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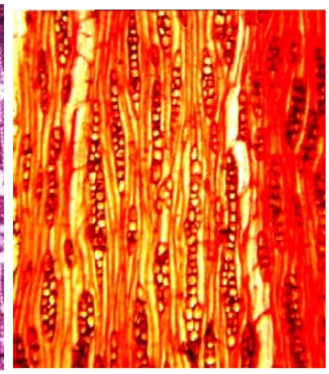
Comparison on wood anatomical characteristics of infected and non-infected agarwood from *Aquilariamalaccensis*Lamk.





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သစ်မွှေး(ခေါ်)အကျော်ပင်၏သစ်မွှေးဖြစ်သောသစ်သားပိုင်းနှင့်သစ်မွှေးမဖြစ်သော သစ်သားပိုင်းတို့၏သစ်အင်္ဂါဗေဒလက္ခဏာများအားနှိုင်းယှဉ်လေ့လာခြင်း

ကျော်ဝင်းမောင်၊ လက်ထောက်သုတေသနအရာရှိ၊ သစ်တောသုတေသနဌာန

ကြူကြူသင်း၊လက်ထောက်ကထိက၊ သစ်တောတက္ကသိုလ်

စာတမ်းအကျဉ်း

အရည်အသွေးမြင့်သော သစ်မွှေးထွက်သည့် *Aquilariamalaccensis*Lam.သည် မြန်မာနိုင်ငံ အပါအဝင် အရှေ့တောင်အာရှနိုင်ငံများတွင် သဘာဝအလျောက် ပေါက်ရောက်ပါသည်။ အရည် အသွေးကောင်းမွန်ပြီး စစ်မှန်သောသစ်မွှေးကို ၎င်းမျိုးစိတ်ရှိအပင်တိုင်း၌ သော်လည်းကောင်း၊ အပင်အစိတ်အပိုင်းတိုင်း၌ သော်လည်းကောင်းမတွေ့ရှိနိုင်ပါ။ ထိုကဲ့သို့ သစ်မွှေးပမာဏ အနည်း ငယ်သာဖြစ်ပေါ်စေသည့် အကြောင်းရင်းကို သေချာစွာနားမလည်နိုင်သေးပါ။ ထို့အပြင် မျက်စေ့ အမြင်ဖြင့် စစ်မှန်သောသစ်မွှေးကိုခွဲခြားရန် ခက်ခဲပါသည်။ ဤစာတမ်းတွင် သစ်မွှေးဖြစ်သော သစ်သားပိုင်းနှင့် သစ်မွှေးမဖြစ်သော သစ်သားပိုင်းတို့၏ သစ်အင်္ဂါဗေဒလက္ခဏာများကို လေ့လာ ခဲ့ပါသည်။ သစ်မွှေးဖြစ်သော သစ်သားပိုင်းနှင့် သစ်မွှေးမဖြစ်သော သစ်သားပိုင်းတို့၏ အဓိက ခြားနားမှုများမှာxylem cells and interxylary phloemဆဲလ်များတွင် cell deposits များ အမြောက်အများ တွေ့ရပြီးXylem cells များပုံပြောင်းသွားခြင်းဖြစ်ပါသည်။ Cell deposits များသည် Interxylary phloemတွင်စတင်ဖွဲ့စည်းခဲ့ပြီး Xylem tissue ရှိ အခြားဆဲလ်များ ထံသို့ပျံ့နှံ့မှုကိုသစ်အင်္ဂါဗေဒလက္ခဏာများအရတွေ့ရှိခဲ့ပါသည်။ ထို့ကြောင့် Interxylary phloem သည်သစ်မွှေးဖြစ်ပေါ်စေသောအမွှေးနံ့သာဆီများစတင်ထုတ်ပေးသည့် Xylem tissue များဖြစ်နိုင်ပါသည်။

Comparison on wood anatomical characteristics of infected and non-infected agarwood from *Aquilariamalaccensis*Lamk.

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ABSTRACT

*Aquilariamalaccensis*Lamk.produced high quality agarwood called true agarwood naturally grows in South and Southeast Asia including Myanmar.True agarwoods are not found in all trees of this species as well as all parts of tree and the factors responsible for it occurrence in only small percentage of trees or tree parts are not properly understood. In addition the diverse trade forms of agarwood lead to difficulties in distinguishing between agarwood from *A. malaccensis* and that from other species in trade because visual identification of wood to the species level is difficult. In this study wood anatomical characteristics are investigated by comparing the anatomical structure of infected and non-infected wood sample of *Aquilariamalaccensis*Lamk. after safranin staining method and direct observations under the light microscope. A number of differences in their anatomical structure are found. Major differences in wood structure between infected and non-infected wood are heavy occlusion of cell deposits in xylem cells and interxylary phloem and deformation of xylem cells. Furthermoremicroscopic observations revealed the trace that cell deposits initiate in interxylary phloem from which these are distributed to other cells constituting in xylem tissue. Thus it was concluded that interxylary phloem is appear to be responsible to produce the aromatic resin for the agarwoodformation in *Aquilariamalaccensis*Lamk.

Keywords: ***Aquilariamalaccensis*, Agarwood, Infected wood, Non-infected wood, Interxylary phloem**

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Comparison on Wood Anatomical Characteristics of Infected and Non-infected Agarwood from *Aquilariamalaccensis*Lamk.

1. Introduction

Agarwood is highly prized as source for medicine, perfumes and incense especially in Middle East and Asia countries (Barden *et al*2000). This wood is traded under several names including agarwood, aloeswood, eaglewood, gaharu and kalamabak (Anon 2003). In Myanmar this wood is famous as Thithmwe. In Myanmar traditional pharmacopeia it is recorded as Akyaw andhas local use in traditional medicineutilized by Myanmar's ethnic Chinese population.The information regarding domestic utilization is very little. Agarwood from Myanmarhas high demand in India trade and Middle East owing to its high quality (Barden *et al* 2000).

Agarwood is the resinous, fragrant and highly valuable heartwood of trees belonging to genus*Aquilaria*. There are 15 agarwood producing species of genus *Aquilaria*(Matsui 2005) and *A. malaccensis*Lamk is major source of agarwood in international trade (Rao and Dayal 1992). *A. malaccensis*Lamk.produced high quality agarwood naturally grows in South and Southeast Asia including Myanmar. In Myanmar, this species is mainly found in evergreen forests and rainforests of Tanintharyi and Sagaing Region, and Chin, Shan and Kachin State (Anon 2003). This species is protected under Appendix II of CITES and classified as vulnerable species on the IUCN Red List because this species is highly threatened due to overexploitation (Barden *et al* 2000; Anon 2003; Novriyanti and Santosa 2011; Puri 2004).

Market demand for agarwoodis continued to rise for its highly appreciated and priced fragrant. Currently, it therefore, remains the excessive and illegal exploitation and trade of this species although there are national controls. Agarwood is traded in several forms ranging from wood section, flakes, chips, oil and powder to finished products like incense and perfumes. These form lead to difficulties in distinguishing between agarwood from *A. malaccensis* and that from other species in trade. Visual identification of wood to the species level is difficult as well. The difficulties in identifying the species in trade hinder to control the indiscriminate harvesting and trade. In order to develop more effective harvest and trade controls the better tools for identification of species in trade are required (Barden *et al* 2000).

The knowledge in wood anatomy has assisted in identification of timber sample to the species level (Nair 1998).*Aquilaria*has unusual anatomical characteristic and specialized cells known as included phloem are found within xylem. The literature (Compton and Ishihara 2001; Novriyanti and Santosa 2011; Suharti*et al* 2011; Azah*et al* 2013)has suggested that aromatic dark resin was formed in heartwood of stem and large branches of agarwood producing tree after tree was wounded and infected by fungus. These infected heartwood concentrated by resin become the precious agarwood. In agarwood formation it involves anatomical response of xylem to injury and invasion by fungi. Parenchyma cells take part in resin formation as the cells showed abrupt changes in sapwood after mechanical wounding (Mohamed *et al* 2013). It hence requires understanding its anatomy to induce agarwood in young trees (Paoli 2001).Although a large number of publication concerning agarwood trading, formation and inoculation can be available there are a few studies on anatomical structure of agarwood from *A. malaccensis*Lamk. However no report has been made on the anatomy of infected wood and its comparison to non-infected wood. In this study anatomical structure of infected wood and non-infected wood of *A. malaccensis*Lamk were investigated and compared.The main objectives of this study is contribute the easier identification of true agarwood and the better understanding on the agarwood formation process based on differences in wood structure between infected and non-infected wood of *Aqualariamallaccensis*Lamk.

1.1 Literature Review

*A. malaccensis*Lamk is one of fifteen species of genus *Aquilaria* producing agarwood. It isbelonging to family Thymelaeceae and found mainly in lowland and on hill at 200-750m altitude with annual rain fall of 1500-6500 mm and mean annual maximum temperature of 22-28˙C. This species stated by synonymous with *A. agallocha* in Myanmar forestry literature (Anon 2003 and Matsui 2005). Itis widely distributed in south and Southeast Asia including Myanmar. The 2002 IUCN Red List has classified this species as vulnerable and it has been listed in CITES Appendix IIsince 1995 (Anon 2000; Blanchette and Beek 2005 and Anon 2003). It is large evergreen tree growing up to 40 m tall and reaches a diameter of 60cm. The tree usually grows straight but is sometimes fluted or with buttress up to 2 meters high (Anon 2003).When tree of *A. malaccensis*Lamk is infected with a type of mold it produces a dark aromatic resin in heartwood as a response to attack to invasive fungi. This process result a very dark, dense and resin impregnated heartwood (Anon 2003; Blanchette and Beek 2005 and Matsui 2005). Such resinous heartwood is traded by such various names as agarwood, egalwood, aloeswood, oudwood, gaharu, kalamabak and etc. Agarwood is well known in international trade.

Agarwood is most expensive wood in the world. This valuable wood has been traded for thousands of years throughout Asia. It is traded in several raw forms ranging from large sections of stem, branch and root, wood chip flakes and powder to agarwood oil. Large sections command a high price and are rarely in trade. Agarwood chips and flakes are most common forms in trade. Agarwood oil is highly valuable and frequently traded product of agarwood. Agarwood is not a uniform product and classified according to various grading systems. The highest quality agarwood is obtained from *A. malaccensis* found in Bangaldesh, Bhutan, Myanmar and India. Agarwood from Myanmar is high demand in India and Middle East market (Barden 2000).

Agarwood is principally used as medicine, incenses and perfume. It has been used in traditional medicine for thousands of years and continues to be used in Ayurvedic, Tibetan and traditional East Asian medicinal practices. It is used as carminative, tonic, stimulant, for heart palpitations, to relieve pain, vomiting and asthma. It is used to make incenses stick or coil for indoor fragrance and for religious purpose by Buddhist, Hindus and Muslim. The use of agarwood for perfumery extends back several thousands of years and is customarily used as perfume in the Middle East. Agarwood essences have recently been used as a fragrance in soaps and shampoos (Barden *et al* 2000).

Formation of agarwood occurs in the trunk and root of tree that have been infected by a parasitic ascomycetous mold. Naturally agarwood is not found in all trees and found in only small percentage of trees (Rao and Dayal 1992). In addition, agarwood is formed around wounded or rotting part of the trunk. A relatively small portion of tree changing to agarwood is difficult to be identified and visual inspection is impossible to identified agarwood producing trees as well (Barden *et al* 2000 and Anon 2001). Consequently, numerous uninfected trees are often cut down indiscriminately in search for resinous partand number of natural stocks becomes depletion (Barden *et al* 2000 and Matsui 2005).

The high prices for agarwood and local depletion of resources in the wild have resulted in a variety of effort to stimulate the agarwood formation.The most common stimulate method is inoculation of certain fungi species within an artificial wound of tree. Currently *Aquilaria*cultivation have been drawn much attention in such countries as China, India, Malasia, Indonesia, Thailand and Vietnam. In these countries the current methods used by farmers include partial trunk pruning, burn-chisel-drill, and fungi inoculation to stimulate production of agarwood (Xu*et al*2013). The plantation of *A.malaccensis*Lamk has been begun in Bangladish, Bhutan, and Myanmar (Anon 2003)

Agarwood formation naturally occurs in xylem. The artificial wounds are formed to a depth of at least about 1-10 cm into the xylem (Blanchette and Beek 2005). According to Matsui (2005) and Mohamed *et al* (2013) parenchyma cells in xylem are likely to be only responsible cell for agarwood formation. In addition, *Aquilaria* trees have unique anatomy. Unlike most trees in Angiospermae*Aquilaria* trees produce bundle of phloem cells throughout the xylem as well as external to xylem. Thus xylem tissues contain groups of phloem cells called included phloem or interxylary phloem in diffuse pattern (Blanchette and Beek 2005 and Matsui 2005). Interxylaryphloem is called foraminate when it appears in diffuse pattern within xylem tissue, concentric when it appears in a layer alternating with xylem layer (Esau 1965 and Eames).

Aquilaria trees produce interxylary phloem cells within xylem and form a network of phloem and xylem parenchyma cells. Cells in this network produce and distribute resins around infected area as a defense reaction (Blanchette and Beek 2005). As a result, resins are more concentrated within interxylary phloem tissue, xylem parenchyma and ray parenchyma (Matsui 2005 and Mohamed *et al* 2013). However factors responsible for occurrence of agawood in only small percentage of trees are not properly understood. The differences on the anatomical structure of infected and uninfected wood will contribute a better understanding of agarwood formation (Rao and Dayal 1992).

2. Materials and Methods

The wood samples used for this research were taken from mature plant growing in Myitkyina Township in 2012. The wood sample of 15cm thick contains both infected portion and non-infected portion. Both portions of wood sample were subjected to sectioning for microscopic observations. For light microscopy, serial transverse, tangential longitudinal and radial longitudinal sections of 20-25 µm thick were cut by using a microtome knife on sliding microtome. These sections were stained with safranin after these were fixed with ferric ammonium sulphate for 15 seconds and hamaltoxylin for overnight, anddehydrated with serial 50%, 70%, 90%, 95%of ethanol and two changes of xylene. Then sections were cleaned with clove oil and mounted with Canada balsam. These were observed under light microscope and microphotographed by using biological microscope model: MT 4300H.

3. Results and Discussion

The wood of *Aquilariamalaccensis* is diffuse porous and has fine texture and interlock grain. It cannot distinguish into heartwood and sapwood. Its color is whitish grey in sound wood (Fig 1.A) but dark brown to black in infected wood (Fig 1.B). The literature revealed that aromatic dark resins was produced in heartwood as response to fungal attack when *Aqualaria* trees were damage and infected by fungi (Compton and Ishihara 2001; Novriyanti and Santosa 2011; Suharti*et al* 2011; Azah*et al* 2013). The production of dark resins imparted changing in wood color.Thus the impregnation of the resin was more intense and the wood more resembled black stone. In trade agarwood is therefore graded into four categories. The wood like black stone is regarded as grade I. The wood in grade II is brown in color without any black tone. The wood referred to grade IV is mostly yellow with scattered brown or black streaks. The color of grade III wood resemblesgrade IV and contains more scattered brown or black streaks (Matsui 2005). Although odor of non-infected wood is not distinct, faintly distinct aroma is found in infected wood. Sound wood is soft and infected wood is easy to disintegrate when it is cut or crush.

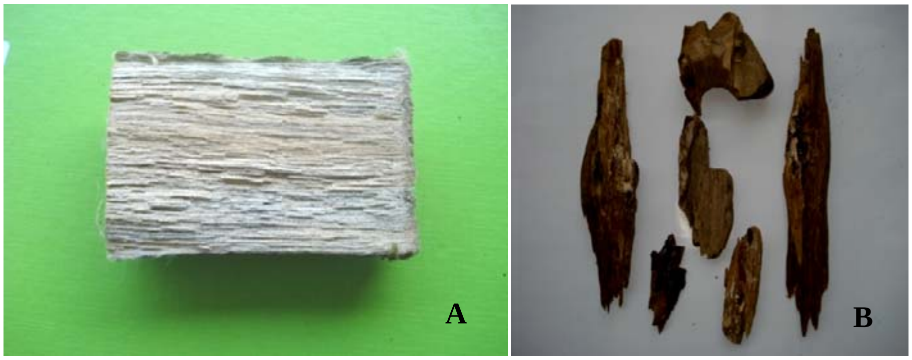

Figure 1. Wood of *Aquilariamalaccensis*Lamk.

A. Non-infected wood, B. Infected wood

The sections of both infected and non-infected wood sample were examined under light microscope. The microscopic survey revealed elements constituting xylem parts as vessel elements, axial and ray parenchyma cells and wood fibers. Transverse sections revealed the wood structure with microscopic characters such as distribution pattern of vessel pores and axial parenchyma, arrangement of vessel pore, cell deposits in xylem cells and interxylary phloem. In both sections diffuse porous vessels are found in solitary, radial multiple or occasionally cluster arrangement (Fig 2.A and B).Distribution of axial parenchyma is scanty paratracheal type and occasionally the parenchyma cells form an incomplete sheath around the vessel (Fig 2.C and D).

In both sections foraminate interxylary phloem irregularly distributes among the xylem tissues (Fig 2.A and B). This is unique wood anatomical feature of *Aquilaria* tree as in statement by Blanchette and Beek 2005 and Matsui 2005.In infected wood section the trace of initial dark substances production is found in interxylary phloem from which these substances are distributed to other xylem tissue (Fig 2.D). This finding is incidence to statement that the phloem and parenchyma cells produce and distribute resin toinfected areas as tree defense reaction proposed by Blanchette and Beek 2005. In fact, dark cell deposits are observed almost all xylem cells including interxylary phloem when transverse section derived infected wood sample are examined under light microscope (Fig 2.B). This finding agrees with the detection of Mohamea*et al* 2011 that brownish substances was found in the interxylary phloem, ray parenchyma, vessels and fiber of wood section collected from part of mature trunk with wounding marker while these did not in wood section of juvenile trunk without wounding marker.

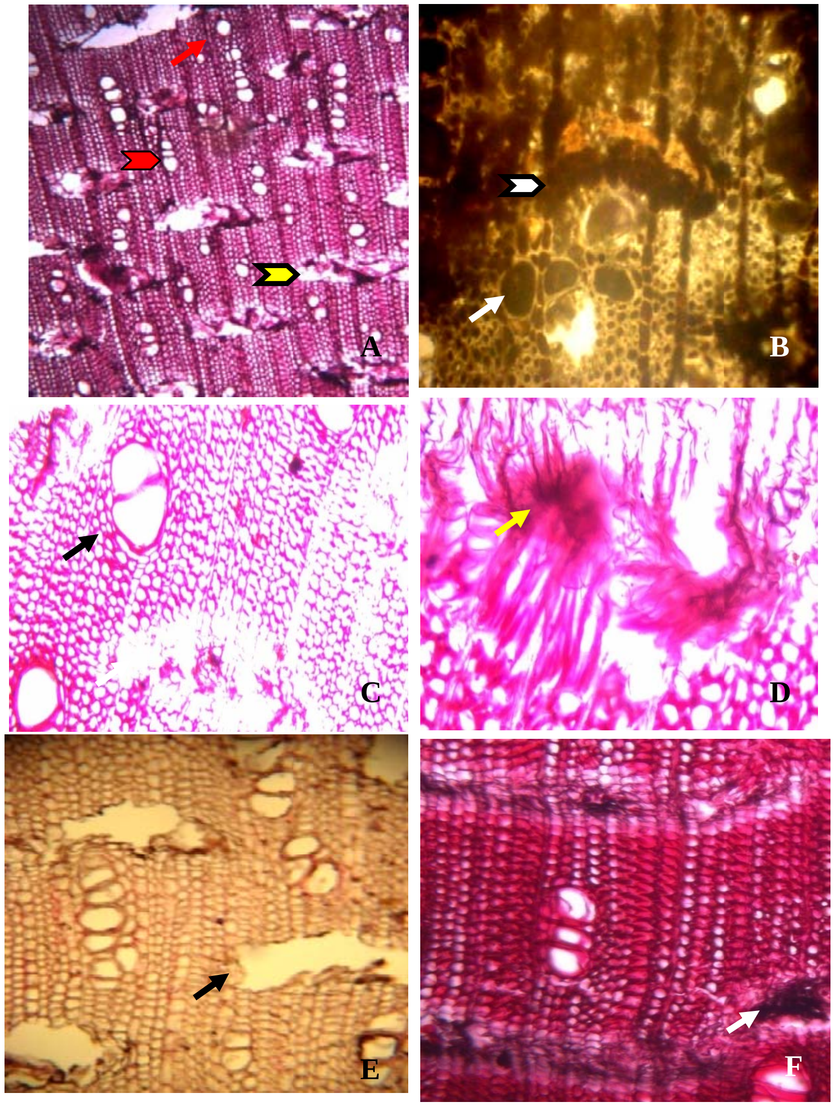

Figure 2.Transverse section of wood sample *Aquilariamalaccensis*Lamk.

A. Non-infected wood section showing diffuse porous distribution of solitary (red arrow) and radial multiple vessel pores (red arrow head) and irregularly distributed interxylary phloem (yellow arrow head); B. Infected wood section showing diffuse porous distribution of solitary occluding with dark cell deposits (white arrow) and interxylary phloem with dark cell deposits (white arrow head); C. Transverse section of non-infected wood showing axial parenchyma forming incomplete sheath around the vessel (black arrow); D. Transverse section of infected wood showing interxylary phloem producing dark resin (yellow arrows); E. Transverse section of non-infected wood showing interxylary phloem without dark resin (Black arrows); F. Transverse section of infected wood showing interxylary phloem producing dark resin (white arrows).

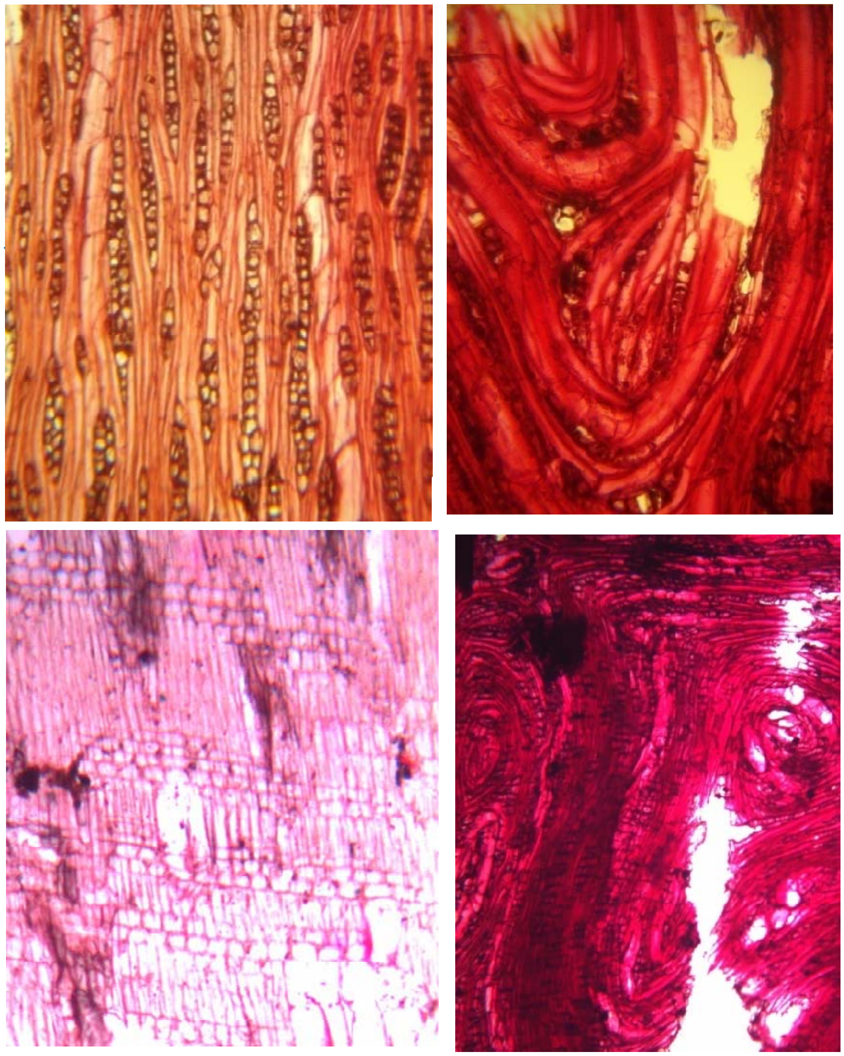

Figure 3 Tantential and radial longitudinal section of wood sample of *Aquilariamalaccensis*Lamk.

A. Tangential longitudinal section of non-infected wood showing vessel elements (Black arrow) and uniseriate and biseriate ray cells (Double head arrow); B. Tangential longitudinal section of infected wood showing deformed xylem cells impregnating dark cell deposits; C. Radial longitudinal section of non-infected wood showing heterogeneous ray cells (Black arrow) and libriform fibers (Blue arrow); D. Radial longitudinal section of infected wood showing deformed xylem cells impregnating dark cell deposits

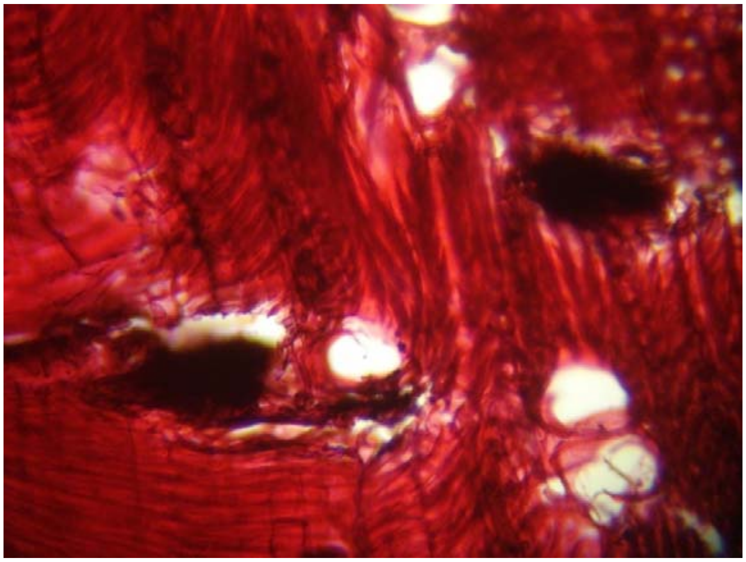

Figure.4. Transverse section of wood sample *Aquilariamalaccensis*Lamkshowing deformed xylem cells and dark colour deposits. (white arrow).

The orientation and types of rays and fibers are more distinct in radial and tangential longitudinal section than transverse section. The rays are uniseriate to biseriate and heterogenerous. Libriform fibers are non-septate and thin-walled (Fig 3.A and B).In the section of non-infected wood sample orientation and types of ray parenchyma and fibers are sharp and any substances are not found in these cells (Fig 3.A and C). In the section of infected wood sample these cells are deformed and occluded by dark cell deposits (Fig 3.B and D). Rao and Dayal (1992) concluded this deformation of xylem cell found in infected wood as the formation of abnormal tertiary tissue form secondary cambium.They also assumed that the occurrence of cell deposits and formation of abnormal tertiary tissues may be caused by some physiological disturbance like wounding, insect and fungal attack.

In this study the darker color in infected wood was appeared the more distinct in incense was emitted. Microscopic examination revealed difference in xylem tissue structure between infected woods with different wood color apart from the already mentioned differences in wood anatomical structure of non-infected wood. The darkest colored infected wood showed the deformation of xylem cells in transverse section while several dark colored cell deposits are observed in almost all xylem cells in darker color infected wood instead of deformation (Fig 4).

4. Conclusions

A number of differences are found in wood anatomical structure of infected and non-infected wood samples undertaken in the current study. According to examination of transverse section several dark colored cell deposits which may be aromatic resin are detected in almost all cells of infected wood section whereas any substances are not in non-infected wood section. Although the xylem cells are normal structure in tangential and radial longitudinal section of non-infected wood these are deformed in that of infected wood. Comparison study of each infected wood sample revealed cell deposits initiated in interxylary phloem from which distributed into other xylem cells. For this reason interxylary phloem is appear to be responsible to produce the aromatic resin for the agarwoodformation in *Aquilariamalaccensis*Lamk. Further more, microscopic observations highlight the occurrence of cell deposits and deformation of xylem cells as indicators of agarwood formation.

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