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# PROGRAMME BOOK

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## Agarwood Resin Inducement in *Gyrinops Walla*: Beyond Fungal Inoculations

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### Abstract

*Gyrinops walla* is the only naturally growing agarwood producing tree species in Sri Lanka. It had no commercial recognition until its agarwood producing ability was scientifically identified in 2012. This paved avenues for numerous researches. This paper illustrates some studies conducted on different inoculation methods using fungal species and their substances.

The first attempt was made in 2014 to identify the potential fungal species that can be used at commercial scale for agarwood formation in *G. walla*. The inhabiting fungal species were identified from naturally formed agarwood tissues *G. walla* trees of the wet zone of Sri Lanka. Those tissues were size reduced, surface sterilised and placed in agar media to grow the inhabiting fungi which were then isolated and identified with colony and morphological characteristics. Altogether, 4 *Fusarium*, 4 *Trichoderma*, 3 *Aspergillus*, 2 *Botryosphaeria* species and 1 species of each of *Diplocladium*, *Mucor* and *Sarcinomyces* were identified. When re-inoculated as pure cultures, *A. niger* and *F. solani* produced the highest agarwood resin contents ( $0.82 \pm 0.07\%$  and  $0.73 \pm 0.06\%$  respectively) in *G. walla* up to 50 cm above the inoculation point in 6 months. 16 and 15 key resin constituents were identified from those resins formed by *A. niger* and *F. solani* respectively. Once 100 ml of spore suspensions of *A. niger* and *F. solani* in nutrient broth media were inoculated, the resin contents were  $0.37 \pm 0.06\%$  and  $0.40 \pm 0.18\%$  respectively after 4 months with pale yellow to brown in colour. Then potential of agarwood inducement using secondary metabolites was tested using different strains of the same fungal species. For this reason, the above two fungal strains (ASP-U and FUS-U) and two other strains (ASP-N of *A. niger* and FUS-N of *F. solani*) were grown in agar media and their secondary metabolites were extracted by filtering through a series of ceramic filters of descending pore sizes. A bioassay conducted on *G. walla* leaves confirmed the toxicity of secondary metabolites only for ASP-U and FUS-U strains. Then 50 ml of those were inoculated to *G. walla* and after 7 months, only ASP-U and FUS-U which were obtained from the naturally formed agarwood produced resins while the other two strains were not productive. ASP-U produced resins ( $2.27 \pm 0.39\%$ ) only up to  $\pm 20$  cm from the inoculation point while FUS-U produced resins ( $4.21 \pm 0.47\%$ ) up to  $\pm 60$  cm. The oil colour was yellow with 14 key constituents and dark yellowish brown with 24 key constituents for ASP-U and FUS-U respectively. The results lead to assume that only some strains of the same fungal species are capable of inducing agarwood. Secondary metabolites of the effective fungal species can also be used for agarwood inducement which can be chemically manufactured at commercial scale.

**Keywords:** *Gyrinops walla*, *Fusarium solani*, *Aspergillus niger*, Fungal strains, Agarwood



**Figure 1:** Agarwood tissues formed by FUS-U and ASP-U strains after 7 months.