclo[3.2.1]octane, 5* = pseudofischerine, 6* = pyripyropene A and 7 = 3-Hydroxy-5-methylphenyl 2,4-dihydroxy-6-methylbenzoate

Flow cytometry analysis confirmed the lack of cytotoxicity of chevalierine because we failed to demonstrate pro-apoptotic effects with this compound in human U373 GBM and A549 NSCLC cells (Figure 68). The positive control narciclasine (Dumont *et al.*, 2007) induced marked pro-apoptotic effects in human apoptosis-sensitive PC-3 prostate cancer cells (Figure 69). Flow cytometry analysis demonstrated that chevalierine did not modify cell cycle kinetics, such as the distribution of cells into the G1, S, and G2 phases of the cell cycle of A549 NSCLC and U373 GBM cells (Figure 70). We used quantitative videomicroscopy to accurately determine the percentages of mitoses under control and 2-treated conditions while taking into account that chevalierine is cytostatic (Figure 67). Figures 71, 72 showed that treatment with chevalierine resulted in a compelling decrease in the percentages of mitotic cells in both A549 NSCLC and U373 GBM cells. Thus, the cytostatic effects induced by chevalierine relate specifically to the inhibition of mitotic rates.

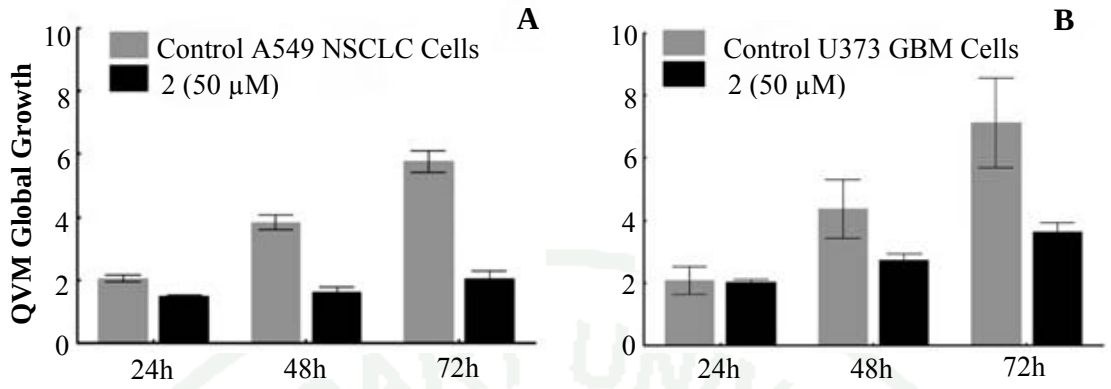
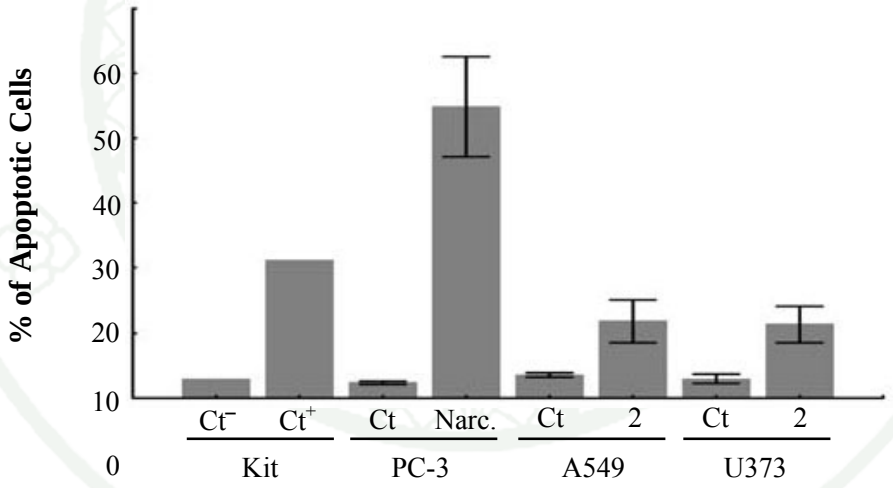


Figure 68 Determination of A549 NSCLC (**A**) and U373 GBM (**B**) growth rates when untreated (grey bars) or treated with 50 µM of chevalierine (black bars). Each experimental condition was carried out in triplicate, and the data are presented as the mean ± SEM values.



*Ct^- = negative control, Ct^+ = positive control available in the kit were used along with 1 µM compound narciclasine-treated human PC-3 prostate cancer cells as control for this experiment.

Figure 69 Determination of the percentages of A549 NSCLC and U373 GBM cells undergoing apoptosis during 72 h of treatment with 50 µM of chevalierine**.** The data are presented as the mean ± SEM values replicated four times, except for the Ct^- and Ct^+ controls, which were performed once.

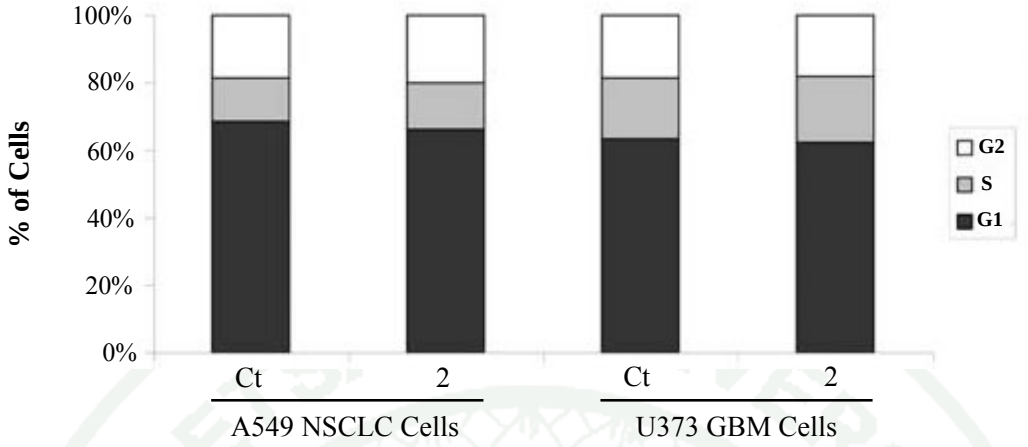


Figure 70 Determination of the percentages of U373 cells in G1 (black bars), S (grey bars), and G2 (open bars) stages of the cell cycle during 72 h of treatment with 50 µM of compound chevalierine**.** Each experimental condition was performed four times

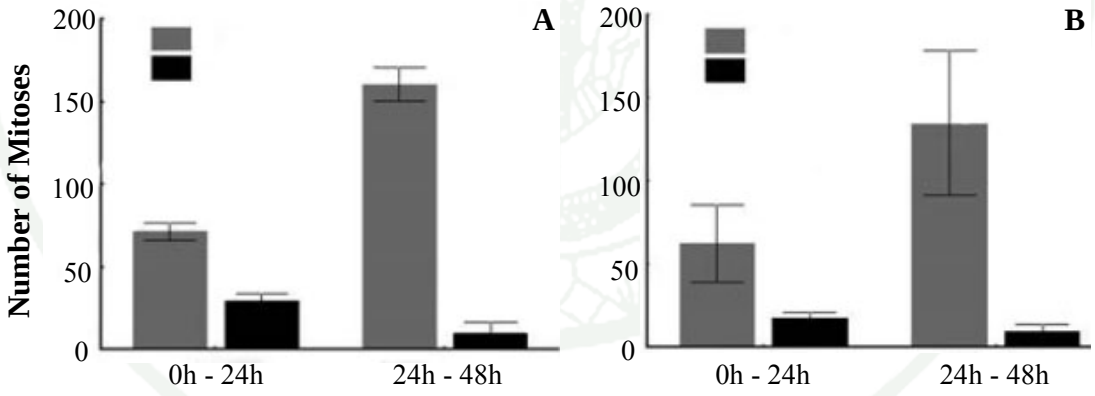


Figure 71 Determination of human A549 NSCLC (**A**) and U373 GBM (**B**) mitotic rates. The number of cells plated at the beginning of the experiments in control and chevalierine-treated cells were indentical.

Taken together, the data in this study emphasize that chevalierine represents a novel chemical scaffold from which derivatives for anticancer cytostatic compounds could be hemisynthesized to combat apoptosis-resistant cancer cells. Compounds of natural origin, such as roscovitine that display marked cytostatic activity and such as eurochevalierine, have entered phase II clinical trials for aggressive cancers (Krystof and Uldrijan, 2010).

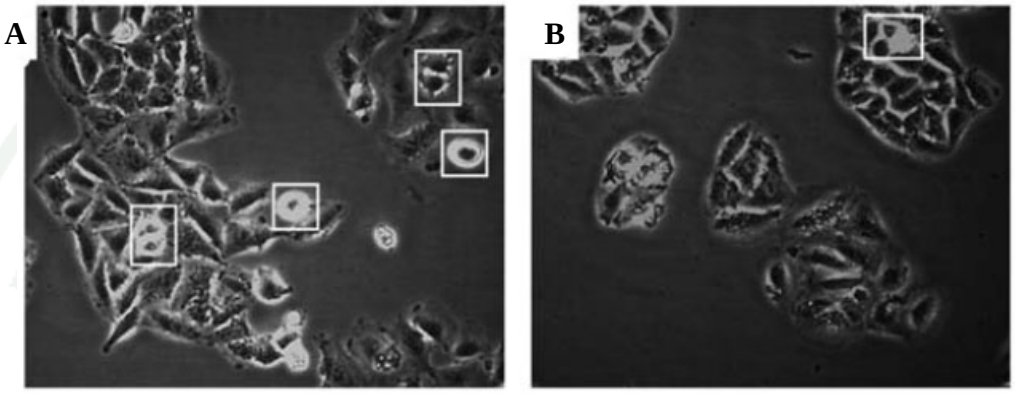


Figure 72 The mitotic rate in untreated A549 NSCLC cells (**A**). The mitotic rate in A549 NSCLC cells treated with 50 μM of chevalierine for 48 hrs. The white rectangles indentify mitoses (**B**).

CONCLUSION

Two hundred and forty-eight isolates of the genus *Neosartorya* comprising seven known species, one new species and three unidentified species were found from two hundred and forty-nine soil samples collected from 16 provinces in Thailand. Seventy-nine isolates of *Neosartorya spinosa* were most frequently occurred in 11 provinces followed by *N. glabra* (45)*, N. siamensis* sp. nov. (45), *N. fischeri* (41)*, N. tatenoi* (26)*, N. laciniosa* (5)*, N. takakii* (3) and one isolate each of *N. pseudofischeri, Neosartorya* sp.1 (KUFC6341), *Neosartorya* sp.2 (KUFC6513) and *Neosartorya* sp.3 (KUFC6579), were found.

Neosartorya siamensis Manoch & Eamvijarn sp. nov. was a new finding for the genus *Neosartorya*, whereas *N. laciniosa* and *N. pseudofischeri* were new records to Thailand. Based on morphological characteristics, three unidentified species comprising *Neosartorya* sp.1 (KUFC6341), *Neosartorya* sp.2 (KUFC6513), *Neosartorya* sp.3 (KUFC6579) might be new species.

DNA sequences of the β-tubulin gene of the strains KUFC 6331, 6341, 6342, 6344, 6349, 6377, 6397, 6412, 6422, and 6513 were obtained and new sequences have been deposited in the DNA Data Bank of Japan (DDBJ). Sequence comparison of the KUFC strains based on the β-tubulin gene was done with BLAST search (http://www.ncbi. nlm.nih.gov). As a result, these strains were found to be related with the species of *Neosartorya* and *Aspergillus* section *Fumigati* by NJ analysis. Four *Neosartorya* strains were identified as known species including, *N. fischeri* (KUFC 6344), *N. pseudofischeri* (KUFC 6422), *N. spinosa* (KUFC 6342), *N. tatenoi* (KUFC 6377). The strains KUFC 6331, 6349, and 6412 were clearly separated and had similarities of 96.0-96.9% at the β-tubulin gene sequence and with the evidence from morphology and these phylogenetic analyses, these strains should be considered a distinct new species. The sequence data of the strain *Neosartorya* sp.2 (KUFC 6341) was not identical to those of known *Neosartorya* spp. and clearly separated and had similarities of 93.7 and 94.5 % at the β-tubulin gene sequence.

In vitro antagonistic activities test of *Neosartorya* spp. against 9 species of plant pathogenic fungi as dual culture indicated that *Neosartorya fischeri* (KUFC 6344) inhibited 53.9-58.3% of radial growth of *Alternaria brassicicola, Colletotrichum capsici, Curvularia oryzae* and *Fusarium oxysporum,* whereas *N. tatenoi* (KUFC 6377) showed highly inhibition of 62.2 and 51.1% mycelial growth of *Phytophthora palmivora* and *Pythium aphanidermatum*. However, all strains of *Neosartorya* failed to inhibit mycelial growth of *Lasiodiplodia theobromae*, *Rhizoctonia oryzae* and *Sclerotium rolfsii.* For the efficacy of six *Neosartorya* spp. crude extracts revealed that crude extract of *N. fischeri* at 1,000 ppm completely inhibited mycelial growth of *Pythium aphanidermatum, Phytophthora palmivora and S. rolfsii*, whereas crude extracts of six *Neosartorya* species at 10,000 ppm strongly suppressed 100% mycelial growth of eight species of plant pathogenic fungi but failed to control *Lasiodiplodia theobromae*.

For inhibitory activity of *Neosartorya* crude extracts at 10,000 ppm on weed germination and growth, *N. pseudofischeri* (KUFC6422) crude extract showed the strongest germination and growth inhibition (91.6% and 81.0%) on *Phaseolus pathyroides*, whereas crude extract of *N. siamensis* sp. nov. (KUFC6349) at 10,000 ppm inhibited germination and growth on *Mimosa pigra* and *Corchorus* sp. (98.7%, 86.3% and 100%, 99.3), respectively.

For secondary metabolites investigation, four known compounds including cadinene , eurochevalierine, brasiliamide B, and pyripyropene A and three other new metabolites including the 1,4-diacetyl-2,5-dibenzylpiperazine 3,7′′-oxide, pseudofischerine, and a previously unreported ester of 2,4 dihydroxy-6-methylbenzoic acid were isolated from the fungus *Neosartorya pseudofischeri* (KUFC 6422). The *in vitro* growth inhibitory activity of cadinene, eurochevalierine, pyripyropene A, 1,4-diacetyl-2,5-dibenzylpiperazine 3,7′′-oxide, pseudofischerine and 2,4 dihydroxy-6-methylbenzoic acid have been compared to two reference compounds, etoposide and carboplatin. The result showed that eurochevalierine displayed growth inhibitory activity similar to carboplatin followed by cadinene, whereas pyripyropene A, 1,4-diacetyl-2,5-dibenzylpiperazine 3,7′′-oxide, pseudofischerine and 2,4 dihydroxy-6-methylbenzoic acid were found to be inactive in six cancer cell lines.

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APPENDIX

Culture media for isolating fungi

1. Gauchnaur's glucose ammonium nitrate agar (GAN)

NH_4NO_3	1.0	g
K_2HPO_4	1.0	g
$MgSO_4.7H_2O$	0.5	g
Rose Bengal	0.03	g
Yeast extract	1.0	g
Glucose	5.0	g
Agar	15.0	g
Streptomycin solution*	30.0	ppm
Distilled water	1,000	ml

* Add after media autoclaved and decrease temperature for 45-50 ˚C

2. Water agar (0.2% WA)

Agar	20.0	g
Distilled water	1,000	ml

Culture media for cultivating fungi

1. Potato Dextrose Agar (PDA)

Potato	200.0	g
Dextrose	20.0	g
Agar	15.0	g
Distilled water	1,000	ml

2. Potato Dextrose Broth (PDB)

Potato	200.0	g
Dextrose	20.0	g
Distilled water	1,000	ml

3. Czapek-Dox Agar (CZA)

Czapek Solution Agar (Difco™)	49.0	g
Distilled water	1,000	ml

4. Malt Extract Agar (MEA)

Malt Extract Agar (Difco™)	33.6	g
Distilled water	1,000	ml

5. Czapek Yeast Autolysate Agar (CYA)

$NaNO_3$	3.0	g
K_2HPO_4	1.0	g
KCl	0.5	g
$MgSO_4.7\ H_2O$	0.5	g
$FeSO_4.7\ H_2O$	0.01	g
Yeast extract	5.0	g
Sucrose	30.0	g
Agar	15.0	g
Distilled water	1,000	ml

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