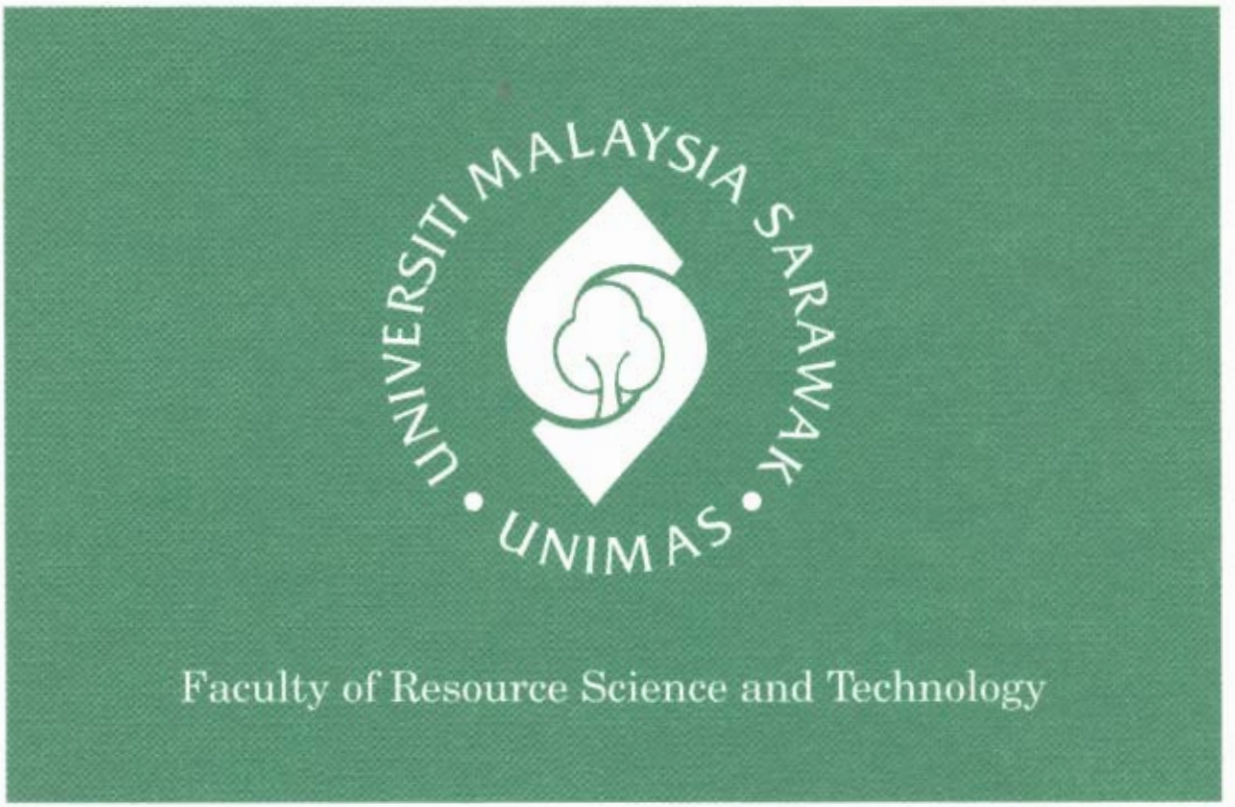

GROWTH OF *PYCNOPORUS* SPP. IN DIFFERENT ENVIRONMENTAL CONDITION

Shirley Maurice Labanjun

Bachelor of Science with Honours
(Plant Resource Science and Management)
2005

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**This project is submitted in partial fulfillment of
the requirements for the degree of Bachelor of Science with Honours
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Growth of *Pycnoporus* spp. in different environmental conditions.

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ABSRACT

Growth of *Pycnoporus* spp. in different environmental conditions were studied. Samples were collected from Kuching, UNIMAS and Bintulu area. Each sample was identified based on their macromorphological and micromorpholofical characteristics. The growth of *Pycnoporus* spp. on PDA, PCA and MEA were tested. This fungus grew significantly faster, at P=0.05 on MEA compared to growth on PCA and PDA. Effect of temperature was studied by growing the *Pycnoporus* spp. on PDA and incubated at 15°C, 20°C, 25°C, 30°C, 35°C and 40°C. Optimum growth of the fungus was at 30°C and the average growth rates decreased at higher and lower temperature. The growths of the *Pycnoporus* spp., in dark and light condition were also tested. The fungus grew significantly faster, at P=0.05 in light condition. The effect of pH were studied by growing the *Pycnoporus* spp. in Potato dextrose broth (PDB), which had been adjusted to pH4.0, pH5.0, pH6.0, pH6.5, pH7.0, pH8.0 and pH9 with Hydrogen chloride (HCl) and Natrium hydroxide (NaOH) and incubated at 25°C in dark condition. The highest mycelium dried weight was obtained at pH7.0 from Kuching sample, pH4.0 from UNIMAS and at pH 8.0 from Bintulu sample. Effect of five different elements on growth of *Pycnoporus* spp. was tested by using complete media and in the absent of the macro elements. There were significant effects at (P<0.05) of the macro elements on growth of *Pycnoporus* spp.

Key words: Pycnoporus, environmental factor, environmental condition, media and mycelium.

ABSTRAK.

Pertumbuhan Pycnoporus spp dalam keadaan persekitaran yang berbeza telah dikaji. Sampel kulat telah diambil dari Kuching, UNIMAS dan Bintulu. Setiap sampel di kenal pasti berdasarkan kepada macromorfologi dan micromorfologi kulat. Pertumbuhan Pycnoporus spp. pada media PDA, MEA dan PCA di uji. Kulat in didapati tumbuh dengan lebih cepat pada P=0.05 di atas media MEA berbanding dengan media PCA dan PDA. Kesan suhu terhadap pertumbuhan Pycnoporus spp. dikaji dengan menumbuhakan kulat di atas media PDA dan di eram pada suhu yang berbeza-beza iaitu 15°C, 20°C, 25°C, 30°C, 35°C dan 40°C. Pertumbuhan optimum kulat adalah pada suhu 30°C dan kadar pertumbuhan akan menurun apabila suhu meningkat atau menurun. Pertumbuhan Pycnoporus spp. dalam keadaan cahaya dan gelap juga dikaji. Kulat ini tumbuh lebih cepat keadaan dalam keadaan gelap pada P=0.05. Kesan pH dikaji dengan menumbuhkan Pycnoporus spp. di dalam ekstrak dektros kentang (PDB) yang telah diatur pada pH4.0, pH5.0, pH6.0, pH6.5, pH7.0, pH8.0 and pH9 dengan Hydrogen chloride (HCl) dan Natrium hydroxide (NaOH) dan dieram pada suhu 25°C dalam keadaan gelap. Purata berat mycelia kering yang paling tinggi diperolehi adalah pada pH7.0 pada sampel Kuching, pH4.0 pada sampel UNIMAS dan pH8.0 pada sampel Bintulu. Kesan lima elemen terhadap pertumbuhan kulat dalam lima elemen dikaji dengan menggunakan media lengkap dan tanpa kehadiran elemen yang diuji. Terdapat kesan yang ketara (P<0.05) kehadiran elemen pada pertumbuhan kulat Pycnoporus spp.

Kata kunci: Pycnoporus, factor persekitaran, keadaan persekitaran, media dan miselia.

INTRODUCTION

Pycnoporus species is a fungus in phylum of Basidiomycota, class of *Basidiomycetes;* order of *Polyporales* and in the family of *Polyporacea*. It exist in the absent of gills but in the present of pores that is the tiny pore at below of the cup (Martin and Pamela, 1990). In temperate region it is known as the over winter species (Lepp, 2003). Etymology from Greek says that this species is "like cinnabar" or "dragon's blood," because of its bright reddish-orange in color. There are three common types of *Pycnoporus* species; there are *P. coccineus*, *P. sanguineus,* and *P. cinnabarinus* (Buchanan and Ryvarden, 2000). These three common of *Pycnoporus* species basically have the same appearance where people might be confused in differentiated between them and people may be confused in the usage of this three species. This *Pycnoporus* species varies from each other even the color seems to be alike.

Pycnoporus species are mainly found on fallen dead tree. The fruiting bodies of this polypore genus look like bright reddish-orange brackets and are widespread on the dead wood. In Australia there are two species – *P. coccineus* and *P. sanguineus*, with overlapping distributions (Buchanan and Ryvarden, 2000). Moreover, the two species are similar in appearance, so without specimens there will be doubt as to which *Pycnoporus* species is meant in any particular account.

We have to know the essential condition for this species to grow in order to grow or to culture them. Although the species of the fungus is same, different isolates might have different characteristic. So, other information are also required to differentiate between them.

The objective of this research is to determine the effect of environmental factors on growth of *Pycnoporus* species.

LITERATURE REVIEW.

Species of *Pycnoporus*.

There are three common species of *Pycnoporus* - *P. coccineus, P. cinnabarinus* & *P. sanguineus* (Buchanan and Ryvarden, 2000). *P. coccineus* has red colour basidiocarp. This species is a common white-rot-fungi. And after degradation the wood surface will turn in light color. The tubes of this species are very small (Lepp, 2003). For *P. cinnabarinus,* the size usually varies from 2.5-10 cm across. The spore powder is white and the fruiting body of this species usually found on dead trunks and branches of deciduous trees (Eriksson *et al.*, 1980). As for *P. sanguineus*, it have an antidote for toxins and used usually for rheumatism, arthritis, gout, styptic, anti-bacteria and anti-fungus disease (Hobbs, 2001).

Growth of *Pycnoporus* spp.

The growth of mycelial is a unique property of fungi that will allows them to spread saprophytically and capture food resources in a nutritionally heterogeneous environment (Giligan and Bailey, 1997). The conditions essential of fungal to grow are food supplies, adequate moisture, suitable temperature and air or oxygen supplies on any substrate. Moisture is the most limiting factor among them (Zabel and Morell, 1992, Annon, 1989). In the presence of moisture, fungi decompose wood by secreting acids and enzymes (ferments) which, rendering soluble some of the cellulose and other constituents of the wood (Findlay, 1985). These are then absorbed and used as nutrients by the fungus. Much of the wood substances may thus be changed in composition and texture before it is actually absorbed by the fungus. If moisture did not remain

about 20% of the oven dry weight regularly for prolonged periods, fungi might not be develop on the wood (Annon, 1989).

The growth and development of fungal mycelium are also known to occur on artificial media such as agar or cellophane in which carbon resources are homogeneously (Trinci, 1997) or even heterogeneously distributed (White *et al.*, 1998). As been stated by Jones *et al.* (1994), the development of the mycelium after 24 hours of growth on the previously defined nutrient medium containing various concentrations of Remazol Brilliant Blue R [RBBR] (Sigma) dye + wood (Jones *et al.*, 1994; 1995a,b; 1996a) do have its various morphologies. Spores of fungi can also successful germinate if they land on a suitable substrate such as damp wood. The germinating spores produce thin threadlike hyphae, which collectively form mycelium. The hyphae making up the mycelium penetrate the wood, breaking down the wood cell walls and feeding on them. If the suitable conditions, the mycelium could produce a fruiting body (Annon, 1989).

Pycnoporus as Degradable Fungus.

P. sanquineus was found mainly in the tropics and under natural conditions. It causes a considerable deterioration even in supposedly durable timber (Cardias, 1992). This fungus attacks a wide range of timber, including angiosperms, gymnosperms and palms. This fungus causes white rot and it is common on standing and fallen timber.

Members of *Basiodiomycetes* including *Pycnoporus* are especially important in the forest ecosystem since they are the only fungi capable of degrading all cell wall components (cellulose,

lignin, hemicelluloses) of wood. White-rot fungi are known to secrete lignin peroxides (LiP), manganese peroxides (MnP), and laccases (Bourbonnais *et al.*, 1995). Some *Pycnoporus* have been found to remove all cell wall components, hemicelluloses and lignin simultaneously while others were found to remove lignin and hemicelluloses. Some investigators identified these fungi as white rot and corrosive rot respectively. Some authors categorized the first one as simultaneous rot and the later one as white rot instead of corrosive rot (Otjen and Blanchette, 1986). *P. cinnabarinus* is a common fungus that can cause white rot. Eggert *et al.* (1997) reported that in *P. cinnabarinus*, only one laccase was found during growth under ligninolytic conditions and laccase of this fungus is thought to degrade lignin using a low molecular weight mediator.

Eriksson *et al.* (1980), showed that the hyphae of a fungus propagate in the spruce wood mainly by utilising the naturally existing connections between wood cells. The tracheids in the wood are joined by pits and the fungus grows through these from one cell to another. In the same manner, growth of the fungus occurs in the pits between tracheids and ray cells. Concentrated mycellial growth occurs in the pit regions of the tracheids. Another feature is the orientation of the hyphae in a wood cell. They not only grow parallel to the tracheid axis but have also been seen to make their way at an angle to the long axis of the cell. Furthermore the fungus does not necessarily need to be mechanically supported by the wood cell wall all the time. It can either grow diagonally across the lumen, supported at its points of attachment with the cell wall, or in the central part of the lumen, where it is held in place by hyphal branches extending from the main hyphae to the cell wall (Eriksson *et al.*, 1980).

White rot *basiodiomycetes* vary greatly in their ability to cause different micromorphological patterns of decay. Individual white rot fungi can cause different types of

decay in different cell types within a host. According to Otjen and Blanchette, (1986), the reasons for these differences are due to the capacities in its ability to removed lignin, cellulose and hemicelluloses from wood. Studies of fungal hyphae associated with white rot fungi indicate an extensive hyphal sheath around the hyphal tip (Highley and Murmanis, 1984). Scanning electron microscopy showed that *P. sanguineus* penetrated the cellulose fibers by means of bore holes (Highley and Murmanis, 1984).

***Pycnoporus* as Medicine.**

Species of *Pycnoporus* are basically known as medicinally in a variety of ways. Some of the used are to cure ulcer, wound and eye problem. To cure ulcer, the part of the *Pycnoporus* species is rubbed inside the mouths especially in baby's mouth, with oral thrush or rubbed on sore lips (Lepp, 2003). It has also been used as a teething ring. Out of curiosity, one person in Canberra chewed on a *Pycnoporus* specimen to see if it would have any effect on a small mouth ulcer (Lepp, 2003). Then the ulcer soon disappeared then this at least approve that the fungus had no detrimental effect. Of course, in this case, there is still the question of whether chewing the fungus cured the ulcer or whether its disappearance was coincidental. In previous research two antibiotic compounds have been found in *P. coccineus*. As for *P. sanguineus*, cinnabarin is an antibiotic substance that been produced by this species. This compound is an orange pigment, which has a basic phenoxazin-3-one structure, with a carbonyl group at C-1, an amino group at C-2 and a hydroxyl group at C-9 (1,3). Not many researchers have been done about this species because there is less information about the fungus.

MATERIALS AND METHODS

Source of samples

Samples were collected from Kuching, UNIMAS campus area and Bintulu.

Specimen identification

The newly collected specimens of *Pycnoporus* species were identified in laboratory based on morphological structures. The basidiomata, pileus, pore surface, pore tube, context, stipe of the fungus were measured and color was recorded. Odor and taste of this fungus were also taken into concern for identification purpose.

The tissues from the various part of freshly collected fruiting body were partially digested on a glass slide with 10% solution potassium hydroxide. The tissues then left to be sufficiently soft for 15 minutes and washed with water. The access water then removed with blotting paper. The tissues were teased by fine needle under light microscope. For clear examination, these tissues were mounted with 5% KOH. The tissues were then stained with cotton blue. The hyphae and basidiospores tissues were stained with Congo Red for observation. Types of hyphae and basidiospores were identified.

Pure Culture Preparation.

Culture was obtained by using fungal tissues and inoculating on Potato Dextrose Agar (PDA). The fungal tissue of the basidiocarp of the mushroom was cut to the size about 3 mm^2 and transfer carefully into a small plate. These tissues were washed with 10% Clorox for 5 minutes then, washed again with sterilized distilled water 2 to 3 times, dried with filter paper and before inoculated on PDA. The inoculated plates were incubated at room temperature. After 5 to 7 days the growth of mushroom was transferred to a new media to get the pure culture.

Effect of media on growth of the fungus.

Three different media were used. The media were Potato Dextrose Agar (PDA), Potato Carrot Agar (PCA) and Malt Extract Agar (MEA). Agar bocks, 3 mm diameter, containing mycelia of *Pycnoporus* species cut from 5 – 7 day-old pure culture were inoculated on these prepared media. The fungus was inoculated at the center of 9 cm diameter of petri dishes. Three replicates were prepared for each media. The inoculated plates were incubated at room temperature. Growth of the fungus on media PDA, PCA, and MEA were determined by measuring the diameter of the colony. Diameter on the agar plates were measured everyday until day 7. The growth rates for each media were calculated as bellow.

The media that gave the faster growth was chosen as the growing media of the fungus in further study.

$$\text{Growth rate} = \frac{(d_n - d_{n-1}) + (d_n - d_{n-1}) + (d_n - d_{n-1}) + \ldots\ldots\ldots + (d_n - d_{n-1}) + (d_n - d_{n-1})}{n - 1}$$

where,

d = average colony diameter

n = total number of days

Effect of temperature on growth of the fungus.

Based on the result of effect of media on the growth of *Pycnoporus* species, PDA was used. Agar blocks, 3 mm diameter, containing mycelia of *Pycnoporus* species cut from 5 – 7 day-old pure culture were inoculated on the agar in Petri dishes. The plates were incubated at 15°C, 20°C, 25°C, 30°C, 35°C and 40°C. Three replicates were prepared for each temperature. The average colony diameter of the mycelium growth was determined. This measurement was obtained everyday for 7 days. The growth rate of the fungus at each temperature was calculated as below.

$$\text{Growth rate} = \frac{(d_n - d_{n-1}) + (d_n - d_{n-1}) + (d_n - d_{n-1}) + \ldots\ldots\ldots + (d_n - d_{n-1}) + (d_n - d_{n-1})}{n - 1}$$

where,

d = average colony diameter

n = total number of days

Effect of light intensity on growth of the fungus.

PDA were used to carry out this study. Agar blocks, 3 mm diameter, containing mycelia of *Pycnoporus* species cut from 5 – 7 day-old pure culture were inoculated on the agar in Petri dishes. All the Petri dishes were labeled with date of inoculation, type of species and light or dark condition. Six Petri dishes were prepared. Three Petri dishes were fully covered with aluminum foil and kept in a cover box and the left three were kept under bright light condition for 7 days. All these Petri dishes were incubated at room temperature. The growths of the fungus were determined by measuring the colony growth diameter of the fungus after 24, 48, 72, 96, 120 144 and 168 hours of inoculation.

Effect of pH on growth of the fungus.

Potato dextrose broth (PDB) was used to investigate the effect of pH on growth of *Pycnoporus* species. The pH values were adjusted to 4.0, 5.0, 6.0, 6.5, 7.0, 8.0 and 9.0 with Hydrogen chloride (HCl) and Natrium hydroxide (NaOH). After the pH was adjusted in the broths, it was poured into Erlenmeyer flask, where each flask contained 25ml broth. Three replicates were prepared for each pH value. All the media in the flask were autoclaved for 20 minutes at 15psi and left it cold in a before used. The pH was adjusted again after the autoclaving.

Agar blocks, 3 mm diameter, containing mycelia of *Pycnoporus* species cut from 5 – 7 day-old pure culture were inoculated in these broths. All the inoculated flasks were incubated at

25°C in the dark condition. After 12 days, the liquid was drained through filter paper Whatman No 1. The mycelia were dried for 2 days in oven at 60°C.

Dried weights of mycelium were calculated as follows:

Mycelia Dry Weight = b – a

where,

a = weight of dried empty filter paper

b = weight of dried mycelia + filter paper

Effects of macro elements on growth of the fungus.

Effects of macro elements on growth of *Pycnoporus* were determined by using the following media:

Complete Medium

Glucose (40g/L), Magnesium sulphate, $MgSO_4$ (1.25g/L), Potassium dihidrogen Orthophosphate, KH_2PO_4 (2.5g/L), Potassium nitrate, KNO_3 (5g/L).

Without Potassium

Glucose (40g/L), Magnesium sulphate, $MgSO_4$ (1.25g/L), Sodium dihidrogen Orthophosphate, Na_2HPO_4 (5g/L), Sodium nitrate, $NaNO_3$ (5g/L).

Without Nitrogen

Glucose (40g/L), Magnesium sulphate, $MgSO_4$ (1.25g/L), Potassium chloride, KCl (5g/L), Potassium dihidrogen Orthophosphate, KH_2PO_4, (2.5g/L).

Without Phosphate

Glucose (40g/L), Potassium sulphate, K_2SO_4 (2.5g/L), Potassium nitrate, KNO_3 (5g/L), Magnesium sulphate, $MgSO_4$ (1.25g/L).

Without Magnesium

Glucose (40g/L), Potassium sulphate, K_2SO_4 (2.5g/L), Potassium nitrate, KNO_3 (5g/L), Potassium dihidrogen Orthophosphate, KH_2PO_4, (2.5g/L)

20 g of agar were added in all media before autoclaved. The autoclaved media was poured into petri dishes.

Agar block containing mycelia 5 -7 day-old pure culture was inoculated onto the media in Petri dish. Three replicates were prepared for each isolate. All the inoculated plates were incubated at 25°C. Colony diameter was measured everyday for seven days and average radial growth rates were calculated as below.

$$\text{Growth rate} = \frac{(d_n - d_{n-1}) + (d_n - d_{n-1}) + (d_n - d_{n-1}) + \ldots\ldots\ldots + (d_n - d_{n-1}) + (d_n - d_{n-1})}{n - 1}$$

where,

d = average colony diameter

n = total number of days

Data analysis

All the data were analyzed with SPSS windows version 11.0 statistical software. Two-way ANOVA and Tukey HSD test were used.

RESULT

Samples were collected from Kuching, UNIMAS and Bintulu area. They were listed in Table 1:

Table 1: Samples of *Pycnoporus* collected from different locations.

UNIMAS MIN*	Sampling Locality
1210	Kuching area.
1211	Near Plant Laboratory, UNIMAS.
1212	Sarawak Borneo Seeds, Bintulu.

* - UNIMAS Mycological Index Number.

The description of the samples are as following:

UNIMAS MIN : 1210
Species name : *Pycnoporus cinnabarinus*
Date of collection : 3rd August 2004.
Locality : Kuching area.
Habitat : Dead wood on fallen tree.

Fruit body bracket-form, 6.5 cm long, 4 cm wide, semicircular to flabellate, habitually mostly attached to the substrate. Stipe 0.5-1.0cm from the upper side of the substrate to the fruiting body. Upper surface tuberculate, veruccose, dull and appressed-tomentose to smoth with indistinct concentric undulation, orange to orange-red, older fruiting body also orange to orange-wine-reddish, margin sharp and concolorous. Lower surface fine-pored; pores rounded, 6-7 per 0.1cm, up to 0.2cm deep, deep orange-red, corky, tough, fibrous, orange-red, without odor or taste.

The asexual spores are elliptical and smooth, 4 – 5um, hyphal systems are trimitic; generative hyphae 9 - 10um across, septate clamps; skeletal hyphae, 10um across and binding hyphae are thick wall to solid.

The pure culture of this fungus after 5 days of inoculation on PDA was white to orange in colour on the top surface of media. However at the bottom of petri dish the colour of the mycelium was light yellow (Figure 1a and 1b).

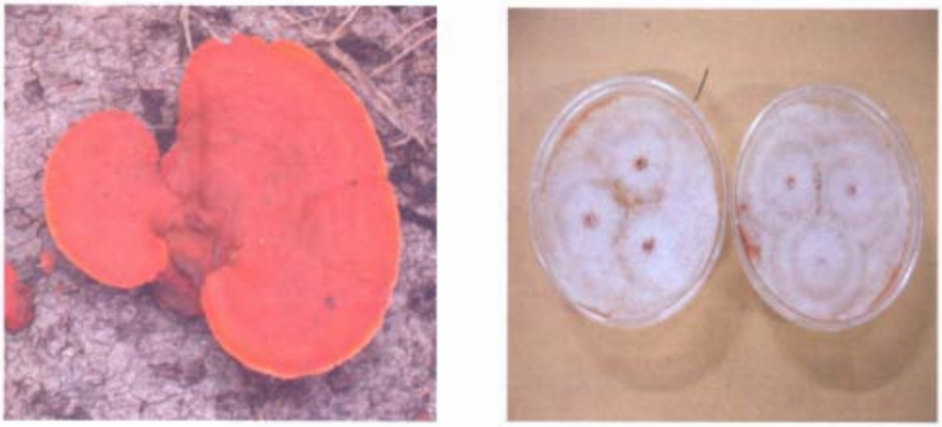

Figure 1a. The fruiting body and pure culture of isolate 1210.

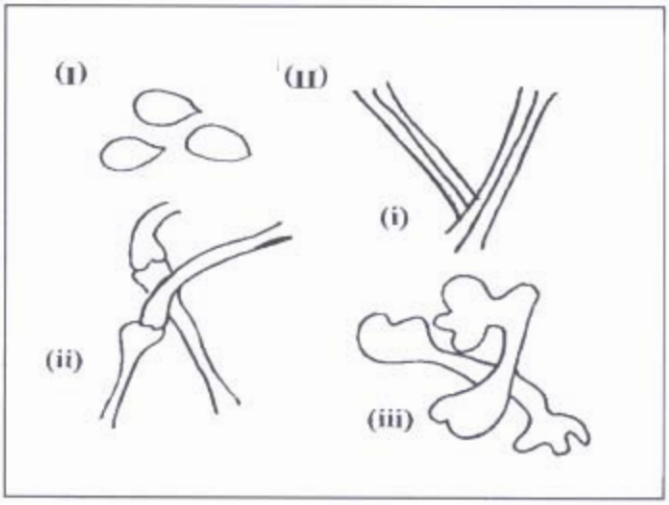

Figure 1b. (I) Asexual spores of isolate 1210 (II) Hyphae of isolate 1210: (i) Generatives hyphae, (ii) Skeletal hyphae and (iii) Binding hyphae.

UNIMAS MIN : 1211
Species name : *Pycnoporus cinnabarinus*
Date of collection : 1st October 2004.
Locality : UNIMAS Campus area, near plant laboratory.
Habitat : Dead wood on fallen tree.

Fruit body bracket-form, 4cm long, 2.3 cm wide, semicircular to flabellate, habitually mostly attached to the substrate. Stipe 0.7 cm from the upper side of the substrate to the fruiting body. Upper surface tuberculate, veruccose, dull and appressed-tomentose to smooth with indistinct concentric undulation, orange to orange-red, older fruiting body also orange to orange-wine-reddish, margin sharp and concolorous, lower surface fine-pored; pores rounded, 5-6 per 1cm, up to 0.15cm deep, deep orange-red, corky, tough, fibrous, orange-red, without odor or taste.

The spores are elliptical and smooth, 4 – 5um, hyphal systems are trimitic; generative hyphae 9 - 10um across, septate clamps; skeletal hyphae, 10um across and binding hyphae are thick wall to solid.

The pure culture of this fungus after 5 days of inoculation on PDA was white to orange in colour on the top surface of media. However at the bottom of petri dish the colour of the mycelium was light yellow (Figure 2a and 2b).

Figure 2a. The fruiting body and pure culture of isolate 1211.

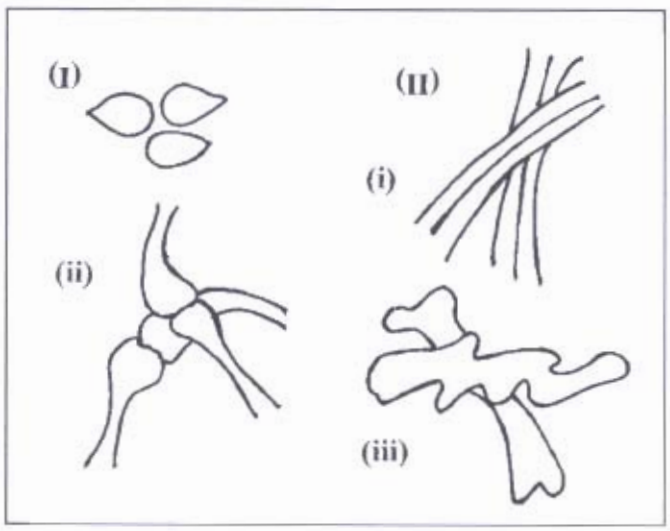

Figure 2b. (I) Asexual spores of isolate 1211 (II) Hyphae of isolate 1211: (i) Generatives hyphae, (ii) Skeletal hyphae and (iii) Binding hyphae.

UNIMAS MIN : 1212
Species name : *Pycnoporus cinnabarinus*
Date of collection : 11th January 2005
Locality : Borneo Tree Seeds and Seedlings Suplies Sdn. Bhd, Bintulu.
Habitat : Dead wood on fallen branches.

Fruit body bracket-form, 3.7 cm long, 2.3 cm wide, semicircular to flabellate, habitually mostly attached to the substrate. Stipe 0.8 cm from the upper side of the substrate to the fruiting body. Upper surface tuberculate, veruccose, dull and appressed-tomentose to smooth with indistinct concentric undulation, orange to orange-red, older fruiting body also orange to orange-wine-reddish, margin sharp and concolorous. Lower surface fine-pored; pores angular, 5-6 per 1cm, up to 0.1cm deep, deep orange-red, pores, corky, tough, fibrous, orange-red, without odor or taste.

The spores are elliptical and smooth, 4 – 5um, hyphal systems are trimitic; generative hyphae 9 - 10um across, septate clamps; skeletal hyphae, 10um across and binding hyphae are thick wall to solid.

The pure culture of this fungus after 5 days of inoculation on PDA was white to orange in colour on the top surface of media. However at the bottom of petri dish the colour of the mycelium was light yellow (Figure 3a and 3b).

Figure 3a. The fruiting body and pure culture of isolate 1212.

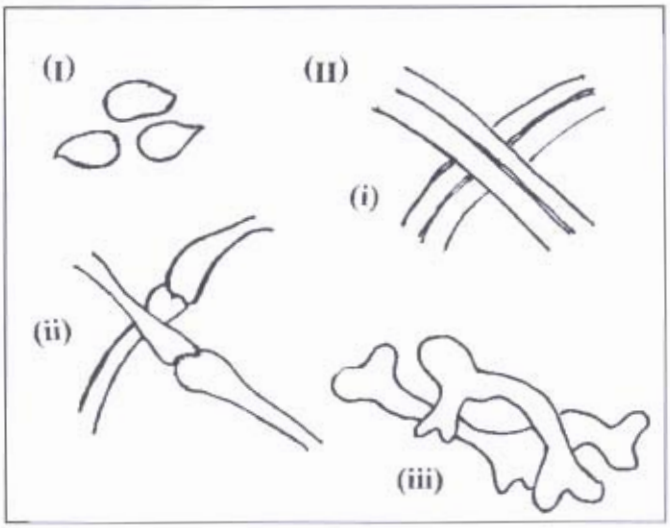

Figure 3b. (I) Asexual spores of isolate 1212 (II) Hyphae of isolate 1212: (i) Generatives hyphae, (ii) Skeletal hyphae and (iii) Binding hyphae.

Morphological characteristics of samples of *Pycnoporus* spp. collected from the three different places does not differ enormously when similarity were found between them. Based on the morphological characteristics of the samples collected from the different locations, the fungus was identified as *Pycnoporus cinnabarinus*.

Effect of media on different growth of the fungus.

There was no significant difference at P = 0.05 in growth rate of the *Pycnoporus* isolates on all the tested media. Table 2 showed the growth rates of *Pycnoporus* isolates on different media.

Table 2. Average growth rate (cm day^{-1}) of *P. cinnabarinus* on different media.

	Average Growth Rate (cm day^{-1})		
Isolate	**PDA**	**PCA**	**MEA**
1210	1.26 ± 0.00a	1.26 ± 0.00a	1.28 ± 0.02a
1211	1.26 ± 0.00a	1.27 ± 0.01a	1.28 ± 0.02a
1212	1.26 ± 0.01a	1.28 ± 0.01a	1.27 ± 0.01a

Mean ± s.d. For each media, values in a row followed by the same letter indicate no significant difference of growth rate at $\alpha = 0.05$ level by Tukey's test.

There were different in formation of mycelia at the surfaces of media. On PDA media the formation of mycelia was smooth and uniform. The colour of mycelia was white. After 7 days the inoculation seems to produce an orange colour at the surfaces of the media. The formation of mycelia on MEA, showed that there was a little bit orange colour that appear on the surfaces of media after 7 days of inoculation. Apart from that also the mycelia produce a very good uniformity and fluffy formation. Whereas at PCA the formation of mycelia was not very clear and not very uniform. The formation of mycelia at the surfaces was not as good as mycelia formation on PDA and MEA.