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Use of *Actinomycetes* Species for Agarwood Formation in *Gyrinops walla* Tree Species

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Abstract

Agarwood is a highly valuable, aromatic substance derived in the heartwood of certain tree members of Thymelaeaceae family. It is formed to prevent internal tissue damages from injuries and diseases. *Gyrinops walla* is the only agarwood producing tree species, naturally growing in Sri Lanka. Healthy heartwood of this species is light colour, odourless, soft and low in density. Once resin-impregnated, it becomes darker in colour, dense and highly fragrant. Several studies were conducted on agarwood formation in *Aquilaria* and *Gyrinops* trees by fungal species. The present study tested agarwood formation in *G. walla* by three *Actinomycetes* species of *Nocardia*, *Pseudonocardia* and *Streptomyces* genera. Those species were isolated from fresh tissues of *G. walla* by culturing in the laboratory. Identification was done by morphological and microscopic characteristics. 100 ml of spore suspension of each selected species in starch casein broth was inoculated to mature *G. walla* tree stems with three replicates. The trees were harvested after three months and discoloured tissue samples were collected at 5 cm intervals above (+) and below (-) each inoculation point. Agarwood resin extraction and constituent identification were done by solvent extraction and GC-MS analysis respectively. The spread of the resinous tissues were the highest in the trees inoculated with *Nocardia* (length=+6.10 to -5.50 cm; width=3.20 cm) followed by *Pseudonocardia* (length=+5.80 to -5.00 cm; width=1.20 cm) and *Streptomyces* (length=+4.60 to -4.30 cm; width=1.10 cm). *Nocardia*, *Pseudonocardia* and *Streptomyces* produced respectively brown, strong brown and gray colour tissues. Extracted resin colour was olive brown, olive yellow and yellow respectively. *Pseudonocardia* produced the highest resin content (4.28±2.36%) followed by *Nocardia* (2.33±1.09%) and *Streptomyces* (1.86±0.61%). GC-MS analysis of extracted resins revealed the presence of agarofuran, agarospirol, valecane, vetispirane, guaiene, eremophilane, selinene and fatty acids, which produce the characteristic agarwood aroma. The highest number of key compounds were found in the resin extracted from the tissues formed using *Nocardia*, followed by *Pseudonocardia* and *Streptomyces*. Therefore, this study confirmed the use of selected *Actinomycetes* species for artificially inducing agarwood resin formation.

Keywords: *Gyrinops walla*, Agarwood, *Actinomycetes*, Resin induction, Chemical constituents