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Radial variations in anatomical properties of *Melia dubia* cav. at five different ages

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The differences in anatomical characteristics *viz.,* vessel length, vessel diameter, vessel arrangement, vessel frequency, ray height, ray width, ray frequency, fibre length, fibre diameter, fibre wall thickness and fibre lumen width of one, two, three, four and five year old *Melia dubia* cav. respectively were investigated. The wood samples were systematically collected from the pith, middle and periphery wood sections of the radial positions. The mean values of vessel length (235.68, 250.72, 272.76, 292.01 and 329.57 µm), vessel diameter (192.57, 207.11, 231.10, 247.54 and 276.96 µm), vessel frequency (4.00, 4.00, 4.00, 5.00 and 5.00 mm^2), ray height (336.65, 356.56, 377.82, 399.15 and 438.23 µm), ray width (71.73, 77.00, 84.53, 91.73 and 98.66 µm), ray frequency (7.00, 8.00, 8.00, 10.00 and 11.00), fibre length (647.00, 825.00, 892.78, 1093.92 and 1159.30 µm), fibre diameter (24.00, 24.90, 26.01, 26.75 and 27.52 µm), fibre wall thickness (4.07, 5.29, 6.49, 7.62 and 9.08 µm) and fibre lumen width (15.87, 14.32, 13.03, 11.52 and 9.35 µm) for one, two, three, four and five year old *M. dubia* wood respectively. All parameters showed significant increment with respect to increase in age and also all the anatomical characteristics examined in this study increased significantly from pith to periphery except lumen width. The effects of age and radial positions contributed significantly to variations in anatomical characteristics of *M. dubia* wood.

Key words: *Melia dubia*, vessels, rays, fibre, age gradation.

INTRODUCTION

Melia dubia cav. belonging to the family Meliaceae has its trade name as Malabar Neem. It is a large deciduous and fast growing tree with wide spreading branches on a stout, straight, tall bole; it also has young shoots with inflorescence covered with mealy stellate hairs. It is indigenous to the Western Ghats of Southern India and is common in moist deciduous forests of Kerala (Gamble, 1992). Outside India, it is found in Sri Lanka, Malaysia, Java, China and Australia*.* *M. dubia* with its multi-various uses like pulpwood, timber, fuelwood and plywood can fit as a suitable species for agro and farm forestry plantation programme. The wood is also used for packing cases, cigar boxes, ceiling planks, building purposes, agricultural implements, pencils, mach boxes, splints and kattamarans. It has been screened as an alternate species for pulpwood (Parthiban et al., 2009).

Wood consists of matrix of walls and air spaces. Its structural properties vary from pith to bark, from the tree base to the top and from the stem to the branches and roots. The primary structural block of soft wood is the tracheids or cells. These cells vary from 16 to 42 µm in diameter and from 870 to 4000 µm long (Panshin and

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Zeeuw,1980). The variations in wood properties are attributable to the different distribution patterns of its micro structures, its arrangement, size and dimension of components cells. In hard woods, the cells that make up the anatomical organization are the vessels, parenchyma cells and the wood rays (Desch and Dinwoodie, 1983). These anatomical properties according to Ocloo and Laing (2003) have a positive correlation on the strength characteristics of wood. Fibre length is one of the quality parameters for pulpwood (Jorge et al., 2000). It has been extensively studied in relation to tree age and within tree position (Hudson et al., 1995; Sandercock et al., 1995). During their formation, these cells are affected by many factors such as site, ecological conditions, management, genetics**,** and age of the trees growing in plantation conditions (Zobel and Van Buijtenen, 1989). The anatomical features are modified within trees during their growth in order to adjust physiologic and water stress; thereafter they maintain the existence of the species (Baas, 1982; Metcalfe, 1989). However such anatomical studies pertaining to *M. dubia,* a fast growing multiple utility species have not been attempted so far. Hence the current study was carried out to determine the anatomical properties of *M. dubia* at different ages.

MATERIALS AND METHODS

M. dubia cav. plantations raised at different age gradation *viz.,* one, two, three, four and five years old located at Kollegal, Samraj Nagar District, Karnataka at 12˚04'N latitude and 77˚09'E longitude were chosen for studying the variation in anatomical properties of the wood sample at different age gradations. The investigations were carried out in the Laboratory of Forest College and Research Institute, Tamil Nadu and College of Forestry, Kerala Agricultural University, Kerala, India during 2010 to 2012. Three replicated samples with dimensions of 2 × 2 × 2 cm^3 were sliced out from the pith, middle and periphery region of *M. dubia* wood disc of 2.5 cm thickness which was sawn at the breast height (1.37 m) of the main trunk from five different ages of trees and thin microscopic sections of size 15 to 20 µm were taken using '*Leica* SM 2000 R Microtome'. Temporary slides were made by staining these sections with safranin stain and subjected to measurements and photography using Image analysis system (Motic). Measurement of various parameters was done using the Motic software. Observations were recorded on at least three fields on each section.

Maceration

Maceration of the wood samples was done using Jeffrey's method (Sass, 1971). For maceration, Jeffrey's solution was used which was prepared by mixing equal volumes of 10% potassium dichromate and 10% nitric acid. Radial chips of wood shavings were taken from the 1 cm^3 wood blocks separately from the three radial positions *viz*., pith, middle and periphery. These chips were boiled in the maceration fluid for 15 to 20 min so that the fibre individuals were separated. Then these test tubes were kept for 5 to 10 min so that the fibres settled at the bottom. The solution was discarded and the resultant material was thoroughly washed in distilled water until traces of acid were removed. The fibre samples were stained using saffranin and mounted on temporary slides using glycerin as the mountant.

Vessel morphology

Parameters viz., length (µm) and tangential diameter (µm) of the vessels were measured on the transverse sections (TS) using the Motic Image Analysis software. At least ten observations per sample were recorded for measuring vessel diameter. The vessel arrangement in heartwood of the species was identified by studying the transverse sections as per Rao and Juneja (1971). Frequency of vessels per squared millimeter was calculated by counting the number of vessels in randomly selected area per field and using the formulae given below. In each field, three count areas were taken.

$$\text{Vessel frequency} = (\text{Number of Vessels} / \text{Area in } \mu^2) \times 10^6$$

Ray morphology

Ray height and width (µm) were measured on the tangential longitudinal sections (TLS) for each tissue type. Frequency of rays per squared millimeter was calculated by counting the number of rays intersect with a transect line drawn randomly in each field. Observations were recorded by counting the rays at three different lines using Motic Image Analyser.

$$\text{Ray frequency} = (\text{Number of rays} / \text{Area in } \mu^2) \times 10^6$$

Fibre morphology

Different measurements were taken from the macerated wood samples viz., fibre length (µm), fibre diameter (µm), fibre wall thickness (µm) by measuring thickness of the wall cross sectional area and fibre lumen width (µm) by measuring width of the lumen at cross sectional area through Motic Image Analyser.

RESULTS AND DISCUSSION

Vessel morphology

The vessel morphology showed significant variations in the vessel length among the five different age gradations of wood samples at 5% level. The mean vessel length of fifth year wood samples (329.57 µm) was significantly higher than the general mean (276.15 µm) and the lowest value (235.68 µm) was recorded in first year wood samples (Figures 1 and 4). The highest mean value was obtained in the radial position (Pith, Middle and Periphery) for fifth year [pith (306.81 µm), middle (330.25 µm), and periphery (351.64 µm)] wood samples. All the five different ages of *M. dubia* wood showed diffuse porous type of vessel arrangement in their heartwoods. It is evident from the Table 1 that the vessel frequency of the heartwood of *M. dubia* was not significantly different among the different age gradation. There was an increase in vessel length and vessel diameter from pith to periphery and also increase in vessel frequency from one year to five year old sample; this was due to the older age of the tree. Rao et al. (2003) and Gimenez and Lopez (2000) reported that the vessel length and vessel diameter were interrelated and also significantly varied from pith to periphery. On the other hand, Florsheim et al. (1999) reported that in *Myracrodruon urundeuva*, the

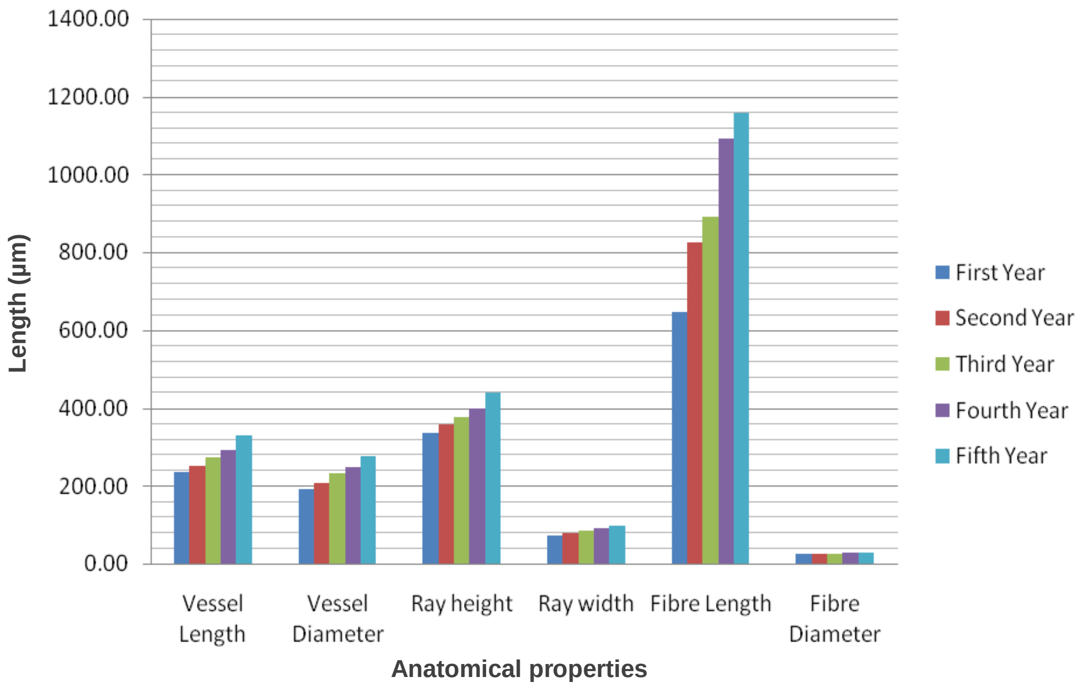

Figure 1. Variation in wood anatomy of *Melia dubia* at five different age gradations.

Table 1. Comparative profile of wood anatomical properties of *Melia dubia* at five different age gradations.

S.No	Anatomical properties	Age in years							
		1	2	3	4	5	Mean	SEd	CD(0.05)
1.	Vessel length (µm)	235.68	250.72	272.76	292.01	329.57	276.15	17.89	39.86
2.	Vessel diameter (µm)	192.57	207.11	231.10	247.54	276.96	231.06	23.55	NS
3.	Vessel frequency (mm^2)	4.00	4.00	4.00	5.00	5.00	5.00	0.40	NS
4.	Ray height (µm)	336.65	356.56	377.82	399.15	438.23	381.68	18.18	40.50
5.	Ray width (µm)	71.73	77.00	84.53	91.73	98.66	84.73	0.82	1.82
6.	Ray frequency (mm^2)	7.00	8.00	8.00	10.00	11.00	8.80	0.59	1.31
7.	Fibre length (µm)	647.00	825.00	892.78	1093.92	1159.30	923.60	46.21	102.96
8.	Fibre diameter (µm)	24.00	24.90	26.01	26.75	27.52	25.84	0.35	0.77
9.	Fibre wall thickness	4.07	5.29	6.49	7.62	9.08	6.51	0.17	0.38
10.	Fibre lumen width	15.87	14.32	13.03	11.52	9.35	12.82	0.56	1.26

Significant at 5% level; CD – Critical difference; NS – non significant.

vessel frequency decreased towards bark whereas, the vessel diameter increased. Vessel morphological study of *M. dubia* revealed that it exhibited diffuse porous vessel arrangement. The diffuse porous nature of *Albizia odoratissima* (Anoop et al., 2005) and *Samanea saman* (Sahu, 2005) are also in conformity with the results of current study.

Ray morphology

Ray height and ray width were studied along the radial position *viz.,* pith, middle and periphery. Ray height showed statistically significant difference among the different ages of wood samples (Figures 1 and 5). The mean ray height of fifth year (438.23 µm) wood samples showed significantly higher value than the general mean (381.68 µm). Similarly, fifth year wood sample values of ray height for pith, middle and periphery were significantly higher than the general mean value [pith (359.47 µm), middle (382.50 µm), and periphery (403.08 µm)] and on par with the fourth year wood sample value (Table 2). The mean ray width of fifth and fourth year samples were significantly higher than the general mean (84.73 µm)

Table 2. Mean wood anatomical properties of pith, middle, periphery of *Melia dubia* at five different age gradations.

Anatomical properties	Radial position	Age in years							
		1	2	3	4	5	Mean	SEd	CD (0.05)
Vessel length (µm)	Pith	218.89	237.08	259.02	272.65	306.81	258.89	17.22	34.67
	Middle	225.36	238.15	265.03	288.16	330.25	269.39	13.23	26.65
	Periphery	262.80	276.94	294.24	315.23	351.64	300.17	14.62	29.44
Vessel diameter (µm)	Pith	168.48	175.56	199.31	219.35	252.50	203.04	10.04	20.23
	Middle	193.74	218.75	238.40	246.16	262.25	231.86	11.17	22.49
	Periphery	215.50	227.03	255.58	277.13	316.13	258.27	20.22	40.72
Ray height (µm)	Pith	312.38	337.60	353.88	377.88	415.60	359.47	25.31	50.98
	Middle	340.75	359.63	372.75	402.75	436.63	382.50	23.44	47.20
	Periphery	356.84	372.45	406.84	416.84	462.45	403.08	27.03	54.44
Ray width (µm)	Pith	61.02	65.28	71.00	76.80	83.25	71.47	3.56	7.17
	Middle	72.15	78.48	85.45	92.25	98.48	85.36	3.45	6.96
	Periphery	82.03	87.25	97.13	106.13	114.25	97.36	3.53	7.12

and the lowest mean value was recorded in first year samples. The ray frequency mean value showed statistically significant difference at 5% level among the five different age gradation of wood samples. The mean values of ray frequency obtained from first, second, third, fourth and fifth year wood samples were 7.00, 8.00, 8.00, 10.00 and 11.00 rays per squared millimeter respectively (Table 1).

The study revealed that the age and position could significantly influence the ray morphology. Ayaz and Nasir (1992) reported ray height as 8192 µm for *Pinus eldarica*, 6169 µm for *Pinus geradiana*,7310 µm for *Pinus roxburghii* and 6162 µm for *Pinus wallichiana*. Generally, ray height changes periodically as tree grows. This increase in ray height with tree age is as a result of transverse division of ray cell initials and fusiform initials of adjacent rays or addition of segments from fusiform initials. Environment has a greater influence in deciding the size of ray height. This is because of the fact that an environmental stress reduce rate of cambial growth thereby height of the ray may be reduced (Larson, 1994). In this study a gradual increase of ray height radially, that is, from pith to periphery was absorbed. Similar result was earlier reported by Lev (1998) in *Pinus halepensis* and *Pinus pinae*. The ray height of *M. dubia* ranged from 336.65 (one year old wood) to 438.23 µm (Five year old wood). This result showed that the ray height increases with increase in the age of the tree (Table 1).

In general, ray width was also found to show periodic changes as the tree grows. This increase happens by anticlinal division of initial cells within rays or by merging of rays (Larson, 1994). The ray width increases with increase in tree age in very young trees and stabilize thereafter. Iqbal and Ghouse (1987) also reported similar changes in ray width with tree age. Environment has a greater influence on ray width. This accounts for the variation in genetic makeup of species and increase in vessel frequency was also due to the older age of the tree.

Fibre morphology

The variation in fibre length was found to be statistically significant at 5% among the different age gradations of wood sample. The maximum mean fibre length (1159.30 µm) was observed in the five year old *M. dubia* wood samples (Figures 1, 2 and 3). The fifth and fourth year wood sample values of fibre length for pith, middle and periphery were significantly higher than the general mean values [pith (884.47 µm), middle (924.27 µm), and periphery (962.06 µm)] (Table 3). This result establishes that wood fibre length increases with increase in age. The same line of findings had been reported by Jorge et al. (2000) who found that with increase in age there was an increase in fibre length from pith to periphery. The radial variation of fibre length showed an increasing trend from pith to the periphery although of small magnitude. This is an indication that age, radial position from where the wood samples were collected contributed to the variation in fibre length. Therefore wood fibre length increased with increase in age. Generally, there was decrease in fibre length from the base to the top and an increase from inner wood to outer wood (Izekor et al., 2011).

The mean fibre diameter was maximum in the five year old (27.52 µm) *M. dubia* wood samples and minimum in one year old samples (24.00 µm) (Table 1). In the radial positions (pith, middle and periphery), the mean values of

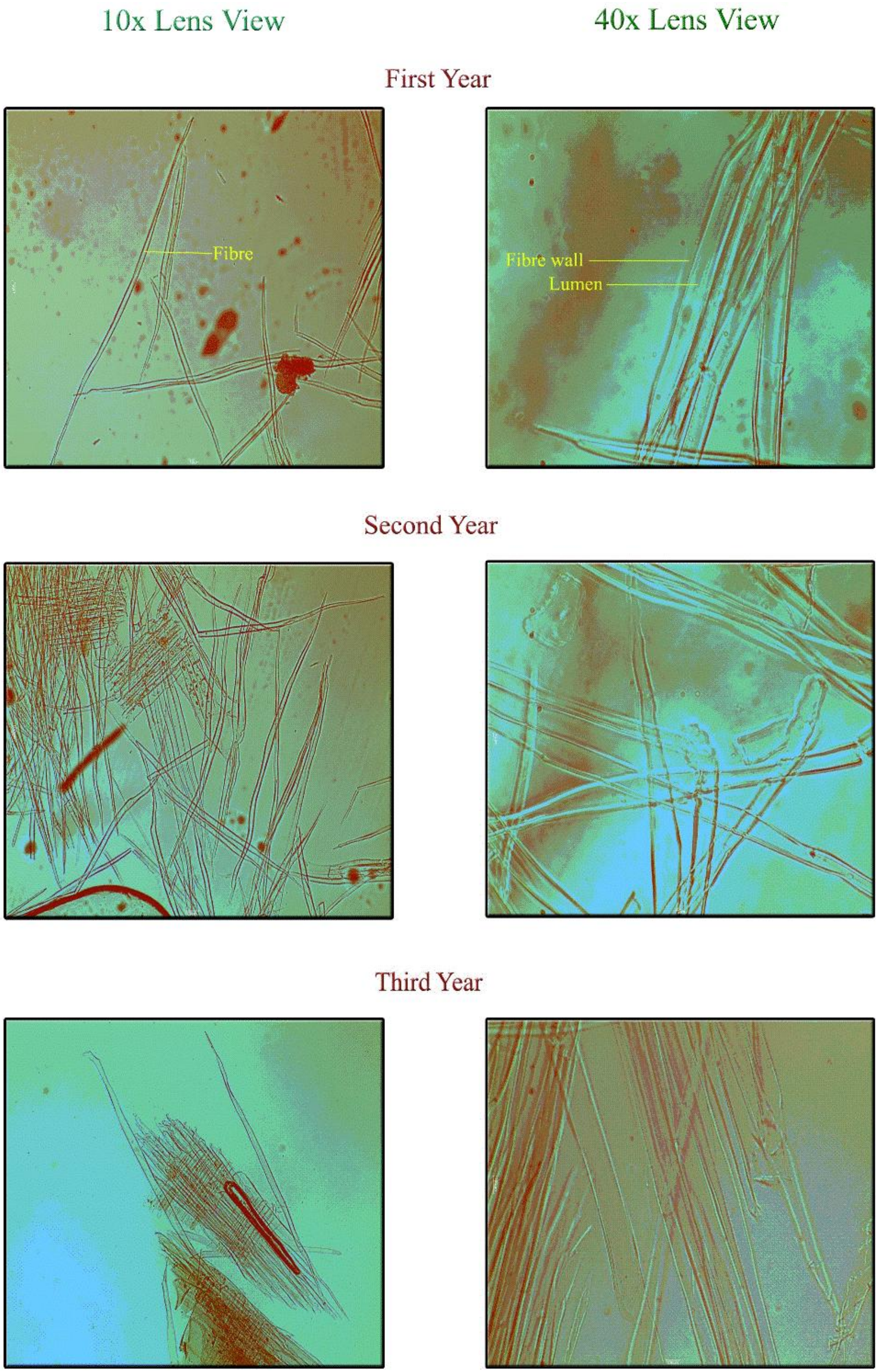

Figure 2. Fibre morphology of first, second and third year wood of *Melia dubia.*

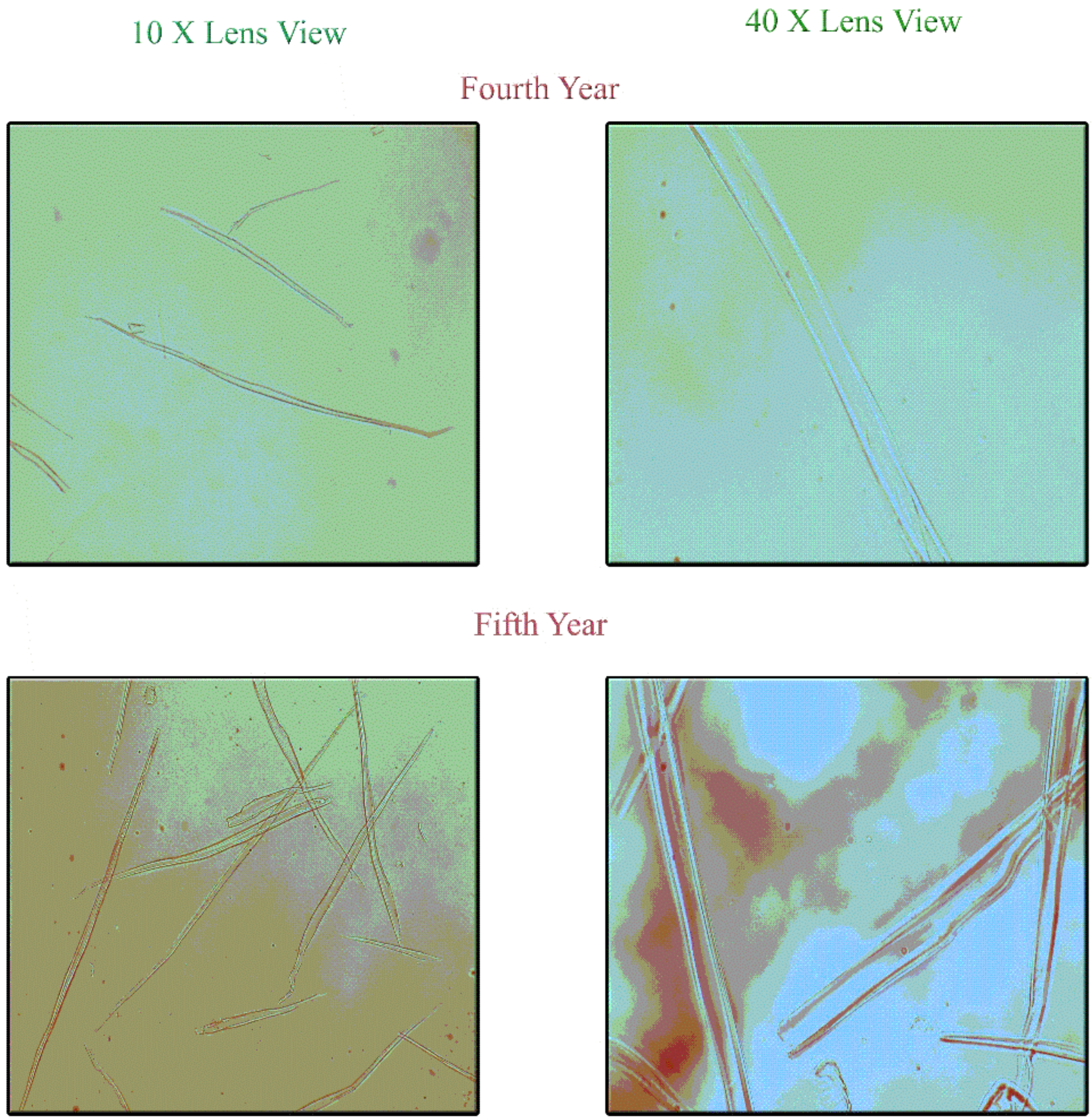

Figure 3. Fibre morphology of fourth and fifth year wood of *Melia dubia.*

fibre diameter obtained were maximum in fifth year [pith (25.95 µm), middle (27.40 µm), and periphery (29.20 µm)] wood samples (Table 3). This result establishes that with increase in age of the wood, the fibre diameter also increases (Figure 1). The observed increase in fibre diameter associated with the increasing age of the tree may be due to many molecular and physiological changes that occur in the vascular cambium as well as the increase in the wood cell wall thickness during the tree ageing process (Plomion et al., 2001; Roger et al., 2007).

The fibre wall thickness was maximum in fifth year wood samples (9.08 µm) followed by fourth year (7.62 µm) (Table 1). The mean values of fibre wall thickness recorded in the radial position (pith, middle and periphery) were lowest in first year [pith (3.50 µm), middle (4.05 µm), and periphery (4.65 µm)] wood samples (Table 3). The mean wall thickness obtained in the radial position was increased from pith to periphery of wood for all age gradations. This showed that fibre wall thickness increases with age. It also increases from pith to periphery. Analysis of variance at 5% probability showed

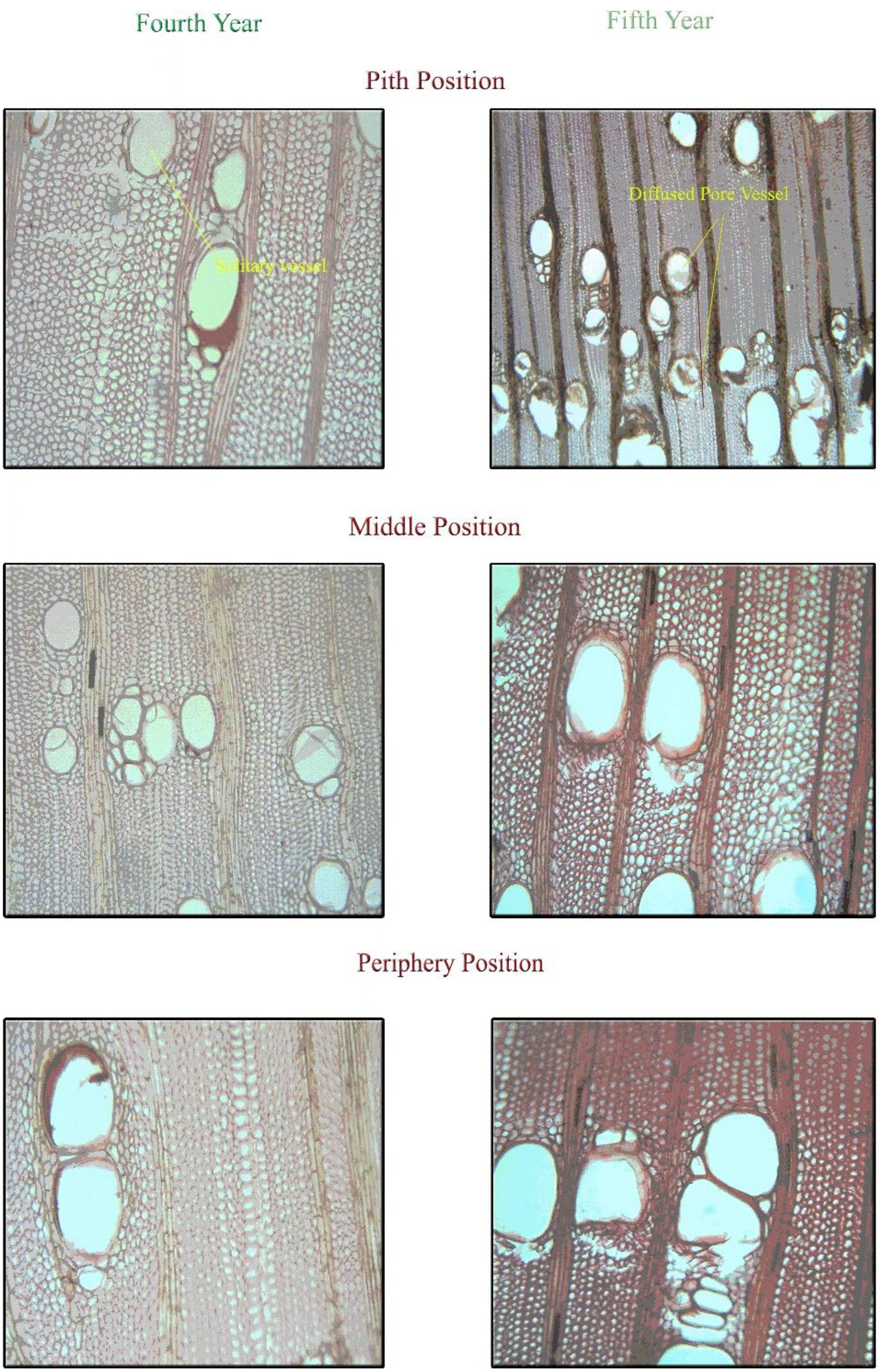

Figure 4. Vessel morphology of Fourth and Fifth year wood of *Melia dubia*.

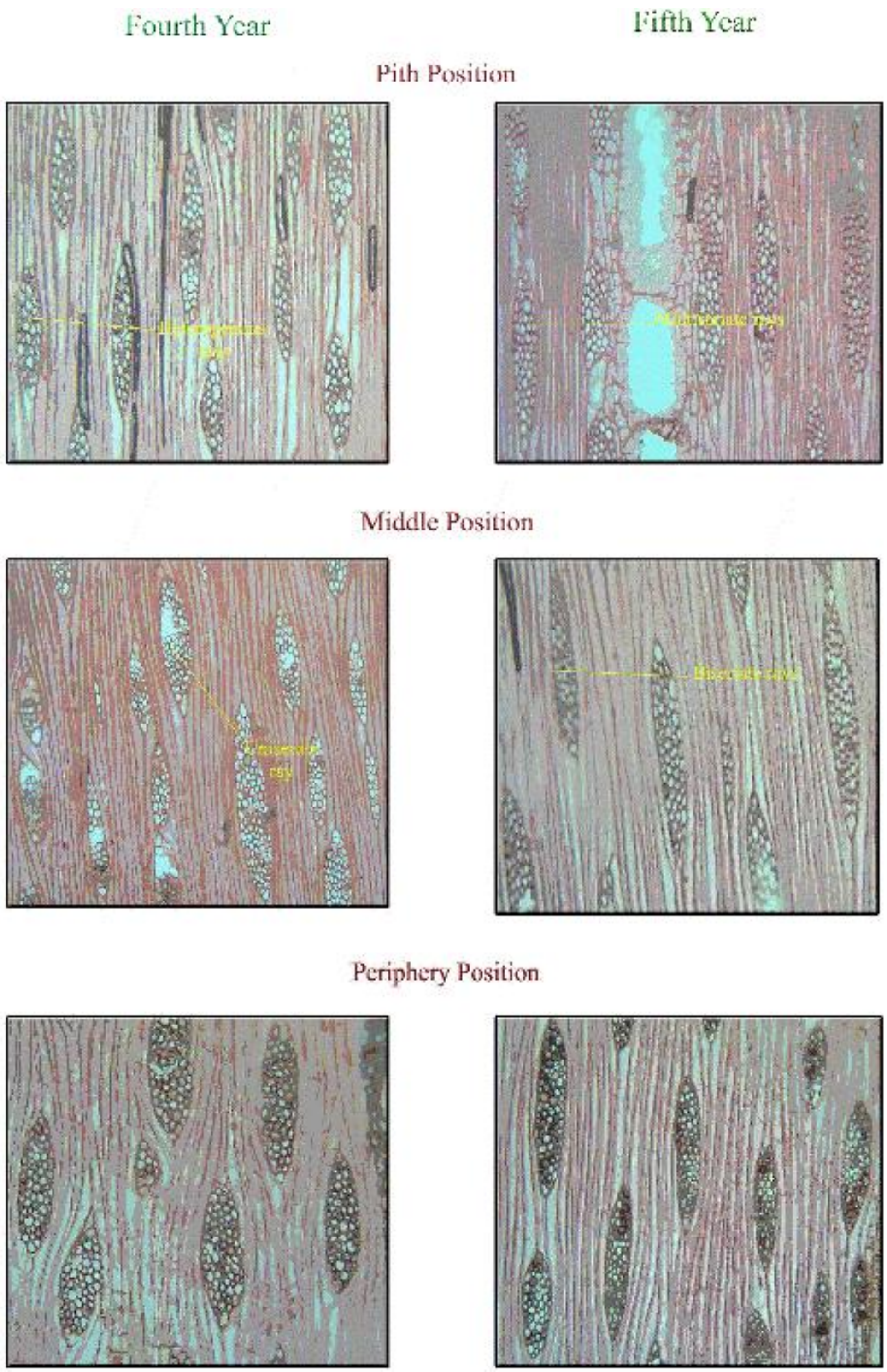

Figure 5. Ray morphology of Fourth and Fifth year wood of *Melia dubia.*

that, the effects of the different age classes and radial positions from where the samples were collected contributed significantly to variations in cell wall thickness. Current findings are in concurrence with studies on *Tectona grandis* by Izekor and Fuwape (2011). Akachuku (1980) also attributed the increase in cell wall thickness of *Gmelina arborea* to changes in cell size that are associated with annual and periodical growth cycles and increase in the age.

Fibre lumen width recorded significant difference

Table 3. Mean wood anatomical properties of Pith, Middle, Periphery of *Melia dubia* at five different age gradations.

Anatomical properties	Radial position	Age in years							
		1	2	3	4	5	Mean	SEd	CD(0.05)
Fibre Length (µm)	Pith	615.00	790.00	866.00	1004.35	1092.00	884.47	30.61	68.21
	Middle	656.00	830.00	890.75	1103.70	1140.90	924.27	28.96	64.54
	Periphery	670.00	855.00	921.00	1173.70	1245.00	962.06	41.49	92.45
Fibre Diameter (µm)	Pith	22.30	23.45	24.36	24.85	25.95	24.18	0.21	0.47
	Middle	23.75	24.40	25.70	26.80	27.40	25.61	0.15	0.34
	Periphery	25.95	26.86	27.96	28.60	29.20	27.71	0.18	0.40
Fibre Wall Thickness (µm)	Pith	3.50	4.80	5.93	6.95	8.65	5.97	0.25	0.55
	Middle	4.05	5.20	6.50	7.65	9.05	6.49	0.22	0.50
	Periphery	4.65	5.88	7.03	8.25	9.55	7.07	0.20	0.44
Fibre Lumen Width (µm)	Pith	15.30	13.85	12.50	10.95	8.65	12.25	0.32	0.70
	Middle	15.65	14.00	12.70	11.50	9.30	12.63	0.76	1.70
	Periphery	16.65	15.10	13.90	12.10	10.10	13.57	1.64	3.66

among different age gradations. Fibre lumen width values for pith, middle and periphery were highest in first year wood [pith (15.30 µm), middle (15.65 µm), and periphery (16.65 µm)] and lowest in fifth year wood samples from the radial position (Tables 1 and 3). The fibre lumen width ranged from 15.87 µm (First year) to 9.35 µm (Five year) and also lumen width increases from pith to periphery for all age gradations. This showed that fibre lumen width decreases with age, which may be attributed to the increase in the length of fibre initials associated with increasing age of the cambium (Jorge et al., 2000). The observed differences in lumen width with increasing age of the tree may also be due to increase in cell size and physiological development of the wood as the tree grows in girth. Roger et al. (2007) reported positive relationship between variations in lumen width and age of the cambium. Fibre lumen width increased from pith to the periphery at any particular height. Generally, the trend of variations in fibre lumen width showed a decrease from base to top and an increase from pith to periphery.

Conclusion

The anatomical characterisation studies in *M. dubia* at different ages exhibited significant differences in vessel, ray and fibre morphology. *M. dubia* has been identified as a diffused porous wood through anatomical characterisation. The anatomical characteristics such as vessel length, vessel diameter, vessel arrangement, vessel frequency, ray height, ray width, ray frequency, fibre length, fibre diameter, fibre wall thickness increases with age, however fibre lumen width decreases with age. The observations on radial morphology indicated that anatomical characteristics increased from pith to periphery of the wood. The effects of age and radial position contributed significantly to variations in anatomical characteristics of *M. dubia* among different age gradations.

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