

vulnerable species due to its over exploitation from natural vegetation. IUCN (2014) Red List listed this species as vulnerable based on the decrease of at least 20% over three generations because of over exploitations that lead to the declined in the population of natural vegetations⁵. So, the knowledge of agarwood plant regeneration, propagation, cultivation, and management biology is extremely desired for the rising protocols for developing significant plantations⁶. The first step for increasing the supplies of agarwood is to boost the accessibility of *Aquilaria* plant to meet the demand. Successful seed storage of *Aquilaria* is difficult because it bears recalcitrant seeds which are cold as well as drying sensitive and lose viability rapidly once exposed to the outside environment. Agar plants are important when plants are infected naturally by the insect *Zeuzera conferat* Walker which leads to the development of resins in plant stems. When resins are developed in the plant stems, it becomes black in color called as black wood or infected wood which has a high demand in the market. Until and unless infections have not occurred wood remains white in color. In some places, where a natural infection does not occur, people go for artificial infection/wounding such as scratching on the stem etc., *i.e.*, by giving external stress; to overcome which, the stressed plants develop some secondary metabolites called as stress aroma resulting in good fragrance, and thus great demand. Thus, our present study is primarily focused on the standardization of packages and practices of agarwood cultivation in Assam. Agarwood is

propagated through seeds due to its high market demand. For the prorogations of agarwood cultivation in Assam, development of good quality nursery practices, producing good quality seedlings, and management of agarwood farm is utmost important.

Materials and Methods

Study site

A comprehensive survey of agarwood plant was conducted during 2019-2021. The survey sites were in different places of Golaghat district of Assam, as shown in the Map, Fig. 1 (26 °41'60.00"N to 93 58'12.00"E; 26° 31'00.1" N to 93 ° 58'00.1" E; 26 ° 35'57.48"N to 94 °1'28.2" E) India. The study sites were selected where large-scale agar plantations were found in each location. 25 number of selected household were visited for survey and data collection. Some people used to cultivate purely *Aquilaria* and some other people preferred mixed cultivation *i.e.* along with agar they cultivated other plants such as tea, areca nut, banana, etc. Standardization of agarwood cultivation was done by interacting with the local growers and was also authenticated through field visits at different areas of the Golaghat district of Assam. Although it is a perennial plant, complete study of its life cycle is not possible within a short duration as plants take almost 8-15 years for maturation. So, data was collected from the traditional practices used by the agarwood growers. Data was also collected by some secondary resources like review of literature, electronic and nonelectronic media.

Optimization of appropriate climatic conditions and land required for cultivation of *Aquilaria*

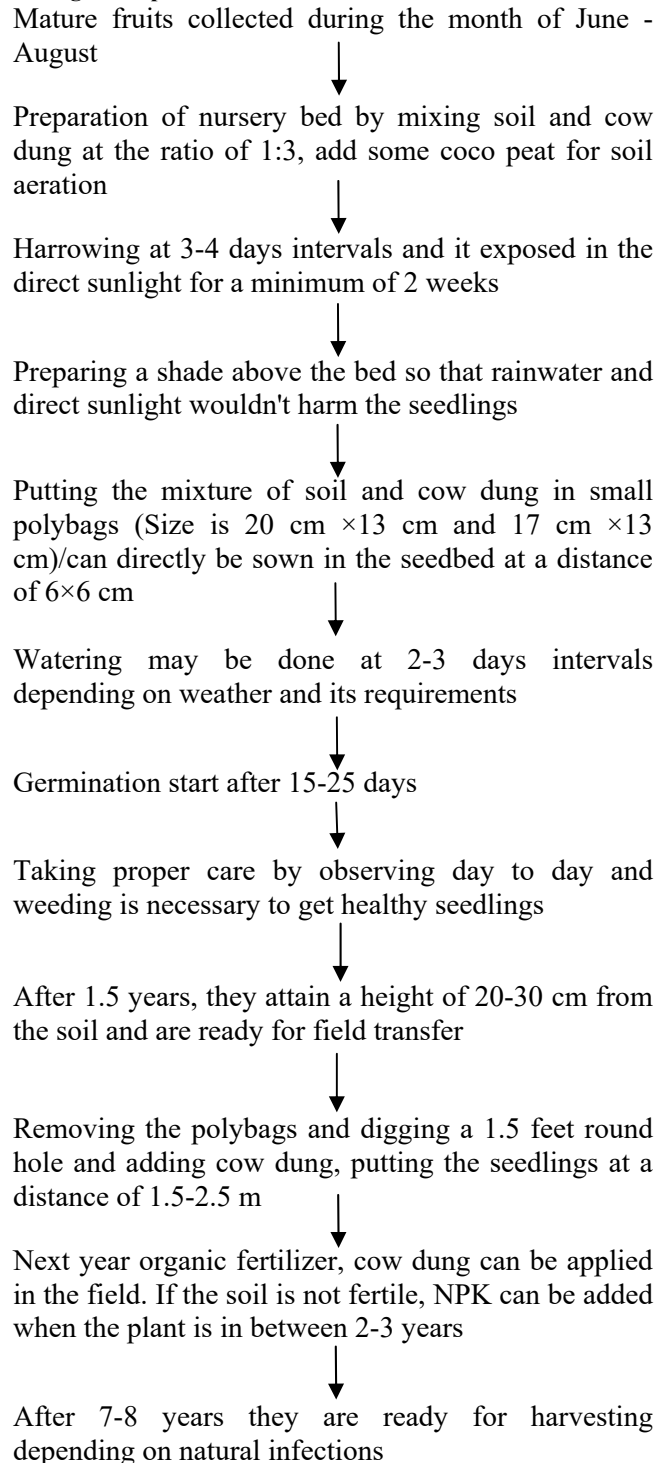
The Agarwood plant cultivation prefers high humid conditions. These are grown well in sub-tropical climatic conditions and require average annual rainfall in the range 1800-3500 mm⁷. Although it grows well above the sea level up to 1000 m altitudes, it is also a sun-loving plant and requires a lot of sunshine for its proper growth and development. It prefers well-drained deep sandy loam which is rich in organic matter but can also profitably be grown in marginal soils and also in shallow soils over rocky beds with cracks and crevices. They prefer to grow well in hill slopes as water logging conditions are not recommended for their cultivation. They prefer to grow in the acidic soil conditions, with the average temperature range from 20°C to 33°C required for their proper development⁷. Although *Aquilaria* trees can be grown well in different forests and ecosystems but the environmental conditions such as characteristics and fertility of the soil influence their growth and development. The required average temperature for growing the sapling ranges from 20-33°C, required relative humidity ranges from 77-85% and required light intensity is between 56-75%⁷. Soil is one of the components which influence the growth of agar plants and affect initiation of natural infections. Some previous experiments reveal that there is an interrelation between the natural infections of agar trees with different soil parameters. Natural infection was primarily found in soils with pH range in between 4.3-5.2, and soil texture also plays an important role for natural infections. Finer the soil texture primarily clay loam to loam and medium to high soil organic carbon content, favorable is the conditions for natural infections. Soil organic carbon was recorded medium range in both Nahoroni in Golaghat district and Namti in Sivsagar District soils and it showed a significant difference in natural infections⁸.

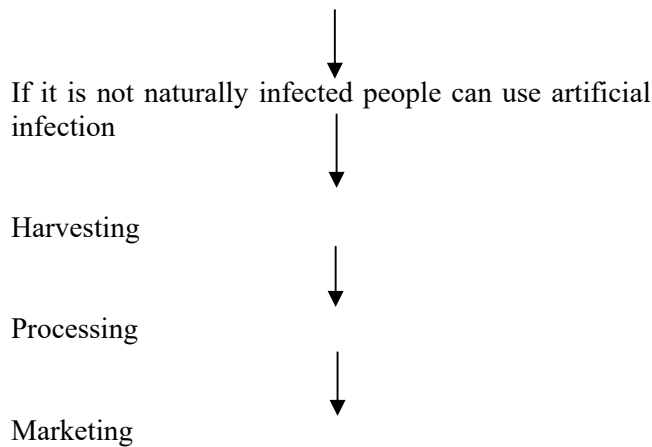
Aquilaria plant propagation

Aquilaria is propagated through seeds. So the collection of good quality seeds and their propagation is of utmost concern. The species can also be propagated through cuttings and tissue culture methods⁹. However, it is a costly and laborious process for common people. Traditionally people follow the seed germination procedure which is easy and cost-effective. Preparing good quality of seedlings and their maintenance is important for the

development of good quality agar. Mature fruits were collected from the plant and seeds were taken out by splitting the fruit. Seeds were sown immediately after collection as they lost their viability within a short time.

Flow diagram of growing *Aquilaria* seedlings and their management practice





Seed collection

Aquilaria starts flowering in the month of March-April, starts producing seeds in the month of April-June, and fruits mature in the months of July-August. In the months of July/Aug fruits are ready for collection. Fruits should be collected from mature *Aquilaria* trees. When fruits are mature they burst and seeds come out. The maturity of the capsule can be judged by observing the ease of splitting of the capsule by applying pressure between two fingers. It was recorded that 70.5% of fruits contain two seeds and the rest are one-seeded¹⁰. Fruits should be dried under shade for two days before the extraction of seeds. When seeds are transported to long distances, well-ventilated cotton cloth bag should be used. One kg of fruit yield almost 1300 seeds. Fruit length of one and two-seeded fruits are 26.82 mm and 30.30 mm, and breadth is 17.60 mm and 19.09 mm, respectively. Seed length, breadth, and weight also vary in one and two-seeded fruits. Single seed obtained from a fruit is smaller with 15.26 mm in length, 5.34 mm in breadth and 0.113 g in weight, while in case of two seeds per fruit, the length of seed is 16.15 mm, breadth is 5.53 mm and weight is 0.112 g. Length of one and two seeded seed was found 15.26 mm to 16.15 mm, breadth is 5.34 mm to 5.53 mm and seed weight is 0.113 g to 0.112 g Individual seed weight ranges from 29 to 135 mg, and seed weight has a strong effect on germination. The shelf life of the seed is only 5-14 days¹¹. On a fresh weight basis, the average seed moisture content was found to be 56.3%. Some researchers reported that the heavy seeds had a higher germination rate in comparison to the light seeds. In many tropical species, superior and earlier germination found in heavier seeds^{12,13}.

Sometimes, ambiguous outcome have also been reported in many other species and germination might be not dependent on the seed weight¹⁴. Therefore, sowing of heavy seeds of over 80 mg fresh weight is recommended¹⁵. In another study, physical viability test was done by its weight and floating technique and it was found that germination percentage increases with increase in seed weight; heavier seeds also need less time for germination likewise seedling growth parameters are also found better with heavy seeds as compared to those seeds which floated and considered as nonviable. Germination started after 7 days of sowing and continued up to 45 days¹⁰.

Preparation of seedbed and its handling

Aquilaria seeds are recalcitrant, and rapidly lose viability below 20% moisture content. The seed cannot be stored for a longer period as its endosperm and embryo dry up and become nonviable and should be sown within 2-5 days after collection. Storing in the refrigerator at (4°C) may prolong the viability up to 20-25 days. The seeds do not require any pre-treatment. As mentioned earlier, seeds are highly recalcitrant and are highly sensitive to the moisture and drought. Seeds beds are prepared by mixing the soil, dry cow dung, and coco peat. Coco peat helps in proper oxygenations and aerations. Seeds are shown in 6×6 cm distance from each, otherwise, can be directly placed into the polybags (as per choice). Seeds placed at top layers of the soil should be slightly pressed into the soil. Seeds are propagated in the monsoon season to avoid excess water. The seedbed is prepared under a shade to obtain more effective result. After 10-15 days, seeds start to germinate with emergence of plumule (shoot) and radicle (root).

Plantations procedure

After 1-1.5 years seedlings attain a height of 20-25 cm and are considered ready for plantation. The plantation is done by the following procedure:

- By digging 1.5 feet round hole under the ground and applying organic manure up to the level of 0.5 feet and planting the seedlings.
- Seedlings are transplanted before attaining the height of 60-100 cm, after that root coiling is found in the polybags, and the survival rate decreases. So older seedlings are not advisable for plantation.

- The standard spacing of the planting of seedlings is 1.5-2.5 m.
- To avoid water logging conditions, plantation should be done in incline areas; otherwise, proper drainage system must be maintained.
- They are the fast-growing plants and within 5-6 years can achieve 10 cm Diameter at breast height (DBH).
- They require adequate moisture and are also sun-loving trees.
- Preferable seasons for plantations are in April-June.

Manure and fertilizer

Organic manures are a good source of plant nutrients that are obtained from the plant and animal wastes. Plant and animals release their nutrients after decomposition and it is mixed with the soil and again plants absorb those nutrients for their growth and development. Collecting, processing and using wastes from different sources like animals, human, and plants for increasing crop production is an old process in agricultural system. Manures that are derived from animal, human, and plant residues called as organic manure which contains plant nutrients in complex forms. Major sources are:

1. Cattle shed wastes, dung, urine, etc.
2. Agricultural wastes product such as rice, sugarcane trash, stubbles, and other related material
3. Water hyacinth, different types of weeds etc.

Plant protection

Agarwood plants are attacked by leaf pests known as *Heortia vitessoides* Moore during summer season (June-August). *H. vitessoides* Moore cause damages on the agar plant leaves and tender stalk. For removing pests, people commonly use some biopesticides. Chemical pesticides are harmful to the plants as well as to the environment.

The cost-benefit ratio of the seedlings

Growing *Aquilaria* seedlings is not a costly process. People can sell the seedlings at Rs. 10-15/- (INR). 1000 seedlings can Rs. 10,000-15,000 (INR). Labor cost and other requirements such as buying polybags etc. will cost Rs. 2,000-3,000 (INR). So, net profit will be at least Rs. 8000-12000 (INR) for 1000 seedlings.

Harvesting and yield

For harvesting, it takes a minimum of 8 years. Natural infections start at the age of 7-8 years and it

will continue up to 25-35 years after that infection rate decreases. Harvesting is generally done when the plant is infected naturally by the insect *Zeuzera conferata*. The life cycle of the insect is confined in the *Aquilaria* plants only. The average yield from a single resinous tree is 4 kgs of resinous wood depending on the rate of infections. The current price of agarwood is Rs. 50,000.00 to 2,00,000.00 depending on their quality and quantity. In some areas, natural infection does not occur; in that case people use artificial inoculation techniques. Among the artificial infection, the most common method is by nailing the plants at the age of 8-10 years after that they are kept in the field for 2-3 years for the development of stress aroma. But the quality of agar is different from the naturally infected plants. Artificially infected agar is considered as an inferior quality.

Intercropping of *Aquilaria* with tea and other plants in Upper Assam for doubling of farmers income

Assam is known as the tea producing state of India. Along with tea, if people can cultivate agarwood, it will increase their economy. Although the Agar tree is evergreen so the canopy is spreading well and it allows partial penetration of sun rays through it, so it is widely used in tea gardens as a shading tree. It is a medium-sized tree and can also be cultivated with any other horticultural crops. Agar is a fast growing plant rapidly-rising plant, easy to cultivate and also cost-effective. Does not require much effort but annual weeding is necessary for growing healthy plants.

Basic information of Agar plantation (Source: Field survey 2019-2021)

- Season of seed sowing: March - April
- Season of seedling plantation: June - September
- The maturity period of agar plant harvested in natural method: 15 - 30 years
- The maturity period of agar plant harvested in nail method: 8 - 15 years
- The average height of a mature tree: 10 - 15 meter
- Favorable season of harvesting: October – March
- Processing, oil extraction throughout the year
- Marketing throughout the year

Only 30% of people are involved in agar cultivation. People who are generally involved in agarwood cultivation belong to the age group of 30-45 years, and also only men are involved in agar

cultivation in rural areas. Only 10% of people are taking up agar cultivation as their primary source of income. Also, we have seen less participation among the women. If women can be involved in agar cultivation then it may play a major role in woman empowerment. Educated and experienced people who are related to the agar cultivation and business should do awareness programmes so that common people can get benefited and their socio-economic status can be enhanced.

Problems faced by agar cultivar are discussed below

- ✚ Scarcity of good quality seedlings
- ✚ Lack of knowledge and training
- ✚ Credit in case of large scale plantation
- ✚ Risk factor sometimes because demands increase or decrease depending on the needs of international and national markets
- ✚ Takes a long time for infections, so farmers have to wait a long period for selling the plants

Discussion

Assam is considered as the capital of agarwood as Assam's topography is mostly suitable for agar cultivations. For promoting the cultivation of agarwood and considering its industrial importance, Government of Assam made a policy regarding promotion of Agarwood cultivation. Recently in the Golaghat district of Assam, "Assam Agar International Trade Center" was established. So it is a revolutionary initiative for the industries & commerce department of Assam. As per a report on "Agarwood Resource in Non-Forest Areas of Assam and its industry", published by Forest Department, Government of Assam and Green Initiatives Certification and Inspection Agency India Pvt. Ltd, Noida, the total number of Agarwood trees estimated in the non-forest areas of Assam is 1.433 million, and 91% of Agarwood trees are concentrated in 4 districts namely Jorhat, Golaghat, Sivsagar, and Hojai. The targeted area to be covered under Agar Block Plantation on Farmer's field is 1000 Ha per year which means 5000 Ha within 5 years. The targeted number of saplings to be distributed freely each year is 20 Lakh leading to 100 Lakh free sapling distribution within the policy validity period of 5 years. It is expected to plant at least 250 Lakh (2.5 Crore) saplings of Agar within 5 years³. However, germination time, germination percentage survival, and seedling growth are highly influenced by the quality of seed and seedling. *Aquilaria* seeds are

recalcitrant types of seeds that are greatly susceptible to drying conditions. Even if they are preserved, they are also cold-susceptible in storage and may result in failure of viability and germination as well as reduction in the physical growth and development of the saplings¹⁵. *Aquilaria* trees might be cultivated commercially in farms, home gardens and conventional for reforestation and afforestation purposes in the different regions such as hill forests and also other marginal soil lands where other plants may not survive. *Aquilaria* grew on marginal soil areas and below a broad range of soil conditions in which water logging condition must be avoided during plantation so it is recommended to do plantation in the degraded land as well. *Aquilaria* is a rapidly growing plant, require enough moisture for its survival, also grows up to the height of 10 cm DBH within 4 to 6 years. *Aquilaria* might be cultivated by intercropping with economically important wood trees such as rubbers, teak, tea, and any short term cultivated cash crops such as bananas, corns, black pepper, pineapples (Table 1,2,3,4 and Fig. 2) and lemongrass in many Southeast Asia countries¹⁶

Table 1 — *Aquilaria* intercropping along with herbs shrubs and tree

Herbs (English Name)	Scientific names
Ginger	<i>Zingiber officinale</i>
Termeric	<i>Curcuma longa</i>
Lemongrass	<i>Cymbopogon citratus</i>
Garlic	<i>Allium sativum</i>
Coriander	<i>Coriandrum sativum</i>
Cauliflower	<i>Brassica oleracea</i>
Cabbage	<i>Brassica oleracea var. capitata</i>
Onion	<i>Allium cepa L.</i>
Colocasia	<i>Colocasia esculenta var. esculenta</i>
Potato	<i>Solanum tuberosum</i>
Peppermint	<i>Mentha arvensis</i>
water hyssop	<i>Bacopa monnieri</i>

Table 2 — Intercropping with shrubs

Shrubs (English Name)	Scientific names
Tea	<i>Camellia sinensis</i>
Chilli	<i>Capsicum annum</i>
pineapple	<i>Ananas comosus</i>
Citrus	<i>Citrus sps.</i>

Table 3 — Intercropping with trees

(English Name)	Scientific names
Betel nut	<i>Areca catechu</i>
Guava	<i>Psidium guajava</i>
Areca Nut	<i>Areca catechu</i>
Teak	<i>Tectona grandis</i>

Table 4 — Intercropping with climbers

Climbers (English Name)	Scientific names
Betel leaf	<i>Piper betel</i>
Black pepper	<i>Piper nigrum</i>
Giloi	<i>Tinospora cordifolia</i>
Skunk Vine	<i>Paederia foetida</i>

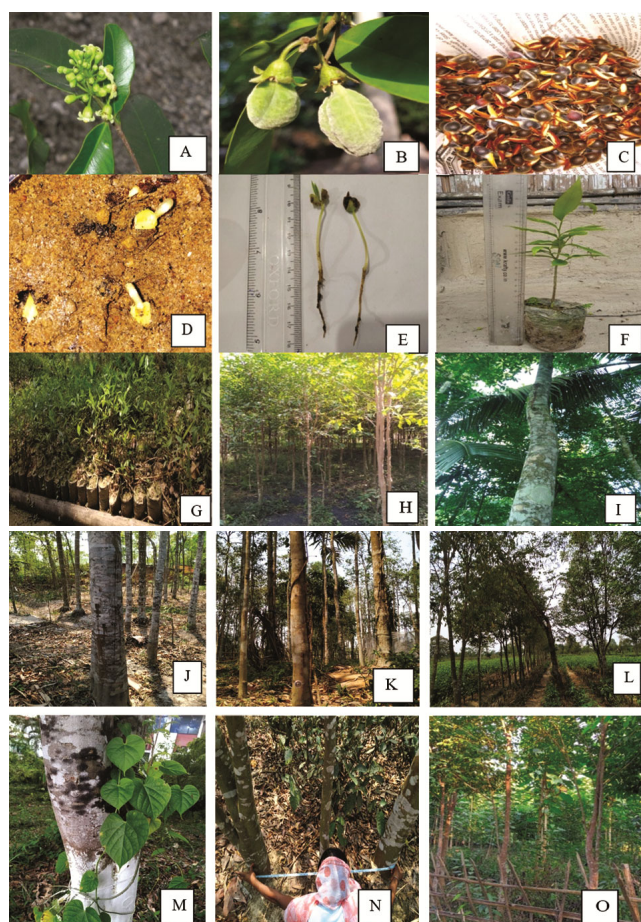


Fig. 2 A) — *Aquilaria malaccensis* flowering B) Fruits C) Seeds D). Germination initiations E) Showing Plumule and radicle F) Minimum size of the seedlings required for plantations G) Seedlings in the polybags H) Seedlings after three years I) Intercropping with areca nut J) Intercropping with beetle nut K) Intercropping with piper betel L) Intercropping with Tea O) Intercropping with Teak, M) Intercropping with Giloi N) Minimum Spacing required to maintain in *Aquilaria* plantations. (Source: Golaghat District Missamora).

Conclusion

Aquilaria plantation for agarwood production has been considered as a green ‘gold mine’ by agar planters. *Aquilaria* by-products are considered as black gold and its oils are considered as the liquid gold due to its high demand in the international markets. Many households of upper Assam are mainly based on Agarwood cultivation so it will encourage people to cultivate and regenerate more plants and it will help in

the conservation of this plant as well as in increasing rural economy. *Aquilaria* seeds are recalcitrant i.e. they are not able to survive dehydration condition^{17,18}. Seeds should be sown directly after harvesting because its loose moisture content after storage which will result in the declined germination percentages. Some recalcitrant seeds found in the tropical and temperate region cannot be stored for longer periods without losing their viability¹⁹. 55.56% of the people in the area of Naharani in Golaghat District are involved in agarwood trading and cultivation, different age groups of the people involved in cultivation and marketing are in the age group of 20-40 years, income varies in each household from INR 3000/- to 50,000/- per month²⁰. Traditional knowledge serves as the basic tool for agricultural and scientific research, and based on the existing knowledge further research can be performed²¹. Cultivation practices of agar are easy, cost-effective, and highly profitable due to its high demand in the national and international markets. People can earn money every year by producing seedlings from the plants. So agar cultivation can increase the annual income of the agarwood growers. Assam government has taken up the initiative to promote the agar cultivation in different regions of Assam. Along with its by-product agar also has a lot of ethnomedicinal, ethnobotanical and pharmaceutical importance. To maintain the agar genotypic diversity, production of good quality seedlings is more important for the growers and for the researchers for maintaining diversity and conservation. Future researchers can go for molecular study to improve the high yielding with high resin content plant so that it will help the economy of Assam as well as North East in near future.

Acknowledgments

The authors acknowledge the help and cooperation received from CSIR-NEIST, Jorhat and also sincere acknowledgment and thanks to the Director, CSIR-North East Institute of Science and Technology, Jorhat, Assam, India for his kind encouragement in carrying out the works. Authors are grateful to Council of Scientific and Industrial Research (CSIR), New Delhi, India for financial support through the project CSIR-Aroma Mission an R& D project no. HCP0007.

Conflict of Interest

The authors declare that there is no conflict of interest.

Authors' Contributions

Both the authors conceptualized and designed, verified the information and drafted the final manuscript. First author (JD) visited different location of Golaghat District, surveyed and collected the information, for preparation of the manuscript.

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