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Optimization of Fungal Extract Based Inoculation for Agarwood Induction in *Aquilaria crassna*

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Abstract

Agarwood is a highly valued non-timber forest product formed as a defense response in *Aquilaria* species following biotic or abiotic stress. Fungal based biological solid media inoculation is constrained by contamination and sterilization challenges, while liquid spore suspensions require precise timing and are difficult to prepare and transport at large scale. This study aimed to identify effective fungal extracts and determine optimal dosages for agarwood induction using *Aquilaria crassna*, to develop an inoculation protocol, and to evaluate agarwood yield and oil content over short and medium term periods. Four fungal species; *Trichoderma* sp., *Lasiodiploidea* sp. (01), *Lasiodiploidea* sp. (02), and *Aspergillus* sp. were selected based on their abundance, toxicity and extract producing potential to induce resin formation. Fungal extracts were prepared using Yeast extract Glucose Broth and Czapek Dox Broth and produced at three volumes of 25, 50 and, 100 mL via dichloromethane extraction. Field experiments were conducted in a 3 ha *Aquilaria crassna* plantation in Galle District, Sri Lanka, using 10 to 12 year old trees. Extracts were inoculated at different stem heights (top, bottom, middle) as four inoculation points per level using a drilled-hole method. Resin samples were collected after 3 and 6 months through tissue chiselling and later tree felling. Inoculation with 100 mL of *Trichoderma* sp., extract resulted in the longest resin formation (235.0 cm) after three months, indicating rapid resin development. In contrast 100 mL of *Lasiodiploidea* sp. (01), extract resulted in the highest resin length, at six months (180.0 cm), indicating slower, but robust development. Statistical analysis confirmed that both fungal extract type and inoculation volume influenced the induced resin formation length. Furthermore, resin length varied significantly between harvesting intervals of three and six months, with greater development observed at six months ($p=0.0023$). The relative resin formation area varied with fungal extract type, inoculation volume, and tree diameter. The extract of *Lasiodiploidea* sp. (01) showed the highest effectiveness at all tested volumes (26.81% at 25 mL, 32.63% at 50 mL, and 38.71% at 100 mL). Extract of *Lasiodiploidea* sp. (02), consistently yielded the highest oil content, with slight increases observed from three to six months for 25 mL (3.70% to 3.81%) and 50 mL (3.26% to 3.29%) treatments. Accordingly, the application of 25 mL and 50 mL of *Lasiodiploidea* sp. (01) and *Aspergillus* sp. is recommended to achieve higher resin yields in *A. crassna* within six months. Further, it is recommended to increase inoculation points from 6 to 8 for trees with diameter at breast height between 16 cm to 25 cm for optimal yield.

Keywords: Optimal dosage, Fungal extracts, Defense response, Yield, Protocol