

Culturable Endophytic Microbial Community Structure in Healthy and Die-Back Infected *Callophyllum walkeri* in Horton Plains, Sri Lanka

**Perera, M.T.N.M.¹, Karandeniya, K.D.P.D.T.¹, Nigesh, V.², Samarasinghe, D.P.³,
De Costa, W.A.J.M.², De Costa, D.M.^{1*}**

¹ Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka

² Department of Crop Science, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka

³ Postgraduate Institute of Archaeology, University of Kelaniya, Kelaniya, Sri Lanka
*dmdcosta@agri.pdn.ac.lk

Abstract

The cause of forest dieback in the upper montane forest at Horton Plains (HP) remains unexplained despite several past investigations. Shifts in the structural profile of endophytic microbes due to conditions experienced by plants could cause beneficial or detrimental effects on plant health. This study aimed to compare the structural composition of the endophytic microbiome between healthy and die-back affected *Callophyllum walkeri* trees at different stages of die-back progression using culture dependent approaches to elucidate the role of endophytic microbes in the dieback process. Sampling was done from two 0.4 ha plots in the forest which represented healthy trees (H) and die-back infected *C. walkeri* trees at four stages of die-back progression, namely, S1 (foliage discolouration), S2 (defoliated), S3 (stem defects) and S4 (bark damage). Leaves and core samples of roots and stems were collected in triplicate per plot from H, S1, S2, S3 and S4 trees. Endophytic fungi, bacteria and actinomycetes were isolated from all samples using specific culture media. Endophytic actinomycetes were not observed even after one month's incubation. Relative abundance (RA) and percentage isolation frequency (PIF) were computed for endophytic fungal and bacterial morphospecies. Gram positive bacteria were present in all plant parts of healthy and die-back infected stages. Fungal RA and PIF differed significantly among plant parts ($p < 0.05$) with roots and leaves showing the highest and lowest RA and PIF respectively. However, there was no significant variation in fungal RA or PIF between healthy and die-back affected trees. *Truncatella* sp., *Neopestalotiopsis* sp., *Pestalotiopsis* sp., collectively termed as *Pestalotioids*, were present as endophytic fungi in roots and stems of both healthy and die-back affected *C. walkeri* at all stages. Similarly, they were present in leaves at S1 stage. Relative abundance of bacteria differed significantly ($p < 0.05$) among plant parts and stages of die-back development, where leaves and stem samples reported the highest and the lowest values respectively. The highest bacterial RA was observed at S3 stage whereas the lowest was at H, S1 and S4 stages which did not differ significantly among themselves. The PIF of bacteria was not significantly different among plant parts or stages of die-back. This study revealed that RA of the endophytic fungal community did not differ between healthy and die-back affected *C. walkeri* trees. Even though RA of the endophytic bacterial community showed variation with stage of die-back development, the observed pattern of variation did not reveal a clear role in the die-back process.

Keywords: Endemic, Morphospecies, Progression of Die-back, Pestalotioids