

DESIGN, DEVELOPMENT AND PERFORMANCE EVALUATION OF PNEUMATIC TYPE AGARWOOD INOCULUM INJECTOR (PAII)

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ABSTRACT

Agarwood, a fragrant resin, is produced by certain tree species in the *Thymalaeaceae* family as a defense mechanism against stress. In commercial plantations, artificial inoculation of the fungal mix is used to induce the said stress, which is crucial for a fruitful harvest. The paste, containing viscous agar to be applied into drilled holes in the tree trunks and branches. Yet, an efficient tool to deliver this paste is lacking. Currently, inadequate industrial caulking guns are being used, proving time-consuming and ergonomically subpar design. To overcome these limitations, a Pneumatic type Agarwood Inoculum Injector was developed. A 6L capacity cast iron chamber was used as the core pneumatic body of the injector. An HCFE 3/8" non-return valve served as the air inlet. The delivered inoculum passed through a 1mm stainless steel strainer to prevent nozzle blockage. A pressure safety valve, set at 15 bars, released excess pressure. Inoculum compression relied on ambient air, filling up to 5L. A comparison of performance between the new device with the existing caulking gun was done for the inoculum filling rate, time taken to complete a single inoculation point, and the prevented volume of inoculum wastage. With the new device, the average filling volume of inoculum inside the drill holes was increased up to 85.35% and reduced the inoculum wastage from 1.325ml to 0.47ml per drill hole and both improvements were significantly different at the 5% probability level ($p < 0.05$). The results concluded that the developed device is capable of delivering a smooth flow of inoculum paste to the target when the impending pressure thrust range is within 5 to 15 bars. Moreover, within this pressure range, the total volume of 5L was delivered at a fairly consistent rate. The invented new device was found to be highly successful in assisting the critical process of inoculation in the commercial-level agarwood industry.

Keywords: Agarwood, Fungal Inoculum Paste, Inoculation, Oud, Pneumatic Injector

INTRODUCTION

Agarwood, a prized and opulent forest product, holds a position of unparalleled prestige in the realm of plant-based aromatics, commanding unparalleled market demand and value (Kanazawa, 2017; López-Sampson & Page, 2018). Its alluring fragrance arises as a result of a self-defense mechanism observed predominantly in the genera *Aquilaria* and *Gyrinops* within the *Thymalaeaceae* family. This captivating phenomenon, however, is not universal across all species, emphasizing the intricacies of agarwood formation and its association with tree species, environmental factors, and genetic variations (Ngadiran *et al.*, 2023).

The inception of agarwood formation is intrinsically linked to the stress and adversity that the trees endure. Natural occurrences like wildfires, grazing, and insect attacks inflict wounds upon the trees, setting in motion the self-defense response responsible for the production of this extraordinary resinous material (Liu *et al.*, 2019; Fitriyasi *et al.*, 2021). In the wild, only a modest fraction of *Aquilaria* trees around 10% undergo this transformative process, underscoring the rarity and unpredictability of natural agarwood formation (Chowdhury *et al.*, 2016).

Acknowledging the challenges posed by the erratic nature of natural formation and the pressing demand for agarwood, artificial inoculation methods have emerged as a pivotal means to stimulate resin production in a controlled and deliberate manner (Liu *et al.*, 2013).

In commercial-scale production, various stress-inducing methods are practiced to generate agarwood inside trees other than the natural inducing. Considering these artificial methods Nailing, Drilling, Aeration, Agar-wit, partially-trunk pruning, burning-chisel drilling, and fungal inoculation are common techniques among farmers (Liu *et al.*, 2013). Among these techniques, fungal inoculation has risen to prominence as a dominant method within the agarwood industry, offering a reliable and effective means of inducing resinous development (Chowdhury *et al.*, 2016).

The fungal inoculation technique, at the heart of this study, represents a sophisticated and targeted approach to agarwood induction. It harnesses the interaction between the tree's self-defense mechanism and fungi to provoke the resinous response (Turjaman *et al.*, 2016). This technique involves the introduction of beneficial fungal species into strategically drilled holes in the tree trunk (Rasool & Mohamed, 2016).

The fungi, thriving within these microenvironments, trigger the defense mechanism and promote the biochemical reactions inside the tree, culminating in the development of the coveted agarwood resin (Rohlf and Churchill, 2011).

The choice of fungal species and the intricacies of inoculation significantly influence the quality and yield of the resin produced (Zhang *et al.*, 2012). Researchers have explored variations in fungal strains, combinations, and culture conditions to optimize the process and maximize resin output (Justin *et al.*, 2020; Ma *et al.*, 2021). As such, fungal inoculation stands at the forefront of modern agarwood production, offering a nuanced and sophisticated means to replicate and enhance the resinous properties found in nature.

One of the common methods for practicing fungal inoculation is the agar-based fungal inoculum injected into 10mm (diameter) holes 4 cm (deep) and drilled holes in the tree trunk by caulking guns that are using in the construction field as a temporary basis.

The bores in the tree trunk will begin 50 cm above ground level. Holes should be 20 cm apart in the vertical distance, with about 2-3 holes in a horizontal line around the perimeter (Liu *et al.*, 2013). After the holes have been drilled, the inoculation can be carried out using the culture medium used for fungi growth. After inserting the culture into the hole, it should be wrapped in a rubberized fabric or clay (Chowdhury *et al.*, 2016).

The Agarwood industry is facing numerous challenges in the above-mentioned inoculation operation. The inoculation process should often be carried out staying either in a ladder or in a twigs often 10-20m above the ground. The inoculation paste should be with high viscosity and thicker enough to avoid the paste discharging back from the hole opening. Therefore, an efficient and effective device for performing this inoculation task is one of the priorities in the agarwood production industry.

As a solution, a Pneumatic type agarwood inoculum injector was developed and tested for its performance in contrary to the existing method to increase the productivity of a critically important inoculation process in the agarwood industry.

MATERIALS AND METHODS

Design and Construction of the Pneumatic Type Agarwood Inoculum Injector

The pneumatic type agarwood inoculum injector was designed and constructed with the primary goal of enhancing the efficiency of the inoculation process by injecting the inoculum paste into drill holes. The

device consists of two main components: a pressure-regulated pneumatic storing tank and an inoculum injecting gun, connected by a conveying hose.

A cast iron chamber with a 6 L capacity was chosen as the foundation for the pneumatic tank. The tank's design incorporated a standard HCFE 3/8 non-return valve as the air inlet. The outlet for delivering the inoculum was fitted with a 1mm stainless steel strainer to prevent nozzle blockage. The delivery line from the strainer filter was connected to the inoculum injector gun.

The inoculum-injecting gun, an integral part of the device, featured a fixed needle at its end. This needle possessed a diameter of 5mm and a length of 80mm. The pressure safety valve was an essential addition, designed to release excess pressure when it reached a limit of 15 bar. Compressed ambient air was utilized to compress the inoculum inside the tank, which was filled up to a loading capacity of 4.5L.

The overarching objectives of the device's design were to enhance the inoculum filling rate, improve labor efficiency, and minimize inoculum wastage.

Performance Evaluations

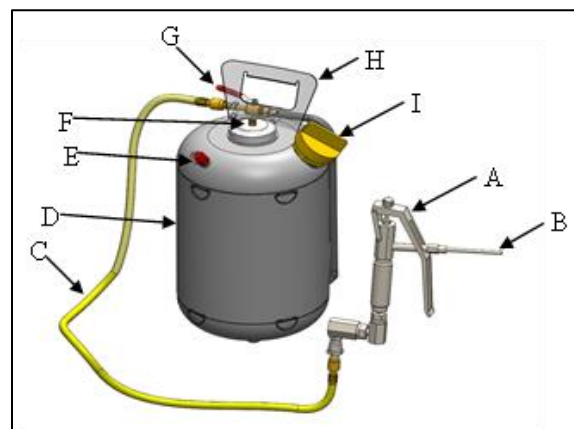


Figure 1: Design model of Pneumatic type Agarwood Inoculum Injector (PAII). (A) Inoculation gun, (B) Needle, (C) Rubber hose, (D) Storing tank, (E) Pressure relief valve, (F) Non-return air inlet valve, (G) Bowl valve, (H) Handle, (I) Airtight lid

Inoculum Paste Discharge Testing with Different Pressure Levels

A laboratory trial was conducted at the Department of Agricultural Engineering, Faculty of Agriculture, University of Ruhuna, Sri Lanka, to assess the discharge characteristics of the inoculum paste at various pressure levels using the pneumatic type agarwood inoculum injector. The tested pressure range spanned from 0.5 to 15 bar, and each pressure level was subjected to four replicate trials. The resulting average discharge values were recorded.

From the collected data, a relatively stable rate of inoculum discharge was identified, aiding in determining the optimal pressure range for the device's operation.



Figure 2: Discharge Testing with Different Pressure Levels

Figure 3: Practicing inoculation by PAII

Inoculum Paste Injecting Performance Evaluation

The performance evaluation of the inoculum injecting process was conducted within the maintained pressure range of the device (5-15 bars) in a commercial agarwood plantation located in Galle, Southern Province, Sri Lanka. The evaluation parameters included the time taken to complete a 10-drill hole operation in seconds, the average volume of inoculum injected per drill hole by collecting them back using a spatula, and the average volume of wasted inoculum due to overflow per drill hole by collecting them.

For the evaluation, twenty *Aquilaria crassna* plants were selected at the appropriate inoculation stage. Drill holes, created near breast height, were introduced using an electric hand drill equipped with a 10mm drill bit, maintaining a depth of 4cm in each hole. A total of 20 inoculation points were established per tree using the specified method. Ten of the selected plants were inoculated using the novel pneumatic type agarwood inoculum injector, while the remaining ten were inoculated using the conventional caulking gun method.

These measurements were then subjected to the paired t-test to determine if there were statistically significant differences in these parameters between the two inoculation methods.

This experimental setup aimed to provide comprehensive insights into the efficiency and effectiveness of the pneumatic type agarwood inoculum injector in comparison to the conventional caulking gun method.



Figure 4: Practicing inoculation process by conventional caulking gun

RESULTS AND DISCUSSION

Selection of optimum pressure range for the inoculum discharge

Laboratory trials were conducted to assess the impact of different pressure levels on the discharge of inoculum using the Pneumatic type agarwood inoculum injector. The objective was to identify the optimum pressure range for achieving consistent and efficient inoculum delivery. The findings offer valuable insights into the influence of pneumatic pressure on the discharge process and contribute to the refinement of the injector's operational parameters.

It was observed that there exists an optimal pressure range within which the inoculum delivery is both consistent and smooth. Specifically, this optimal pressure range was identified as lying between 5 to 15 bars. Within this range, the inoculum discharge exhibited a balanced and effective performance, ensuring reliable delivery without wastage or inefficiencies. When the pressure falls below this threshold, it indicates inadequate inoculum discharge due to insufficient force to elevate the highly viscous inoculum paste against the frictional resistance of the tubes and the inoculum itself.

At pressure levels below 5 bars, the inoculum discharge was characterized by a lower volume, which was insufficient to fully fill the delivery hose up to the discharge nozzle. This indicated a limitation in the effectiveness of the injection process at lower pressures. On the other hand, when the pneumatic pressure exceeded 15 bars, the discharge level increased, but an undesirable consequence emerged: excess inoculum paste was spilled away from the drill holes, leading to wastage. This highlights the importance of maintaining pressure within the optimal range to achieve optimal results.

The pressure discharge curve, constructed based on the experimental data, demonstrated a consistent upward trend. This positive correlation between pressure and average inoculum discharge reaffirms the direct influence of pressure on the injection process. The results indicate that as pressure increases within the optimal range, the average inoculum discharge also increases. For instance, the average inoculum discharge was measured at 839.5 ml/min at 5 bars pressure and improved to 983.5 ml/min at 15

bars pressure. This indicates that the injector maintains a fairly consistent and efficient inoculum delivery rate within this pressure range.

Importantly, the laboratory trials successfully demonstrated that a total volume of 5L could be delivered at a reliable and consistent rate within the identified optimal pressure range of 5 to 15 bars. This substantiates the suitability of this pressure range for practical applications of the device. Consequently, based on these results, it can be confidently concluded that the optimal working pressure range for the Pneumatic type agarwood inoculum injector lies within this range.

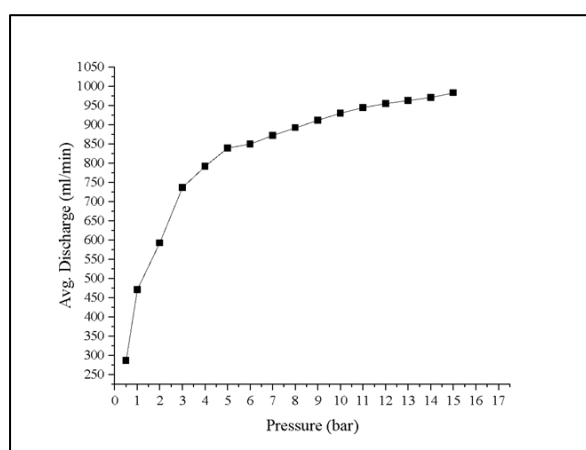


Figure 5: Average inoculum discharge for different pressure levels

Percentage of inoculum filling

The study focused on the comparison between a newly developed pneumatic type agarwood inoculum injector and the traditional caulking gun method for the inoculation of tree trunks. The objective was to assess the efficiency of the new injector in filling the drill holes with agarwood inoculum and to determine whether it outperforms the existing method in terms of filling volume. The results of this investigation provide insights into the efficacy of the new injector and its potential implications for agarwood cultivation.

In the conventional inoculation process, cylindrical holes with a diameter of 10mm and a length of 4cm were created in the tree trunk, resulting in an approximate internal volume of 3.14ml per hole. However, due to the presence of potential fibrous tissues within the hole (Idroes, 2019), it was hypothesized that the actual filling volume would range between 2.5ml and 3.00ml. The initial assessment revealed that the new pneumatic injector exhibited a substantial enhancement in terms of inoculum filling volume per drill hole compared to the traditional caulking gun method.

The data analysis demonstrated a noteworthy increase in the average inoculum filling volume per drill hole when using the new injector. Specifically, the average filling volume achieved with the new injector was determined to be 2.68ml per hole. In contrast, the average filling volume attained with the traditional caulking gun method was found to be 2.19ml per hole. This discrepancy in average filling volumes between the two methods is of significant importance.

The observed improvement in the filling volume of the drill holes with the new injector can be attributed to its pneumatic mechanism, which allows for a more controlled and consistent injection process. The enhanced precision of the new injector likely contributes to a more efficient use of the available space within the drill hole, resulting in a closer approximation to the total volume of the hole. Consequently, the new injector facilitates a filling process that is more aligned with the theoretical capacity of the drill hole (~3.14ml) compared to the caulking gun.

The statistical analysis conducted on the obtained results further strengthens the significance of the observed differences between the two methods. The paired T-test indicated a statistically significant difference at the 5% significance level ($p < 0.05$) between the new pneumatic injector and the traditional caulking gun method. This finding underscores the robustness of the evidence supporting the superiority of the new injector in achieving higher inoculum filling volumes per drill hole.

Labour and time saving

In the context of time efficiency, the performance evaluation demonstrated a significant improvement in the time taken to fill a set of 10 drill holes using the novel device as compared to the caulking gun. Specifically, the average time required to fill 10 drill holes using the novel method was 37.55 seconds, whereas the caulking gun exhibited an average time of 40.65 seconds for the same task. The statistical analysis, utilizing a paired T-test, substantiated these findings by revealing a significant difference at the 5% significance level ($p < 0.05$) between the two methods in terms of consumed time.

This reduction in time taken for the inoculation process when using the novel device can be attributed to its pneumatic mechanism, which offers enhanced precision and speed in the injection process. The consistent and controlled injection facilitated by the Pneumatic Type Agarwood Inoculum Injector likely contributes to the observed time savings. The shorter duration required for injecting a similar number of drill holes implies that the Pneumatic Type Agarwood Inoculum Injector can streamline the inoculation

operation, potentially leading to increased efficiency and productivity in agarwood cultivation practices.

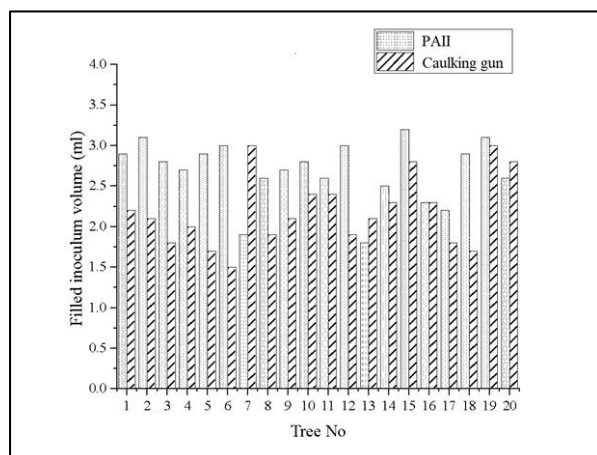


Figure 6: Average volume of inoculum filled in drill holes using PSAII and caulking gun



Figure 7: A segment of an inoculated *A. crassna* branch

Furthermore, the study also considered practical aspects of nozzle congestion and blocking during the inoculation process. The results indicated that neither the Pneumatic Type Agarwood Inoculum Injector nor the caulking gun experienced inoculum paste congestion or nozzle blocking when injecting into a total of 20 trees. However, in real-world field operations, the caulking gun exhibited a notable issue of nozzle blocking, occurring up to five to six times per day. This recurrent problem necessitates frequent nozzle cleaning and refilling time, contributing to increased operational downtime.

The Pneumatic Type Agarwood Inoculum Injector addresses this concern by incorporating a stainless-steel strainer, which effectively prevents nozzle blocking and congestion issues. This design feature enhances the operational reliability of the injector and contributes to its efficiency, particularly in scenarios involving prolonged or continuous use. The absence of a similar provision in the caulking gun's design

leaves it susceptible to nozzle-related challenges, further highlighting the advantages of the novel device in real-world applications.

Reduction of inoculum waste volume

The focus of this investigation was to evaluate the wastage of inoculum during the agarwood inoculation process using a Pneumatic type agarwood inoculum injector in comparison to the conventional caulking gun method. The study aimed to quantify and compare the amount of wasted inoculum due to overflow between the two methods, and to ascertain the potential benefits of the newly developed injector in terms of inoculum utilization efficiency and cost-effectiveness.

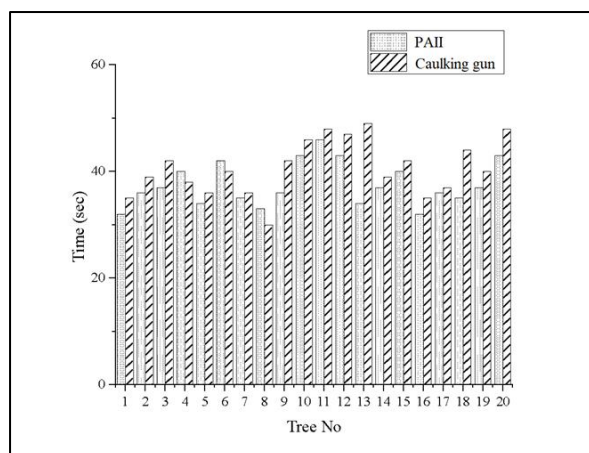
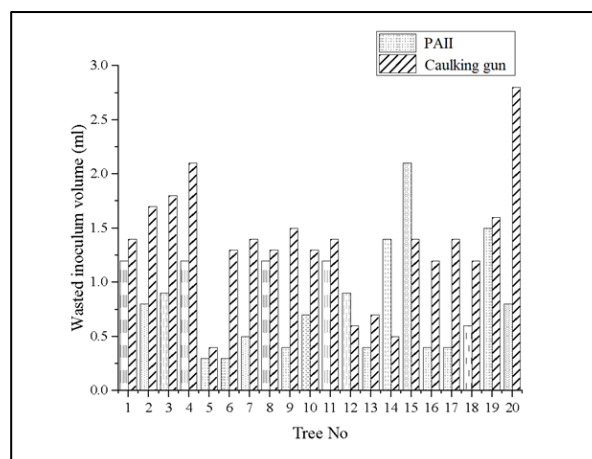
The analysis of wastage revealed noteworthy differences in the amount of inoculum overflow between the Pneumatic type agarwood inoculum injector and the caulking gun. The results indicate that the Pneumatic Type Agarwood Inoculum Injector exhibited a substantially lower average wasted inoculum volume of 0.86ml per drill hole due to overflow. In contrast, the caulking gun method was associated with an average wasted inoculum volume of 1.325ml per drill hole. This discrepancy in wasted inoculum volumes is of considerable significance.

The observed reduction in wasted inoculum volume when using the Pneumatic Type Agarwood Inoculum Injector can be attributed to its pneumatic mechanism, which facilitates a more controlled and precise injection process. The enhanced accuracy of the injector likely minimizes the occurrence of excessive overflow, resulting in more efficient utilization of the inoculum. As a result, It demonstrates a notable advantage, with approximately 0.47ml less wasted inoculum volume per drill hole compared to the caulking gun.

Furthermore, the statistical analysis using a paired T-test reaffirmed the significance of the differences in wasted inoculum volumes between the two methods. The test revealed a significant difference ($p < 0.05$) between the Pneumatic Type Agarwood Inoculum Injector and the caulking gun in terms of wasted inoculum volume due to overflow. This statistical evidence supports the contention that the Pneumatic Type Agarwood Inoculum Injector is more effective in terms of inoculum usage, resulting in reduced wastage and potential cost savings. Nevertheless, operators require some training in operating the new device, as mishandling may lead to the wastage of inoculum. In this experiment, we observed instances of such wastage, particularly in trees numbered 12, 14, and 15, where the new device resulted in greater wastage compared to the existing one.

Table 1: Pneumatic pressure Vs. Inoculum discharge

Pressure (bar)	Replicates				Avg. Discharge (ml/min)
	R1 (ml/min)	R2 (ml/min)	R3 (ml/min)	R4 (ml/min)	
0.5	270	256	260	360	286.5
1	470	469	471	474	471
2	592	590	593	596	592.75
3	739	737	735	734	736.25
4	791	788	795	792	791.5
5	839	837	840	842	839.5
6	848	852	847	854	850.25
7	874	876	868	870	872
8	896	892	894	889	892.75
9	916	912	909	910	911.75
10	932	933	928	927	930
11	951	945	942	940	944.5
12	957	954	952	956	954.75
13	964	967	959	962	963
14	972	974	968	970	971
15	982	988	979	985	983.5

**Figure 8:** Consumed time to fill a set of 10 drill holes using the PAII and Caulking gun**Figure 11:** Average wasted inoculum per drill hole by caulking gun and PAII**Figure 9:** External view after inoculation process by caulking gun**Figure 10:** External view after inoculation process by PAII

The implications of these findings are important for agarwood cultivation practices. Minimizing the wastage of inoculum is not only environmentally responsible but also economically advantageous. The reduced wastage associated with the Pneumatic Type Agarwood Inoculum Injector can contribute to cost savings and improved resource utilization, making the inoculation process more economically viable. Moreover, the more efficient inoculum utilization provided by the Pneumatic Type Agarwood Inoculum Injector could potentially lead to better results in terms of inoculation success rates and agarwood production.

CONCLUSIONS

The invented new inoculum injector Pneumatic Type Agarwood Inoculum Injector was successfully operated under the internal pressure range between 5 to 15 bars while achieving smooth and continuous

flow of the inoculum delivery. The performance evaluation has concluded the capabilities of the novel approach with a significant increment of inoculum filling volume and at the same time significantly reducing the wasted inoculum volume and time taken to complete single-hole inoculation. The inoculum filling performance of the Pneumatic Type Agarwood Inoculum Injector was with significantly ($p < 0.05$) high rate (2.68ml per drill hole) to the rate of the caulking gun method (2.19ml per drill hole) which the previous one is closer to the theoretical drill hole capacity of ~3.14ml. The time taken to complete single hole inoculation of the Pneumatic Type Agarwood Inoculum Injector device was with significantly ($p < 0.05$) low (3.75s/ drill hole) to the time taken to complete it by the caulking gun method (4.06s/per drill hole) highlighting the inoculum delivery efficiency and the possibility of speeding up the inoculation process. The Pneumatic Type Agarwood Inoculum Injector was capable of maintaining a significant reduction of average wasted inoculum volume of (0.86ml per drill hole) to the caulking gun (1.325ml per drill hole). The wastage reduction was a critical consideration in designing the Pneumatic Type Agarwood Inoculum Injector to ensure maximum resource utilization. Moreover, the new device was having with five times more capacity than the existing approach ensuring the reduction of refilling time. Weightless backpack-type design and freehand agronomy were among the other advantages of the device. As such, the newly invented device demonstrated excellent capabilities in fulfilling the task associated with the inoculation process in commercial level Agarwood plantations as a practically viable solution to bring the inoculation process to a new level of business instead of inefficient and ineffective existing caulking gun-based operation environment.

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REFERENCES

- Chowdhury, M., Hussain, M.D., Chung, S.O., Kabir, E. and Rahman, A. 2016. Agarwood manufacturing: a multidisciplinary opportunity for economy of Bangladesh-a review. *Agricultural Engineering International: CIGR Journal*, 18(3), pp.171-178.
- Fitriasari, P.D., Soetarto, E. and Surata, I.K. 2022. Induction of Agarwood Formation by Lignocellulolytic Bacteria. *International Conference on Engineering, Technology and Social Science (ICONETOS 2020)*, pp. 345-351.
- Idroes, R. 2019. May. Isolation and identification of Endophytic Fungus *Fusarium* sp from Agarwood (*Aquilaria* sp) population originated from the forest of Aceh Tamiang district, Indonesia. In *IOP Conference Series: Materials Science and Engineering* 523(1) pp. 012013. IOP Publishing.
- Justin, S., Lihan, S., Elvis-Sulang, M.R. and Chiew, T.S. 2020. Formulated microbial consortium as inoculant for agarwood induction. *Journal of Tropical Forest Science*, 32(2), pp.161-169.
- Kanazawa, K. 2017. Sustainable harvesting and conservation of agarwood: A case study from the Upper Baram River in Sarawak, Malaysia. *Tropics*, 25(4), pp.139-146.
- Liu, Y., Chen, H., Yang, Y., Zhang, Z., Wei, J., Meng, H., Chen, W., Feng, J., Gan, B., Chen, X. and Gao, Z. 2013. Whole-tree agarwood-inducing technique: an efficient novel technique for producing high-quality agarwood in cultivated *Aquilaria sinensis* trees. *Molecules*, 18(3), pp.3086-3106.
- López-Sampson, A. and Page, T. 2018. History of use and trade of agarwood. *Economic botany*, 72(1), pp.107-129.
- Ma, S., Fu, Y., Li, Y., Wei, P. and Liu, Z. 2021. The formation and quality evaluation of agarwood induced by the fungi in *Aquilaria sinensis*. *Industrial Crops and Products*, 173, p.114129.
- Ngadiran, S., Baba, S., Nor, N.F.A., Yahayu, M., Muhamad, M.F., Kushairi, A.K.A., Zaidel, D.N.A. and Muhamad, I.I. 2023. The induction techniques of resinous agarwood formation: A review. *Bioresource Technology Reports*, p.101337.
- Rohlf, M. and Churchill, A.C. 2011. Fungal secondary metabolites as modulators of interactions with insects and other arthropods. *Fungal Genetics and Biology*, 48(1), pp.23-34.
- Roslee, M.N., Mohd Muji, S.Z., Talip, B.A. 2018. An automatic transfusion set for accelerating inoculation process of agarwood artificial inducer. *International Journal of Engineering & Technology* 7 (4.30), pp. 397–402.
- Turjaman, M., Hidayat, A. and Santoso, E. 2016. Development of agarwood induction technology using endophytic fungi. *Agarwood: science behind the fragrance*, pp.57-71.
- Zhang, X.L., Liu, Y.Y., Wei, J.H., Yang, Y., Zhang, Z., Huang, J.Q., Chen, H.Q. and Liu, Y.J. 2012. Production of high-quality agarwood in *Aquilaria sinensis* trees via whole-tree agarwood-induction technology. *Chinese Chemical Letters*, 23(6), pp.727-730.