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Wood anatomical features and natural resistance of planted *Azadirachta excelsa* and *Khaya ivorensis*

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Plantation species such as *Azadirachta excelsa* and *Khaya ivorensis* has gained traction as alternative raw materials for timber industry in Malaysia. This present study,was conducted to examine the anatomical features and resistance of the 10-year-old planted *A. excelsa* and *K. ivorensis* to white rot fungus (*Lentinus sajor-caju*). Both *A. excelsa* and *K. ivorensis* were easily identified by its anatomical features which were parenchyma, ray, fibre and prismatic crystal. *A. excelsa* and *K. ivorensis* wood were classified as resistant, with *K. ivorensis* being more resistant to white rot fungus, *Lentinus sajor-caju*. The resistance of the wood to fungal decay decreased towards the top and higher resistance was shown in the heartwood than the sapwood portion. Based on this finding, density could not be an indicator of decay but the resistance of wood is related to the presence of extractives.

Keywords: ***Azadirachta excelsa*; *Khaya ivorensis*; anatomical features; resistance; white rot fungus; plantation species.**

INTRODUCTION

Forest plantations were established in Malaysia circa 1900, aiming to alleviate pressure on natural forests while ensuring a steady wood supply for the local wood products industry (Ratnasingam, 2020). The Malaysia Timber Industrial Board has recommended eight fast-growing tree species for plantation purposes in Malaysia, encompassing both local and introduced species. Among the local species are *Azadirachta excelsa* (sentang), *Neolamarckia cadamba* (kelempayan/laran), *Paraserianthes falcataria* (batai) and *Octomeles sumatrana* (binuang). Meanwhile, the introduced species include *Khaya ivorensis* (African mahogany), *Tectona grandis* (teak), *Acacia* spp., and *Hevea brasiliensis* (rubberwood) (Abdul Rasip et al. 2004; Anon, 2007). Stated by Ahmad Zuhaidi and Mohd Noor (2002) *Azadirachta excelsa* or known as sentang locally and *Khaya ivorensis* also known as African Mahogany have significant potential for the furniture industry.

Since plantation species were introduced to the wood industry, there's been a necessity to study wood properties to assess wood quality (Zhang et al. 2020). Among the important studies is examining the anatomical features, which not only for wood identification but also as indicators of other significant properties and for utilisation (Rajput 2023;Mensah et al. 2024). As asserted by Tageldin et al. (2019) and Lima et al. (2023), there exists a significant correlation between anatomical properties and density as well as shrinkage. Besides, anatomical properties could also serve as indicators of strength. For instance, thicker fiber walls may signify higher density and strength (Hamdan et al. 2020) where the timbers were potential for the heavy duty purposes.

Additionally, comprehending wood's resistance to fungal decay holds significance as an indicator of wood quality, as highlighted in studies by Zaidon et al. (2002), Nzokou et al. (2005), Syofuna et al. (2012) and Roszaini et al. (2016). This information gains particular relevance when considering wood for outdoor applications (Eaton and Hale 1993; Bowyer et al. 2003), encompassing potential hazards and protective measures outlined in utilization guidelines (Zaidon et al. 2002). As highlighted by Salmiah (2001), wood serves as a critical substrate for microfungi, with wood degradation due to microfungi occurring significantly faster in tropical regions than in temperate regions. Two prevalent types of fungal decay are observed: white-rot fungi capable of completely breaking down woody cells, and brown-rot fungi that primarily target hemicellulose and cellulose.

The aim of this study is to assess the wood anatomical features and resistance of 10-year-old planted *A. excelsa* and *K. ivorensis* from the family of Meliaceae to white rot fungus (*Lentinus sajor-caju*).

Samples for this study consist of the sapwood and heartwood taken along the tree height. Wood anatomical features and durability data obtained from the study will be used as basis for utilization of *A. excelsa* and *K. ivorensis*.

MATERIALS AND METHODS

Field Sampling

Plantation timber species aged 10 years, i.e *A. excelsa* and *K. ivorensis*, were harvested from the Forest Research Institute Malaysia (FRIM). The field sampling was followed the procedures that outlined by Tan et al. (2010). Three trees of each species were felled at 15 cm above ground level. Two discs, about 5 cm thick, were then extracted from each tree at diameter breast height (DBH), wrapped in plastic, and stored in a freezer for the study of anatomical characteristics and natural resistance. The measurements of these trees are summarised in Table 1.

Table 1: Measurements of the 10-year-old *A. excelsa* and *K. ivorensis* trees from plantation.

Species	Tree	DBH (cm)	Total bole height (m)	Clear bole height (m)
	1	16.60	18.00	7.00
A. excelsa	2	18.00	15.00	6.50
	3	22.30	14.20	6.70
	1	19.00	15.00	7.60
K. ivorensis	2	17.00	14.00	7.30
	3	17.00	15.00	7.10

Determination of The Anatomical Features

The anatomical features analysis followed the procedure described in Wheeler et al. (1989). A 1cm^3 wood block was extracted from each wood disc and immersed in distilled water until fully soaked and sank. Thin sections from the transverse, tangential, and radial surfaces of each block were obtained using a sledge microtome, with section thickness approximately 25 µm. These sections were placed separately in petri dishes for staining, utilizing 1% safranin-0. After staining, the sections underwent washing with 50% ethanol and dehydration through a series of ethanol solutions ranging from 70% to 95% concentration. A drop of Canada Balsam was applied to each section, which was then covered with a cover slip and oven-dried at 60 °C over several days. Measurements and terminology adhered to the guidelines by Wheeler et al. (1989) and also other related references by Menon (1993) and Ogata et al. (2008).

Decay Study Using White Rot Fungus

Wood discs were harvested from 10-year-old planted *A. excelsa* and *K. ivorensis* trees at bottom, middle, and top positions. These discs were then transformed into smaller samples (25 x 25 x 9 mm, radial x tangential x longitudinal) following the specifications outlined in ASTM D2017-05, with 20 replicates (10 sapwood, 10 heartwood) obtained from each disc and height position. Weight-loss experiments were conducted in accordance with ASTM D2017-05 using the white-rot fungus *Lentinus sajor-caju*. The bottles were loosely capped and steam sterilized at 121 ºC for 30 minutes. After cooling overnight, feeder strips were inoculated with *L. sajor-caju* fungus and incubated at 27 ºC for 1-2 weeks until the fungal mycelium fully covered the feeder strip, signaling readiness for the introduction of test blocks. Before testing, all blocks were oven-dried overnight at 105 ºC, then weighed and conditioned to a constant weight in a conditioning room at 27 ºC and 70% relative humidity. A sterilized test block was then placed on each prepared feeder strip, and the bottles were incubated for 12 weeks in a controlled environment at 27 ºC and 70% relative humidity. A total of 360 replicate test blocks from each height and section type i.e sapwood or heartwood were prepared. Weekly observations ensured no contamination and monitored any basidiome formation. By the end of the 12-week incubation period, the test blocks were removed from the culture bottles, cleaned of soil and fungal mycelium, re-weighed, and the mean weight loss was calculated as detailed in Table 2.

Table 2: The degrees of fungal resistance of the test samples were grouped into four classes based on the weight loss.

Mean weight loss (%)	Resistance class
0-10	Highly resistant
11-25	Resistant
26-50	Moderately resistant
>50	Slightly resistant

Source: ASTM D2017-05 (ASTM, 2005)

The resistance of the species to the fungus was calculated based on the percentage weight loss as follows:

$$\text{Weight loss (\%)} = \left(\frac{W_a - W_b}{W_a} \right) \times 100$$

Where,

W_a = weight before exposure, g

W_b = weight after exposure, g

Statistical Analysis

Statistical analysis utilized SAS software. A two-way analysis of variance (ANOVA) was conducted to assess variations in wood resistance to the white rot fungus across stem positions and between sapwood and heartwood. Significant differences among samples were identified using the Least Significant Difference (LSD) test with a significance level of $p \leq 0.05$.

RESULTS

Wood Anatomical Features Descriptions

Figures 1 to 3 illustrate the wood anatomical features of *A. excelsa* and *K. ivorensis*. These figures depict the wood anatomical features and other timber characteristics essential for species identification. The identification of *A. excelsa* and *K. ivorensis* wood is important to describe and document since the wood from these plantation forests is also widely used by the timber industry.

Table 3: Comparative of wood anatomical features between *A. excelsa* and *K. ivorensis*.

Anatomical features	Species	
	Azadirachta excelsa	***Khaya ivorensis***
Parenchyma	Vasicentric and in bands of 3 to 6 cells wide	vasicentric
Ray		
Ray width	2 to 3 cells	3 to 7 cells
Cellular composition	Heterocellular rays with one row of upright/square marginal cells (type III)	Heterocellular rays with 2 to 4 rows of upright/square marginal cells (type II)
Fibre	Fibre non-septate	Fibre septate
Mineral inclusions		
Prismatic crystals	Prismatic crystals present in chambered axial parenchyma cells	Prismatic crystals present in upright/ square ray cells

Azadirachta excelsa (Figure 1 and 2)

The sapwood is pale red and distinct from the heartwood, which is reddish brown (Figure 1a). The results showed that vessels are diffuse, solitary or in multiples of up to 2 to 4 or rarely more, clustering common, up to 3 to 4 vessels (Figure 2a). Tangential vessel diameter ranged from 125 to 175 µm and 6 to 10 vessels per mm^2. Perforation plates were simple. Intervessel pits were alternate and polygonal. Vessels filled with dark-coloured deposits of dried extractives are present in the heartwood (Figure 2b), whereas in the sapwood the vessels are usually devoid of extractives. Wood parenchyma is vasicentric and in bands of 3 to 6 cells wide (Figure 2c). Rays are moderately fine to medium sized (Figure 2d), mostly 2 to 3 cells wide and height of 325 to 650 µm. Heterocellular rays (Figure 2e) with one row of upright/square marginal cells (type III). Fibre was non-septate (Figure 2f). Fibre length ranged 880 to 1500 µm,fibre lumen diameter was 11.2 µm and fibre wall thickness was 2.83 µm. The fibre was classified as thin to thick walled. Prismatic crystals were present in chambered axial parenchyma cells (Figure 2f). Silica was absent.

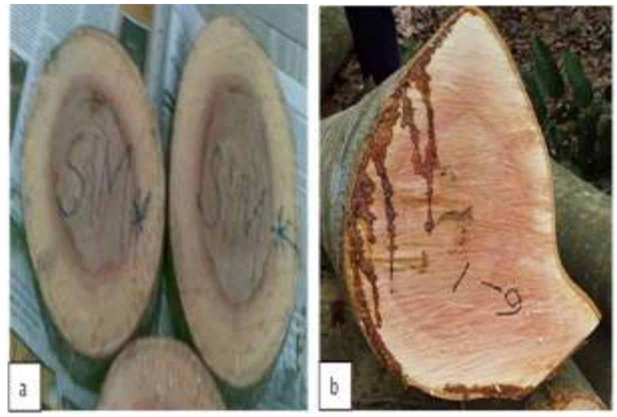

Figure 1: Disc of (a) *Azadirachta excelsa* and (b) *Khaya ivorensis*

Khaya ivorensis (Figure 1 and3)

The sapwood is whitish to yellowish distinct from the heartwood, which is reddish brown (Figure 1b). The results showed that vessels are diffuse, solitary or in multiples of up to 2 to 4 or rarely more, clustering common, up to 3 to 4 vessels (Figure 3a). Tangential vessel diameter ranged from 111 to 157 µm and 6 to 14 vessels per mm^2. Perforation plates were simple. Intervessel pits were alternate and polygonal. Vessels filled with dark-coloured deposits of dried extractives are present in the heartwood (Figure 3b), whereas in the sapwood the vessels are usually devoid of extractives. Wood parenchyma is vasicentric. Rays are moderately fine to medium sized (Figure 3c), mostly 3 to 7 cells wide and height of 427 to 603 µm. Heterocellular rays (Figure 3d) with 2 to 4 rows of upright/square marginal cells (type II). Fibre was septate (Figure 3e). Fibre length ranged from 1202 to 1398 µm,fibre lumen diameter was 13.3 µm and fibre wall thickness was 3.15 µm. The fibre was classified as thin to thick walled. Prismatic crystals were present in upright/square on the ray cells(Figure 3f). Silica were absent.

Resistance of *A. excelsa* and *K. ivorensis* to Fungal Decay

Mean percentage weight loss of planted *A. excelsa* and *K. ivorensis* against white rot fungus (*Lentinus sajor-caju*) are given in Table 4. Results shows that weight loss of *A. excelsa* was in the range from 6.4 to 27.6 % with a mean of 17 %. On the other hand, weight loss of *K. ivorensis* was in the range of 5.91 to 22.0% with a mean of 14 %. Both species was classified as resistance. Based on the Table 4, lower in weight loss was observed at the bottom and heartwood indicated that this part is more resistance than at the top part and sapwood.

Table 4: Mean percentage weight loss of planted *A. excelsa* and *K. ivorensis* along the tree height.

Species	Height level of the tree			Wood disc proportion		Properties		
	B	M	T	S	H	Mean weigh t loss (%)	Mean Density (kg/m^3)	Decay resistance class
A. excelsa	11.26^b (11.20)	13.62^b (5.39)	25.37^a (5.32)	19.92^a (8.61)	14.31^b (9.31)	17.00^a (10.6)	501^a (40)	Resistance
K. ivorensis	12.21^b (8.88)	12.03^b (3.58)	17.25^a (11.9)	15.81^a (10.02)	11.85^b (7.21)	14.00^b (8.09)	464^b (48)	Resistance

Note: Means followed by the same letter in the same row are not significantly Different at $p \leq 0.05$
Values in parentheses are standard deviations
B=Bottom, M=Middle, T=Top, S=Sapwood, H=Heartwood

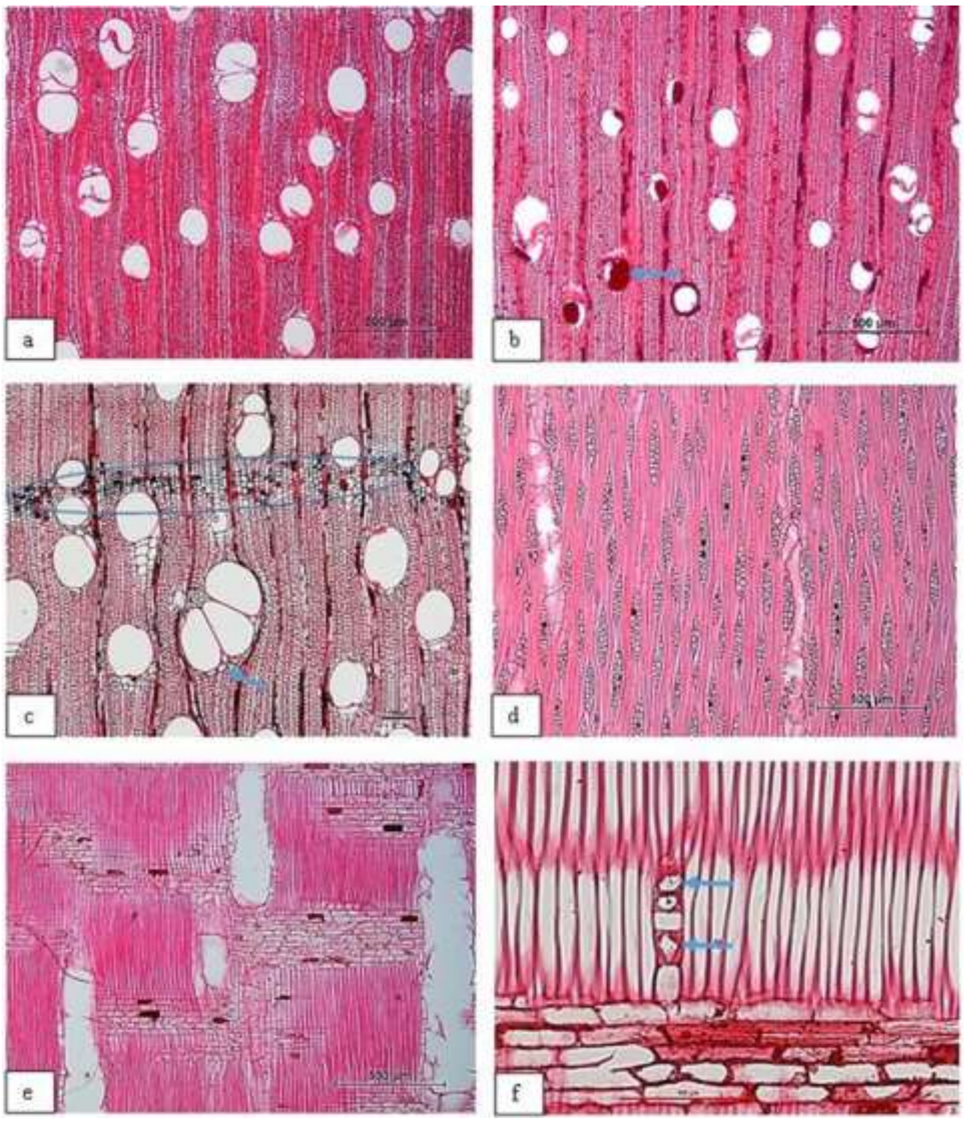

Figure 2: *Azadirachta excelsa* (a) Transverse section in sapwood: vessels solitary or in multiples and clustering common, vessels are usually devoid of extractives. (b) Transverse section in heartwood: vessels filled with dark-coloured deposits of dried extractives (arrow). (c) Transverse section: wood parenchyma is vasicentric (arrow) and in bands of 3 to 6 cells wide (circle) (d) Tangential section: rays are moderately fine to medium sized, mostly 2 to 3 cells wide. (e) Radial section: heterocellular rays (type III). (f) Radial section: fibre non-septate, crystals present in chambered axial parenchyma (arrow).

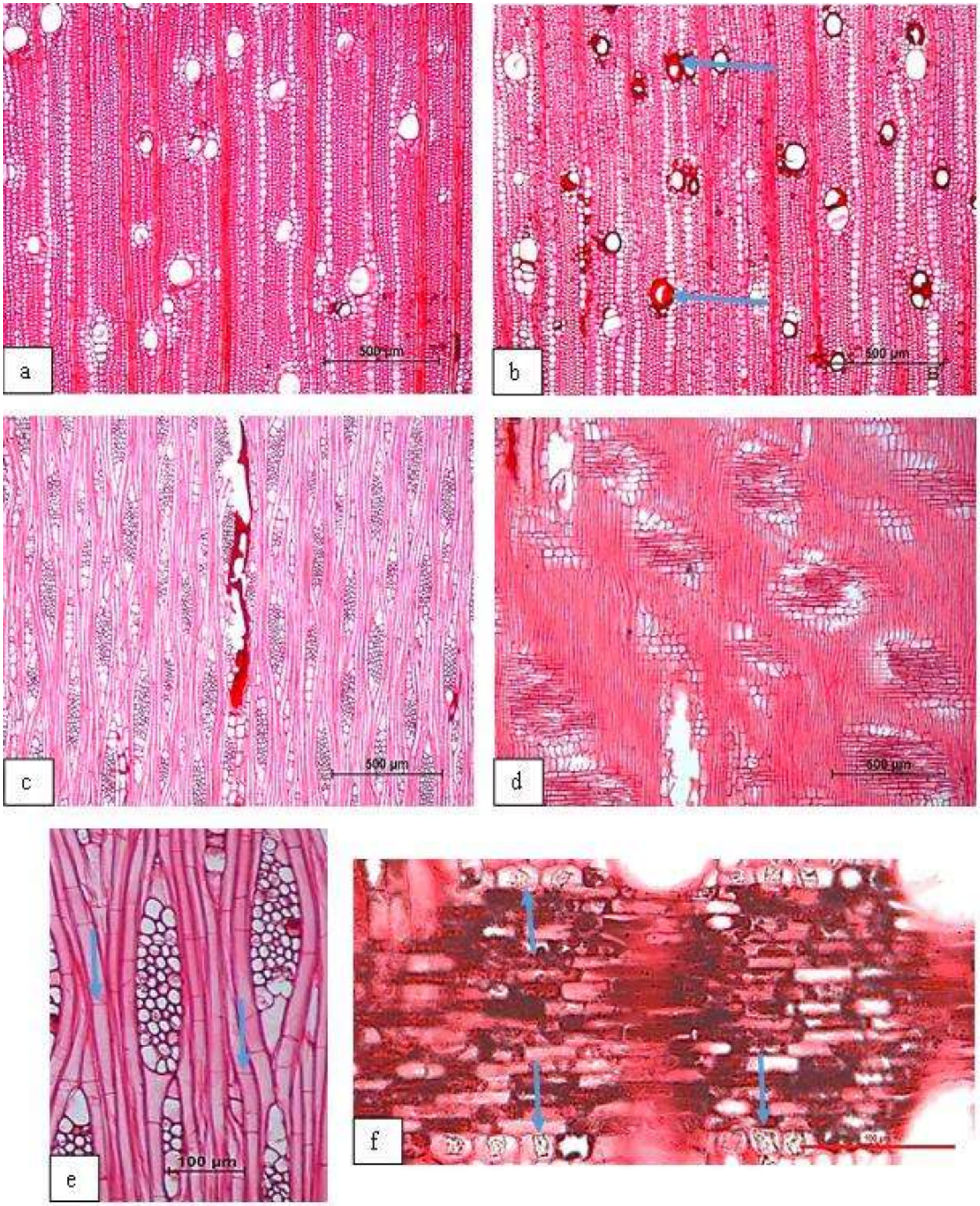

Figure 3: *Khaya ivorensis* (a) Transverse section in sapwood: vessels solitary or in multiples and clustering common, vessels are usually devoid of extractives. (b) Transverse section in heartwood: vessels filled with dark-coloured deposits of dried extractives (arrow). (c) Tangential section: rays are moderately fine to medium sized, mostly 3 to 7 cells wide. (d) Radial section: heterocellular rays (type II). (e) Tangential section: fibre septate (arrow). (f) Radial section: crystal present in ray cells (arrow).

DISCUSSION

Wood Anatomical Features of *A. excelsa* and *K. ivorensis*

There are significant differences in microscopic wood anatomical features between *A. excelsa* and *K. ivorensis* that can be used to identify these two species. A comparison on the wood anatomy of *A. excelsa* and *K. ivorensis* are presented in Table 3. *A. excelsa* could be differentiate from *K. ivorensis* by the parenchyma which *A. excelsa* shows present of both vasicentric and parenchyma in band of 3 to 6 cells wide, whilst *K. ivorensis* shows vasicentric parenchyma. On the other hand, *K. ivorensis* was found to have larger rays which was 3 to 7 cells wide compare to *A. excelsa* was 2 to 3 cells wide. Ray cells of *A. excelsa* consist of one row of upright/square marginal cells (type III). Whilst *K. ivorensis* consist of 2 to 4 rows of upright/square marginal cells (type II). Septate fibre was present in *K. ivorensis*, whilst non-septate fibre was observed in *A. excelsa*. Stated by Krishna *et al*. (2003), mineral inclusions such as crystals, druses, and silica represent distinctive wood anatomical features that possess diagnostic significance in distinguishing between wood species, genera, or even families. In this present study, the mineral inclusion i.e prismatic crystals could be diagnostic feature to distinguish the wood of *A. excelsa* and *K. ivorensis*. Prismatic crystals were found in chambered axial parenchyma in *A. excelsa*. Whereas, prismatic crystals were present in upright/square ray cells in *K. ivorensis*.

Wood anatomy serves as an indicator for other important wood properties. In this present study, it shows that both timbers have medium-sized vessels filled with deposits of dried extractives, particularly in the heartwood, suggests that these two timbers are challenging to treat with preservatives. Additionally, the presence of deposits indicates that these timbers are unsuitable for plywood production, according to Adeniyi et al. (2013), as plywood timber should ideally be devoid of such deposits to ensure proper wood bonding. Furthermore, the absence of silica in these timbers implies that they can be easily processed.

Resistance of *A. excelsa* and *K. ivorensis* to Fungal Decay

Zabel and Morrell (1992) and Roszaini et al. (2016) have noted that there exists no correlation between wood resistance and density or specific gravity. Certain species with lower density or specific gravity exhibit greater resistance compared to those with higher density. Similarly, to the present result (Table 4) that shows higher density in *A. excelsa* resulted to the lower resistance, compare lower density in *K. ivorensis* that shows higher in resistance. Eaton and Hale (1993) observed that, in general, species with natural durability tend to have darker hues than less durable ones, the darker hues often indicating a higher extractive content, which is commonly viewed as a sign of superior natural durability (Bowyer et al., 2003). According to Zabel and Morrell (1992), extractives are the primary factor influencing wood resistance. This finding aligns well with the conclusions of Humar et al. (2008), Roszaini et al. (2016), and Nusirat et al. (2021), who reported that the extractive content significantly affects wood resistance to fungal decay, surpassing other parameters such as density and ring width.

This study observed a slightly higher resistance to white rot fungus in *A. excelsa* compared to a prior study involving 10-year-old *A. excelsa* from Universiti Putra Malaysia (UPM), which exhibited a 15.44% weight loss when exposed to the white rot fungus *Pycnoporous sanguineus* (Zaidon et al., 2002). Similarly, seven-year-old *A. excelsa* sourced from Forest Research Institute Malaysia (FRIM) showed a 15.57% weight loss when exposed to the white rot fungus *Coriolus versicolor* (Othman et al., 1999). Discrepancies between the current and earlier studies could be attributed to variations in the fungi used, as elucidated by Sukartana and Highley (1997), Roszaini et al. (2016), and Nusirat et al. (2021), who suggested that the decay resistance of wood species may vary depending on the fungus employed. Additionally, differences in site and age could also influence wood resistance to white rot fungus (Salmiah and Amburgey, 1993). In the present study, *A. excelsa* and *K. ivorensis* were classified as resistant when exposed to *Lentinus sajor-caju*. These current findings were compared to those of a study conducted by Salmiah (2001), where similar with Yellow pine which exhibited resistance to the same fungus, resulting in a weight loss of 15.6%. In contrast, rubberwood displayed moderate resistance with a weight loss of 35.2%, while sweetgum showed slight resistance with a weight loss of 50.2% when exposed to *Lentinus sajor-caju*.

Samples of *A. excelsa* and *K. ivorensis* that taken from the bottom to the top of the tree were classified as resistant (Table 4). The resistance of the two species varied across different heights of the tree, with an increase in weight loss observed towards the top, as tabulated in Table 4. This indicates a decrease in resistance for both species with increasing tree height. The declining resistance towards the top can be attributed to a higher proportion of sapwood at the top part, which sapwood is generally considered non-resistant, as noted by Menon (1993) and Lim et al. (2016). Additionally, as mentioned by Choong et al. (1989), the content of wood extractives decreases from the base upwards, contributing to lower resistance in the upper part. This present finding was also support by other researchers i.e Terrasse et al. (2021) and N’Guessan et al. (2023), highlighting variations in wood extractive compounds were observed among trees, part of trees, and tree ages that influencing the natural durability of the wood.

The heartwood was more resistant than the sapwood (Table 4). A similar trend was also reported in 10-year-old plantation *A. excelsa*, *Acacia auriculiformis*, *Gmelina arborea* (Zaidon *et al*. 2002) and *Khaya ivorensis* (Nusirat *et al*. 2021). Although the sapwood of *A. excelsa* has been reported as non-resistant by Mungkordin (1995), the sapwood of both wood species in the present study was classified as resistant. In the case of the heartwood, Mungkordin (1995) rated that the heartwood of *A. excelsa* as non-resistant to moderately resistant, but in this present study both wood species were classified as resistant with *K. ivorensis* more resistant to fungus. The heartwood in both wood species is more resistant due to the higher extractives content compared to the sapwood as shown in Figure 2b and 3b. This is supported by the findings of Mohd Tamizi (2003) that found extractives in the heartwood was significantly higher (5.66 %) than sapwood (3.33 %) in 8-year-old plantation *A. excelsa*. Rayner and Boddy (1988), Syofuna et al. (2012) and Tumirah et al. (2020) have also emphasized the inherently greater resistance of heartwood due to its elevated extractives content. According to Eaton and Hale (1995) and Kirke et al. (2024) heartwood extractives are known to be toxic to fungi, with many imparting decay resistance, such as hydrolysable and condensed tannins, lignans, alkaloids, terpenoids, flavonoids, limonoids, and others. Cui et al. (1998) noted the presence of limonoids in *A. excelsa*, which play a crucial role in wood protection.

CONCLUSIONS

The reddish-brown heartwood of both *A. excelsa* and *K. ivorensis*, rendering the wood as preferred for high end applications like cabinet making and furniture as the resulting products look attractive and luxurious aesthetic. *A. excelsa* could be easily identified by its vessels are diffuse, solitary or in multiples of up to 2 to 4, wood parenchyma is vasicentric and in bands, fibre non-sepate, fine to medium ray sized with mostly 2 to 3 cells wide, heterocellular rays with type II and present of crystal in chambered axial parenchyma cells. Besides, *K. ivorensis* easily recognised by its vessels are diffuse, solitary or in multiples of up to 2 to 4, wood parenchyma is vasicentric, fibre sepate, fine to medium ray sized with mostly 3 to 7 cells wide, heterocellular rays with type III and present of crystal in upright/square ray cells.

A. excelsa and *K. ivorensis* wood were classified as resistant, with *K. ivorensis* being more resistant to white rot fungus, *Lentinus sajor-caju*. The weight losses of both species increased with tree height indicating that the resistance of the wood decreased towards the top. There was also a significant difference at $p \leq 0.05$ in resistance between the sapwood and heartwood, with the sapwood being lower resistant than the heartwood. Based on this finding, density could not be an indicator of decay but the resistance of wood is related to the presence of extractives as clearly observed in the vessels of heartwood in both species.

Supplementary materials

The supplementary material / supporting for this article can be found online and downloaded at: https://www.isisn.org/article/

Author contributions

NDAS devised the project, the main conceptual ideas and proof outline. NDAS contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript, with help from SL. MKAU helped supervise the project. NSSMY, NT, NAA and CNACA conceived the study and helped to draft the manuscript.

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Institutional Review Board Statement

The study was approved by the Bioethical Committee of the Forest Research Institute Malaysia (FRIM).

Informed Consent Statement

Not applicable.

Data Availability Statement

All of the data is included in the article/Supplementary Material.

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Conflict of interest

The authors declared that present study was performed in absence of any conflict of interest.

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