Research Article

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Influence of crop combinations and soil factors on diversity and association of arbuscular mycorrhizal fungi in arecanut based cropping systems

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Abstract

Arbuscular mycorrhizal (AM) species diversity and extent of association were investigated in arecanut based cropping systems differing in crop combinations. The study was carried out in farmers' fields under acidic soil conditions at three locations representing low land (Maneikkara), midland (Cheruvanjeri) and high land (Nedumpoyil) regions of Kannur district in Kerala. The cropping systems in Maneikkara, Cheruvanjeri and Nedumpoyil had arecanut-banana, arecanut-banana-black pepper and arecanut-banana-black pepper-cardamom as component crops. AM spore load and root colonization differed significantly in arecanut in the three cropping systems. Highest spore load was recorded in Maneikkara followed by that in Nedumpoyil and Cheruvanjeri regions. Crops which formed components of the cropping system differed in root colonization levels, with banana recording the highest level, followed by arecanut, black pepper and cardamom. Colonization pattern was *Paris* type in all crops, but varied with respect to predominance of arbuscules in arecanut and vesicles in banana. Arecanut-black pepper-banana system at Cheruvanjeri in midland was superior with respect to species diversity and species richness as evidenced by Shannon–Weiner index (Hs), Simpson's index of diversity (Ds) and species richness index. Arecanut-banana cropping system in Maneikkara in low land had low level of species diversity and species richness, indicating the combined influence of crop combinations and soil factors such as N and P on AM diversity and distribution. *Rhizophagus fasciculatus, Funneliformis geosporum, F. mosseae, Glomus macrocarpum, G. aggregatum, G. multicaule, G. glomerulatum* and *Acaulospora bireticulata* were the AMF species identified from the arecanut cropping systems. *F. geosporum* was the most abundant (29-50%) species in the cropping system. The relative occurrence and abundance of AM species varied significantly with respect to the crops and locations.

Keywords: Arecanut cropping systems, AMF species, diversity, relative species distribution

Introduction

Arbuscular mycorrhizal fungi (AMF), an important component of soil microbial community, form symbiotic association with majority of the plants and influence plant health and above ground productivity (Jeffries *et al*., 2003). With more than eighty per cent of the plant species growing on land depending on the AMF for phosphorus and water uptake, this association of roots of higher plants with Glomeromycota fungi remains the most wide spread symbiosis on earth (Brachmann and Parniske, 2006). Among the many tree crops, arbuscular mycorrhizae association in palms, particularly coconut (Karunasinghe *et al*., 2009; Rajesh Kumar *et al*., 2015), oil palm (Phosri *et al*., 2010), date palm (Al-Yahya'ei *et al*., 2011) and native ornamental palms of Florida in United States (Fisher and Jayachandran, 2008) have been studied in some detail for application of the fungi in improving the cultivations of these palms.

In India, besides coconut and date palm, arecanut (*Areca catechu* L.), a highly profitable

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plantation crop, is also cultivated in nearly half a million hectare area in many parts of southern and north-eastern states and in Andaman and Nicobar islands (http://dasd.gov.in/index.php/statistics.html). The palm yields close to 0.7 million tonnes of arecanut which is used in several food-based products. Livelihood of nearly six millions Indians is dependent upon arecanut production, processing and its trade. This palm is generally cultivated in soils that are lateritic, alluvial or sandy loam and is seen growing up to 1000 m height above mean sea level. To enhance the income from arecanut cultivation, it was recommended to grow intercrops as well as adopt mixed farming in the spaces between the palms (Thomas *et al*., 2011). Plants that are recommended to be grown as intercrops in arecanut gardens include cocoa, black pepper, banana, pineapple, tapioca, ginger, turmeric, elephant foot yam, arrow root, sweet potato, *etc.* Recently, vanilla had also become a promising intercrop in arecanut garden (Sujatha and Bhat, 2010). Though AM research in all the above mentioned crops has been done to some extent, it has been relatively scarce in arecanut (Bopaiah, 1991, Ambili *et al*., 2012). Given the choice, farmers may select a combination of crops to be cultivated in arecanut interspaces depending upon their socio-economic conditions, soil type and water availability, public demand for the crop, *etc*. Different crop combinations could have varied impact on arbuscular mycorrhizae diversity and root colonization potential as observed in coconut and arecanut based cropping systems (Ambili *et al*., 2012, Rajesh Kumar *et al*., 2015). In date palms inter-cultivated with natural vegetation too, it was reported that the AMF diversity differed among the different plants growing in the area in Southern Arabia (Phosri *et al*., 2010). Not only the plant communities growing together, the geographic distances along with soil factors such as pH also significantly impact the AM association (Xu *et al*., 2016). In addition to above factors, AM association is also determined to large extent by the type of agronomic interventions taken up by the farmers, particularly nutrient application (Gryndler *et al*., 2006). Arecanut cultivation is mostly a low-input type of cultivation with farmers generally applying some organic manures available through milch animals they maintain in their farm. Such low-input cultivation can be immensely benefitted through the AMF association as has been reported in sesame cultivation in Kerala (Harikumar, 2015). Keeping this in mind, the present investigation was undertaken to study the impact of crop combinations and soil factors on the diversity of AM species and extent of association in arecanut-based cropping systems in farmers' fields under acidic soil conditions.

Materials and methods

Sampling sites

Arecanut palm (*Areca catechu* Linn.) based cropping systems in farmers' fields in three topographically different regions- highlands, midlands and lowlands of Kannur district of Kerala, under organic management practices, were selected for the study (Table 1). The highland region comprises mainly of mountains and is the area of major plantations like coffee, rubber, tea, cardamom and other spices. The midland region, lying between the mountains and the low lands are mostly undulating hills and valleys, where intense agricultural activities are followed. The lowland is comparatively narrow and comprises of rivers, deltas and seashore. The district lies between

Table 1. Details of the location, soil and intercrops of arecanut based cropping systems selected for the study

Location	Geographical coordinates	Soil type	Land type	Intercrops	Inputs
Maneikkara	11°45' 11" N, 75°33' 08" E	Red sandy loam	Coastal land (60 m, AMSL)	Banana	Cow dung with banana leaf, husk
Cheruvanjeri	11°51' 11.91" N, 75°34' 26.04" E	Clay	Mid land (200 m, AMSL)	Banana, Pepper	Cow dung
Nedumpoyil	11°52' 40" N, 75°51' 08" E	Forest loam	High land (600 m AMSL)	Banana, Pepper, Cardamom	Cow dung

AMSL - Above mean sea level

latitudes 11°40'-12°48' N and longitudes 74°52'-76°07' E. It is bound by the Western Ghat in the east (Coorg district of Karnataka State), Kozhikode and Wayanad districts in the south, Arabian Sea in the west and Kasaragod district in the north. Soil samples were collected from arecanut based cropping systems in three different regions of the district from the three locations; Nedumpoyil (N), Cheruvanjeri (C) and Maneikkara (M). Nedumpoyil lies in the highland region of the district, rich in vegetation and guarded by a natural forest reserve (Kannavam forest). Cheruvanjeri lies in the midland part of the district. Maneikkara region can be included in the lowland part of the district. Cropping systems at the three locations differed in type and number of intercrops, with Maneikkara plot having banana alone as the intercrop, Cheruvanjeri plot with banana and pepper as intercrops and Nedumpoyil with banana, pepper and cardamom as intercrops.

Soil sampling and analysis

Soil and root samples were collected from the three cropping systems during the period October to November 2012. Samples were collected from three equidistant points at a lateral distance of 0.5 m from the base of each crop to a depth of 25 cm from the surface after scraping out the top layer of the soil. Then, using a clean auger, soil was scooped out from a depth of 25 cm from the cleared surface. The soil along with the root bits scooped out from three points from the palm basin was thoroughly mixed to make a composite sample. Approximately 300 g of the composite sample was packed in clean polythene bag, labelled and secured with rubber band and transported to microbiology laboratory in an ice-box. The samples were then stored in refrigerator at 4 °C for further studies. For soil analysis, samples were passed through a sieve having two mm mesh size to remove large soil particles and were mixed thoroughly.

Mycorrhizal spore isolation

A sub-soil sample of 50 g was taken from the main sample for the study. AM fungal spores were extracted by wet sieving (45, 100, 250, and 355 µm sieve openings of Endecotts Test Sieves, Endecotts Ltd, England) and decanting method (Gerdemann and Nicolson, 1963). The spores extracted through sieves with mesh size ranging between 45 and 355 µm filtered using a Whatman No.1 filter paper were observed under stereomicroscope (Leica WILD M8, Germany) and the total number of spores was counted. Spores exhibiting morphologically similar characters were then clustered into one group. Number of spores in each morphotype was recorded in order to construct spore community composition. Spore count was calculated as the total number of spores in each soil sample (spores per 50 g soil). Further, the spores were isolated, intact and crushed spores were mounted on slides in polyvinyl-lactoglycerol (PVLG) and PVLG mixed with Melzer's reagent and examined under a compound microscope (Nikon Eclipse Ni, Japan) at 10x to 60x magnification. The identification was done based on the key provided by International Collection of Vesicular-Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu) and the original species descriptions (Schüßler, 2001).

Root staining for mycorrhizal infection

Roots were cut into one centimeter long segment and preserved with formalin-acetic acid-alcohol (FAA) in 90:5:5 ratio. Fixed roots were processed by the method given by Phillips and Hayman (1970). Root colonization parameters like frequency and intensity of hyphal, vesicular and arbuscular colonizations were calculated as per the formula given below:

$$\text{Frequency of root colonization, F (\%)} = \frac{\text{Number of root bits having colonization}}{\text{Number of root bits observed}} \times 100$$

Intensity of mycorrhizal colonization in the root system (M) was determined by the following formula (Trouvelot, 1986):

M (%) = (95n5+ 70n4+ 30n3+ 5n2+ n1) / nb total

Where, n5, n4, n3 and n2 are proportions of root cortex colonized by the fungus,

nb total = total number of root bits showing AMF colonization

Chemical analysis of soil

Root region soil samples were analyzed for soil chemical characteristics. Soil pH was measured in

1:2.5 soil water suspension using pH meter (Eutech instruments, pH tutor) and electrical conductivity was measured at room temperature in 1:5 soil suspension using conductivity meter (Eutech instruments). Soil analysis techniques *viz*., Walkley and Black's rapid titration method (Walkley and Black, 1934), Kjeldahl method (Kjeldahl, 1883) and Bray and Kurtz (1945) method were employed for determination of organic carbon, total nitrogen and available phosphorus, respectively. Available potassium was estimated by ammonium acetate method (Hanway and Heidel, 1952).

Diversity analysis of AM fungi

The diversity of AM fungi in the cropping systems was assessed based on diversity indices:

Simpson's index of dominance (Ds) = 1- (Σni2-N)/ N(N-1) (Simpson,1949)

Shannon's index of general diversity (Hs) = NC {(N log 10N) - Σn*i*/log10n*i*}

Where, C=3.321929 (constant used in converting log 10 to log 2). "n*i* is the number of species in the 'i^{th}' species and "N" is the total number of individual, (Lloyd *et a1*., 1968)

Statistical analysis

Pearsons's correlation analysis was done to determine relationship between AMF spore count, root colonization and soil physico-chemical factors using SPSS Base 20.00 (SPSS, Cary, N.C.). One way ANOVA was used to test for the spore count, root colonization diversity indices and soil physico-chemical factors in arecanut based mixed cropping system using SAS software version 9.2 (Statistical Analysis System Institute, Cary, NC, USA).

Results

Arbuscular mycorrhizal (AM) parameters and physico-chemical properties of soil in arecanut based cropping systems in different locations of Kannur district are presented in Table 2. AM spore load in soil and root AM colonization frequency differed significantly in arecanut based cropping systems in different locations and in relation to crops which formed the components of the cropping system. The highest spore load was observed in cropping systems of Maneikkara in the low land region, followed by that in high land (Nedumpoyil) and midland (Cheruvanjeri) plots. Root AM colonization was highest in cropping systems of Cheruvanjeri located in the midland region, followed by that in Maneikkara and Nedumpoyil. The soil pH was acidic in all locations and ranged from 4.7 to 4.9, electrical conductivity varied from 142.6 µS cm^{-1} (Maneikkara) to 177.7 µS cm^{-1} at Nedumpoyil. Organic carbon content was higher in the soils collected from Cheruvanjeri (1.39% ± 0.104) and least at Maneikkara (1.04% ± 0.09). There was marked variation in the total nitrogen (0.076 ± 0.002 - 0.2 ±0.01%) and available phosphorus (P) content (8 - 41ppm) of soils in cropping systems in different locations. N content was the highest at Nedumpoyil plot and the available P content at Cheruvanjeri plot. The K content of the soil did not differ significantly in the three cropping systems studied.

Pearson correlation analysis was done to study the relationship between mycorrhizal parameters and physico-chemical properties of soil in cropping systems of different locations (Table 3). There was significantly positive correlation between AM spore load and N content of soil in two locations with highest correlation at Maneikkara site (0.887 at $P \leq 0.05$). The AM root colonization was positively correlated with K and P content of soil in one location each. AMF spore load was found to be negatively correlated with soil pH, but a positive correlation was observed between AMF spore load and organic carbon and electrical conductivity.

Table 2. AM spore load in soil, root colonization and physico-chemical parameters in arecanut based cropping systems

Location	AM spore load in soil (Nos. 10 g^{-1} soil)	Root AM colonization (F %)	OC (%)	N (%)	P (ppm)	K (ppm)	pH	EC (µS cm^{-1})
Maneikkara	81.6 ± 2.75	50.8 ± 3.24	1.40 ± 0.10	0.13 ± 0.003	8.9 ± 0.8	91.6 ± 2.80	4.95 ± 0.08	156.2 ± 2.48
Cheruvanjeri	28.6 ± 2.94	77.5 ± 4.84	1.05 ± 0.09	0.08 ± 0.002	41.7 ± 6.2	91.2 ± 1.64	4.70 ± 0.04	142.6 ± 1.27
Nedumpoyil	48.8 ± 4.23	54.2 ± 2.99	1.33 ± 0.12	0.20 ± 0.01	14.4 ± 3.2	96.1 ± 7.88	4.75 ± 0.11	177.6 ± 2.14

OC- Organic carbon; N- Total nitrogen; P- Available phosphorus; K- Available potassium; EC- Electrical conductivity

Table 3. Correlation between AMF parameters and soil physico-chemical properties in arecanut-based cropping systems

Location	pH		EC		OC		N		P		K	
	AM S	F%	AM S	F%	AM S	F%	AM S	F%	AM S	F%	AM S	F%
Maneikkara	-0.464	-0.866	0.488	0.426	0.165	0.023	0.881 *	0.651	-0.269	0.711	-0.119	0.366
Cheruvanjeri	-0.176	-0.151	0.130	85	0.230	-0.516	0.428 *	0.311	-0.215	0.389 *	0.017	-0.596
Nedumpoyil	-0.187	0.253	0.468	0.351	0.287	-0.008	0.303	0.306	-0.058	-0.118	0.128	0.683 *

OC- Organic carbon; N- Total nitrogen 0; P- Available phosphorus; K- Available potassium 0; EC- Electrical conductance; AM S – AM spore load, F % - Frequency of AM colonization in root
*Significant at $p<0.05$

AMF root colonization was characterized by arbuscules, arbusculate coils, vesicles and hyphae (Table 4 and Fig. 1). Among the AMF colonization patterns, hyphal pattern of AMF infection was the most abundant in cropping systems. AMF root colonization frequency in the cropping systems was maximum in arecanut-banana intercropping system (77.5%) followed by arecanut-banana-pepper-cardamom intercropping system (48.8%). *Paris* type of colonization was the most predominant one found in four plant families. Hyphal colonization pattern was uniformly present in all the crops irrespective of the cropping system. Among the crops studied, frequency of hyphal pattern of root colonization was the maximum in banana (66.7%), followed by arecanut (50.6%), pepper (49.5%) and cardamom (46%). Arbuscular and vesicular colonization pattern was also recorded in the observed samples. The frequency of arbuscules was more in arecanut (43.2%), when compared to its component crops in

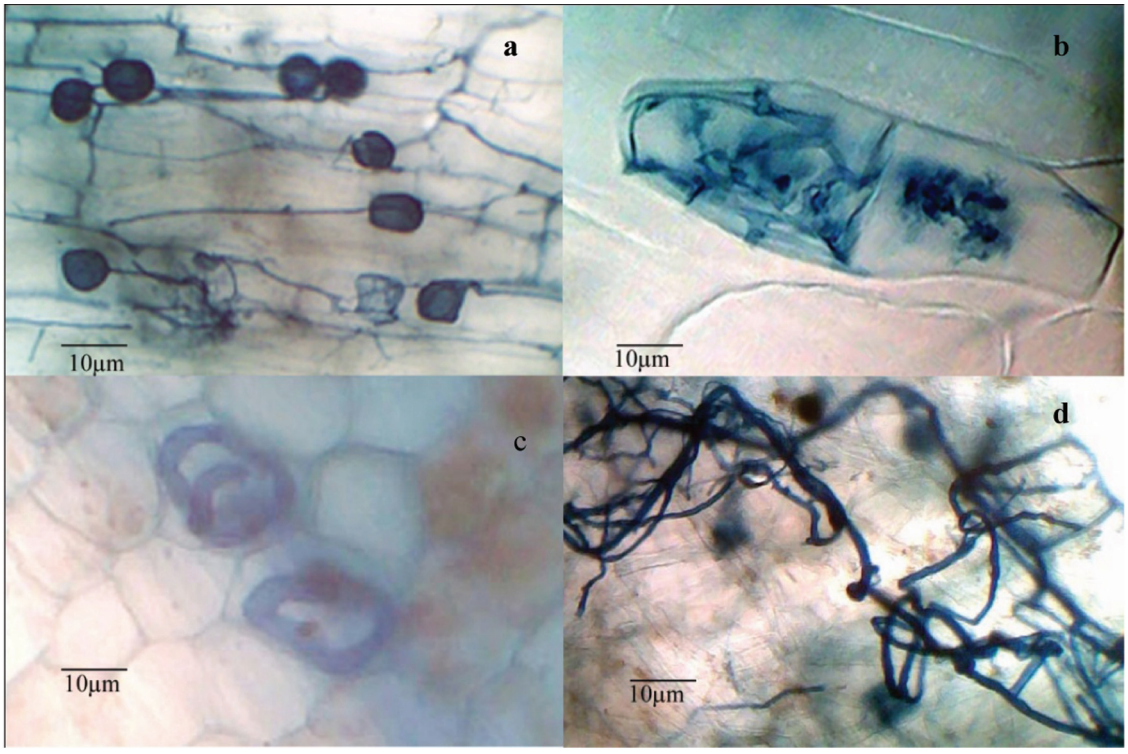

Fig. 1. AMF infection patterns in component crops of arecanut cropping systems (a to d; clockwise from top left)
(a) Vesicle formation in banana root
(b) Cross section of an arecanut root cell with arbuscular pattern of infection
(c) Hyphal coiling in arecanut root
(d) Branched hyphae in arecanut root

Table 4. AMF infection pattern in component crops of arecanut based cropping systems in different locations

Plant family/ Species	Hyphal pattern of infection						Arbuscular infection pattern						Vesicular infection pattern					
	F %			M %			F %			M %			F %			M %		
	M	C	N	M	C	N	M	C	N	M	C	N	M	C	N	M	C	N
Arecaceae																		
Arecanut	74.8	41.6	35.6	17.2	39.6	30.6	54.6	36.0		71.6	44.0		24.5	37.8		15.8	65.2	
(Areca catechu L.)	±0.3	±0.6	±3.0	±0.3	± 0.3	±0.6	±2.6	±0.3	—-	±0.8	±0.3	—-	± 0.5	±0.5	—-	±0.4	±0.3	—-
Musaceae																		
Banana	80.1	54.6	70.8	21.2	58.2	51.6		23.0		30.5	30.2		81.1	35.6	19.2	75.9	72.6	12.5
(Musa indica)	±0.1	±0.9	±0.4	±0.4	± 0.6	± 0	—-	±1.2	—-	±0.8	± 0.3	—-	± 0.7	±4.5	±2.6	±0.4	±0.3	±0.4
Piperaceae																		
Pepper		57.0	44.8		57.3	35.0								38.5	21.3		54.6	10.5
(Piper nigrum L.)		±4.9	±9.0		± 0.6	±0.3		—-	—-		—-	—-		±0.9	± 0.4		±0.9	±0.3
Zingiberaceae																		
Cardamom			45.9			34.3									13.4			9.2
(Eletteria cardamomum)			±0.4															

Values are means of three replications; ± Standard error

F % - Frequency of root colonization; M % - Intensity of root colonization

M – Maneikkara (Arecanut+Banana), C- Cheruvanjeri (Arecanut+Banana+Pepper), N- Nedumpoyil (Arecanut+Banana+Pepper+Cardamom)

'—-' No infection was observed in the samples collected

Blank column indicates absence of crop in the cropping system at a particular location

a particular cropping system. However, vesicular colonization pattern was more in banana (61%). Apart from the AMF structures, special endophytic structures known as microsclerotia formed by dark septate endophytic hyphae (DSE) were also observed in banana and pepper.

The relative distribution of AMF species in the main crop arecanut and the component crops in a cropping system is presented in Table 5. The AMF species from the cropping systems belonged to the Glomineae order, and is represented by Glomaceae, Claroideoglomeraceae, and Acaulosporaceae families (Fig. 2). However, *Glomus* was the common and widely distributed genus in all the cropping systems. *Claroideoglomus etunicatum* and *Funneliformis geosporum* were the species uniformly observed in all the cropping systems. However, certain species were restricted to a particular crop in the cropping system. *Glomus aggregatum* and *C. maculosum* were observed in arecanut rhizosphere and were not observed in banana and pepper rhizosphere in the same cropping system. Arecanut-banana-pepper mixed cropping system exhibited the maximum AMF diversity (Table 6), with the highest relative abundance of *F. geosporum* (28.5±0.33%) followed by

Table 5. Crop wise distribution pattern of AMF species in arecanut-based cropping systems in different locations of Kannur district of Kerala

Plant Family/	**AMF species observed**		
Species	**Maneikkara**	**Cheruvanjeri**	**Nedumpoyil**
Arecaceae	*G. glomerulatum*	*G. multicaule*	*G. multicaule*
Arecanut	*G. macrocarpum*	*G. boreale*	*G. boreale*
(Areca catechu L.)	*G. boreale*	*G. aggregatum*	*C. etunicatum*
	C. etunicatum	*G. constrictum*	*F. geosporum*
	F. geosporum	*C. etunicatum*	*R. fasciculatus*
		C. maculosum	*A. delicata*
		F. geosporum	
		R. fasciculatus	
		A. delicata	
		A. lacunosa	
Musaceae	*G. macrocarpum*	*G. multicaule*	*G. multicaule*
Banana	*G. boreale*	*G. boreale*	*G. boreale*
(Musa indica)		*G. globiferum*	*C. etunicatum*
		G. constrictum	*F. geosporum*
		C. etunicatum	
		F. geosporum	
		A. scorbiculata	
Piperaceae		*G. boreale*	*G. multicaule*
Pepper		*C. etunicatum*	*G. boreale*
(Piper nigrum L.)		*F. geosporum*	
		F. mossae	
		R. fasciculatus	
Zingiberaceae			*G. multicaule*
Cardamom			
(Eletteria cardamomum)			

M – Maneikkara (Arecanut+Banana), C- Cheruvanjeri (Arecanut+Banana+Pepper**),**
N- Nedumpoyil (Arecanut+Banana+Pepper+Cardamom**)**
Blank column indicates absence of crop in the cropping system at a particular location

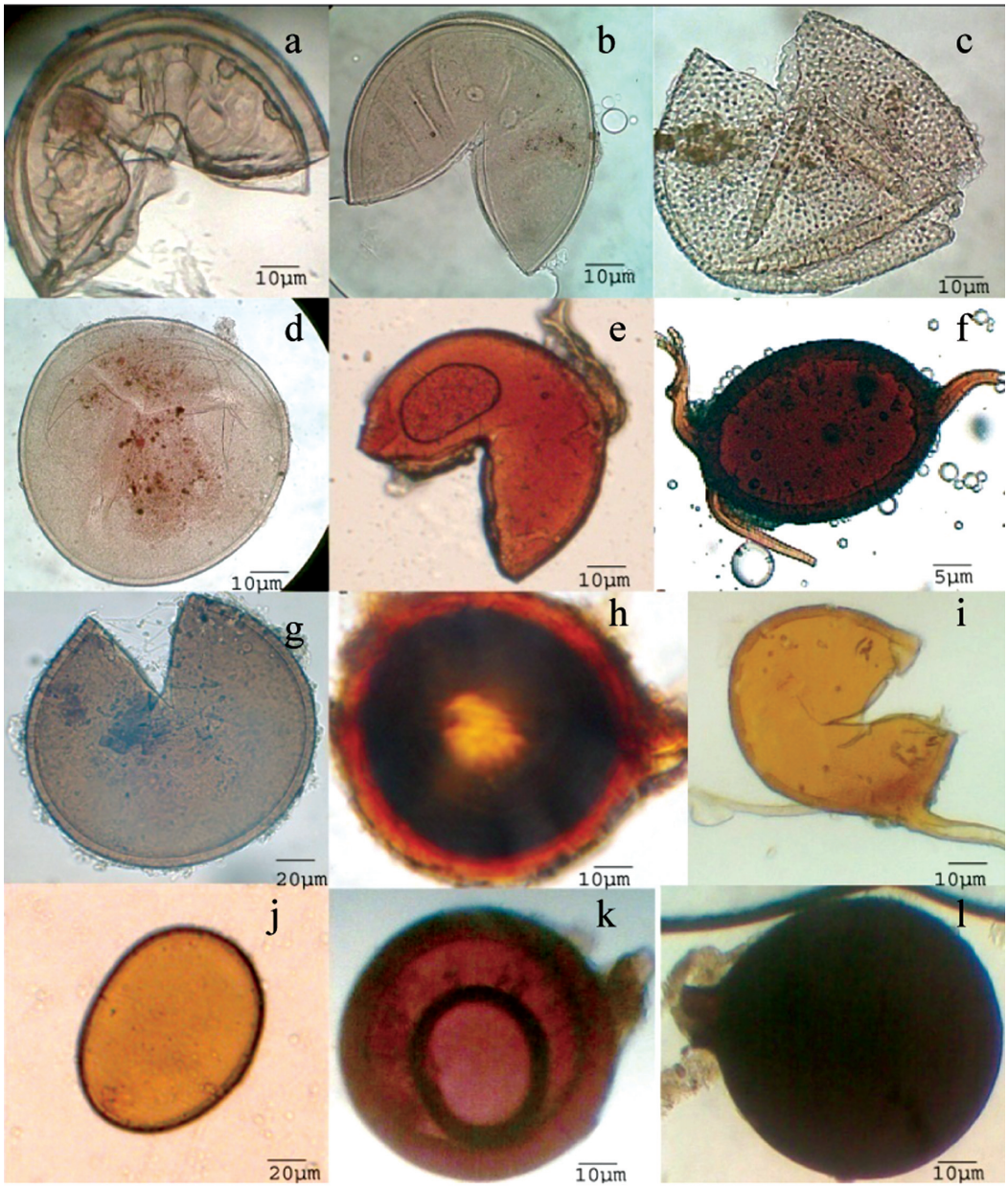

Fig. 2. AMF species prevalent in arecanut cropping systems at different locations (a to l; clockwise from top left)

(a) *A. lacunosa*, arecanut rhizosphere, Cheruvanjeri
(b) *Acaulospora* sp., arecanut rhizosphere, Cheruvanjeri
(c) *A. scorbiculata,* banana rhizosphere, Cheruvanjeri
(d) *Acaulospora* sp., arecanut rhizosphere, Nedumpoyil
(e) *Glomus* sp., arecanut rhizosphere , Cheruvanjeri
(f) *G. multicaule*, arecanut rhizosphere, Nedumpoyil
(g) *C. etunicatum*, banana rhizosphere, Cheruvanjeri
(h) *G. glomerulatum*, arecanut rhizosphere, Maneikkara
(i) *R. fasciculatus*, arecanut rhizosphere, Nedumpoyil
(j) *G. boreale*, banana rhizosphere, Maneikkara
(k) *G. constrictum* , banana rhizosphere, Cheruvanjeri
(l) *G. macrocarpum*, arecanut rhizosphere, Maneikkara

Table 6. Species wise distribution of AMF species in arecanut-based cropping systems of different locations in Kannur district of Kerala

Family/AMF spp.	Frequency of occurrence (%)*			Relative abundance (%)**		
	Maneikkara	Cheruvanjeri	Nedumpoyil	Maneikkara	Cheruvanjeri	Nedumpoyil
Glomaceae						
G. aggregatum		24.2±13.9			11.6 ± 6.6	
G. multicaule		66.6 ± 2.04	70.2 ± 0.5		11.4 ± 0.30	29.4 ± 0.32
G. macrocarpum	61.2 ± 0.3			29.4 ± 0.32		
G. boreale	50 ± 0.5	40.7 ± 0.24	58.3± 0.3	4.3 ± 0.2	8.4 ± 0.1	18.5 ± 0.02
G. glomerulatum	6.5 ± 0.1			3.5 ± 0.6		
G. globiferum		41.6 ± 0.09			6.7 ± 0.19	
R. fasciculatus		22.2±0.12	25.0 ± 0.6		5.8 ± 0.9	9.5 ± 0.08
C. maculosum		11.1± 0.43			6.8 ± 0.30	
C. etunicatum	33.3 ± 0.8	74.3 ± 0.03	75 ± 0.03	6.2 ± 0.38	5.7 ± 0.51	7.3 ± 0.19
F. geosporum	66.6 ± 0.6	70.3 ± 0.21	32.3± 0.27	15.3 ± 0.33	28.5 ± 0.3	29.3 ± 0.15
F. mossae		8.2 ± 0.30			0.9 ± 0.06	
Acaulosporaeceae						
A. scorbiculata		3.7 ± 0.1			0.2 ± 0.01	
A. delicata		14.4 ± 0.4	33.3 ± 0.5		6.8 ± 0.04	5.3 ± 0.14
A. lacunosa		2.3 ± 0.08			0.6 ± 0.03	-

Values are means of three replications; ± Standard error

M - Maneikkara (Arecanut+Banana), C - Cheruvanjeri (Arecanut+Banana+Pepper**),** N - Nedumpoyil (Arecanut + Banana + Pepper + Cardamom)

Blank column indicates absence of a particular AMF species at a particular location

$$\text{*Frequency of occurrence (\%)} = \frac{\text{Number of soil samples possessing spores of a particular species}}{\text{Total number of samples analysed}} \times 100$$

$$\text{**Relative abundance (\%)} = \frac{\text{Number of spores of a particular species}}{\text{Total number of spores}} \times 100$$

G. multicaule (11.4±0.3%), *G. boreale* (8.4±0.1%), *Acaulospora delicata* (6.8±0.04%), *C. etunicatum* (5.74±0.51%), *Rhizophagus fasciculatus* (5.8±0.9%) and *A. scorbiculata* (0.23±0.01%) in Cheruvanjeri . However, less diversity was observed in Maneikkara plot with *G. macrocarpum* (29.4±0.32 %), *F. geosporum* (15.3±0.33%), *C. etunicatum* (6.2±0.38%), *Glomus boreale* (4.30±0.2%) and *G. glomerulatum* (3.5± 0.6%). The relative abundance of *G. multicaule* was at par with *F. geosporum* (29.3±0.15%) followed by *G. boreale* (18.5±0.02%), *R. fasciculatus* (9.5±0.08%), *C. etunicatum* (7.30±0.19%), and *A. delicata* (5.3±0.14%) at Nedumpoyil site.

The AM population showed high levels of diversity in arecanut based cropping systems as revealed by diversity indices (Table 7). The

Table 7. AM fungal species diversity in arecanut- based cropping systems in different locations of Kannur district of Kerala

Location	Shannon–Weiner index (Hs)	Simpson's index of diversity (Ds)	Species richness
Maneikkara	2.2	0.634	5
Cheruvanjeri	4.5	0.952	13
Nedumpoyil	3.5	0.915	8

Shannon-Wiener diversity index (Hs) of AMF species varied in different locations. Arecanut-banana-pepper mixed cropping system gave the maximum Hs value of 4.5 at Cheruvanjeri, followed by Nedumpoyil (3.5) and Maneikkara (2.2). The lowest value of index of dominance was shown in Maneikkara (0.634). There was not much variation

in dominance index at Nedumpoyil and Cheruvanjeri cropping systems. The AMF species richness between different cropping systems followed a similar trend as that of diversity. Cheruvanjeri plot has maximum species richness, followed by that in Nedumpoyil and least in Maneikkara.

Discussion

Plant health and productivity are rooted in the soil, and the quality of soil depends on the viability and diversity of its biota which determine the structures that support a stable and healthy agro-system (Doran and Linn, 1994). Among the wide range of biota in the rhizosphere, the role of AMF in providing nutrition to the plant is significant. The role of AM fungi in enhancing the plant growth through improved nutrient and water uptake and suppression of soil- borne pathogens have been reported (Jeffries *et al*., 2003; Sanchez-Diaz and Honrubia, 1994). AM association assumes great significance in arecanut cultivation as farmers traditionally follow the organic way of farming without the use of chemical fertilizer inputs. It was observed in the present study that AM association as evidenced by spore counts in root region soil and colonization in roots varied significantly in arecanut based cropping systems at different locations in Kannur district of Kerala. Differences in AM spore densities with same plant species, papaya, had been reported in agro-based ecosystem of Goa (Khade and Rodrigues, 2010). This difference was noticed because of several factors such as the soil type, plant communities associated with main crop and agronomic practices (Gaidashova *et al*, 2010). The increase in plant species diversity also contribute to increase in AMF sporulation and species numbers as well as changes in AMF community composition (Iyer, *et al*., 1986). These increases may be due to the direct effects of increased numbers of plant species on the AMF community. The variation of AM fungal root colonization in different locations could be due to the change in the habitat, soil fertility and acclimatization of a particular AM fungal genus/ species to a particular location (Brundrett, 1991). The cropping systems included in the present study are low input systems with decreased use of fertilizers, pesticides and tillage and had organic manures as the major source of nutrients. In these cropping systems, all components have positive influence on AMF symbiosis. Such situations provide a conducive environment for the development and functioning of AMF.

Correlation studies between AM parameters and soil factors in the present study revealed significantly positive correlation between AM spore load and nitrogen content of soil in two locations and positive correlation of AM root colonization with K and P content of soil in one location. Root colonization and AMF diversity were higher in the soils of Cheruvanjeri plot, which had higher available P content. Earlier reports suggested AM colonization of roots in soils with high available P, indicating that AMF colonization is influenced by other factors also in addition to P, such as pH (Valsalakumar *et al*., 2007). In the present study, the soil pH was acidic in arecanut based cropping systems in all locations and was in the range of 4.7-4.9. It has been reported that AMF distribution in soils of low pH (4.1- 4.7) supports the adaptability of AMF in acidic environment (Manoharachary, 2004).

Identification of AMF from arecanut- based cropping systems revealed that genus *Glomus* was more abundant compared to *Acaulospora* in all locations. Among the species identified, *C. etunicatum* and *F. geosporum* were more abundant and present in all the three locations. The predominance of *Glomus* under varying soil conditions may be due to the fact that they are widely adaptable to the varied soil conditions and can survive in acidic soils. It was reported that the *Glomus* sp. usually produces more spores than other AMF species in the same environment (Bever *et al*., 1996). An increase in number of intercrops increased the AMF diversity, which may be due to the fact that above ground diversity in a cropping system increases the below ground microbial diversity in the system owing to the greater availability and variability of root exudates for microbial growth and multiplication. The present results are in agreement with earlier reports on coconut agroforestry system where the distribution of mycorrhizal species was influenced by the diversity of intercrops (Iyer *et al*., 1986). Earlier results also indicated differential rate of multiplication of each species in different host plants (Bever, 2002). The AMF species distribution in the arecanut based cropping system was thus

influenced by the crops which formed the components of the system and the soil factors specific to a particular habitat.

AM colonization of the *Paris* type was the predominant one in the four plant families which formed component of arecanut based cropping system in the three locations. This type of colonization pattern was reported earlier in Piperaceae and Musaceae families (Muthukumar *et al*., 2003). AMF spore load was also found to be directly correlated to frequency of root colonization which was in accordance with previous reports (Giovannetti and Nicholson, 1983). Shannon-Wiener species diversity index and Simpson's index of diversity varied in cropping systems from different locations. Both these indices were very high in Cheruvanjeri. The richness of AM fungal populations or their functional diversity has consequences for the equilibrium of natural plant community structure (Francis and Read, 1994). Beena *et al*. (2000) reported that soil disturbance rather than seasonal or climatic factors influence the diversity and species richness. The comparatively less diversity of AMF obtained at Maneikkara site points to the fact that abundance and species richness are affected by the crops which form the components of the cropping system and the soil factors.

Conclusion

The arecanut palm harboured AMF to different levels in the arecanut-based cropping systems established in three different locations in Kannur district of Kerala as evidenced by spore load in rhizosphere soil and colonization frequency in roots, indicating the influence of the component crops and soil factors in determining the extent of the AM association. The root colonization frequency was in the following order in the crops which formed the components of the cropping system; banana > arecanut > pepper > cardamom. Arecanut-banana-black pepper mixed cropping system at Cheruvanjeri had the highest diversity of AM fungi with the maximum Shannon–Weiner index value and Simpson's index of diversity. Hyphal pattern of AMF infection predominated in all the crops irrespective of the cropping systems, followed by vesicular and arbuscular type of infection. The arbuscular type of infection was predominant in arecanut, while vesicular type predominated in banana. *Glomus* spp. was prevalent in all the cropping systems, followed by *Acaulospora* sp. *Funneliformis geosporum* was the most abundant species irrespective of the crop in a cropping system. The information on the influence of component crops and the soil factors on AM association and diversity in cropping system will greatly help to develop strategies to utilize the symbiotic partnership to achieve sustainable crop production in the context of organic farming avoiding the chemical inputs.

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