DIVERSITY OF FUNGI ASSOCIATED WITH FALLEN DEAD SPADIX OF *Cocos nucifera* L.

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Abstract

Present paper deals with the occurrence and diversity of the fungi on coconut spadix. A total of 17 species of fungi was found to be associated with the fallen dead spadix of *Cocos nucifera* L. (coconut) during a tenure from 2010 to 2016. The associated fungi were *Aspergillus flavus* Link., *A. fumigatus* Fresenius, *A. niger* Van Tieghem, *A. terreus* Thom, *Cladosporium cladosporioides* (Fresen.) de Vries, *C. elatum* (Harz) Nannf., *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., *Fusarium* Link., *Lasiodiplodia theobromae* Pat., *Pestalotiopsis* Pat., *Stachybotrys* Corda., *Tetraploa aristata* Berk. & Br., *Torula* Pers. *Trichoderma* Pers., *Zygosporium minus* Hughes and two unidentified species. Association of *Stachybotrys* sp., *Tetraploa aristata*, *Torula* sp. and *Zygosporium minus* with coconut spadix is a new record.

Key words: Biodiversity, *Cocos nucifera*, fallen, fungi, spadix.

INTRODUCTION

Fungi are important heterotrophic organisms and so distinct from the plants and animals that they have been allotted a 'kingdom' of their own in our classifications of living organism. Fungi are tremendously important to human society and the planet we live on. They provide fundamental products including foods, medicines, and enzymes important to industry. They are also the unsung heroes of nearly all terrestrial ecosystems, hidden from view but inseparable from the processes that sustain life on the planet. Knowledge of the diversity of fungi is essential for anyone collecting and/or monitoring any fungus. Fascinating and beautiful, fungi are vital components of nearly all ecosystems and impact human health and our economy in a myriad of ways. Standardized methods for documenting diversity and distribution have been lacking. A wealth of information, especially regarding sampling protocols, compiled by an international team of fungal biologists, make the diversity of fungi an incredible and fundamental resource for the study of organismal biodiversity (Mueller *et al*. 2004). Present research has been carried out on the fungal diversity of the fallen dead spadix of *Cocos nucifera*.

Cocos nucifera is a member of the family Arecaceae. It is grown throughout the tropics for decoration, as well as for its many culinary and non-culinary uses; virtually every part of the coconut tree can be used by humans in some manner and has significant economic value. Per 100 gram serving with 354 calories, raw coconut meat supplies a high amount of total fat (33 grams), especially saturated fat (89% of total fat) and carbohydrates (24 grams) (USDA 2015). Micronutrients in significant content include the dietary minerals, manganese, iron, phosphorus and zinc. Coconut milk has a total fat content of 24%, most of which (89%) is saturated fat, with lauric acid as a major fatty acid (USDA 2015).

During storage the food commodities are spoiled by biotic and abiotic agents. Among the microorganisms fungi play a significant role in deteriorating the aesthetic and nutritive value of stored food commodity (Christensen and Kaufmann 1965, Neergard 1977). Under storage conditions coconut is susceptible to attack by fungi, insects and other microorganisms because they have a rich source of stored nutrients conducive to the growth of numerous fungi.

The diseases of coconut palms are well-documented (Onuegbu 2002). Anthracnose, bitten leaf, black scorch, bud rot, butt rot, damping-off, die back, dry basal rot, leaf blight, leaf break, leaf rot, leaf spot, nut fall, powdery mildew, root rot, root wilt, shoot rot, stem bleeding, stump rot, thread blight are common diseases of *C. nucifera*. But, little information is available on the fungi of spadix and their deteriorative ability. Chuku *et al*. (2007) reported that most of the fungi of coconut spadix are seed borne. Bangladesh exports coconut (Anonymous 2016) and since coconut needs to be preserved in good quality for storage purposes it is important to identify the fungi which are associated with the deterioration process. The reason for this study is to enrich the mycofloral study in Bangladesh as well as to highlight the fungal diversity in a specified habitat that is the fallen spadix of *C. nucifera*.

MATERIAL AND METHODS

The fallen dead spadix of coconut tree was collected from the Botanical Garden of the Curzon Hall campus of Dhaka University, Dhaka and Mohakhali DOHS area, Dhaka during the tenure from the year 2010 to 2016 to record the occurrence and diversity of fungi. A total of 30 samples was examined in search of fungi. Samples were collected in separate sterile polyethylene bags, labeled properly and then brought to the laboratory for isolating associated fungi following "Tissue planting method" on PDA medium. From the fallen spadix of coconut showing fungal growth, thirty inocula each measuring 2 mm^2 sized were cut separately with a pair of sterilized scissors and kept in a separate sterilized Petri plate. The inocula were washed with sterile water and then surface sterilized by dipping in 10% chlorox solution for three minutes. The inocula were again washed with sterile water. A total of 30 inocula was placed separately on 10 sterilized Petri plates containing 15 ml of PDA medium with an addition of one drop (ca 0.03 ml) of lactic acid to check the bacterial growth and incubated in an incubator (25±2˚C) for seven days. Fungal population growing from the plated inocula was sub-cultured and maintained on PDA slants. Percentages of the fungi were calculated by the following formula:

$$\text{Percentage of infected inocula} = \frac{\text{Number of inocula infected}}{\text{Total number of inocula}} \times 100$$

Morphological studies of the fungal isolates were made in order to determine their identity. The microscopic structural view of the fungi was taken with a high resolution digital camera. Conidia and conidiophores of the important fungi were drawn with the aid of a Camera Lucida. Identification of the isolates was determined following standard literatures (Barnett and Hunter 2000, Booth 1971, Ellis 1971, 1976, Sutton 1980).

RESULTS AND DISCUSSION

A total of 17 species of fungi was found to be associated with the fallen dead spadix of *Cocos nucifera* during the tenure from 2010 to 2016. The associated fungi were *Aspergillus flavus* Link., *A. fumigatus* Fresenius, *A. niger* Van Tieghem, *A. terreus* Thom, *Cladosporium cladosporioides* (Fresen.) de Vries, *C. elatum* (Harz) Nannf., *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., *Fusarium* Link., *Lasiodiplodia theobromae* Pat., *Pestalotiopsis* Pat., *Stachybotrys* Corda., *Tetraploa aristata* Berk. & Br., *Torula* Pers. *Trichoderma* Pers., *Zygosporium minus* Hughes and two unidentified species (Fig. 1-2).

Table 1 shows that six species of fungi, viz. *A. flavus*, *A. fumigatus*, *A. niger*, *C. cladosporioides*, *C. elatum* and *Stachybotrys* sp. were associated with the fallen dead spadix of coconut in 2010. Among them, *A. niger* was predominant. In 2011, six species of fungi, namely *A. fumigatus*, *A. niger*, *C. cladosporioides*, *C. elatum*, *Fusarium* sp., and *Trichoderma* sp. were recorded while in 2012, four species of fungi, namely *C. cladosporioides*, *C. elatum*, *Fusarium* sp., and *L. theobromae* were recorded. Five species of fungi namely, *A. flavus*, *A. niger*, *C. elatum*, *Fusarium* sp., and *Trichoderma* sp. were recorded on coconut spadix in 2013. In case of *A. niger* and *C. elatum* moderately developed fruiting structures were found.

In 2014, seven species of fungi, namely *A. flavus*, *A. fumigatus*, *A. niger*, *C. cladosporioides*, *C. elatum*, *Fusarium* sp. and *Stachybotrys* sp. were recorded. Among the fungi, fruiting structure of *A.*

niger, *C. cladosporioides* and *Fusarium* sp. were moderately found. During 2015, nine species of fungi namely *A. flavus*, *A. terreus*, *C. cladosporioides*, *Fusarium* sp., *L. theobromae*, *Pestalotiopsis* sp., *Stachybotrys* sp., *T. aristata* and *Trichoderma* sp. were recorded. *Stachybotrys* sp. and *Trichoderma* sp. showed abundant fruiting structures. *Pestalotiopsis* sp. and *T. aristata* were not found in previous years. In 2016, maximum eleven species of fungi, namely *A. flavus*, *C. cladosporioides*, *C. elatum*, *Colletotrichum gloeosporioides*, *L. theobromae*, *Stachybotrys* sp., *Torula* sp., *Trichoderma* sp., *Zygosporium minus* and two unidentified species of fungi were recorded. Among the fungi, *Stachybotrys* sp. and *Trichoderma* sp. showed abundant fruiting structures. *Colletotrichum gloeosporioides*, *Torula* sp. and *Zygosporium minus* were exclusively found in the year 2016.

Table 1. Occurrence of fungi on the fallen dead spadix of *Cocos nucifera* during the years 2010-2016.

Name of fungi	Year						
	2010	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**
Aspergillus flavus	+	-	-	+	+	+	+
A. fumigatus	+	+	-	-	+	-	-
A. niger	++	+	-	++	++	-	-
A. terreus	-	-	-	-	-	+	-
Cladosporium cladosporioides	+	+	+	-	++	+	+
C. elatum	+	+	+	++	+	-	+
Colletotrichum gloeosporioides	-	-	-	-	-	-	+
Fusarium sp.	-	+	+	+	++	++	-
Lasiodiplodia theobromae	-	-	+	-	-	+	+
Pestalotiopsis sp.	-	-	-	-	-	+	-
Stachybotrys sp.	+	-	-	-	+	+++	+++
Tetraploa aristata	-	-	-	-	-	+	-
Torula sp.	-	-	-	-	-	-	+
Trichoderma sp.	-	+	-	+	-	+++	+++
Zygosporium minus	-	-	-	-	-	-	+
Unidentified species 1	-	-	-	-	-	-	+
Unidentified species 2	-	-	-	-	-	-	+

'–' = no fungal spore, + = fruiting structure present, ++ = fruiting structures developed moderately, +++ = fruiting structure abundant.

From India Mukerji and Bhasin (1986) reported the leaf spot of coconut caused by *Alternaria alternata* (Fr.) Keissler (= *A. tenuis* Nees), *Ascochyta cocoina* Cz. Frag., *Colletotrichum* sp. [State of *Glomerella cingulata* (Stonem.) Spauld. & Schrenk.], *Curvularia lunata* (Wakker) Boedijn, *Diplodia epicoccos* Cooke, *Exoxporium palmivorum* Sacc., *Gloeosporium* sp., *Pestalotiopsis palmarum* (Cooke) Stey., *Phomopsis cocoes* Petch., *Phyllostica cocos* Cooke, *Physalospora transversalis* Syd. H. & P. & Butler, *Pleospora bataanensis* Petrak; the leaf rot caused by *Drechslera halodes* (Drechsler) Jain & Subram.; the powdery mildew caused by *Oidium cococarpum* Stev. & Pierce; anthracnose caused by *Colletotrichum paucisetum* Petch.; bud rot caused by *Phytophthora palmivora* Butler; the kernel rot caused by *Penicillium frequentas* West.; the root rot caused by *Botrydiplodia theobromae* Pat.; *Ganoderma lucidum* (Leyss.) Karst.; the root wilt caused by *Cylindrocarpon effusum* Wr., *Fusarium equiseti* (Cda.) Sacc.; the stump rot caused by *Daedalea flavida* Lev.; *Fomes senex* Nees & Mont.; *Macrophomina phaseolina* (Tassi) Goid.; *Polyporus ostreiformis* Berk.; *Trametes cubensis* (Mont.) Sacc.; the stem bledding caused by *Ceratostomella paradoxa* (de Seynes) Dade. They also reported *Hendersonia palmigena* Ponnappa, *Mycosphaerella gastonis* (Sacc.) Lindau on leaves, *Ciliochorella* *indica* Kalani on dying leaves, *Phytophthora arecae* (Colem.) Pethybridge on nuts and infloresence. *Phytophthora colocasiae* Racib. Emend Thomes & Ramakr. on nuts, *Sphaeropsis palmarum* Cooke on petiols and *Vectria bulbophylli* P. Henn. on trunk.

Chuku *et al*. (2007) isolated three major fungi, *viz. A. niger*, *Penicillium italicum* and *Rhizopus stolonifer* from the endosperm of *C. nucifera*. Srivastava *et al.* (2014) reported 19 species of fungi associated with the kernels of *C. nucifera* during different seasons in India.

Dead and fallen plant parts are excellent habitat of mycoflora and most of these fungi play a significant role in the decomposition and recycling of organic matter in soil. Leaf litters are major component at the top layer of natural soils caused by fallen leaves. Leaves are chemically composed of lignocellulosic substances that have to be broken down by microorganisms to maintain the carbon cycle (Hammel 1997). Phylloplane microorganisms are chemoorganotrophic species requiring organic nutrients for growth (Dickinson 2012). Fungi play a central role in leaf litter decomposition through nutrient cycling and humus formation in soil because they colonize the lignocellulose matrix in litter that other organisms are unable to decompose. It has been described that cellulase is an adaptive enzyme in most fungi capable of degrading lignocellulosic materials and the most common carbohydrate on earth having a wide range of applications (Kjøller and Struwe 1982, Cooke and Rayner 1984).

Microbial enzymes, involved in the degradation and transformation of plant cell-wall polysaccharides, have found many biotechnological applications (De vries and Visser 2001). Since these polysaccharides are widely used in various industries, such as food and dairy, pulp and paper, textile, animal feed, pharmaceutical, detergent, cosmetic, and chemical-synthesis processes, the development of enzyme systems that break down the polysaccharides has been looked for.

Fungi are essential part of the ecosystem because they play an important role in the decomposition of organic materials such as plant residues. Fungi feed on dead organic matter and return the nutrients back into the soil. However, the occurrence of fungi growing on the leaf litters which are responsible for decomposition are not yet fully studied and documented.

Waing *et al.* (2015) reported different species of fungi present in the leaf litters of three species of forest trees, namely rain tree (*Samanea*), orchid tree (*Clitorea*) and paper tree (*Gmelina*) in Central Luzon State University (CLSU) campus. These species of forest trees are abundant in the campus and the major contributors of the fallen leaf litters. Isolated phyllosphere fungi were further evaluated for their cellulose degrading ability. These fungal species could be potentially used to hasten the decomposition of enormous leaf litters of forest trees. The isolated fungal species were screened for their ability to produce cellulase. A total of these 30 species, namely *Absidia* sp., *Aspergillus flavus*, *A. fumigatus*, *A. japonicus*, *A. niger*, *A. niveus*, *A. tamarii*, *Colletotrichum gloeosporioides*, *Curvularia lunata*, *Eurotium repens*, *Fusarium acuminatum*, *F. semitectum*, three unidentified species of *Fusarium*, *Monascus ruber*, *Mucor piriformis*, *Neosartorya fischeri*, *Penicillium chrysogenum*, *P. citrinum*, *P. decumbens*, *P. hirsutum*, *P. implicatum*, *P. olsonii*, *P. oxalicum*, *P. purpurogenum*, two unidentified species of *Penicillium*, *Rhizopus microsporus* and *Trichoderma hamatum*. Out of 30 species of phyllosphere fungi isolated, 22 can degrade cellulose as shown by the formation of clear zone around the colony of the organism. The five species of fungi that produced the largest clear zone were *P. citrinum*, *P. olsonii*, *P. purpurogenum*, *A. niveus* and *P. chrysogenum*.

Many fungal species have been reported to be associated with the decomposition of organic matter such as plant residues. Kannangara and Deshappriya (2005) reported that the species of *Acremonium*, *Alternaria*, *Aspergillus*, *Botrytis*, *Broomella*, *Cladosporium*, *Curvularia*, *Cylindrocarpon*, *Fusarium*, *Penicillium*, *Rhizopus* and *Trichoderma* were associated with the decomposition of Michelia and Semercarpus leaves. Out of these species, cellulolytic fungi were *Aspergillus*, *Fusarium*, *Alternaria* and *Penicillium*. Moreover, Manoch *et al.* (2008) identified 29 species of fungi from various fallen leaves of bamboo, jack fruit, lanthom, banana, Kaffir lime (ma-krut), star gooseberry and rose apple. These were *Arthrinium phaeospermum*, *Bipolaris maydis*, *Beltrania rhombic*, *Chaetospermum camelliae*, *Cladosporium cladosporioides*, *Colletotrichum capsici*, *Corynespora* sp., *Curvularia eragrostidis*, *Cylindrocladium* sp., *Ellisiopsis gallesiae*, *Fusarium semitectum*, *Gilmaniella humicola*, *Gyrothrix* sp., *Helicomyces* sp., *Lophodermium* sp., *Memnoniella echinata*, *Myrothecium verrucaria*, *Nigrospora sphaerica*, *P. ericonia digitata*, *Pestalotiopsis guepinii*, *Pithomyces* sp., *Pseudorobillarda* sp., *Stachybotrys nephrospora*, *S. kampalensis*, *Tetraploa aristata*, *Torula herbarum*, *Volutella concentric* and *Wiesneriomyces javanicus*

Aspergillus terreus Thom also known as *Aspergillus terrestrius*, is a fungus (mold) found worldwide in soil. Although thought to be strictly asexual until recently, *A. terreus* is now known to be capable of sexual reproduction. This saprotrophic fungus is prevalent in warmer climates, such as tropical and subtropical regions. Aside from being located in soil, *A. terreus* has also been found in habitats, such as decomposing vegetation and dust. *A. terreus* is commonly used in industry to produce important organic acids, such as aconic acid and *cis*-aconitic acid, as well as enzymes, like xylanase. It was also the initial source for the drug mevinolin (lovastatin), a drug for lowering serum cholesterol (Wikipedia 2015).

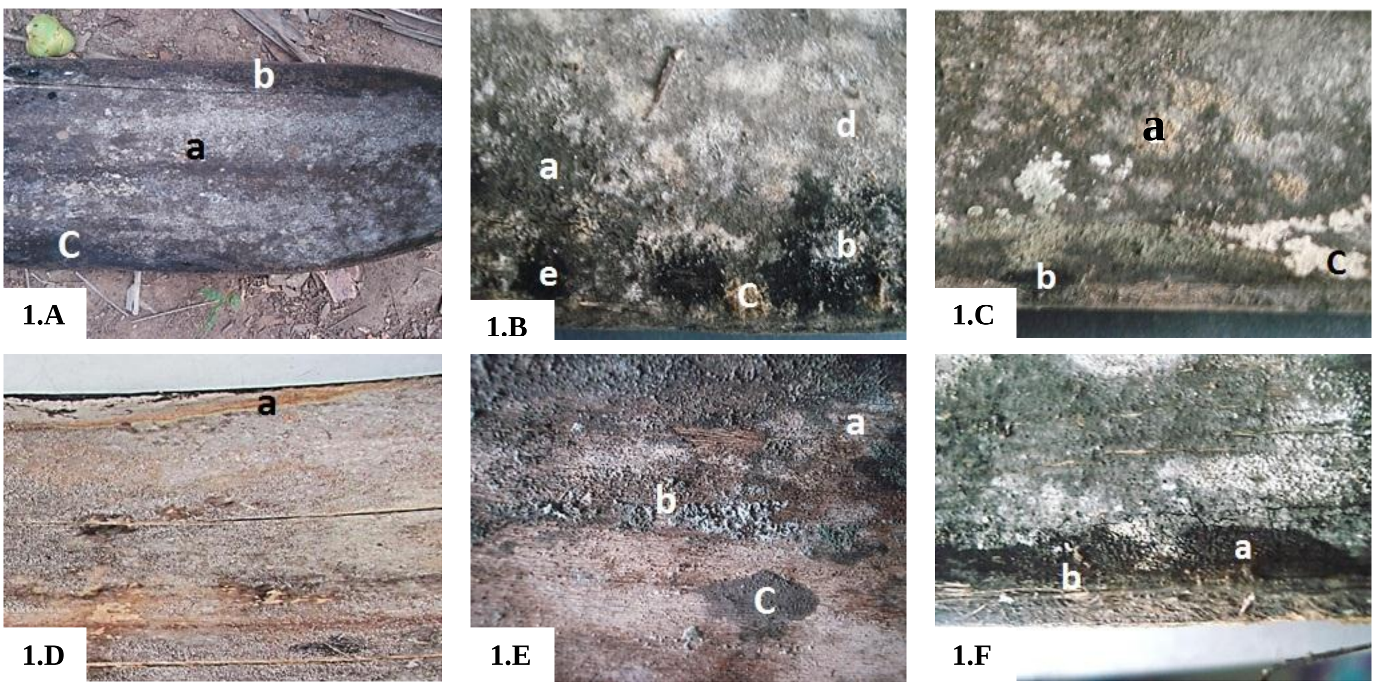

Fig. 1. **A**. Colonies of (a) *Aspergillus fumigatus*, (b) *A. niger* and (c) *Cladosporium cladosporioides;* **B**. Colonies of (a) *A. flavus*, (b) *L. theobromae* (c) *T. aristata*, (d) *Pestalotiopsis* sp. and (e) *Stachybotrys* sp.; **C**. Colonies of (a) *A. terreus*, (b) *C. elatum* and (c) *Fusarium* sp.; **D**. Colony of unidentified species no. 1(a); **E.** Colonies of (a) *C. gloeosporioides*, (b) *Trichoderma* sp. and (c) *Torula* sp.; **F.** Colonies of (a) *Zygosporium minu* and (b) unidentified species no. 2(b).

Seventeen species of fungi were found to be associated with the fallen dead spadix of *Cocos nucifera* during the tenure of present investigation. The associated fungi were *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Cladosporium cladosporioides*, *C. elatum*, *Colletotrichum gloeosporioides*, *Fusarium* sp., *Lasiodiplodia theobromae*, *Pestalotiopsis* sp., *Stachybotrys* sp., *Tetraploa aristata*, *Torula* sp., *Trichoderma* sp., *Zygosporium minus* and two unidentified species. Out of these 17 fungi, the species of *Aspergillus* and *Trichoderma* are thought to be cellulase producers and crude enzymes produced by these microorganisms are commercially available for agricultural use.

Microorganisms of the genus *Trichoderma* produce relatively large quantities of endo-ß-glucanase and exo-ß-glucanase, but only low levels of ß-glucosidase, while those of the genus *Aspergillus* produce relatively large quantities of endo-ß-glucanase and ß-glucosidase with low levels of exo-ß- glucanase production. *Colletotrichum gloeosporioides*, *Fusarium* sp., *L. theobromae*, *Pestalotiopsis* sp. and *Stachybotrys* sp. are facultative parasites having a wide host range. Rest of the fungi are saprophytic in nature.

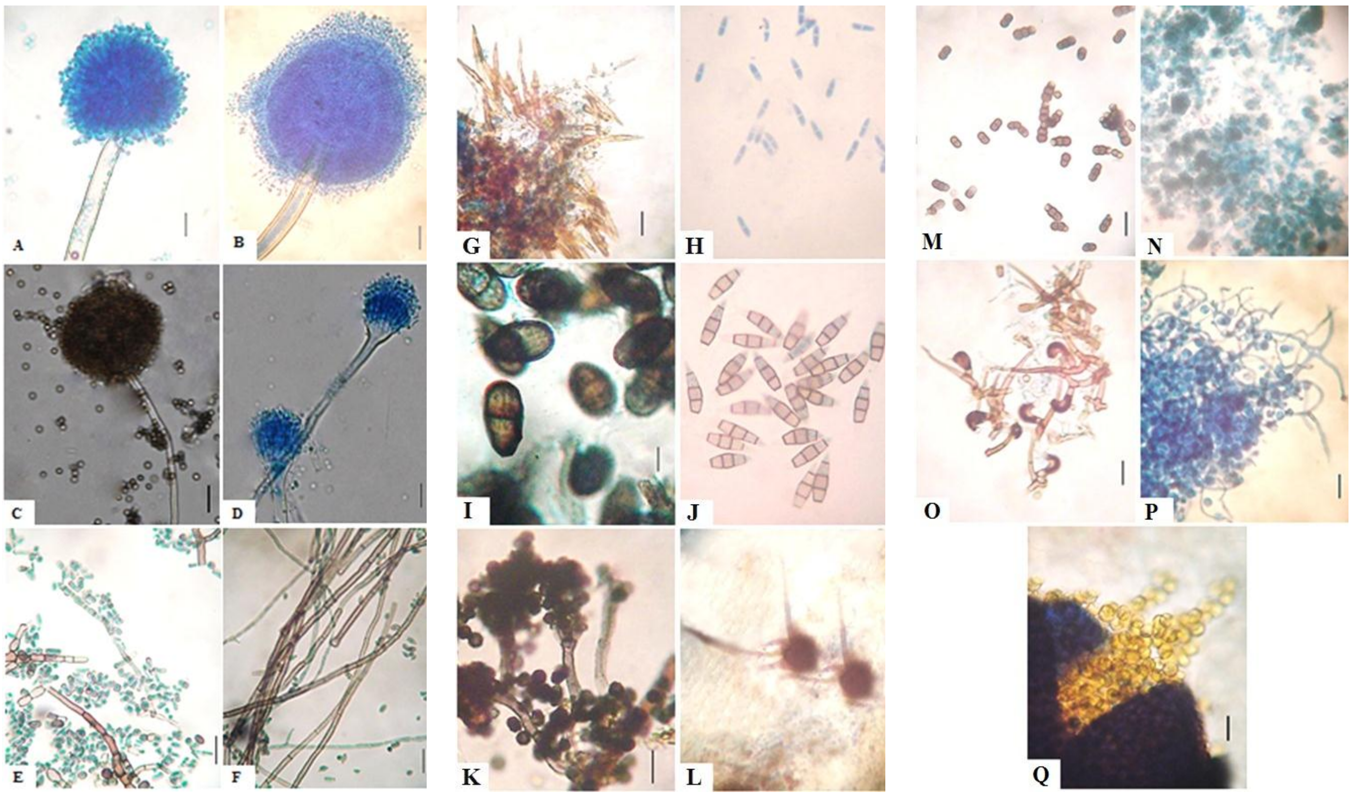

Fig. 2. A. *Aspergillus flavus*, B. *A. fumigatus*, C. *A. niger*, D. *A. terreus*, E. *Cladosporium cladosporioides*, F. *C. elatum*, G. *Colletotrichum gloeosporioides*, H. *Fusarium* sp., I. *Lasiodiplodia theobromae*, J. *Pestalotiopsis* sp., K. *Stachybotrys* sp., L. *Tetraploa aristata*, M. *Torula* sp., N. *Trichoderma* sp., O. *Zygosporium minus*, P. Unidentified species 1 and Q. Unidentified species 2 (Bar = 50 µm).

From the results it appears that the fungi associated with the dead fallen spadix mostly remain as saprophytes and perhaps complete overwintering and over summering stage of their life cycle. The occurrence and abundance of fungi were different in every year of the investigation. The association of *Stachybotrys* sp., *Tetraploa aristata*, *Torula* sp. and *Zygosporium minus* with coconut spadix is a new record. This finding clearly exhibited the diversity of the fungi on *C. nucifera* as well as their decomposing ability.

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