7.0 SUMMARY

_Cryptococcus neoformans_ species complex is the causal agent of meningitis in humans. Increasingly, this opportunistic fungal pathogen has been reported in immunocompromised patients resulting in serious fatalities. In India, reports of clinical manifestations of this condition has been available but few reports on the fungal biology or characterization has been made. This study was attempted to obtain a detailed pattern of occurrence of this serious human pathogen both in patients and in the environment. The salient results obtained based on this study were:

1) Both pulmonary (8%) and meningeal (43 %) cryptococcosis was detected in patients with classical symptoms.

2) The yeast pathogen was detectable under microscopy (negative staining) in 66% of CSF samples and in 33% of pulmonary samples.

3) In culture test using selective media SAB, for cryptococci the 43 % of isolates recovered was the highest being (3/38) in pulmonary samples and 61/ 143 CSF samples. All these patients also had other conditions such as AIDS, TB or other predisposing factors.

4) These yeasts were also isolatable on SAB media from the environment (37%) samples.

5) Classifying all these isolates into serovars using standard growth media- CGB & GCP was done. Isolates from CSF samples (53/61) serotyped to serovar A (C. grubii) and from extraneural or pulmonary samples (3/38) also were of serotype A. Only 8/61 CSF isolates typed to _C. neoformans_ (type D/AD).
6) Isolates obtained from the environment typed to serotype *C. gattii* (B/C) in 11/13 samples.

7) Four primer sets spanning the rRNA genes of yeast was used to test a PCR based diagnostic method. Of the primers tested ITS1- CN4 primer pair gave (93%) detection of the product (415 bp) from neat samples. The four samples not detectable thus could be detected when DNA was obtained from their cultures.

8) FTIR spectroscopic technique was used for the first time on Indian sampled to obtained spectra of 15 CSF, culture positive samples and compared with control samples from which no Cryptococci were isolated. Unique IR bands at 1746 cm\(^{-1}\) was observed only in patient samples (Fig. 21a)

9) Leaf powder extracts of SM12-E1 & E2 showed antifungal activity to clinical isolates. This report was observed for the first time with the bioactive fractions at \(R_f\) 0.5 & 0.25.

Thus, a systematic study of clinical samples form Chennai tertiary hospital revealed the cause of meningitis as due to *Cryptococcus* with a majority caused by species, *C. grubii*. PCR based diagnosis in South India is reported for the first time. FTIR spectral analysis to obtain markers for raid diagnosis also gave promising results. Plant extracts showing anti-cryptococcal activity was demonstrated for the first time. These important leads need to be followed up in future to address the problem of early accurate diagnosis of this increasingly important opportunistic human pathogen.