

ABSTRACT
OF THE THESIS ENTITLED
INVESTIGATIONS ON FUNGI ASSOCIATED WITH
LEAF ROT DISEASE OF COCONUT PALM (*Cocos nucifera* L.)

SUBMITTED TO THE
MANGALORE UNIVERSITY

By
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IN FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN APPLIED BOTANY IN THE FACULTY OF SCIENCE

RESEARCH GUIDE
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ABSTRACT

INVESTIGATIONS ON FUNGI ASSOCIATED WITH LEAF ROT DISEASE OF COCONUT PALM (*Cocos nucifera* L.)

Root (wilt) and leaf rot diseases are the major constraints to the production and productivity of coconut in Kerala. Root (wilt) disease (RWD) affected palms are also susceptible to leaf rot disease (LRD). RWD and LRD occur in all the districts of Kerala with very high incidence and severity in southern districts. Apart from this, RWD is also prevalent in some districts of Tamil Nadu and Karnataka state adjoining to Kerala. Recently the disease has also been recorded in Goa. Phytoplasma has been identified as the cause of RWD. LRD caused by fungi mainly *Colletotrichum gloeosporioides* and *Exserohilum rostratum*, is noticed in RWD affected palms of all ages. LRD causes reduction in photosynthetic area, disfiguration of the palms and reduction in yield.

No studies on advanced molecular techniques have been conducted so far on different aspects of the pathogen of LRD. Isolates of *C. gloeosporioides* and *E. rostratum* were collected from different locations with varying intensities of the disease. To isolate total DNA from leaf samples of healthy and diseased coconut palms, the method using GenElute Plant Genomic DNA Kit (Sigma) was found to be the best based on several advantages.

In all the *C. gloeosporioides* isolates tested with the standardized PCR conditions, the size of DNA fragment amplified was approximately 590 bp. The size of amplicons produced using the same pair of primers (ITS 4 and ITS 5) was estimated to be 600 bp for six isolates of *E. rostratum* and approximately 590 bp for the isolate from Theni district of Tamil Nadu, where the disease has started recently. *C. gloeosporioides* isolates did not appear to be host-adapted strains, since different coconut isolates grouped with different *C. gloeosporioides* and *C. fragariae* isolates from various hosts. Among the *E. rostratum* isolates also four groups could be observed. RFLP also indicated different groups among *C. gloeosporioides* isolates.

Among *E. rostratum* isolates, the Theni isolate alone formed a separate type. Out of seven isolates, six isolates of *E. rostratum* were very much similar to one another. But Theni isolate was clearly quite different from the other isolates in RAPD-PCR, RFLP and rDNA sequence analysis.

Based on RFLP and sequencing *C. gloeosporioides* isolates were grouped into three types. The closest sequence was that of *C. gloeosporioides* isolate from avocado. Five isolates of *C. gloeosporioides* were close to *C. gloeosporioides* isolates of citrus.

Isolate ErTni 1 from Theni district was quite different from other *E. rostratum* isolates based on RFLP and RAPD analysis and the sequence analysis further confirmed this. These studies indicate that isolate ErTni 1 is not *Exserohilum rostratum*, but is probably related to *Bipolaris tetramera* or *B. specifera*.

The specific primer amplified products of *C. gloeosporioides* and *E. rostratum* from infected leaf material, which exactly corresponded to the PCR amplified products of genomic DNA from respective fungus. Thus, this molecular technique clearly augmented the results of microscopic examination and isolation studies.

With the standard conditions of PCR, RWD phytoplasma was consistently detected from all RWD affected samples as well as some samples from apparently healthy (without any visual symptoms) palms which gave positive reaction in sero-diagnostic test, ELISA.

The present work has clearly revealed that variability exists within *C. gloeosporioides* and *E. rostratum*, the major pathogens of LRD. The results of RAPD, RFLP and rDNA sequence analysis of ITS 4 / 5 region supported this. The molecular techniques employed to characterize the isolates of *C. gloeosporioides* and *E. rostratum* indicated that among the isolates collected from different locations at least five strains of *C. gloeosporioides* and three strains of *E. rostratum* exist in endemic areas (in Kerala state). The fungus, suspected to be *E. rostratum* collected from areas where the disease is of recent occurrence (Theni, Tamil Nadu) was found to be either *Bipolaris tetramera* or *B. specifera*. This finding is important in the etiology and management of LRD as not only different strains of a particular pathogen but also different pathogens may exist in different locations especially in areas with recent outbreak of the disease. Since PCR assays are highly sensitive, these tests can be used to detect root (wilt) and leaf rot diseases of coconut prior to the appearance of visual symptoms of the diseases.

Key words: Root (wilt) disease (RWD), Phytoplasma, leaf rot disease (LRD), *Colletotrichum gloeosporioides*, *Exserohilum rostratum*, molecular detection, PCR, RAPD-PCR, RFLP, rDNA sequence analysis, species-specific primer, species-specific detection.