

Project work on

**IN VITRO ANTIFUNGAL BIOASSAY OF *EUPHORBIA NERIIFOLIA*  
ROOT EXTRACTS ON *CERATOCYSTIS PARADOXA***

Submitted in partial fulfillment of the requirement of  
Bachelor of Engineering in

**Biotechnology**

Prescribed by Visvesvaraya Technological University, Belgaum



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## ABSTRACT

Stem bleeding is known to occur in all coconut growing regions in the tropics. The disease was first reported in India during 1922. In the early stages, there is not much yield loss, however, in the later stages, there is a steady decline in the yield causing considerable amount of loss and in the advanced stages even leads to death of the palm. The characteristic symptoms of the disease are the exudation of rusty brown liquid from the cracks and cuts found on the outer tissue of the trunk. The exuded material turns black in color as it dries up on the bark. In India, trees showing the above mentioned symptoms were proven to be attacked by *Thielaviopsis paradoxa* (De Seynes) Höhn. (anamorphic *Ceratocystidaceae*). The studies in India showed that inoculation of healthy coconut plants with isolates obtained from diseased plants resulted in rusty brown discoloration of the bark within 2-8 weeks, followed by the oozing of a brownish liquid from stems. Re-isolation of *T. paradoxa* was made from such diseased tissues (Nambiar et al., 1986).

The objective of our project was to analyze the antifungal activity of crude extracts from *Euphorbia neriifolia*. The extracts assayed for antifungal activity primarily included crude extracts of stem and roots. Tests performed included techniques like the agar well diffusion technique and food poison technique. Pathogenicity tests were performed to test the ability of the organism *Thielaviopsis paradoxa* to infect the plant. Following the determination of minimum inhibitory concentration, the extracts were subjected to Thin Layer Chromatography with various solvent systems such as Ethyl acetate: methanol: water (40:5:5), Chloroform: ethyl acetate: formic acid (5:4:1), benzene: ethanol: ammonium hydroxide (90:10:1), Chloroform: ethanol: ethyl acetate: acetone: water (50:20:20:5:3.5), Butanol: ethyl acetate: water (5:1:2), Ethyl acetate: acetic acid: formic acid: water (50:5.5:5.5:13), chloroform: ethyl acetate: water.

Effective inhibition was observed by the aqueous root and the stem extracts of the plant. However multiple runs of Thin Layer Chromatography in different solvent systems failed to show clear cut bands but consistent unclear bands were observed in all the runs in chloroform: ethyl acetate: water for both aqueous and methanol root extracts and in Butanol: ethyl acetate: water (5:1:2) for aqueous root extracts.