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Diversity of fungi associated with estuarine sedge *Cyperus malaccensis* Lam.

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Six tissues of mature sedge (rhizome, leaf, basal stem, middle stem, top stem and bract) and middle stem portions of three age groups (mature, senescent and standing dead) of *Cyperus malaccensis* were assessed for fungi on plating without and with surface sterilization. In non-surface sterilized tissues, *Aspergillus niger* and *Fusarium oxysporum* were common, while *Chaetomium* sp., *Nigrospora oryzae*, *Penicillium crustosum* and *Sordaria fimicola* in surface sterilized tissues. In age groups, *A. niger*, *F. oxysporum*, *N. oryzae* and *Trichoderma harzianum* were dominant in non-surface sterilized, while *P. crustosum* in surface sterilized stem segments. The frequency of occurrence of fungi between two methods in tissue and age groups did not significantly differed ($P > 0.05$). The diversity of fungi was highest in basal stem in non-surface sterilized, while in middle stem in surface sterilized tissues. Stem tissues of mature sedge showed the highest diversity than senescent and standing dead plants in both methods. Rarefaction curves of non-surface sterilized rhizome and leaf showed increasing trend, while it was in surface sterilized rhizome and bract. In both methods, tissues of mature plants showed the highest species richness. The Jaccard's index revealed highest fungal similarity between middle stem and top stem in non-surface sterilized (67%) and between middle stem and bract in surface sterilized (67%) tissues. It was least in stem tissues of sterilized mature vs. standing dead plants (17%), while highest between non-sterilized senescent vs. standing dead plants (58%). Estuarine sedge, *C. malaccensis* seems to be collateral host for many pathogenic fungi of paddy and vegetables.

Key words: *Cyperus malaccensis*, endophytes, estuaries, fungal diversity, saprophytes, sedge

Introduction

Mangrove and estuarine flora have been classified into three major groups: (i) True mangroves (about 80 tree and shrub species, which are restricted to intertidal areas between high water levels of neap of spring tides);

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(ii) Minor species (inconspicuous elements of vegetation and rarely form pure communities); (iii) Mangrove associates (salt-tolerant plant species not found exclusively in the vicinity of mangrove and occur only in transitional vegetation landwards and seawards) (Tomlinson, 1986; Field, 1995). Highest mangrove and estuarine plant species diversity is seen in South-East Asian region (two-thirds of all species) (Field, 1995). Mangrove and estuarine vegetation have adapted to harsh conditions (high salinity, tidal extremes, high wind velocity, high temperature and anaerobic soils) (Chapman, 1976). Thus, autotrophic production is more important in maintaining the community structure and function in estuaries and mangroves. In terms of productivity and sustained tertiary yield, mangroves and estuaries constitute the second most important ecosystems after coral reefs in marine habitats (Qasim and Wafar, 1990).

Estuarine and mangrove region of the southwest coast of India encompass a variety of macrophytes including sedges. Among the sedges, *Cyperus malaccensis* is the most dominant photosynthetic agent. This sedge forms a thick mat and occupied about one-third of the area of estuaries and mangroves. It is commonly known as 'mat-grass' (as their stem is used in weaving mats) and has been categorized under mangrove associates. It generates substantial quantity of deritus in the form of standing dead culms and contributes towards the energy flow in estuarine and mangrove habitats. In the Nethravathi estuary near Mangalore, groves of *C. malaccensis* provide shelter and food mainly for the giant snail, *Telescopium telescopium* Linn. (Class, Gastropoda orthogastropoda; Family, Potamididae) (common name: 'top snail' or 'telescope snail'; grow up to 10-12 cm length).

Our main objective was to document the fungal community structure and diversity in *C. malaccensis* at the Nethravathi estuary of the Southwest coast of India. The study has been designed to assess the fungal composition, vertical distribution and diversity in six tissues of mature sedge and stem tissues of three age groups. The fungal composition of sedge has been compared with other mycological studies in southwest mangroves of India.

Materials and methods

Sedge

The study was carried out at the estuary of the Nethravathi River mouth at the southwest coast of India (12º50′N, 74º50′E). Nethravathi estuary possesses a large area of groves of *Cyperus malaccensis* Lam. (Order, Cyperales; Family, Cyperaceae) (Fig. 1). This brackish water sedge has perennial rhizome, sharp triangular stem grow up to a height of 1.5 m with 3-5 leaves (15-20 cm length).

Compound inflorescence of sedge possesses 3 bracts (approx. 15 cm length). During monsoon and post-monsoon seasons, different age groups (mature, senescent and standing dead) of sedge are available at the Nethravathi estuary.

The assemblage and diversity of fungi in six live tissues of mature sedge (rhizome, leaf, basal stem, middle stem, top stem and bract) was assessed without and with surface sterilization during September to November 2004. During December 2004 to March 2005, middle stem portions of three age groups of the sedge (mature, senescent and standing dead) were also assessed for fungi.

Water temperature and pH of the sampling sites was measured using water analysis kit (Water Analyzer 371, Systronics, Gujarat, India). Additional water samples were brought to the laboratory to measure salinity by Argentometric method (APHA, 1995).

Fig. 1. A close view of *Cyperus malaccensis* grove at the backwaters of River Nethravathi.

Tissues and age groups

Mature plants one each from five sedge groves were uprooted and brought to the laboratory. Six tissue portions (rhizome, leaf, basal stem, middle stem, top stem and bract) from each plant were excised into one cm length pieces and washed in sterile distilled water. The tissue pieces were aseptically plated on malt extract agar medium (1.5%) amended with tetracycline (250 mg/l). Similarly processed tissue segments were surface sterilized using 95% ethanol (1 min), 6% sodium hypochlorite (5 min) and 95% ethanol (0.5 min) followed by three rinses in distilled water were plated on antibiotic amended malt extract agar medium. The plates were incubated at 23±2°C up to four weeks at 12 hr light and dark regime. The middle stem portions of sedge of

three age groups (mature, senescent and standing dead) were excised into one cm pieces and processed and plated on the medium as described above. Periodically the segments were screened for the growth of mycelia or discrete colonies on the medium or above the segments. The colonies or mycelial portions were transferred to fresh antibiotic-free malt extract medium and identified based on the colony characteristics, sporulation and spore morphology using monographs and taxonomic keys.

Data analysis

The percent colonization frequency and mean percent frequency of all fungi and core-group fungi (frequency of occurrence, ≥10%) of non-surface sterilized and surface sterilized tissue and age groups were calculated:

$$\text{Frequency of occurrence (\%)} = \frac{\text{Number of segments colonized x } 100}{\text{Total segments screened}}$$

$$\text{Mean \% frequency of occurrence/fungus} = \frac{\text{Total \% frequency of fungi}}{\text{Total fungi on sedge samples}}$$

$$\text{Mean \% frequency/core-group fungus} = \frac{\text{Total \% frequency of core-group fungi}}{\text{Total fungi on sedge samples}}$$

Paired *t*-test was employed to assess the difference in frequency of occurrence between non-surface sterilized and sterilized segments of each tissue and age groups (StatSoft Inc., 1995).

The Simpson and Shannon diversity (Magurran, 1988) and evenness (Pielou, 1975) were estimated for tissue and age groups. The rarefaction index for non-surface sterilized and sterilized segments out of random isolates obtained from the total isolations was calculated (Ludwig and Reynolds, 1988).

Jaccard's index of similarity was calculated pair-wise among the tissue and age groups based on the presence or absence of each fungal species (Kenkel and Booth, 1992).

Results

The average temperature during study period ranged between 24.6 and 26.5°C (Table 1). The pH was slightly alkaline ranging from 7.01-7.4. The salinity was 0.5 ppt in September 2004 gradually elevated up to 30.6 ppt in March 2006.

Table 1. Water parameters in Nethravathi estuary during sampling (n=5, mean and range is parenthesis).

Tissue/age class	Date of sampling	Temperature (°C)	pH	Salinity (ppt)
Tissue class				
Rhizome and leaf	September 10, 2004	26.5 (25.8-26.7)	7.01 (6.92-7.05)	0.51 (0.21-0.62)
Basal and middle stem	October 11, 2004	24.6 (24.1-25.2)	7.09 (6.99-7.12)	5.22 (5.05-5.31)
Top stem and bract	November 12, 2004	24.8 24.5-25.1)	7.12 (7.02-7.32)	9.11 (8.91-9.31)
Age class				
Mature	December 12, 2004	25.0	7.17 (6.92-7.44)	11.9 (11.8-12.0)
Senescent	February 02, 2005	25.7 (25-26)	7.37 (7.31-7.41)	26.17 (26.13-26.19)
Standing dead	March 08, 2005	24.8 (24.5-25)	7.20	30.57 (30.54-30.60)

Fungi in tissues

More number of mitosporic fungi was recovered than other group on non-surface sterilized tissue segments (Table 2). Out of a total of 28 fungi found on non-surface sterilized segments, two were non-sporulating, but showed distinct colony morphology. The number of fungi in different tissues ranged between 10 (bract) and 16 (leaf), while the core-group fungi between 4 (leaf) and 12 (rhizome) (Fig. 2a). *Aspergillus niger* (rhizome and bract, 11.1%; leaf, 25.9%) and *Fusarium oxysporum* (rhizome, 22.2%; bract, 85.2%) were common on all tissues and belonged to core-group. The mean frequency of occurrence per fungus was highest in rhizome for total fungi as well as core-group fungi (Fig. 2b).

In surface sterilized tissues, out of 30 fungi, 24 were mitosporic fungi and three each represented by ascomycetes and non-sporulating fungi. The number of fungi in different tissues ranged between 12 (rhizome) and 18 (basal stem), while the core-group fungi between 6 (basal stem) and 12 (rhizome) (Fig. 2c). *Nigrospora oryzae* (basal stem, 3.7%; rhizome, 66.7%), *Penicillium crustosum* (rhizome, leaf, middle stem and bract, 11.1%; top stem, 22.2%), *Chaetomium* sp. (middle stem, 7.4%; leaf and top stem, 18.5%) and *Sordaria fimicola* (top stem, 3.7%; leaf, 22.2%) were common in all tissues. The mean frequency of occurrence per fungus was highest in basal stem for total fungi, while in rhizome for core-group fungi (Fig. 2d). The frequency of occurrence of fungi

between non-surface sterilized and surface sterilized tissues did not significantly differed (P > 0.05).

Table 2. Frequency of occurrence (%) of fungi in non-surface sterilized and surface sterilized (in parenthesis) six tissues of mature plants of *Cyperus malaccensis* of Nethravathi estuary.

Taxon	Rhizome	Leaf	Basal stem	Middle stem	Top stem	Bract
Mitosporic fungi						
Alternaria triticina Prasada & Prabhu		7.4	7.4	3.7	7.4	
Aspergillus caespitosus Raper & Thom		(25.9)	(3.7)			
A. flavus Link	11.1	(7.4)	18.5	(3.7)		(3.7)
			(3.7)			
A. fumigatus Fresen.				(3.7)		
A. japonicus Saito		3.7	(7.4)	(11.1)	(14.8)	(3.7)
A. niger van Tiegh.	11.1	25.9	14.8	18.5	14.8	11.1
			(14.8)	(14.8)	(7.4)	(7.4)
A. ochraceus G. Wilh.	(11.1)					
A. parasiticus Speare	11.1			(7.4)		(14.8)
A. sclerotiorum G.A. Huber	11.1					
A. terreus Thom		3.7	7.4	3.7	3.7	
					(3.7)	
A. unguis (Wiell & L. Gaudin) Thom & Raper		3.7	7.4			
A. ustus (Bainer) Thom & Church		(14.8)	(33.3)	(29.6)	(29.6)	(25.9)
A. versicolor (Vuill.) Tirab.				(3.7)	(3.7)	
A. wentii Wehmer			3.7			
Aspergillus sp.					3.7	
Cladosporium oxysporum Berk. & M.A. Curtis	66.7	(14.8)	11.1	7.4	(3.7)	(3.7)
			(11.1)	(18.5)		
Codinea sp.		3.7		11.1	11.1	11.1
Curvularia lunata (Wakker) Boedijn	22.2	3.7	3.7			
Cylindrocladium sp.		(7.4)				
Fusarium oxysporum Schltdl.	22.2	59.3	44.4	74.1	66.7	85.2
Gonytrichum sp.	(11.1)	(11.1)	(14.8)	(3.7)		
Neosartorya sp.	(11.1)		(3.7)	(3.7)		
Nigrospora oryzae	(66.7)	(14.8)	18.5	3.7	3.7	3.7
(Berk. & Broome) Petch			(3.7)	(14.8)	(11.1)	(14.8)
Paecilomyces sp.	(11.1)		(3.7)		(3.7)	
Penicillium bilaiae Chalab.	(22.2)		(3.7)	(3.7)		(25.9)
P. crustosum Thom	(11.1)	7.4	3.7	11.1	(22.2)	(11.1)
		(11.1)	(14.8)	(11.1)		
P. echinulatum Fassat.			(3.7)		(14.8)	
P. hirsutum Dierckx	(11.1)	3.7		(14.8)	(11.1)	(11.1)
		(3.7)				
P. oxalicum Currie & Thom		3.7				3.7
						(11.1)
Penicillium sp. 2			(3.7)			
Pestalotia sp.	(55.6)	7.4	18.5	11.1	7.4	
Phialophora sp.	11.1			3.7		
Scytalidium sp.	55.6					
Trichoderma harzianum Rifai	(33.3)	22.2	14.8	25.9	18.5	11.1

Table 2. Continue..

Taxon	Rhizome	Leaf	Basal stem	Middle stem	Top stem	Bract
Ascomycetes						
Chaetomium sp.	(11.1)	3.7 (18.5)	(14.8)	(7.4)	(18.5)	11.1 (3.7)
Sordaria fimicola (Roberge ex Desm.) Ces. & de Not.	11.1 (11.1)	(22.2)	(7.4)	(11.1)	(3.7)	(11.1)
Sordaria sp.		(3.7)	(3.7)			
Mastigomycete						
Pythium sp.				3.7		3.7
Zygomycete						
Mucor sp.		18.5	22.2			
Non-sporulating fungi						
Sp. 1	11.1	(3.7)	(3.7)	7.4 (3.7)	14.8 (3.7)	7.4 (3.7)
Sp. 2	33.3	3.7		3.7	3.7 (3.7)	3.7 (7.4)
Sp. 3						(3.7)
Total taxa	12 (12)	16 (13)	14 (18)	14 (17)	11 (15)	10 (16)
Total core-group taxa	12 (12)	4 (8)	8 (6)	6 (8)	5 (7)	5 (8)

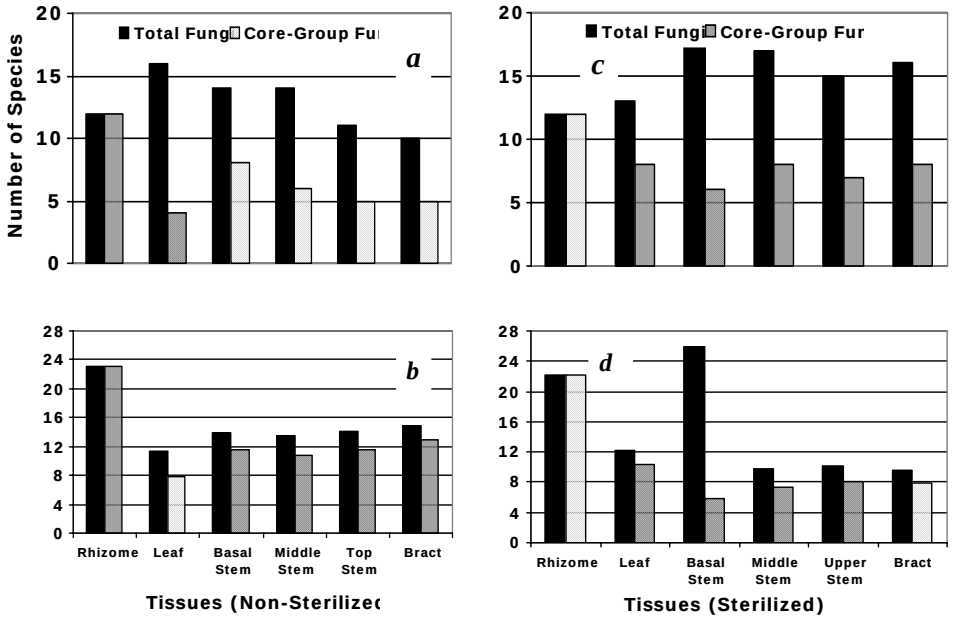

Fig. 2. Number of fungi and mean frequency of occurrence (%) of fungi on non-sterilized and sterilized tissues of *Cyperus malaccensis*.

Fungi in age groups

As seen in tissues, more number of mitosporic fungi was recovered than other group on non-surface sterilized middle stem of different ages (Table 3). Out of a total of 24 fungi found on non-surface sterilized tissues, two were non-sporulating fungi with distinct colony morphology. The number of fungi in different age groups ranged between 13 (mature and standing dead) and 17 (senescent), while the core-group fungi between 2 (senescent) and 6 (mature) (Fig. 3a). *Aspergillus niger* (mature, 4.4%; standing dead, 33.3%), *Fusarium oxysporum* (mature, 55.5%; standing dead, 66.6%), *Nigrospora oryzae* (senescent, 2.2%; mature, 13.3%), *Trichoderma harzianum* (senescent and standing dead, 6.7%; mature, 40%) and non-sporulating sp. 1 (senescent, 4.4%; mature and standing dead, 6.7%) were common to all age groups. The mean frequency of occurrence per fungus was highest in mature culms for total fungi as well as core-group fungi (Fig. 3b).

In surface sterilized age groups, out of a total of 27 fungi, 21 were mitosporic fungi. The number of fungi in different age groups ranged between 11 (senescent) and 15 (mature), while the core-group fungi between 2 (senescent) to 9 (mature) (Fig. 3c). *Penicillium crustosum* (standing dead, 2.2%; mature, 31.1%), non-sporulating sp. 1 and 2 (senescent, 2.2%; mature and standing dead, 4.4%) were common to all age groups. The mean frequency of occurrence per fungus was highest in mature plants for total fungi as well as core-group fungi (Fig. 3d). The frequency of occurrence of fungi between non-surface sterilized and surface sterilized age groups did not significantly differed ($P > 0.05$).

Diversity, richness and similarity

The Simpson and Shannon diversities were highest in basal stem of non-surface sterilized tissues, while in surface sterilized tissues it was highest in middle stem (Table 4). Among age groups, mature sedge showed highest diversity in non-surface sterilized as well as surface sterilized segments.

Rarefaction curves of non-surface sterilized rhizome and leaf tissues showed elevated species richness, while it was in rhizome and bract among surface sterilized tissues (Fig. 4). In both treatments of stem segments of age groups, mature plants peaked in species richness (Fig. 4). However, the extent of expected number of species was higher in surface sterilized than in non-surface sterilized segments.

Out of 15 pair-wise comparisons, in non-surface sterilized segments of tissues, the similarity was ranged between 21% (basal stem vs. bract) and 67% (middle stem vs. top stem) (Table 5). There was high similarity (48-67%) in surface sterilized segments of tissues. In age groups, similarity was least

between sterilized stem of mature vs. standing dead plants, while highest between senescent and standing dead plants (Table 6).

Table 3. Frequency of occurrence (%) of fungi in non-surface sterilized and surface sterilized (in parenthesis) middle stem segments of three age classes of *Cyperus malaccensis* of Nethravathi estuary.

Taxon	Mature	Senescent	Standing dead
Mitosporic fungi			
Alternaria triticina Prasada & Prabhu	13.3	2.2	
Aspergillus caespitosus Raper & Thom		(17.8)	
A. flavus Link	(4.4)	4.4	6.7
		(6.7)	
A. fumigatus Fresen.		(2.2)	
A. japonicus Saito	(28.9)	2.2	
A. niger van Tiegh.	4.4	13.3	33.3
		(4.4)	(22.2)
A. parasiticus Speare	(13.3)		
A. terreus Thom		6.7	4.4
			(2.2)
A. unguis (Wiell & L. Gaudin) Thom & Raper			6.7
A. ustus (Bainer) Thom & Church		(80)	
A. versicolor (Vuill.) Tirab.		(4.4)	
A. wentii Wehmer		2.2	
Aspergillus sp.	2.2		
Cladosporium oxysporum Berk. & M.A. Curtis	11.1		
	(31.1)		
Codinea sp.			22.2
Curvularia lunata (Wakker) Boedijn		4.4	
Cylindrocladium sp.			(4.4)
Fusarium oxysporum Schltdl.	55.5	75.5	66.6
Gonytrichum sp.	(17.8)		
Nigrospora oryzae (Berk. & Broome) Petch	13.3	2.2	4.4
		(6.7)	(28.9)
Neosartorya sp.	(4.4)		
Paecilomyces sp.	(4.4)		
Penicillium bilaiae Chalab.	(17.8)	(2.2)	
P. crustosum Thom	6.7	6.7	(2.2)
	(31.1)	(8.9)	
P. echinulatum Fassat.			(11.1)
P. hirsutum Dierckx	2.2		
	(24.4)		
P. oxalicum Currie & Thom		2.2	2.2
			(6.7)
Penicillium sp.			(2.2)
Pestalotia sp.	26.6		
Phialophora sp.	2.2		2.2
Trichoderma harzianum Rifai	40	6.7	6.7

Table 3. Continue..

Taxon	Mature	Senescent	Standing dead
Ascomycetes			
Chaetomium sp.	6.7 (37.8)	2.2	
Sordaria fimicola (Roberge ex Desm.) Ces. & de Not.	(26.7)		(6.7)
Sordaria sp.	(4.4)		
Mastigomycete			
Pythium sp.		2.2	2.2
Zygomycete			
Mucor sp.		2.2	22.2
Non-sporulating fungi			
Sp. 1	6.7 (4.4)	4.4 (2.2)	6.7 (4.4)
Sp. 2	(4.4)	8.9 (2.2)	2.2 (4.4)
Sp. 3			(2.2)
Total taxa	**13 (15)**	**17 (11)**	**13 (12)**
Total core-group taxa	**6 (9)**	**2 (2)**	**4 (3)**

Table 4. Diversity, evenness and species richness of fungi in non-surface sterilized and surface sterilized (in parenthesis) tissue segments of mature plants and stem segments of three age groups of *Cyperus malaccensis* of Nethravathi estuary.

Tissue/Age class	Diversity		Evenness		Species Richness	
	Simpson	Shannon	Simpson	Shannon	Observed	$E_{(s, 35)}$*
Tissue class						
Rhizome	0.681 (0.867)	3.173 (3.208)	0.936 (0.943)	0.885 (0.895)	12 (12)	19 (19)
Leaf	0.832 (0.905)	3.151 (3.466)	0.892 (0.974)	0.807 (0.937)	16 (13)	19 (14)
Basal stem	0.889 (0.908)	3.462 (3.743)	0.958 (0.955)	0.989 (0.898)	14 (18)	17 (17)
Middle stem	0.802 (0.917)	3.006 (3.761)	0.863 (0.969)	0.789 (0.920)	14 (17)	16 (16)
Top stem	0.772 (0.900)	2.738 (3.520)	0.849 (0.958)	0.792 (0.901)	11 (15)	17 (16)
Bract	0.659 (0.913)	2.306 (3.679)	0.732 (0.968)	0.694 (0.920)	10 (16)	11 (19)
Age class						
Mature	0.834 (0.905)	3.017 (3.532)	0.904 (0.966)	0.816 (0.904)	13 (15)	24 (26)
Senescent	0.728 (0.638)	2.896 (2.215)	0.770 (0.698)	0.694 (0.640)	17 (11)	22 (20)
Standing dead	0.806 (0.839)	2.877 (2.911)	0.873 (0.906)	0.777 (0.834)	13 (12)	23 (20)

*Expected number of species out of 35 random isolates

Table 5. Jaccard's similarity index (%) of non-surface sterilized and surface sterilized (in parenthesis) sedge of six tissues of mature plants of *Cyperus malaccensis* of Nethravathi estuary.

	Leaf	Basal stem	Middle stem	Top stem	Bract
Rhizome	55	57	59	56	58
	(59)	(61)	(62)	(58)	(58)
	Leaf	50	50	47	35
		(56)	(53)	(37)	(50)
		Basal Stem	47	47	21
			(59)	(48)	(55)
			Middle stem	67	47
				(52)	(67)
				Top stem	50
					(55)

Table 6. Jaccard's similarity index (%) of non-surface sterilized and surface sterilized (in parenthesis) stem tissues of three age groups of *Cyperus malaccensis* of Nethravathi estuary.

	Senescent	Standing dead
Mature	36	25
	(24)	(17)
	Senescent	58
		(28)

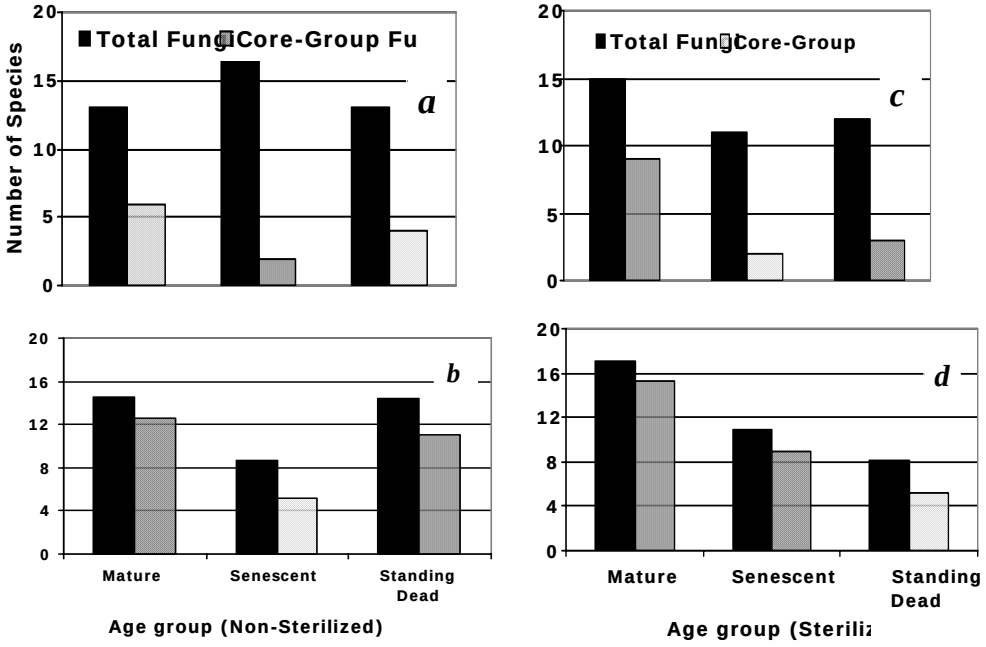

Fig. 3. Number of fungi and mean frequency of occurrence (%) of fungi on sterilized and non-sterilized stem tissues of three age groups of *Cyperus malaccensis*.

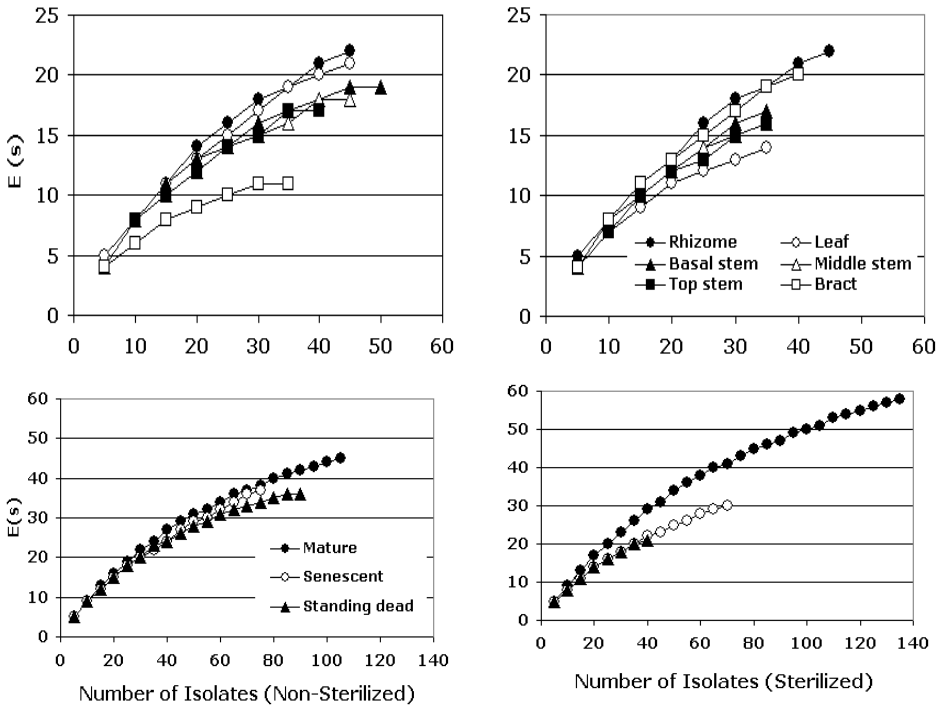

Fig. 4. Rarefaction curves [number of isolates vs. expected number of species, *E*(*s*)] of fungi from randomly sampled segments of six tissues and stem segments of three age groups of *Cyperus malaccensis.*

Discussion

Tropical coastlines are inhabited by a variety of vegetation (e.g. sedges, palms, true mangroves, mangrove associates, mangrove minors) and play a major role in food web and nutrient cycle. The detritus (leaf litter, woody litter, animal remains), marsh vegetation and imported substances in estuarine habitats constitute a major source of organic matter. Elevated productivity in estuaries and mangroves supports abundant benthic communities through high rates of decomposition (Robertson *et al.*, 1992; Alongi *et al.*, 1999). Being detritus-driven ecosystem, the fungal component of estuaries and mangroves deserves special attention to understand the pattern of detritus decomposition and enrichment. Most investigations on fungi of the west coast of India are confined to mangrove leaf or woody litter (e.g. Borse, 1988; Ananda and Sridhar, 2004; Maria and Sridhar, 2004; Ananda *et al.*, 2008). However, a few studies are available on the endophytic fungi of mangrove plants and mangrove associates on the west coast and east coast of India (Kumaresan and

Suryanarayanan, 2001; Ananda and Sridhar, 2002; Maria and Sridhar, 2003; Anita and Sridhar, 2009; Anita *et al*., 2009). Although the emergent sedge, *Cyperus malaccensis* is a major plant resource in the estuaries of west coast of India, no studies have been conducted on the fungal component of live or dead part to understand their contribution to the energy flow.

Fungi in tissues

The current study revealed association of several fungi in different tissues of the mature sedge *Cyperus malaccensis*. As seen in endophytes of mangrove plant species (Ananda and Sridhar, 2002; Maria and Sridhar, 2003; Anita and Sridhar, 2009; Anita *et al*., 2009), the current study also demonstrated more of mitosporic fungi than meiosporic fungi. The fungal flora of the sedge is partly similar to studies on mangrove or estuarine vegetation (Ananda and Sridhar, 2002; Maria and Sridhar, 2003; Anita and Sridhar, 2009; Anita *et al*., 2009). *Cladosporium oxysporum* was found on the whole roots of *Avicennia officinalis* and bark of *Rhizophora mucronata* as endophyte, while *Fusarium oxysporum* on *Acanthus ilicifolius, Avicennia officinalis* and *R. mucronata* (Ananda and Sridhar, 2002). *Fusarium oxysporum* was a core-group fungus on damp-incubated *A. officinalis* roots and whole roots of *R. mucronata* at mid-tide and high tide levels, and also dominant on the root bark (Ananda and Sridhar, 2002). In our study, *F. oxysporum* colonized the entire sedge with frequency of occurrence ranging between 22.2% (rhizome) and 85.2% (bract). It was also a major fungus in sterilized segments of *A. officinalis* and the estuarine fern, *Achrostichum aureum* (Maria and Sridhar, 2003). *Nigrospora oryzae*, common fungus on basal stem of *C. malaccensis* was frequent on sterilized segments of *A. aureum* (Maria and Sridhar, 2003) and decorticated roots of *R. mucronata* (Ananda and Sridhar, 2002). *Aspergillus flavus, A. niger* and *Fusarium oxysporum* were also core-group fungi on surface sterilized and non-sterilized tissues of legumes (*Canavalia cathartica* and *Sesbania bispinosa*) of Nethravathi estuary (Anita and Sridhar, 2009; Anita *et al*., 2009). The difference in fungal composition between non-surface sterilized and surface sterilized segments of rhizome is glaring. Out of 12 fungi recovered, only *Sordaria fimicola* was common on non-sterilized and sterilized segments of rhizome indicate the occurrence of entirely different set of endophytic fungi. Interestingly, all fungi found on rhizome belonged to core-group (>10%) and the mean frequency of occurrence per fungus was highest except for sterilized basal stem. Rhizome has also showed elevated species richness in rarefaction curves. However, Petrini (1986) has pointed out that a few endophytic fungi dominate a single host plant species.

Overall, except a few instances, high percent similarity was seen in associated fungi among the tissues of mature sedge irrespective of treatments. Non-

surface sterilized segments consist of only two fungi in common (*Aspergillus niger, Fusarium oxysporum*), while *Nigrospora oryzae, Penicillium crustosum, P. hirsutum, Chaetomium* sp. and *Sordaria fimicola* in surface sterilized segments indicating the dominance and inhibition of endophytes by *A. niger* and *F. oxysporum.* Several endophytic fungi of rhizome (e.g. *Gonytrichum sp., Neosartorya sp., Nigrospora oryzae, Phaecilomyces* sp., *Penicillium bilaii, P. crustosum, P. hirsutum, Chaetomium, Sordaria fimicola*) were found in other tissues as endophytes. This clearly shows that different sets of fungi associated with sedge either as pathogens/saprophytes or endophytes, however, some fungi seem to have common role. Among the three ascomycetes recorded, *Chaetomium* sp. and *S. fimicola* were common in surface sterilized segments probably have a protective role as endophytes. Interestingly, the percent similarity of endophytes was ranged between 16% and 25% in *Acanthus ilicifolius* (roots, prop roots, stem and leaf), while 21.7% and 25% in *Acrostichum aureum* (root, rhizome, petiole and leaf) of Nethravathi estuary (Maria and Sridhar, 2003). Endophytic fungi of *A. ilicifolius* and *A. aureum* differ up to 75% indicating that they are host-dependent.

Two interesting fungi, *Aspergillus ochraceus* and *Trichodrema harzianum* were recovered as endophytes. The former was confined only to rhizome, while the latter in all non-surface sterilized tissues except for rhizome. As *A. ochraceus* is toxin producing (achrotoxin) and *T. harzianum* has biopesticidal role, these fungi might provide defence to *Cyperus malaccensis* against herbivores. As seen in our study, sterile fungal morphotypes were also common endophytes in mangrove plants (Ananda and Sridhar, 2002) and mangrove associates (Maria and Sridhar, 2003; Anita and Sridhar, 2009; Anita *et al*., 2009). Several fungi showed high frequency of occurrence throughout the sedge (rhizome to bract). Variation in fungal density particularly endophytes in different tissues of sedge reveals some selection pressure besides host specificity as predicted by Kumaresan and Suryanarayanan (2001). However, there was no significant difference in the frequency of occurrence of fungi in non-surface sterilized and surface sterilized tissues ($P > 0.05$).

Fungi in age groups

On non-surface sterilized middle stem segments of different age groups of sedge, *Cladosporium oxysporum, Fusarium oxysporum* and *Nigrospora oryzae* were common, which were also associated with mangroves (Ananda and Sridhar, 2002) and mangrove associates (Maria and Sridhar, 2003). *Aspergillus niger, F. oxysporum, N. oryzae, Trichoderma harzianum* and non-sporulating sp. 1 were common to all age groups of sedge. The most common in all age groups was *F. oxysporum* (mature, 55.5%; senescent, 75.5%), while *T. harzianum* was most common in mature sedge (40%). Among the surface

sterilized segments, *Penicillium crustosum*, non-sporulating sp. 1 and 2 were common to all age groups with maximum occurrence of *P. crustosum* on mature plants (31.1%). Among 23 fungi found on the mature plants, only five species are common in non-surface sterilized and surface sterilized segments indicating their duel role as endophytes and saprophytes. The diversity and species richness (based on rarefaction indices) was also higher in mature than senescent and standing dead sedge. Contribution of total fungi and core-group fungi is consistent in mature than senescent and standing dead sedges. Due to single species dominance in senescent sedge, the overall fungal diversity and evenness is lower than mature and standing dead sedges. The percent similarity in fungi between age groups ranged between 17% and 36% (except for non-surface sterilized segments: senescent vs. standing dead, 58%) indicating different fungi colonize sedge tissues at different age. It is worth noting an elevated percent similarity in tissues of mature sedge segments ranged between 47% and 67% (with two exceptions: 21% and 35%). As seen in tissues, the frequency of occurrence of fungi in non-surface sterilized and surface sterilized age groups did not differed significantly ($P > 0.05$).

Terrestrial and other fungi

Several soil fungi have been recovered in estuarine and mangrove habitats (e.g. leaves, seedlings, wood, foam, sediments, rhizosphere, epiphytes) (Ananda and Sridhar, 2002). They were ignored as invaders of estuaries or mangroves from terrestrial habitats. Occurrence of many typical terrestrial fungi as endophytes in our study indicates their intimate association with estuarine macrophytes. Foliar endophytes of east coast of India were also typical terrestrial fungi (e.g. Suryanarayanan *et al*., 1998; Kumaresan and Suryanarayanan, 2001, 2002). On the contrary, decomposing mangrove litter consists of more of ascomycetes than mitosporic fungi (Kohlmeyer and Volkmann-Kohlmeyer, 2001; Maria and Sridhar, 2002; Ananda *et al*., 2008). Endophytic fungi of mangrove roots composed of a consortium of soil, marine and freshwater fungi (Ananda and Sridhar, 2002). However, typical marine or freshwater fungi have not been recovered in our study probably due to employing only plating technique.

As the estuarine sedges have been adapted to extreme habitats (e.g. muddy, brackish, anaerobic), their association with specific endophytic fungi might prevent their susceptibility to herbivory. For instance, *Trichoderma harzianum* and *Chaetomium* sp. were found in all tissues of the sedge as endophytes and deserves special attention to understand their role. *Trichoderma harzianum* was also endophytic in legumes (*Canavalia cathartica* and *Sesbania bispinosa*) of Nethravathi mangroves (Anita and Sridhar, 2009; Anita *et al*.,

2009). Vast area adjacent to the Nethravathi estuary consists of agricultural fields (paddy and vegetable crops). The sedge, *C. malaccensis* may acts as collateral host for pathogenic fungi of paddy and vegetables. Such delicate balances may be affected severely if the human interference (e.g. fishery activities, sand mining) destroys the sedge groves.

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