NISEB JOURNAL Vol. 11, No. 3 , July 31, 2011
Printed in Nigeria

1595-6938/2011 $5.00 + 0.00
© 2011 Nigerian Society for Experimental Biology
http://www.nisebj.org

NISEB 2011093/11302

Isolation and Identification of Fungi Associated with Teak (*Tectona grandis* L.) Seedlings at the University of Ilorin Teak Plantation, Kwara State, Nigeria

J. E. Amadi[1] and Y. A. Saka[2]

1Department of Botany, Nnamdi Azikiwe University, PMB 5025, Awka, Nigeria
2Department of Plant Biology, University of Ilorin, PMB 1515, Ilorin, Nigeria

(Received May 8, 2011; Accepted June 30, 2011)

ABSTRACT: A disease survey was carried out at the University of Ilorin Commercial Tree Planting site in Ilorin, Kwara State, Nigeria. This site was planted to teak (*Tectona grandis* L.). Different disease symptoms were observed on the teak leaves. Isolations from the leaves yielded eight different fungal species namely *Nigrospora* sp., *Aspergillus nidulans*, *A.* niger, C*urvularia* sp., *Phoma* sp. *Rhizoctonia* sp., isolate G and isolate I. Three of these isolates namely *Curvularia, Phoma*, and *Rhizoctonia*, spp. proved pathogenic on teak leaves under the conditions of this study. The three pathogenic isolates responded differently to different culture media but the best medium for growth was yeast extract agar (YEA). Growth of the test organisms was also affected by temperature.

Key Words: Teak; Tectona grandis; Fungi; Seedlings;Ilorin; Nigeria.

Introduction

Teak (*Tectona* sp.) is a genus of tropical hardwood trees in the mint family, Lamiaceae (formerly Verbenaceae), sometimes included in the subfamily Prostantheroideae (Singh, 2004) and commonly found as a component of monsoon forest vegetation. Teak trees are usually large and grow up to 30–40 meters tall with large, leathery, reddish-green deciduous leaves. There are three species of *Tectona* namely *T. grandis* (Common Teak), *T. hamiltoniana* (Dahat Teak) and *T. philippinensis* (Philippine Teak) (Wikipedia, 2010). Teak (*T. grandis*) grows in most areas between the Tropic of Cancer and the Tropic of Capricorn and is indigenous to India, Myanmar and a few South East Asian countries (Balasundaran *et al*., 1995; Siswamartana 1999; Thaiutsa *et al.*, 2002).

According to Ball *et al*. (1999), an introduction of teak from India to Nigeria in 1902 was the first transfer out of Asia. Now, teak is one of the most widely cultivated hardwood timber species in the world having a total plantation area of over 2.3 million ha with India and Indonesia alone having more than half of the area. Teak is propagated mainly from seeds and germination takes 15-30 days following pre-treatment to remove dormancy arising from the thick pericarp. Teak trees have straight stems and so yield fine timber when processed. The sap has anti-bacterial and anti-fungal properties that make it resistant to parasites and diseases. Teak also contains rubber and silica, which repel water and also give the wood great pliability, making it better able to withstand high pressure. Teak wood is used for ship building, fine furniture, door and window frames, wharves, bridges, cooling-

tower louvres, flooring and paneling. Its desirability has led to severe over-cutting in tropical forests (Robertson, 2002; Wikipedia, 2010).

Teak plantations provide economic and environmental relief and are the major source of income for many villages in tropical areas. Teak increases in value by up to 6% every year and has revenue of up to 30,000 USD per trunk. This makes it a very resourceful cash crop (Smith, 1962). This economic potential is one of the major reasons why the University of Ilorin adopted Teak as one of its mandate crops in its commercial tree farming venture. The planting of teak at the University started in June 2006 and it is perhaps the greatest frontal effort of the University to combat climate change (Oloyede, 2010). By 2008, a total of 70,383 seedlings of Teak (*T. grandis*) have been planted on 57 hectares of campus land. The venture was supervised by the Commercial Tree Development and Management Committee (CTDMC) set up by the Vice Chancellor of the University, Prof. Ishaq O. Oloyede.

Teak production is constrained by a few serious diseases both in nurseries and plantations. Some of these diseases include leaf spot (*Phomopsis* sp., *Colletotrichum gloeosporioides, Alternaria* sp. and *Curvularia* sp.), leaf rust (*Olivea tectonae*) and powdery mildew (*Uncinula tectonae*) (Balasundaran *et al*., 1995). Nursery diseases are very important in teak production because if timely control measures are not taken, the disease spreads to the entire nursery and reduce the number of healthy seedlings available for planting. This in turn disrupts the planting programme and so hampers teak production.

The objective of this study is to carry out a disease survey of the University of Ilorin Teak Plantation with a view to:

1. determining the incidence of disease in the plantation and
2. isolating and identifying the causal agent(s) of any observed disease.

Materials and Methods

Survey and Sample Collection:

A disease survey was carried out on the 57 hectare area planted to *Tectona grandis* seedlings at the University of Ilorin, Kwara State, Nigeria. The survey was entirely on the leaves of the teak seedlings. Infected leaves showing various symptoms were collected into sterile polyethylene bags. Leaves showing same symptoms were collected together in the same polyethylene bag. The samples were taken to the Plant Pathology laboratory in the Department of Plant Biology of the University for isolation of associated organisms.

Isolation:

The infected leaves were cut into small pieces (3mm) and surface-sterilized in 70% alcohol for 3mins and rinsed in 2 changes of sterile distilled water. The cut pieces were inoculated aseptically on sterile potato dextrose agar (PDA) in Petri dishes. The plates were incubated at room temperature ($27\pm1^{o}C$) for the growth of associated organisms. Observed growths were sub-cultured several times to obtain pure cultures. Microscope slides were prepared from the isolates for microscopic observation and identification. The isolates were preserved in stock cultures in bottle slants for subsequent use. Identification of the isolates was carried out with the help of laboratory manuals and texts (Campbell and Stewart (1980)). Photomicrographs of the isolates were also taken to aid identification.

Effect of Media and Temperature on Growth of Isolates:

Studies were carried out to determine the effects of media and temperature on the growth of the isolated fungal organisms. The test media were potato dextrose agar (PDA), malt extract agar (MEA) and yeast extract agar (YEA) while the temperatures tested were $0^{o}C$ (freezer),$15^{o}C$, $20^{o}C$, $25^{o}C$, $30^{o}C$ and $45^{o}C$ (oven). The medium used for the temperature experiment was YEA. Sterile agar plates were inoculated with 3mm-diameter mycelial discs of the appropriate inoculum. Inoculated plates were incubated at room temperature ($27\pm1^{o}C$) and growth measured daily for 7 days. Each test was replicated thrice and the results were the means of the replicates.

Pathogenicity Test

In order to determine the relative importance or otherwise of each of the organisms isolated from the teak leaves, a pathogenicity test was carried out separately with each of the isolates. Artificial inoculation experiments were conducted using the detached leaf method (Amadi, 2003). Healthy mature leaves were collected from the plantation in sterile polyethylene bags and taken to the laboratory for artificial inoculation. Samples were surface-sterilized in 70% ethanol for 3 minutes and rinsed in 2 changes of sterile distilled water. The leaves were pricked (injured) at four points with sterile needle and then placed on layers of sterile moist filter papers in Petri dishes. Spore suspensions (10^{-3} spores per ml) of the different fungal isolates were prepared and 1ml of each suspension was used separately to inoculate each test leaf. Inoculated leaves were incubated at room temperature (27 ± 1^{o}C) and observed frequently for symptom development. Re-isolations were carried out from inoculated leaves showing symptoms. Control plates were inoculated with sterile distilled water and incubated as described earlier.

Results

Isolation and Identification

A total of eight different fungal species were isolated from the University of Ilorin teak plantation in this study. Macroscopic and microscopic observations of the organisms considered their colour, texture, reproductive structures and growth patterns. Observations revealed the organisms to be *Nigrospora* sp., *Aspergillus nidulans*, *A.* niger, C*urvularia* sp., *Phoma* sp. and *Rhizoctonia* sp. Two isolates (G and I) were, however, not identified in the present study (Plate 1-5).

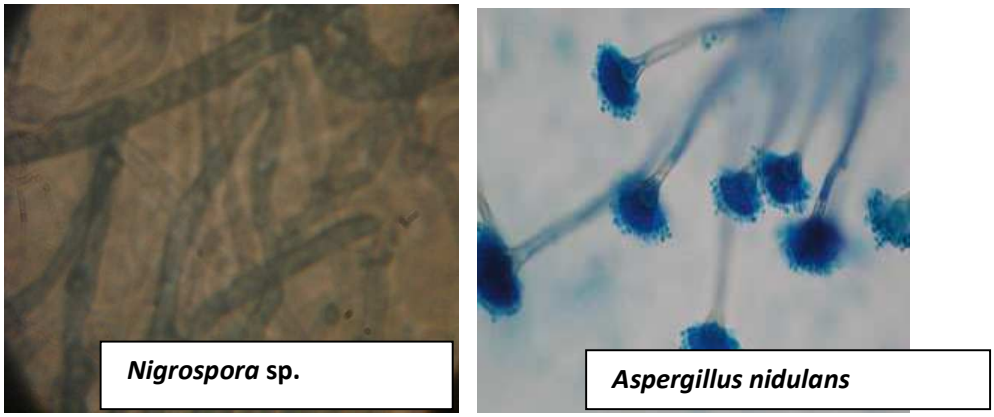

PLATE 1: ***Nigrospora sp.* and *Aspergillus nidulans***

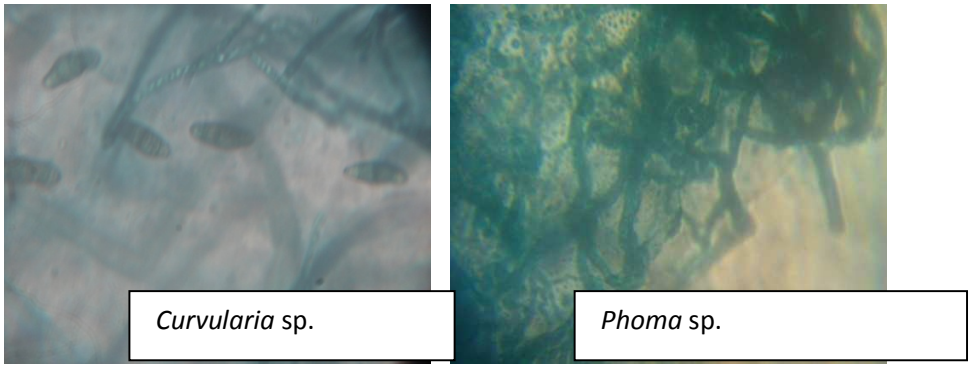

PLATE 2: *Curvularia* sp. and *Phoma* sp.

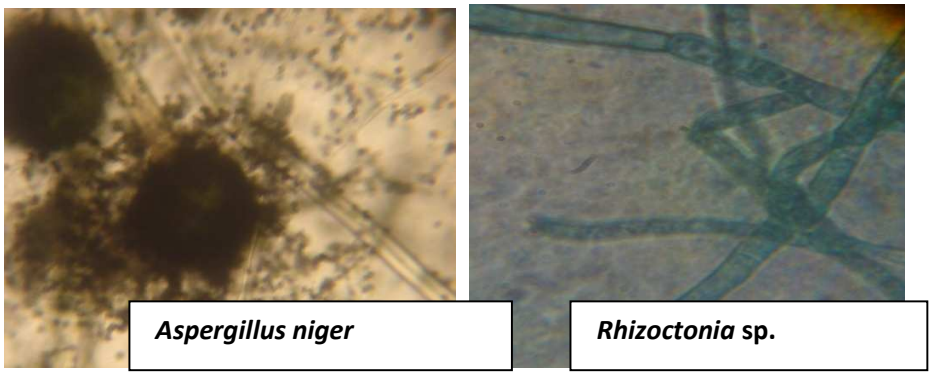

PLATE 3: *Aspergillus niger* and *Rhizoctonia* sp.

Plate 4: Isolate G

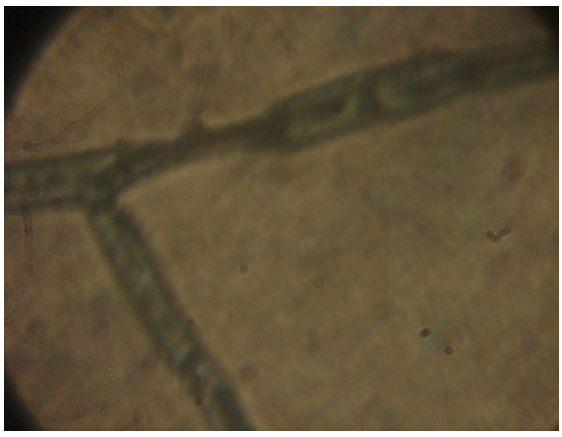

PLATE 5: Isolate I: Not identified

Pathogenicity Test

Results of this study revealed that out of the eight organisms isolated from infected teak leaves, only three isolates produced symptoms on artificially inoculated leaves under the conditions of this study. The three isolates are *Rhizoctonia*, *Curvularia* and *Phoma* species. Inoculated leaves showed soaked brown lesions (Plates 6-8) along the leaf veins and extended to other parts of the leaves. Symptoms started from the injured portions of the leaves and spread throughout the leaf surface. Inoculated leaves also showed white cottony growth covering the initial points of entry of the pathogen. This symptom corresponds to that observed in the field during disease survey. The artificially inoculated organisms were later re-isolated from the inoculated teak leaves.

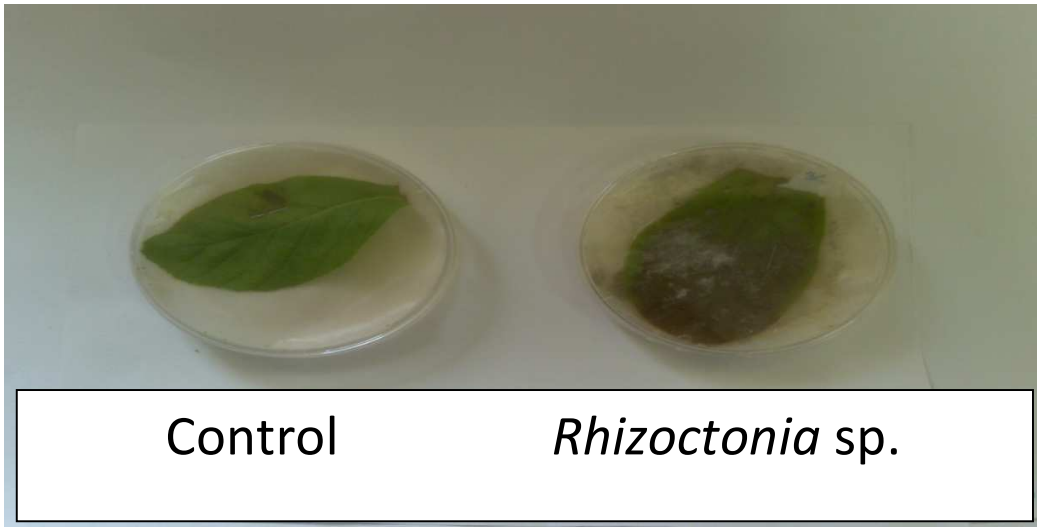

PLATE 6: Leaf Inoculation of *Tectona grandis* with *Rhizoctonia* sp.

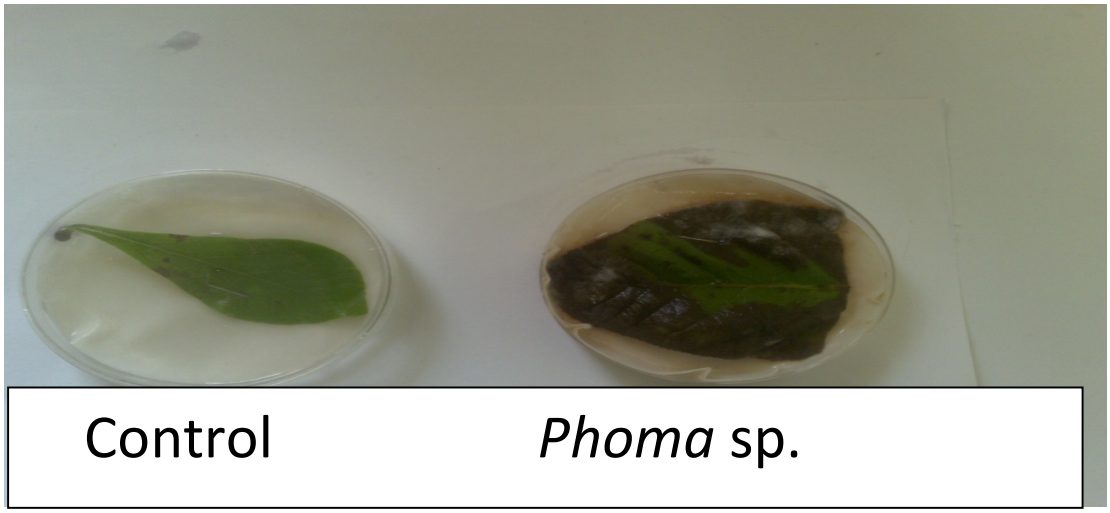

PLATE 7: Leaf Inoculation of *Tectona grandis* with *Phoma* sp.

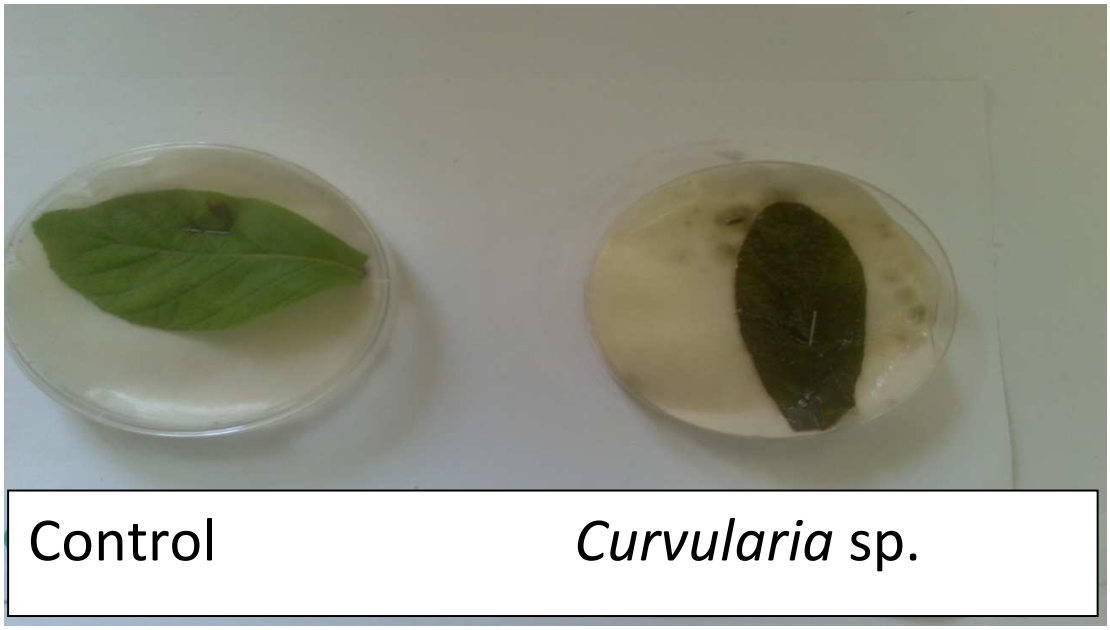

PLATE 8: Leaf Inoculation of *Tectona grandis* with *Curvularia* sp.

Effects of Media and Temperature on Growth of Isolates

It was observed that culture media have a lot of effect on the growth of *T. grandis* leaf isolates under the conditions of this study. The different isolates showed appreciable growth in all the media tested. However, all the isolates grew best in the yeast extract agar (YEA). *Curvularia* sp. grew better than both *Phoma* and *Rhizoctonia* spp. in all the media tested in this study with the best growth (11mm) occurring in YEA. The least growth among all the isolates studied was recorded in *Rhizoctonia* sp. (3mm) in malt extract agar (MEA).

Temperature was also observed to affect the growth of isolates in the present study. Growth of the isolates occurred over a range of temperatures. The medium used was YEA which had proved to be the best medium for all the organisms under the conditions of this study. *Curvularia* sp. recorded the highest growth of 12.2mm at 30^{o}C while *Rhizoctonia* sp. recorded the least growth of 5.7mm. No growth was observed at 0 and 45^{o}C for any of the isolates. Results are means of three replicates.

Discussion

Eight fungal species namely *Nigrospora sp., Aspergillus nidulans, A. niger, Curvularia sp., Phoma sp., Rhizoctonia* sp., Isolate G and Isolate I were isolated from teak seedlings from the University of Ilorin teak plantation. This result supports the understanding that microorganisms commonly occur in plants and other materials in nature. Marciano *et al*., (2005) have reported several endophytic fungi in cocoa (*Theobroma cacao* L.) including *Colletotrichum, Fusarium, Phomopsis, Cladosporium, Trichoderma and Gliocadium* species. Among these fungi, some were identified as potential antagonists of *Crinipellis perniciosa*, the witches' broom pathogen. *Gliocladium catenulatum* reduced the incidence of witches' broom disease in cocoa seedlings to 70%. Amadi and Oso (1996) and Amadi (2002) have reported several fungi including *Aspergillus flavus, A. fumigatus, A. niger, Mucor hiemalis, Colletotrichum* sp., *Curvularia* sp., *Penicillium* sp., *Rhizopus oryzae, Alternaria longissima, Macrophomina phaseolina and Cochliobolus pallescens* in cowpea and sugarcane.

Pathogenicity test carried out showed that of the eight fungi isolated, *Rhizoctonia* sp., *Phoma* sp., and C*urvularia* sp. produced leaf symptoms in inoculated leaves. These organisms were also re-isolated from inoculated leaves. Balasundaran *et al*., (1995) had reported *Phomopsis* sp., *Colletotrichum gloeosporioides, Alternaria* and *Curvularia* spp. as causal organisms of leaf spot disease in *T. grandis* in India. Seedling diseases have been identified as an

impediment to seedling productivity in some indigenous trees of Kerala, India (Nair *et al*., 1996). According to Mohan (2008), different disease problems of teak were recorded in the nursery. *Rhizoctonia solani* was implicated in the leaf blight of teak while different fungal pathogens were responsible for leaf spots. Both blight and leaf spots spread laterally in the nursery through overlapping foliage of the adjoining seedlings and often result in group blighting of seedlings. In each case of severe infection, defoliation is high.

Mohanan *et al.* (2005) reported that *Rhizoctonia solani* rarely occurs in teak nurseries while *Curvularia* sp. and *Phoma* sp. can cause foliage infection in teak. *Aspergillus nidulans* was reported (Campbell and Stewart, 1980) to be rarely a pathogen. *Aspergillus* sp. is a filamentous, cosmopolitan and ubiquitous fungus found in nature commonly isolated from soil, plant debris and indoor air environment. It is advisable to keep phytosanitary measures as some of these fungi are soil-borne and can be opportunistic on teak plants. It is also important to clear plant debris as some of these pathogens survive in them.

From the result of this study, it is obvious that culture media and temperature affect the growth of organisms in the laboratory. YEA supported growth best in all the test organisms while the optimum temperature was 30^{o}C in the present study. This might be due to the preference for the nutritional contents of the medium. Host nutrition is an important factor in fungal physiology and disease development. Organisms generally have preference for one nutrient component or the other and this must have been responsible for the differential growth capabilities recorded in the test media. Temperature affects organism growth by its effect on the metabolism of the organisms. These observations are in agreement with earlier reports on fungal physiology (Campbell and Stewart, 1980; Agrios, 1988).

References

Agrios, N.G. (1988). Plant Pathology. Academic Press, London. 803pp.

Amadi, J.E. and Oso, B.A. (1996). Mycoflora of cowpea seeds (*Vigna unguiculata* L.) and their effects on seed nutrient contents and germination. *Nig. Journal of Science 30*:63-69.

Amadi, J.E. (2002). Studies on the mycoflora of sugarcane (*Saccharum officinarum* L.) seeds and their importance in the nursery. *NISEB Jour. 2*(1): 89-95.

Amadi, J. E. (2003). Isolation and identification of the causal organism of leaf spot of peony (*Paeonia* sp.) in Ferderick, Maryland, USA. *Nig. J. Pure and Applied Science 18*:1364-1368.

Balasundaran, M; Sharma, J.K.; Florence, E.J. and Maria, M.C. (1995). Leaf spot Diseases of Teak and their impact on Seedling Production in Nurseries. http//www.metla.fi/iufro/iufro95abs/d2pap88.htm

Ball, J. B., Pandey, D. and Hirai, S. (1999). Global overview of teak plantations. Paper to regional seminar on site, technology and productivity of teak plantations, Chiangmai, Thailand. 17pp.

Campbell, C. M. and Stewart, J. L. (1980). The Medical MycologyHandbook. Wiley and Sons Inc., New York. 211-345.

Marciano, R.R; Rute, T.S; Alan, W.V.P.; Cristina, S.M; Welington, L.A; Deise,, R.S. and Joao, L.A. (2005). Diversity of endophytic fungal community of cacao (*Theobroma cacao* L.) and biological control of *Crinipellis perniciosa*, causal agent of Witches' Broom Disease. *Int. J. Biol. Sci. 1*: 24-33.

Mohan, V. (2008). Pathological problems of economically important forest tree species in India – An Overview. www.agritech.tnau.ac.in/forestry/forest_disease_teak.html

Mohanan, C.; Ratheesh, N.; Laya P. N. and Rajesh-Kumar, K.C. (2005). Disease problems in root trainer forest nurseries in Kerala State and their management. Working Papers of the Finnish Forest Research Institute 11.

Nair, K.S.S; Sharma, J.K. and Verma, R.V. (Eds.)(1996). Impact of Diseases and Insect Pests in Tropical Forests: Proceedings of the IUFRO Symposium 23-26 November 1993, Peechi, India.

Oloyede, I. O. (2008). Afforestation and Reforestation: The Unilorin Experiment. A presentation at the High Level Technical Workshop on Afforestation and Climate Change in Africa December 15-17, 2008. 33pp

Robertson, B. (2002). Growing Teak in the Top End of the NT. Agnote. No. G26. www.nt.gov.au.

Singh, G. (2004). Plant Systematics: An Integrated Approach. Science Publishers. 120pp.

Siswamartana, S. (1999). Teak plantation productivity in Indonesia. Paper to Regional Seminar on site, Technology and Productivity of Teak Plantation. Chiangmai, Thailand. 9pp.

Smith, D.M. (1962). The Practice of Silviculture. Wiley and Sons, New York.578pp.

Thaiutsa, B.; Puangchit, L.; Yarwudhi, C.; Wacharinrat, C. and Kobayashi, S. (2002). Coppicing Ability of Teak (*Tectona grandis*) after Thinning. *18*:152-156.

Wikipedia (2010). Plant Pathology. *www.wikipedia.com*