

PROJECT REPORT ON INOCULATION OF AGARWOOD



**Submitted to
West Bengal Forest and Biodiversity Conservation
Society**

**By
The Research Wing
Directorate of Forests
Government of West Bengal**



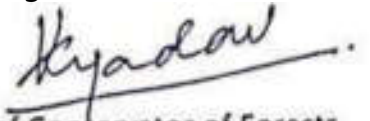
PREFACE

Aquilaria malaccensis Lamk. (Locally known as agar or Sasi) is a precious floral wealth of North-East India and has been identified as a potential aromatic plant of this region. It is a natural component of the evergreen and semi evergreen forest flora of the North East and the tree yields the most expensive wood of the world. Agar is a scented product, oleoresin, obtained from pathological conditions of the wood of live trees containing many aromatic substances. Various bacteria and fungi have been found to be associated with Agarwood formation, although it is still not absolutely clear which are important or even necessary.

Considering the economic significance of the Agarwood, an initiative had been taken during 2009 to 2011 to introduce the plant in North Bengal and introductory trial has been made. In the north eastern states the Agarwood tree got naturally infected with bacteria and fungus and formed Agar subsequently which is not found in the experimental plantations in the North Bengal. Both physical and chemical stresses like mechanical wound and induction have long been practiced to enhance Agarwood yield as well as fungal inoculation. The idea of inoculation of Agarwood Trees conceived by the Research wing to have a future in Agarwood production and develop a model for both forestry and social and farm forestry that would boost economy of fringe population through conservation and benefit sharing mode.

The project was proposed to the West Bengal Forest Biodiversity and Conservation project (WBFBCP) in the year 2020 for inoculation of fungal inoculums to the trial plantations raised during 2009-11 at Rajabhatkhawa and Lataguri. The Rain Forest Research Institute of the Indian Council of Forestry Research and Education, Jorhat (RFRI) had been involved with the Project.

Trial was conducted and out of a total of 976 no. of trees, 155 no. of trees have been treated with inoculums (fungal artificial inoculation). This is an initial attempt to inoculate Agarwood trees in North Bengal. The Research Circle has incorporated the methodology as per objective of the project. However, it is a continuous exercise and need to conduct more such experimental trial for long term management.



Principal Chief Conservator of Forests
Research, Monitoring and Development Wing

ACKNOWLEDGMENT

Agarwood introductory trial started in North Bengal during 2009-10 and since then the trial plots were maintained for natural infection of fungus. As the trees were not showing any symptom, this inoculation project was conceived and a pilot scheme was implemented. Project formulation and its implementation the Officers, field executives and ministerial staffs of the wing contributed to the best of their capabilities and dedication.

Firstly we thank to The Principal Chief Conservator of Forests (Research, Monitoring & Development) Dr. Jose T. Mathew, IFS who first gave the proposal for doing such type of Research Work. Thanks to the Chief Project Director of WBFBCP, Sri Debal Roy, IFS who approved the project. Thanks to The Principal Chief Conservator of Forests, RMD, Shri V.K. Yadav, IFS for his kind support and permission to compile the research finding and compilation of the report. Thanks to the Additional PCCF, R&M, Smt Pratibha Raj, IFS for going through the project document and getting it approved in the Committee for Scrutiny and Approval of the final project report. Thanks to the Chief Conservator of Forests, R&D Shri D. Mallick, IFS and Dr. S. C Das, IFS, CCF, Soil Conservation for guidance and support to compile the findings of the experimental work. Thanks to Shri B. Sarkar, IFS, Conservator of Forest, Research Circle, WB for guidance to the field work and data collection.

I extend my sincere thanks to Dr. R. K. Bora and his associates of the Rain Forest Research Institute of the Indian Council of Forestry Research and Education, Jorhat (RFRI) who played immense role to conduct and to complete the trial Project.

Sri T. T Bhutia, IFS successfully guided the experimental part and data collection at North Bengal which is acknowledged. Sri B. Talukdar, WBFS, ADFO of this Division involved himself for supervising the work. Last but not the least Sri Sanjoy Chaki, FR.

Range Officer, Buxa-Coochbehar Research Range and Sri Manideep Mothay, FR, Range Officer of Jalpaiguri Research Range along with their team are to be acknowledged for carrying out this entire research work up to this level.



Divisional Forest Officer
Silviculture North Division

PROJECT REPORT ON INOCULATION OF AGARWOOD (*Aquilaria malaccensis*)

1. Introduction:

***Aquilaria malaccensis* Lam. Synonym *Aquilaria agallocha* Roxb.**, commonly known as agar, is distributed in all the North-eastern states. It is a natural component of the evergreen & semi-evergreen forest flora of the N-E. However, it is now rarely seen naturally because of over harvesting and indiscriminate felling. Agar tree prefers a subtropical climate with high humidity and rainfall in the range of 1800-3500 mm. It requires reasonable amount of sunshine and well-drained soil but will grow in a situation with other species having longer rotation. Apart from India the species is distributed also in China, Indonesia, Malaysia, Thailand & Vietnam. The species is included in CITES Appendix-II as critically Endangered (IUCN, 2009). Agarwood oil is the resinous substance in the heartwood of the tree which develops against a certain fungal infestation. It is a highly valuable product for medicine & fragrance Industry. Agar tree symbolizes the religious & cultural aroma of the North-eastern region and has become a substrate for the economic prosperity drive in the North-Eastern region of India.

Agarwood develops from the resinification of fermented *Aquilaria* timber over a pathological process of infection by a fungus in the trunk or main branches. A specific fungus (identified as *Phialophora parasitica*) is responsible for it. Fungal species *Fusarium*, *Rhizophora*, *Aspergillus*, *Mucor* were also isolated from the infected host tissue. Natural infection occurs in the wood when trunks of standing trees are bored by the larvae of a stem borer, mainly *Zeuzera conferta*. The Fungus enters the plant through the vertical and zigzag tunnels made by the borer inside the stem. Over a period of time, large volume of wood gets infected with gradual accumulation of the agar oleoresin giving its special odour. Infection may also occur due to mechanical or natural injuries on the agar tree, but are found limited to the localized portions only.

Agarwood is traded in several forms, from being large sections of trunk to finished products such as incense & perfumes. The major constituents of Agarwood oil are sesqui-terpenes. Agarwood oil is the most exalted perfumery raw materials and is known in the east as *agar-attar* imparting a lasting balsamic odour to the product & is much priced in international market for mixing it with quality perfumes. Agar oil is an export oriented product & the demand is very high in the international market especially the Middle East.

2. Objective of the Project:-

- I. To inoculate the standing more than hundred trees of *Aquilaria malaccensis* for development of Agarwood essential oil on pilot basis.
- II. Evaluation of the success rate so as to extend it to other trees of the said species in future.
- III. Training of staff and skill development.

3. Purpose of the Project:-

Considering the economic significance of the Agarwood, an initiative has been conceived by the Research wing to have a future repository of gene pool of Agarwood tree which in turn will boost economy of fringe population through conservation and benefit sharing mode.

4. Proposal of the Project :-

The project was proposed to the West Bengal Forest and Biodiversity Conservation Society in the year 2020. As the objective of the West Bengal Forest and Biodiversity Conservation Project is to improve forest ecosystem and conserve biodiversity by undertaking afforestation, regeneration and wildlife management activities through Forest Management approach, including institutional capacity development, thereby contributing to environmental conservation and harmonized

socio-economic development of West Bengal, this project proposal was initiated by the Research Wing.

5. Funding Agency:-

West Bengal Forest and Biodiversity Conservation Project is the funding agency of this Project. The plantations where this project is to be conducted have been done at Buxa-Coochbehar Research Range and Jalpaiguri Research Range under Silviculture (North) Division, over 1 Ha.each in the year 2009 and 2011 respectively.

6. Financial outlay of the Project:-

Sl. No.	Component	Rate	Amount (Rs.)
A	Payment to ICFRE being the Consultancy related expenditure as provided by RFRI, Jorhat (incl. 18% GST)		
	1. Cost of man days of official deployed (1 Scientist and Research fellow for 06 days)		30,000
	2. Cost of physical inputs /services/utilities/consumables/raw materials		50,000
	3. Travel Expenses (TA/DA)/POL		23,550
	4. Contingencies		10,000
	5. Total expenses (1 to 4)		1,13,550
	6. Intellectual Fees		30,000
	7. Total expenses + Intellectual Fee		1,43,550
	8. GST (18%)		25,839
	9. Total		1,69,389
B	Departmental Expenditures		
	1.Training to Officers and Staff by the expert on artificial induction at Salugarah	2 days (lump sum)	30000
B.	2. Visit to RFRI and site-Travel fare, vehicle etc. for officers, experts and selected field staff.	Lump sum	50000
	3.Cost for mandays for inoculation of trees through drilling, maintenance,etc.	Lump Sum	30000
	4.Cleaning of the plantation site	Lump Sum	5000
	5. Sum of 1, 2, 3, 4.		115000
	6.Contingency@5%		5750
	7. Total		120750
	Grand Total (A+B)		290139

Details of fund release and balance

Works	Allotment	Received	Expenditure	Balance	Balance of total allotment
Payment to ICFRE being the consultancy related expenditure as provided by RFRI, Jorhat(incl.18% GST)	169389	169389	169389	0	0
Training to officers & staff by the expert on artificial inoculation of 100 Aquilaria trees at Salugarah	30000	30000	30000	0	0
Visit to RFRI and site travel, vehicle etc. for officers, expert and selected field staff	50000	50000	35000	15000	15000
Cost of mandays for inoculation of trees through drilling& maintenance etc.	30000	30000	30000	0	0
Cleaning of the plantation site	5000	5000	5000	0	0
Contingency @ 5%	5750	5750	0	5750	5750
Total	290139	290139	269389	20750	20750

7. Involvement of the Institutional Organization :-

Rain Forest Research Institute of the Indian Council of Forestry Research and Education, Jorhat is involved with the project along with following conditions as described in Memorandum of Understanding between the Directorate of Forests, Government of West Bengal and the Rain Forest Research Institute, Jorhat for carrying out artificial inoculation of Agarwood.

- i. RFRI shall take up the artificial inoculation of agarwood in 100 trees in the northern part of West Bengal at the sites identified by the West Bengal Forest Department.
- ii. WBFD shall pay Rs 1,69,389/- (Rupees One lakh sixty nine thousand three hundred and eighty nine only) including GST for this purpose to RFRI.
- iii. RFRI will provide training to the staff and WBFD will arrange and depute them to the venue at its own expense.

- iv. WBFD shall arrange the necessary logistics and man power to carry out the drilling of trees.
- v. RFRI shall arrange the inoculums and training material for carrying out the inoculation as well as conduct the training of staff of WBFD in the process.
- vi. WBFD shall provide accommodation in the forest rest houses at the sites of inoculation, if available, to the Scientists and staff of RFRI on payment.
- vii. RFRI shall complete the work within 90 days from the date of receipt of payment from WBFD.
- viii. All expenditure will be incurred in accordance with General Financial Rules.
- ix. In case of any dispute the matter will be resolved with mutual consultation by Principal Chief Conservator of Forest, Research, Monitoring and Development, Directorate of Forests and Director, Rain Forest Research Institute, Jorhat.
- x. Force Majeure: Non-compliance by a party with this Memorandum of Understanding due to any cause beyond the reasonable control of the party, such as civil commotion, disturbing law and order situation, flood and other natural calamities etc. shall not constitute a breach of this Memorandum of Understanding.

8. Agarwood plantation pattern:-

The plantations were created in the year 2009 and 2011 by Jalpaiguri Research Range and Buxa-Coochbehar Research Range under Silviculture (North) Division respectively in the following locations:-

Sl. No	Plantation Year	Location	Area
1	2009	LT-1 compartment of Lataguri Range under Jalpaiguri Division.	1.00 Ha.
2	2011	SRVK-10 compartment of West Rajabhatkhawa Range under Buxa Tiger Reserve (West) Division.	1.00 Ha.

At Jalpaiguri Research Range the plantation was created with the spacing of 2.5 mt. x 2.5 mt. (Initially 1600 No. of Agarwood saplings were transplanted, currently 667 No. of trees are existing). Presently the plantation is having an average DBH in the range of 40 cm to 65 cm and average height range of 8.00 mt to 10.00 mt.

Whereas the Buxa-Coochbehar Research Range followed the plantation spacing of 3.00mt.x 3.00mt. (initially 1111 No. of Agarwood saplings were transplanted, currently 309 No. of trees are existing). Presently the plantation is having a DBH range of 45 cm to 50 cm and average height range of 6.00 mt. to 8.00 mt.

9. Methodology Applied:-

Agarwood is formed after natural and artificial injury and trees produce oleoresin to prevent or recover the injury in response to plant defense mechanism (Zhang et al., [2012](#)). Naturally the wound is produced by microbial invasion, gnawing of insects, lightning strikes and heavy winds (Xu et al., [2013](#); Zhang et al., [2014](#)). As a defense response to these outbreaks, normal heartwood converts into dark Agarwood. Firstly, Bhattacharyya in 1952 reported role of endophytic fungi in inducing Agarwood production in the tree trunk. Later many scientists isolated several endophytes from the Agarwood tree (Chhipa et al., [2017](#)).

The natural development of Agarwood takes 25–30 years and the yield and probability of occurrence is less, thus unable to meet the demand of the growing market. The increasing commercial demand has led to development of artificial infection methods. Rain Forest Research Institute, Jorhat, Assam has been carrying out research on Agarwood for a decade and developed the technology of artificial inoculation of Agarwood using fungal culture. RFRI has also successfully implemented the technology in farmer fields in Assam, Manipur and Meghalaya. Inoculation will be carried out on the trees of 7-9 years of age (having dbh of 35-40 cm). At least 10-12 holes will be made on these trees at a distance of 1 foot from each other in a circular

fashion with the help of drilling machine and a standard methodology will be followed for fungal artificial inoculation. Since infecting fungus is not naturally available in North Bengal, it needs to be artificially inoculated from sources in Assam. During 2020, artificial inoculation of fungus was made on sample plots in Lataguri and Rajabhatkhawa with the technical support of RFRI, Jorhat, by artificially boring holes on the standing trees. After injection, it is closed with wet cotton and again after fifteen days, jaggery solution is applied on the wound. It takes about two years for maximum infection by the fungus. Infection is discernible with the gradual yellowing of leaves followed by leaf shedding and infected area gets darkened.

10. Kind of treatments applied:-

A. For Jalpaiguri Research Range:

The plantation was inoculated with fungal inoculums on 06th November 2020, 08th November 2020 & 10th November 2020. Out of existing 667 No. of trees 105 No. of trees have been treated with inoculums. The inoculation was done by Dr. Bora and Party from Assam with technical support from RFRI, Jorhat. There were four types of treatments namely T1, T2, T3 & T4. Out of the four treatments T1 is control treatment without any fungal inoculums whereas T2, T3 & T4 were with fungal inoculums, later followed by introduction of molasses for two times (however the composition of fungal inoculums is unknown since the team did not disclose the type of fungi used as inoculums for different treatments). So far the plantation has been treated only once as mentioned above. The number of trees treated with different inoculums is shown below:-

- i. T1 (Control): 02 No.
- ii. T2: 49 No.
- iii. T3: 36 No.
- iv. T4: 18 No.

B. For Buxa-Coochbehar Research Range:

The plantation was inoculated with fungal inoculums on 07th November 2020 & 08th November 2020. Out of existing 309 No. of trees 50 No. of trees have been treated with inoculums. The inoculation was done by Dr. Bora and Party from Assam with technical support from RFRI, Jorhat. There were four types of treatments namely T1, T2, T3 & T4. Out of the four treatments T1 is control treatment without any fungal inoculums whereas T2, T3 & T4 were with fungal inoculums, later followed by introduction of molasses for two times (however the composition of fungal inoculums is unknown since the team did not disclose the type of fungi used as inoculums for different treatments). So far the plantation has been treated only once as mentioned above. The number of trees treated with different inoculums is shown below:-

- i. T1 (Control): 02 No.
- ii. T2 : 16 No
- iii. T3: 16 No.
- iv. T4: 18 No.

11.Observations:-

- i. No treated tree has been found dead till August 2021.
- ii. No treated tree has been found drying up (i.e. all the treated trees are standing green) till August, 2021.
- iii. No treated tree has symptoms like yellowing of leaves or shedding of leaves till August, 2021.
- iv. Prominent black patches have appeared around the drilled holes so made for the purpose of inoculation.

12. Conclusion –

1. Every year at fixed time (November) the treated trees are to be observed and changes noted down.
2. This observation should be continued till fifth year i.e till November, 2025.

Images of treated Agarwood trees under Buxa-Coochbehar Research Range.



Images of treated Agarwood trees under Buxa-Coochbehar Research Range.



Images of treated Agarwood trees under Jalpaiguri Research Range.



Images of treated Agarwood trees under Jalpaiguri Research Range.



Images of treated Agarwood trees under Buxa-Coochbehar Research Range at Rajabhatkhawa.



After six months of inoculation infection is spreading

