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Teak Rust *Olivea Tectona* : Occurrence, Epidemiology, Its Chemical Control *In Vitro* and Recation of Teak Clones and Provenances

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ကျွန်းသံချေးမှို *Olivea tectinae*: ရောဂါဖြစ်ပေးမှု၊ ပျံနှံ့မှု ၊ ရောဂါကာကွယ်နိုင်မှုကို ဓါတုဗေဒပစ္စည်းများဖြင့် ဓါတ်ခွဲခန်းအတွင်း စမ်းသပ်ခြင်းနှင့် ကျွန်း Clones နှင့် ဒေသမျိုးတို့ရဂါအပေါ် တုံ့ပြန်မှုများကို လေ့လာခြင်း

ဒေါ်ဝေဝေသန်း
သိပ္ပံဘွဲ့ (သတ္တဗေဒ)၊ မဟာသိပ္ပံ (ကျမ်းပြု) သစ်တောရောဂါဗေဒ
လက်ထောက်သုတေသနအရာရှိ၊
သစ်တောသုတေသနဌာန၊ ရေဆင်း

စာတမ်းအကျဉ်းချုပ်

ဤစာတမ်းတွင် *Olivea tectonae* ကြောင့်ဖြစ်သော ကျွန်းရွက်သံချေးမှိုရောဂါကို လေ့လာထားပါသည်။ ပူ၍ခြောက်သွေ့သော ရာသီဥတုအခြေနေသည် ရောဂါပြင်းထန်မှုကို ဖြစ်ပေါ် စေသည်။ ဒေသ (၅)မျိုးမှ ဤရောဂါကို လေ့လာ၍ ရောဂါလက္ခဏာ၊ ရုပ်သွင်ဗေဒတို့ကို နှိုင်းယှဉ် ထားပါသည်။ ရောဂါထိုးသွင်း၍ ဖြစ်ပေါ်လာသော လက္ခဏာသည် မူလလက္ခဏာနှင့် တူညီကြောင်း တွေ့ရပါသည်။ ရောဂါသည် အပူချိန် ၂၂ - ၂၆°C နှင့် အမှောင်ထဲတွင် ပို၍ ပေါက်နိုင်စွမ်း ရှိပါသည်။ မှိုသတ်ဆေး (၅)မျိုး၏ ရောဂါအပေါ် ဟန့်တားနိုင်မှုကို လေ့လာရာ အချို့ မှိုသတ်ဆေး တို့သည် အချို့သောပြင်းအား၌ ထိရောက်စွာ ဟန့်တားကြောင်း တွေ့ရပါသည်။ ခံနိုင်ရည် ရှိသော ကျွန်းမျိုး များရွေးချယ်ခြင်းကို ကနဦးလေ့လာထားရာ ထပ်မံ၍ လေ့လာ သုတေသနပြုရန် လိုအပ်ပါသည်။

Teak Rust *Olivea Tectonae* : Occurrence, Epidemiology, Its Chemical Control *In Vitro* and Reaction of Teak Clones and Provenances

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Abstract

The paper dealt with the incidence of teak leaf rust disease caused by the fungus *Olivea tectonae*. The correlation of disease occurrence and the weather conditions indicated that the severity of disease is favoured by hot and dry climatic conditions. Symptomatology and morphology characteristics of 5 isolates of *Olivea tectonae* from different regions were more or less identical. Inoculated seedlings with uredospores of *Olivea tectonae* showed typical rust symptoms and sign. Photoperiod and temperature experiments indicated better germination of uredospore in the dark at 22 - 26 °C. On testing the fungicides effectiveness some chemicals showed in some cases the inhibiting of uredospores germination. The selection of rust resistant of teak varieties is still in its initial stage which needs a projected research program.

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1. Introduction

Teak leaf rust caused by *Olivea tectonae* occurs throughout the range of distribution of the host in warmer areas of the world, such as India, Pakistan, SriLanka, Taiwan, Myanmar, Indonesia and Thailand (Gibson, 1992). In Myanmar, the occurrence of teak rust has recorded by Commonwealth Mycoligical Institute (CMI) (Mulder & Gibson, 1973). It was also reported by Cannon during his consultant period in 1988. It attacks trees growing in the nurseries, young plantations and also road side plantings. It is most prevalent during August to February. The severity of disease is favoured by hot, dry weather and it causes an early defoliation and serious loss in increment of the tree.

Teak rust fungus is classified as belonging to he fungal order Uredinales, constitute one of the largest groups in Basidiomycetes and family Chaconiaceae (Cummin & Y.Harat, 1983).

Teak has been adopted as an exotic plantation species in a number of tropical regions and teak rust is a potentially important disease in this respect. In comparison with many other diseases, published information on teak rust is very few. Therefore the present investigations were carried out at Yezin, Moeswe (Pyinmana township) and Oktwin with the following objectives.

(a) To study teak rust disease distribution and the effect of environmental factors.

(b) To observe the disease symptoms and the causal pathogen.

(c) To assess the *in vitro* inhibition of different fungicides against uredospore germination of teak rust pathogen.

(d) To evaluate the reaction of some teak clones and provenances to the rust.

2. Materials and Methods

2.1 Distribution and environmental factors of teak leaf rust

2.1.1 Occurrence of teak rust and meteorological data

A survey of teak leaf rust was conducted and the occurrence of disease regions were recorded in November and December, 1998. The disease occurrence and the weather conditions were studied. To know the weather conditions of the surveyed regions, the data of 1998 was collected from the Meteorological Department, Kabaaye. The reports monthly rainfall in millimeter (mm) and temperature in Celsius. (°C)

2.1.2 Disease survey

The disease incidence was assessed in different planting types:

(a) Forest nursery in Lewe Township.

(b) Road-side planting of teak along Pyinmana - Yezin highway.

(c) Group planting of teak in Aye Myint Thayar Unclassed Forest.
(Pyinmana Township)

(d) Special teak plantation in Kabaung Unclassed Forest, Coupe XVII. (Oktwin Township)

A hundred tress were randomly selected and the infected leaves were inspected. Height and spacing of the trees were measured. Age of the trees were also obtained from the local forest office.

Of the four blocks, block II of special teak plantation in Kabaung Unclassed Forest was selected for a survey of disease incidence. All the trees in the block were inspected for the incidence of disease. Height, age and spacing were also recorded.

2.2 Symptomatology of teak leaf rust, morphology and pathogenicity of the causal organism

2.2.1 Study on symptom and morphology

Rust infected leaves were collected and symptoms of rust on the leaves were systematically studied. Uredial morphology of 5 isolates from Mandalay, Mawlamyine, Pathein, Yangon and Yezin were checked under the microscope. For each isolate, the length and breadth of 100 spores were measured at random by using an ocular micrometer under high power magnification (400 x). The experiment was of a Completely Randomized Design (CRD) with 4 replications. The statistical analysis was performed and mean data were compared by Duncan's New Multiple Range Test (DMRT).

2.2.2 Uredospore germination at different temperature and photoperiods

Uredospores from the infected teak leaf were dusted on TEA (Teak leaf Extract Agar) plates. The plates were incubated in dark at different temperatures in range of 10-12 °C, 18°C, 20-22°C, 22-26°C, 30°C and 35°C. The other plates were placed under three different conditions of photoperiod , namely (a) in the dark (b) under the fluorescent (2000 lux) and (c) 12 hours dark and 12 hours light alternately. The experiment was carried out with 4 replicates. Observations were made under the microscope after 24 hours of incubation.

2.2.3 Viability of uredospore

The uredospores from infected leaves were dusted daily on TEA plates from the day when rusted leaves were collected for 30 days. There were four plates per treatment in this study. Germination percentage of uredospore was counted after 24 hours of incubation.

2.2.4 Hyperparasites on teak leaf rust

Some uredospores were covered with the parasitic growth. The morphology of the organism were recorded.

2.2.5 Inoculation

Inoculation experiments of teak rust was carried out either in the mist chamber or nursery. The healthy teak seedlings having the same age, and same height (about 1 ft) were inoculated plants were incubated at 25°C with about 65% relative humidity in a mist chamber under 12 hours light and 12 hours dark. Teak seedlings were inoculated and kept in the nursery which had a temperature range of 25-38°C and 20-43% relative humidity. Control teak seedlings were included in every experiments.

2.3 The *in virto* inhibition of different fungicides on uredospore germination of *O. tectonae*

The fungicides used in the study were Bayleton, Daconil, Dithane M-45, Maneb and Zineb. The concentrations used for fungicides were 0.01, 0.1.1, 10, 100, and 1000 ppm (active ingredient) (a.i.). Firstly the fungicides were prepared as concentrated suspensions or solutions. Sterile distilled water was added separately in warm sterile 1.25% TEA to obtain the required concentration. Fifteen ml of TEA medium incorporated with fungicides were then poured into 9 cm diameter petridish. Rust infected teak leaves were collected from the field of Yezin. The uredospores from the infected leaves were dusted on the TEA media in the plate. The plates were then incubated at 25°C in the dark for 24 hours.

The experiment was carried out with 3 replicates. Control plates consisted of the media without fungicides. Observation was made after 24 hours of incubation. The percentage of germinating uredospores was counted under the microscope. Percent inhibition of spore germination in each treatment was calculated by following equation (Vincent, 1927).

$$I = \frac{100\ (\ C-T\ )}{C}$$

Where, I = inhibition over control
C = uredospore germination on untreated agar
T = uredospore germination on treated agar

The results were then subjected to probit analysis (Finney, 1962). The efficacy of the fungicides was compared by calculating the amount of fungicides required for 50% inhibition of growth (ED_{50}). ED_{50} (Effective Dose) values for radial growth inhibition due to fungicides were obtained from regression lines plotting percentage inhibition on a probit scale versus log concentration of fungicides.

2.4 The reaction of teak clones and provenances to rust

For each clone in block A and B of the Moeswe teak clonal seed orchard, rust percentage was estimated on the second limb selected on the southern side of the tree (Cannon, 1988) (Figure 6). Height and girth were also measured for each tree. The Study was initiated in April, 1989 when new leaves were appeared. Observations of the rust occurrence started from April until February. There were 4 replicates per treatment (Clone), and the experiment was of a Randomized Complete Block Design (RCBD). DMRT was used in comparison within clones.
In Oktwin teak provenance trial plot, block II and IV were selected, for measurements and statistical analysis. Disease severity was recorded in November, 1999.

3. Results

3.1 Distribution and environmental factors of teak leaf rust

3.1.1 Occurrence of teak rust and meteorological data

Teak rust was observed in Bamaw, Mandalay, Pyinmana, Taungoo, Pyay, Sittwe, Yangon, Pathein and Mawlamyine. The locations of teak rust occurrence in the country were shown in figure (1). The histogram indicating rainfall and temperature (maximum & minmum) in regions of Myanmar where teak rust occurred were also shown in figure (2). In these regions the maximum temperature were from 27.2 to 35.0°C and rainfall ranged from 0 mm to 44 mm during November and December.

3.1.2 Disease survey

Table (1) showed the incidence of teak rust in different planting types. The infection percentage assessed in nursery seedlings, road-side planting, group planting and special teak plantation were 98, 55, 100 and 100 respectively.

3.2 Symptomatology of teak leaf rust, morphology and pathogenicity of *Olivea tectonae*

3.2.1 Symptom and morphology

The infected leaves had a gray flecked appearance on the upper surface and there were masses of yellowish orange uredia on the underside, in corresponding position. Colour of uredospores changed from yellowish to tan colour. The rusty orange powder can very easily be marked. The spores did not appear on petioles and stems.

Uredia were abundant on the lower surface of the leaves, where they range from 100 to 300 µm in diameter. Uredial paraphyses united cylindric, swollen at apex, incurved and hyaline. Uredia were hypophyllous, pulverulent, sub-epidermal in origin

later on becoming superficial. No telial stage was observed (Plate 5). Uredospores borne singly on pedicels. They were ovoid., globose, echinulate. (Plate 6). The uredospore size of 5 different isolates were shown in table (2). No significant difference was found in length of uredospores, however, they were different in breadth among the isolates tested.

3.2.2 Uredospore germination at different temperatures and photoperiods

Percentage germination of uredospores at different incubated temperatures was shown in table (3) and figure (3). The highest spore germination was found at the temperature range of 22-26°C, followed closely by 20-22°C and 30°C. At 18°C, there was still germination but less than that of the temperatures mentioned above. No uredospore germinated at 10-12°C and 35°C.

Percentage germination of uredospores for photoperiod test was shown in table (4) and figure (4).The highest germination was found in dark, moderate in dark & light and minimal under light.

3.2.3 Viability of uredospore

The results indicated that viability of uredospore reduced with the storage time (Table 5.) However, there was a variation of viability in daily observations. Finally, no germination was observed after 28 days of storage.

3.2.4 Hyperparasites on teak rust

Two fungal hyperparasites covered on uredospores were found on the media. The first one appeared white cottony colony on TEA. Conidiophores were slender, hyaline and conidia were globose, hyaline, 2.5-10 x 2.5-10 µm in size (Plate 8). The second one appeared in the form of greenish gray colony on TEA. Conidiophores were dark, upright and conidia were one-celled, ovoid to cylindrical and 1.25-11.25 x 1.25-5 µm in size (Plate 9). The morphology of the two hyperparasitic fungi were accorded with *Acremonium recifei* and *Cladosporium oxysporum* respectively.

3.2.5 Inoculation

The inoculated seedlings in the mist chamber turned gradually yellow and the lower leaves drooped after two weeks. The seedlings kept in the nursery showed disease symptoms and sign in two weeks after inoculation. (Plate 10).

3.3 The *in vitro* inhibition of different fungicides on uredospore germination of *O. tectonae*

The percentage inhibition of uredospore germination of *O.tectonae* by each fungicide at different concentration was shown in table (6). Dosage response curves for uredospore germination on TEA media containing various fungicides were shown in figure (5). Table (7) summarized the different ED_{50} values of fungicides in

inhibition of uredofpore germination of *O.tectonae*. Under normal condition in the absence of fungicides, about 67.65% of uredospores germination on TEA. Among the fungicides, assessed, Daconil was the most effective. Next to Daconil was Dithane M-45 followed by Maneb, Bayleton and Zineb. All these 5 fungicides started showing inhibitory effect at the low concertration of 0.01 ppm. Daconil at 1000 ppm (a.i.) resulted in complete inhibition of uredospore germination. Dithane M-45, Maneb and Zineb completely suppressed uredospore germination at 10 ppm (a.i.). In the case of Bayleton, complete inhibition could not be obtained even at 1000 ppm (a.i.). Daconil gave very high *in vitro* inhibition effect on uredospore germination reflected by its extremely low ED_{50} value of 0.09 ppm (a.i.). Dithane M-45, Maneb, Bayleton, and Zineb were also toxic with ED_{50} of 0.19, 0.53, 2.5 and 4.9 ppm (a.i.) repectively.

3.4 The reaction of teak clones and provenances to rust

The symptoms of teak leaf rust appeared in August and the sign of disease persists throughout from November to February at Moeswe teak clonal seed orchard. The disease occurred severely from December to February, before the normal shedding of the leaves (Figure 7).

The differences in susceptibility among the teak clones investigated were shown in table (10) and figure (8). Of the teak clones tested, CP was least susceptible followed by 18/4. However, there were no significant differences among 66/1. 17/5, 19/1 and 17/4. Clone 8/1 was most susceptible among the clones tested.

The differences in susceptibility among the provenances tested were shown in table (11) and figure (9). There were no significant differences in all provenances tested. Disease severity was, however, the lowest in Bago provenance whereas the highest in Padaung provenance.

4. Discussion

In Myanmar, there has been very few information on teak leaf rust although the disease has been noticed for a long time. The experiments included the distribution of rust, the collection of meteorological data, survey of disease incidence in different planting types, the observation of disease symptoms, morphology and pathogenicity of *Olievea tectonae*, chemical control *in vitro* and reaction of teak clones and provenances to rust.

The observation of the present disease survey agreed with the reports of many authors. (Khan, 1951; Mulder & Gibson, 1973; Bakshi, 1976 and Sharma et al, 1985) who stated that the prevalence of teak leaf rust in regions with hot and dry climatic conditions. Although the observations made were not a comparison among different types of planting, there was indication that trees planted closely had more infection than trees planted with a wide spacing (Table 1). This can be attributed to the fact that a buffer zone or mixed planting with suitable spacing between teak may reduce the incidence of disease to a certain extent.

In this study the rust infected teak plants showed the typical symptom as described by many workers (Khan, 1971, Mulder & Gibson, 1973; Sharma et al. 1985). There was no difference in uredial morphology of rust pathogen except the size of breadth among the isolates tests. The results showed that *O. tectonae* differed

in uredospore germination with different temperatures and photoperiods. Uredospores were found to be viable for 28 days. However, there has been differences in the viability test. It may depend on the maturity of the spores. Observations of two fungal hyperparasites were similar with those reported by Sharma et al. (1885). The knowledge of biological control of hyperparasites is also necessary. The typical rust symptoms and sign were observed on the inoculated leaves placed in the nursery. This study also attributed to the fact that environmental factors such as temperature and moisture may influence the disease development of rust.

In this study, all fungicides tests were highly toxic to uredospore germination of *O. tectonae*. However, Dacoil was most effective with low concentration. Finally, it can be concluded that a certain fungicides were effective in suppressing uredospore germination of *O.tectonae* under laboratory conditions. Field trials should be undertaken using chemicals which are effective and economical.

Moeswe teak clonal orchard indicated that no clones were found to be resistant to the disease although CP and 18/4 were the least susceptible among the selected clones. There were no significant differences in the severity of rust disease among the provenances planted at Oktwin. To confirm the finding of the reaction of teak clones and provenances to rust further investigations should be carried out at other corresponding clonal seed orchards and provenance trial plots.

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Table 1. The incidence of teak rust in different planting types.

Planting type	**Age**	**Height (m)**	**Spacing (m)**	**No .of trees observed**	**Infection**
Nursery seedling	4 months	0.2	-	100	98
Road-side planting	1 year 4 months	2.6	9.1 - 15.2	100	55
Group planting	1 yeat 4 months	28	3 x 3	100	100
Special plantation	1 year 5 months	2.0	2.7 x 2.7	490	100

Table 2. Morphology of uredospores from different isolates of *O. tectonae*

Isolates	Shape	Length (µm)		Breadth (µm)	
		Range	Mean	Range	Mean
Mandalay	Globose, Ovoid	15.0-27.5	20.4a	12.5-20.0	16.4ab
Mawlamyine	Globose, Ovoid	15.0-27.5	20.4a	11.3-20.0	15.9 b
Pathein	Globose, Ovoid	17.5-25.0	20.7a	12.5-22.5	17.0 a
Yangon	Globose, Ovoid	17.5-25.0	19.8a	12.5-20.0	16.3 ab
Yezin	Globose, Ovoid	15.0-27.5	20.1 a	12.5-20.0	17.0 a
CV%			3.8		3.9

Table 3. Uredospore germination of Yezin isolate after 24 hours incubation on 1.25% TEA at different temperatures.

Temperature (°C)	Mean uredospore germination (%)
10-12	0
18	22.18
20-22	27.42
22-26	31.94
30	26.22
35	0

Table 4. Uredospore germination of Yezin isolate after 24 hours incubation on 1.25% TEA at different photoperiods

Photoperiod	Mean uredospore germination (%)
Dark	39.05
Light & Dark	10.26
Light	3.41

Table 5. Viability of uredospore on TEA after different dusting times.

Day after storage	Mean uredospore germination (%)
1	56.78
4	8.05
7	4.31
10	21.81
13	11.16
16	6.49
19	5.65
22	2.95
25	1.98
28	0

Table 6. Percent inhibition of uredospore germination of *O.tectonae* at different concentrations of fungicides

Concentration (ppm)	Inhibition (%) of germination by				
	Bayleton	Daconil	Dithane M-45	Maneb	Zineb
0.01	30.47	33.24	24.02	32.39	9.37
0.1	35.51	51.37	32.17	45.72	18.29
1	37.18	61.52	73.17	51.32	37.59
10	42.69	90.20	100.00	100.00	100.00
100	48.15	100.00	-	-	-
1000	95.00	-	-	-	-

Table 7. Ed_{50} values of fungicides in inhibition on uredospore germination of *O. tectonae*

Fungicides	ED_{50} (ppm) (a.i.)
Deconil	0.09
Dithane M-45	0.19
Maneb	0.53
Bayleton	2.50
Zineb	4.90

Table 8. Sources of scion material used for grafting in the teak clonal seed orchard at Moeswe

Source of scion	Compartment
Pyinmana, Ngalaik	9, 17, 18, 19, 20
Pyinmana, Palwe	51, 56
Taung Oo, Kabaung	16, 18 (TGO)
Pyay, Nawin	7 (CP)
Kyaukgyi, Madaya	8
Lower Madaya	66

Table 9. Sources of seeds for seedlings in the teak provenance trial plot at Oktwin

Source of seed	Provenance plot number
Pyinmana	1
Kalay	2
Bago	3
Phyu	4
Mudon	5
Padaung	6
Momeik	7
Saw	8
Moenhyin	9
Mabin	10

Table 10. Teak rust diseases severity in Moeswe teak clonal seed orchard

Clone	**Disease severity %**
8/1	63.9 a
66/1	46.9 b
18/6	42.8 bc
17/5	38.5 bc
19/1	37.7 bc
17/4	35.8 bc
18/4	33.8 c
CP	31.6 c
CV%	17.98

Table 11. Teak rust disease severity in Oktwin teak provenance trial plot.

Provenance	Disease severity
Padaung	40.26 a
Mudon	37.87 a
Momeik	36.87 a
Saw	36.07 a
Kalay	35.97 a
Mabein	35.86 a
Moenhyin	35.80 a
Phyu	33.99 a
Pyinmana	33.69 a
Bago	33.59 a
CV%	17.00

Locations of Teak Rust occurrence reported in MYANMAR

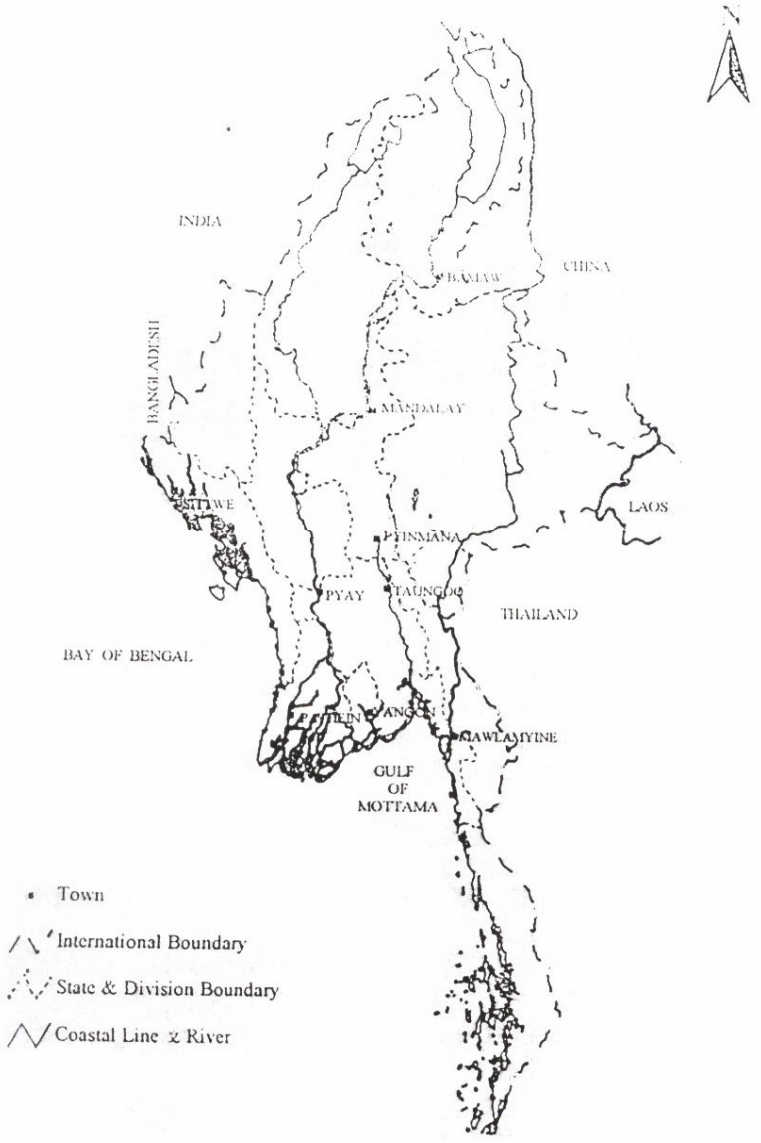
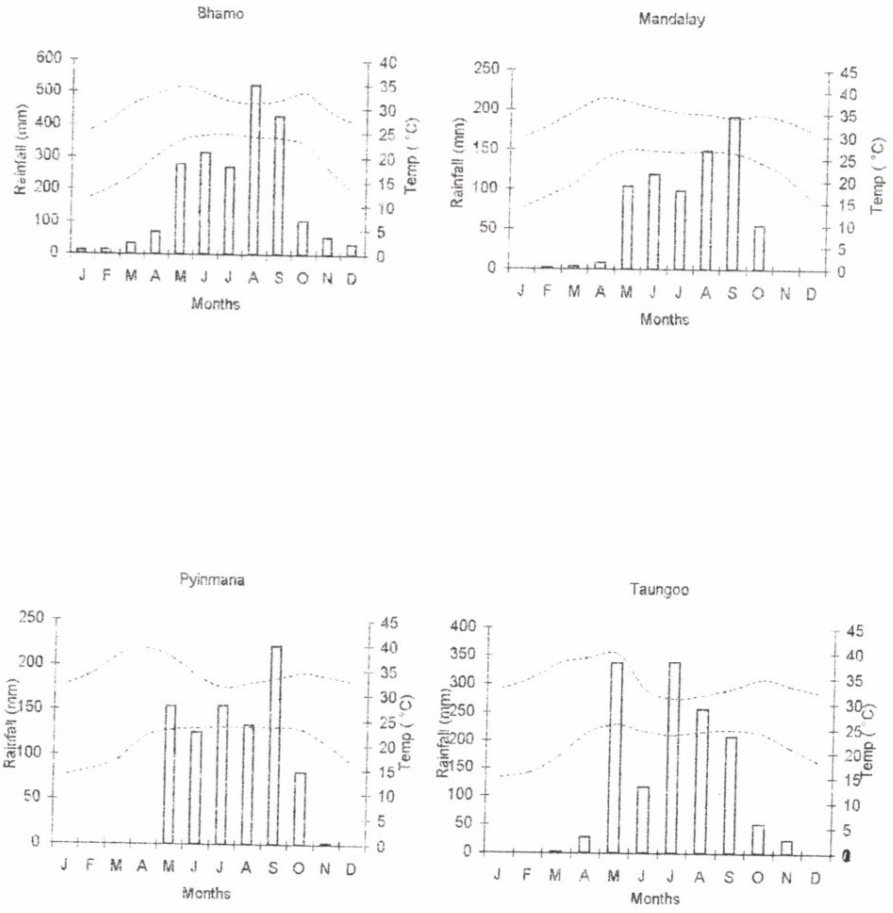
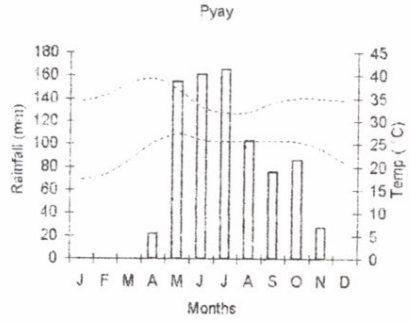
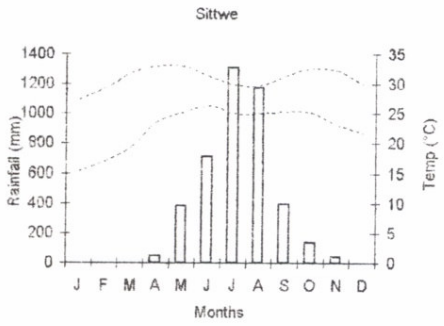
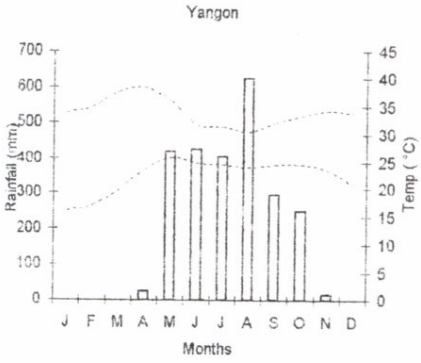


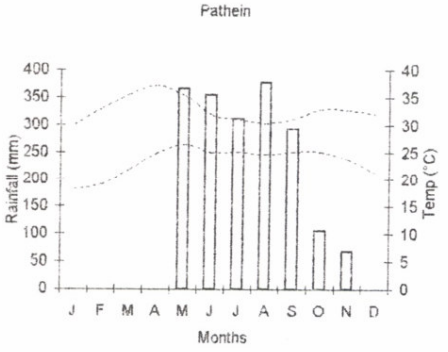
Figure 2. Histogram indicating Rainfall and Temperature (Max-Min) in regions of MYANMAR where Teak Rust occurred.

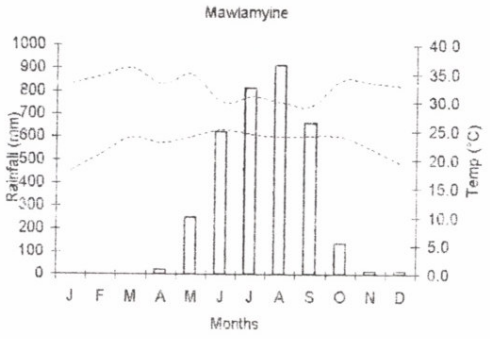



Pyay
Rainfall (mm)
Temp (°C)
Months
J F M A M J J A S O N D


Sittwe
Rainfall (mm)
Temp (°C)
Months
J F M A M J J A S O N D


Yangon
Rainfall (mm)
Temp (°C)
Months
J F M A M J J A S O N D


Pathein
Rainfall (mm)
Temp (°C)
Months
J F M A M J J A S O N D


Mawlamyine
Rainfall (mm)
Temp (°C)
Months
J F M A M J J A S O N D

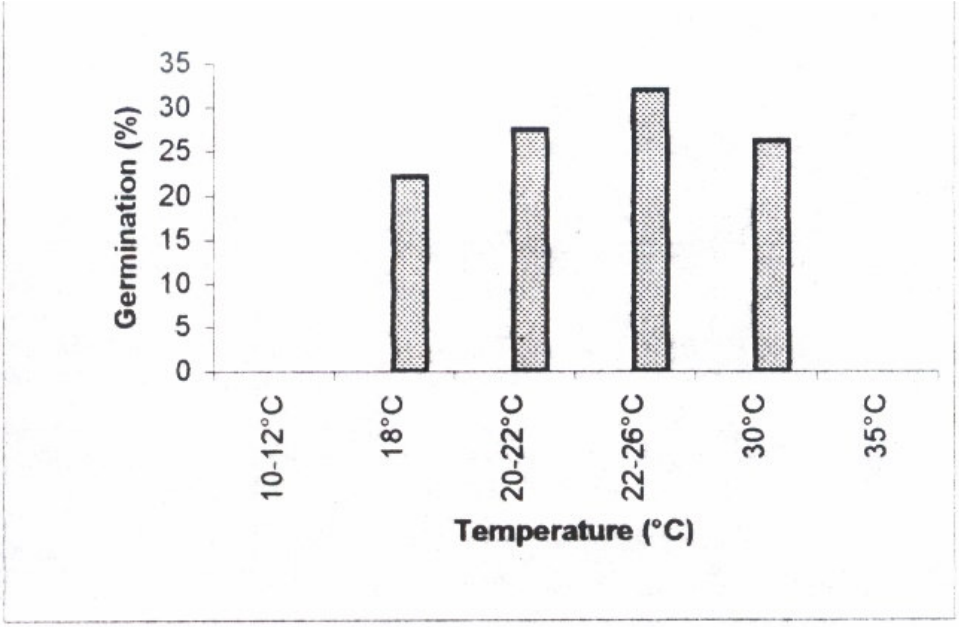


Figure 3. Uredospore germination at different temperature

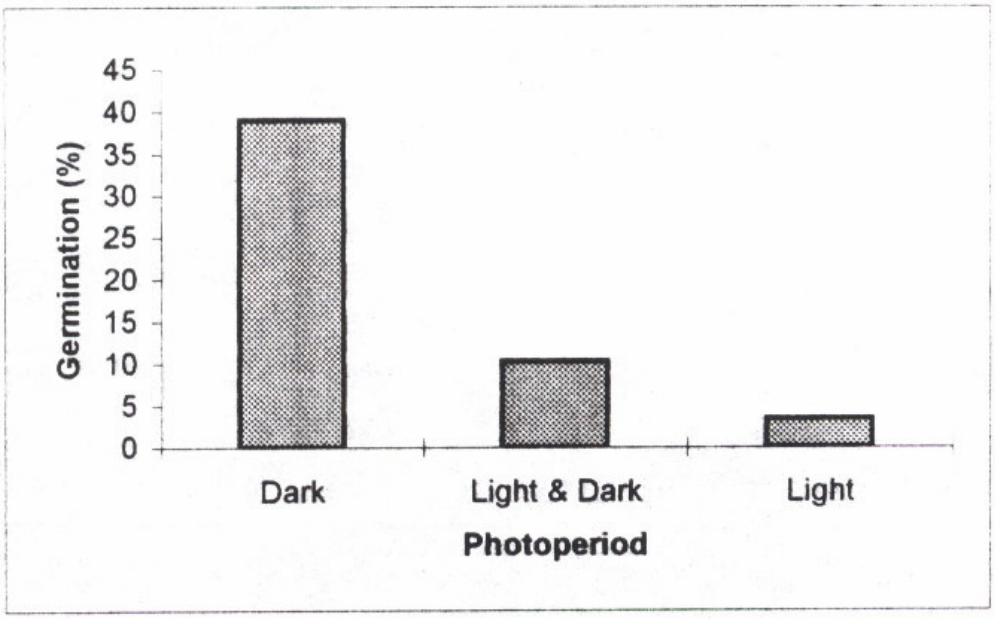


Figure 4. Uredospore germination at different photoperiod

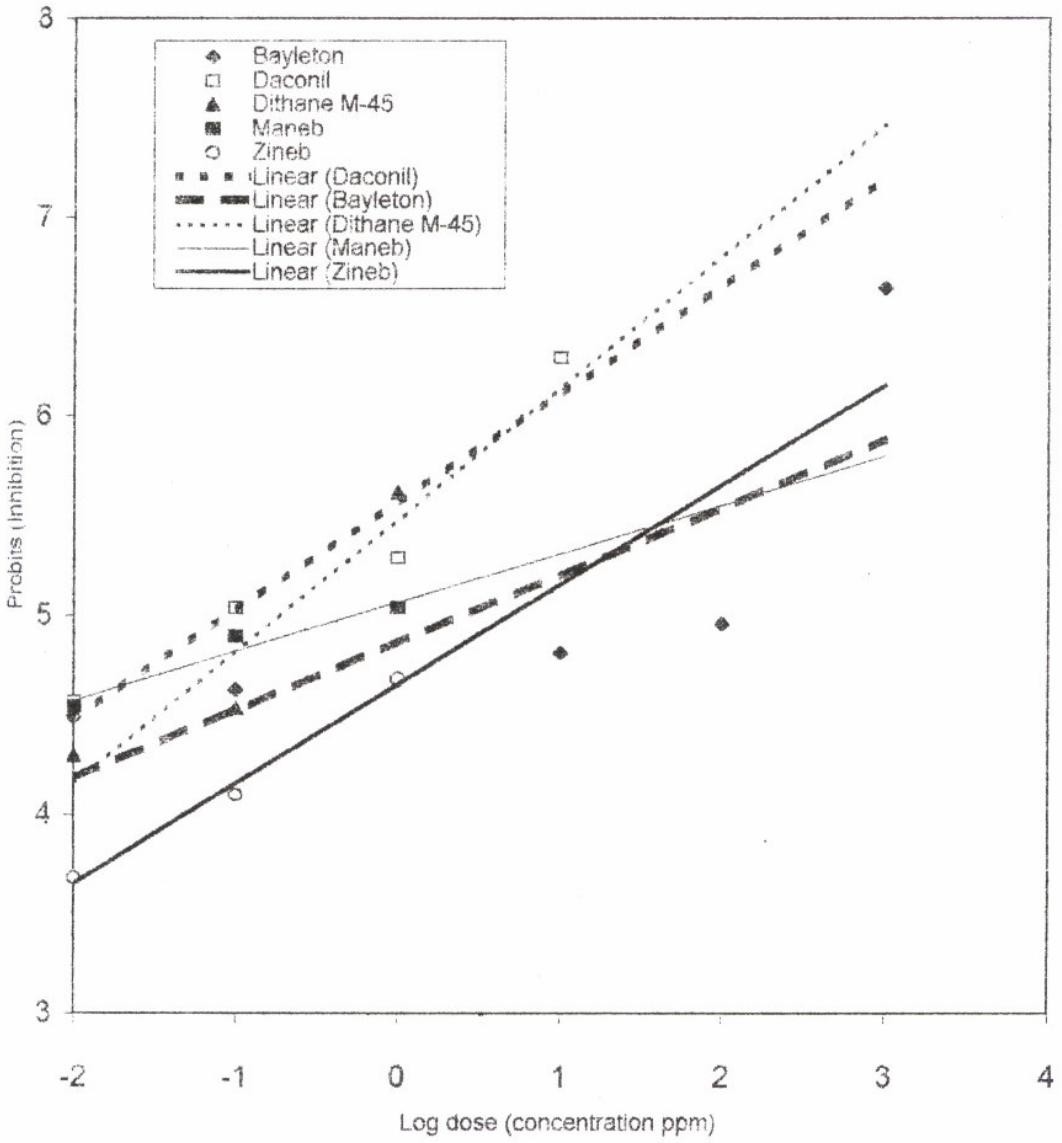
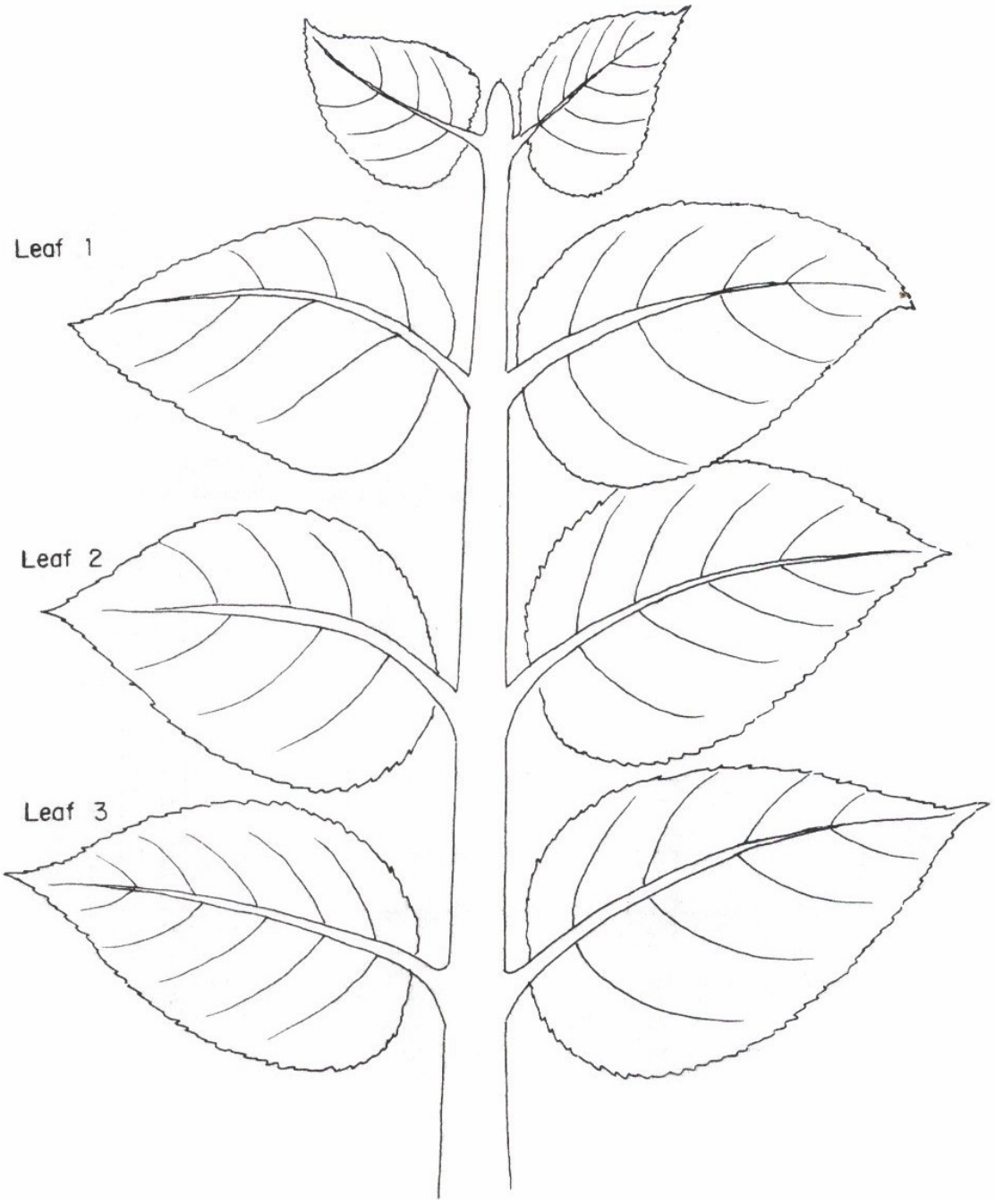


Figure. 5 Probit-log dose plots of effect of fungicides on uredospore germination of O. tectonae in vitro.

Figure 6. Selection of teak in estimation of rust infection.



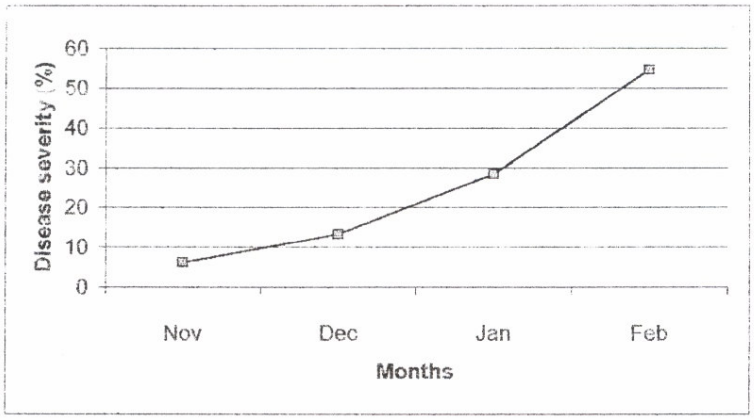


Figure 7. Disease progress curve showing the infection of different month in Moeswe Teak Clonal Seed Orchard

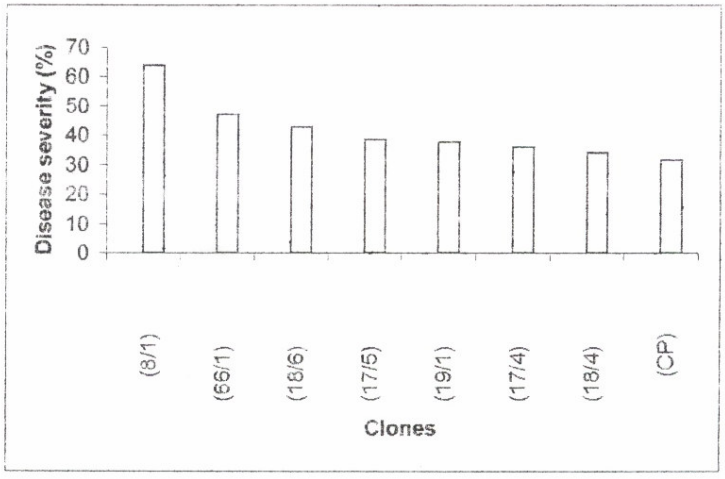


Figure 8. Disease progress bar chart showing the infection of different clones in Moeswe Teak Clonal Seed Orchard

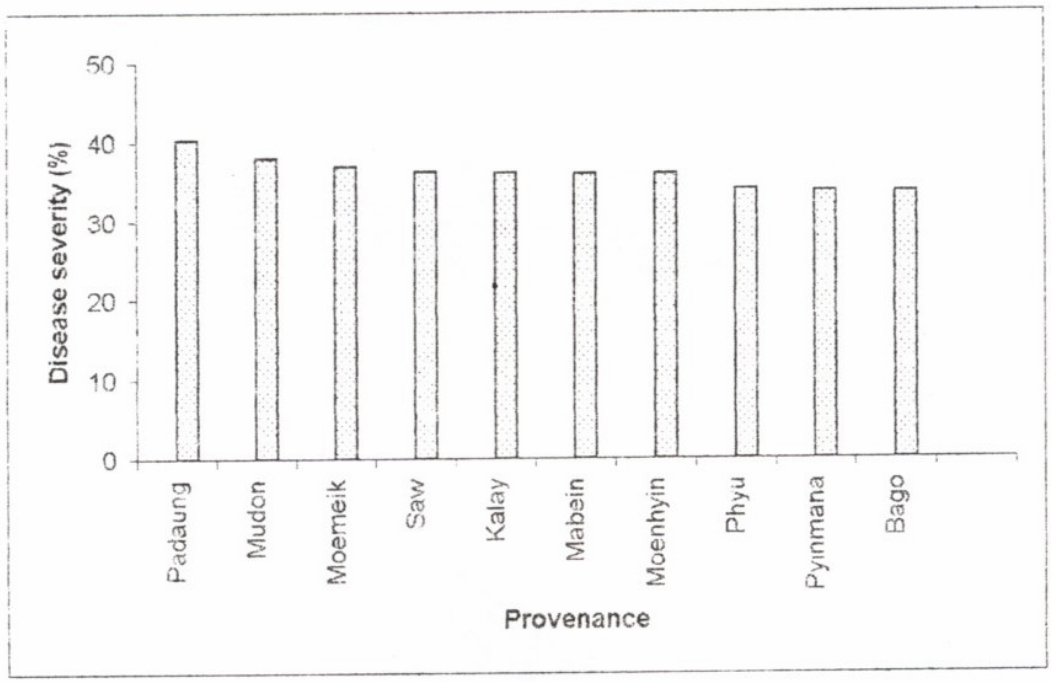


Figure 9. Bar chart showing the disease severity of different provenances in Oktwin teak provenance trial plot.

Plate 1. Rust infected teak seedlings in Lewe forest nursery.

Plate 2. Rust infected teak tree at Aye Myint Thayar group plantation.

Plate 3. Rust symptom on young teak leaf (left), and healthy leaf (right) (Upper surface)

Plate 4. Rust sign on young teak leaf (left), and healthy leaf (right) (Lower surface).

Plate 5. Corss section of uredium of *Olivea* tectonae showing uredospores and paraphyses.

Plate 6. Uredospores of teak leaf rust (200 X).

Plate 7. Germinating uredospore showing germ tube with appressorium (400 X).

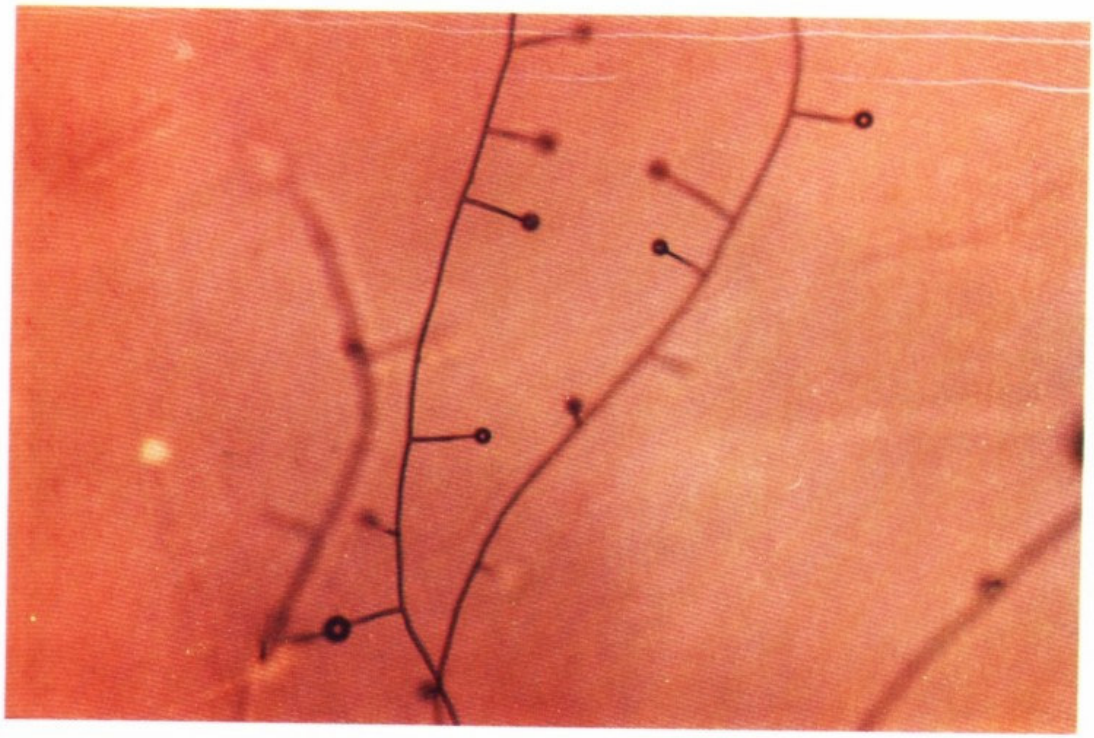


Plate 8. Hyperparasite of teak rust, conidia of *Acremonium recifie* (100X)

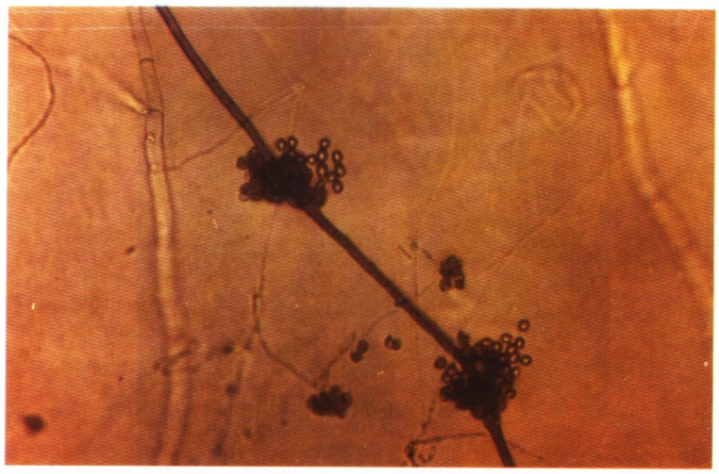


Plate 9. Hyperparasite of teak rust, conidia of *Cladosporium oxysporum* (200 X)

Plate 10. Rust inoculated teak seedling leaf showing the sign two weeks after inoculation.

Plate 11. Moeswe Teak Clonal Seed Orchard.

Plate 12. Oktwin Teak Provenance Trial Plot.