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A review on biological induction of agarwood in *Aquilaria*, with special reference to India

Indrani Gogoi[1], Sneha Choudhury[2] and Rathinam Saviour Caesar Jayaraj[3,4]✉

[1]*Dibrugarh University, Dibrugarh- 786004, Assam, India*
[2]*Rain Forest Research Institute, Jorhat- 785010, Assam, India*
[3]*Former Director, Rain Forest Research Institute; Former Principal Chief Conservator of Forests, Andaman and Nicobar Islands- 744102, India*
[4]*R/o A1-204, Harihar Paradise, P.N. Pudur, Coimbatore- 641041, Tamil Nadu, India*
[✉]rsc.jayaraj@gmail.com

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ABSTRACT

There are thirteen species of *Aquilaria* producing agarwood, a product of plant and microbe interaction. The process of natural formation of agarwood is slow and only 10 percent of the trees in wild get infected forming agarwood. Since commercial cultivation of agarwood has begun, there is a need for a supporting programme of artificial induction to make the programme viable. The paper reviews the methods of biological induction in *Aquilaria* sp., the role of endophytes especially species of *Fusarium* and the availability of inocula for inducing agarwood formation.

INTRODUCTION

Aquilaria is a genus belonging to the family Thymelaeaceae, yielding a resin impregnated heartwood called agarwood, which is a highly valuable, fragrant, dark resinous product used as incense, in perfumery and as medicine in many traditional systems. It is an important non-timber forest product in international trade. Unsustainable logging and smuggling of agarwood from the natural forests is the reason for its decreasing natural population. *Aquilaria malaccensis* is the first species of the genus *Aquilaria* to be listed under Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix II in 1995 and now all the species of *Aquilaria* are listed in the same Appendix since 2004. *Aquilaria* is categorized as 'Vulnerable' by the International Union for the Conservation of Nature (IUCN, 2009) and *Aquilaria malaccensis* was downgraded from 'Vulnerable' to 'Critically Endangered' in 2018 due to continued unsustainable exploitation (Harvey-Brown, 2018).

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Aquilaria species yielding agarwood are found only in the areas ranging from India eastwards throughout southeast Asia and in southern China, with Indonesia and Malaysia being the two major agarwood producing countries. Out of 21 species of Aquilaria, 13 are agarwood-producing, *viz*., *Aquilaria acuminata*, *A. apiculata*, *A. baillonii*, *A. banaensae*, *A. beccariana*, *A. brachyantha*, *A. crassna*, *A. cumingiana*, *A. filaria*, *A. grandiflora*, *A. hirta*, *A. khasiana*, *A. malaccensis*, *A. microcarpa*, *A. rostrata*, *A. sinensis* and *A. subintegra* (Lee & Mohamed, 2016). In India there are two species of *Aquilaria*, namely *Aquilaria malaccensis* widely cultivated and *A. khasiana* having a restricted distribution in Khasi hills of Meghalaya. *Aquilaria malaccensis* occurs naturally mostly in the foothills of northeastern region except Sikkim as well as northern part of West Bengal.

Induction of agarwood production

Production of agarwood is mainly the result of plant-microbes interaction, induced by external factors such as physical injury, insect damage or microbial infection. *Aquilaria* trees may take several years to make agarwood around the wound naturally (Zhang et al., 2012). Further, only about 10% of the trees in a population get infected and form agarwood naturally (Soehartono & Newton, 2000). Due to high economic and medicinal values of agarwood and the resultant demand, different strategies have been devised to produce agarwood artificially in a short period

of time. The causal agents associated with production of agarwood can be physical, chemical and biological. The history and perspectives of induction technology for agarwood production from cultivated *Aquilaria* in Asia have been recently reviewed (Azren et al., 2019). The relative advantages and disadvantages of these methods have also been reviewed recently discussing the current developments and future perspectives (Tan et al., 2019; Shivanand et al., 2022).

Cutting, cauterizing, holing, nailing, axe chopping, wounding using chisels and bark removal have been used as physical modes (Pojanagaroon & Kaewrak, 2003; Chhipa, Chowdhary & Kaushik, 2017). Although this approach is cost effective and requires only one person for handling, it usually results in inferior quality of agarwood and uncertain yield (Tan et al., 2019). A modification of the physical method is wounding and keeping the wound active by aeration through insertion of pipes of plastic, bamboo or wood, to a depth of 1-10 cm into the xylem, as a series of closely spaced wounds in a spiral fashion, from ground to the crown, wounds positioned 5 cm apart. In the same method, chemical induction can also be combined or biological inducers can also be inserted, but keeping the wound fresh by aeration and the tree alive are important (Blanchette & van Beek, 2005).

Chemicals such as sulphuric acid, acetic acid, alcohol, methyl jasmonate, soybean oil, jaggery, sodium bisulphate, yeast extract, iron powder, sodium chloride, formic acid, hydrogen peroxide and salicylic acid have been used as chemical inducers for production of agarwood (Chen et al., 2011; Wei et al., 2012; Ito et al., 2005). An alternate method of agarwood production patented in China is called the whole-tree agarwood induction technology (Agar-Wit) wherein a degradable chemical solution is injected into the xylem of *A. sinensis* trees through the transfusion sets, and it induces high quality agarwood production in just 20 months with very high similarity to wild agarwood in terms of quality, including chemical constituents, ethanol-soluble extract content and essential oil content (Zhang et al., 2012). A combination of both the physical and chemical methods has also been attempted which improved the agarwood yield in comparison to natural process (Pojanagaroon & Kaewrak, 2003).

For biological induction of agarwood production, there are various well-established methods. The advantage of biological induction is that it is progressive and systematic growth of fungal agents results in continuous formation of agarwood (Novriyanti et al., 2010). The fungal inoculation can be induced by drilling the tree and injecting the inoculum. In this method, the wound closes after some time and therefore to keep it open and allow flow of inoculum continuously the bottle drip method, where fungal inoculum is kept in bottles hung upside down, allowing slow seepage of inoculum continuously, has been devised (Tang, 2012; Justin et al., 2020). By suitable selection of fungal strains high grade agarwood can be produced in substantial quantities (Turjaman, Hidayat & Santoso, 2016). A further improvement in biological method is the pinhole infusion (Tian et al., 2013) wherein formic acid is used as a chemical inducer along with culture of *Botryosphaeria dothidea*, a combination of chemical and biological methods to collectively improve the resin formation. In this review, the role of microbes such as the endophytes occurring naturally in the host *Aquilaria* and also the artificially inoculated microbes for production of agarwood, especially in India, have been elaborated.

Artificial induction of agarwood using microbes

Artificial induction using fungi isolated from agarwood was first attempted by Tunstall in 1929 (Gibson, 1977). Schuitemaker (1933) suggested the possibility of a "pathological occurrence of which the cause was unknown" related to formation of agarwood in Borneo. He suggested possibility of inducing agarwood formation by inserting freshly cut agarwood into the stem of a healthy tree. The experiment of Tunstall was reviewed and the nature of agar formation was first discussed by Bose (1938), who also examined the feasibility of agaru formation by fungal inoculation using pure culture of *Cladosporium* sp. (Bose, 1943, 1962). In 1940 he sent the inoculum to the Forest department of Assam and after inoculation fungal hyphae had developed and drops of resin were seen under microscope. By distillation, he was able to obtain oil with agar smell, and this is the first instance of artificial induction of agarwood in Assam (MacKarness, 1941). Bhattacharyya, Datta and Baruah (1952) reported that the formation of agarwood is possibly due to the presence of microbes in the wood, and the fungus *Epicoccum granulatum* is responsible for agarwood production. Saikia (1956) attempted artificial inoculation to promote formation of agarwood, in Assam. Similarly, Jalaluddin (1977) isolated *Cytosphaera mangiferae* from diseased tissues in standing trees of *Aquilaria agallocha* and used them for artificial inoculation.

However, in a study in Sylhet region of Bangladesh, Gibson (1977) observed the presence of *Penicillium* sp. and *Aspergillus* sp. in all the infected and mechanically injured wood and suggested that there is no consistency in the range of fungi observed in the agarwood deposits collected, and therefore agarwood formation may be in response to wounding, followed by invasion of weak fungal pathogens, rather than a specific response to fungal attack. Similarly, Rahman and Basak (1980) also observed that there is no specific fungus which causes agarwood production, but is a general reaction of the host to injury and invasion. They also postulated that presence of exposed open wound is more important than presence of certain species of fungi within the wound, for the formation of agarwood.

The combined effect of physical and biological stress in the form of stick method to improve agarwood production in *Aquilaria malaccensis* has been explored and compared with

Oudino-Universiti Malaysia Pahang

Agarwit-400 by Vanadurgi Foundation, Bengaluru

Agarwood (Oud) Organic inoculum syrup
AWK Researh India Pvt. Ltd., Kerala

Tanali Gaharu Inoculation starter kit, Malaysia

Witasa Agarwood inducer, Thailand

Sasi inoculant produced by Rain Forest Research Institute, Jorhat

Plate 1. Some of the agarwood induction kits available in the market.

well-known artificial fungal infection by syringe method. In total 21 fungal strains were applied alone (syringe method) and with bamboo sticks (stick method). Maximum infection in stick method was by *Penicillium polonicum*. *Penicillium aethiopicum* has shown high potential as agent in stick method for artificial production of agarwood (Chhipa & Kaushik, 2020).

Endophytes and their role in agarwood production

Many microbial endophytes dwell within plant tissues and play an important role in plant physiology. They are generally opportunistic and establish a symbiotic relationship with plants; they can be isolated from the surface or from inside the plant parts. However, some endophytes show latent pathogenicity in plant species, i.e., under specific conditions can turn into pathogens. In *Aquilaria* this latent pathogenicity of endophytes is economically useful, as it induces oleoresin production. The role of endophytes in the formation of agarwood has earlier been reviewed (Enshasy et al., 2019). A recent review has indicated that 59 endophytic fungal strains of 16 genera induce agarwood production, most of which belong to the genus *Fusarium* (Du et al., 2022). Yet another review of endophytic fungi found in species of *Aquilaria* and *Gyrinops*, mainly from China and India has listed 71 species, of which 9 were able to induce agarwood formation, *viz*., *Botryosphaeria* sp., *Chaetomium* sp., *Colletotrichum gloeosporioides*, *Fusarium* sp., *Nemania aquilariae*, *Penicillium* sp., *Pestalotiopsis* sp., *Trichoderma* sp. and *Xylaria* sp. (Zhang et al., 2022).

Besides fungi, bacteria have also been isolated from the infected trees and have been found to play a role in agarwood formation. Bhore, Praveena and Kandasamy (2013) isolated and identified 18 culturable endophytic bacteria associated with 7 *Aquilaria* sp. from Malaysia, *viz*., *A. beccariana*, *A. crassna*, *A. hirta*, *A. malaccensis*, *A. microcarpa*, *A. sinensis* and *A. subintegra*. *Bacillus pumilus* was dominant of all the culturable endophytic bacteria. Others were *Acinetobacter radioresistens*, *Bacillus altitudinis*, *B. anthracis*, *B. arbutinivorans*, *B. arsenicus*, *B. aryabhattai*, *B. cereus*, *B. licheniformis*, *B. megaterium*, *B. methylotrophicus*, *B. stratosphericus*, *B. subtilis*, *B. tequilensis*, *Pantoea agglomerans*, *Rahnella aquatilis*, *Roseomonas mucosa* and *Vibrio cholera*. Nguyen and Nguyen (2014) identified 26 bacterial isolates divided into 7 groups associated with *Aquilaria crassna*, the dominant species being *Bacillus pumilus* and *Alcaligenes faecalis*. Chhipa and Kaushik (2017) studied the fungal and bacterial diversity from *A. malaccensis* tree as well as soil collected from different sites of Assam, and found that in stem Hypocreaceae is the dominant fungal family while Bacillaceae is the dominant bacterial family in both stem and soil isolates. Using bacterium *Pantoea dispersa* and fungus *Penicillium polonicum*, they induced agarospirol, the compound responsible for fragrance in agarwood, successfully. Phakeenuya et al. (2022) identified *Pantoea dispersa* as the dominant bacterium in the healthy wood of *Aquilaria crassna*. Fitriasari, Soetarta and Surata (2020) have suggested that use of lignocellulolytic bacteria can be more effective than the fungi as inoculants, as the bacteria can multiply faster than fungi, based on their study in *Gyrinops versteegii*.

There are several genera of fungi which have been found inducing agarwood development in *Aquilaria*, *viz*., *Acremonium*, *Arthrinium*, *Aspergillus*, *Botryodiplodia*, *Botryosphaeria*, *Chaetomium*, *Colletotrichum*, *Cylindrocladium*, *Diaporthe*, *Diplodia*, *Epicoccum*, *Fusarium*, *Lasiodiplodia*, *Melanotus*, *Penicillium*, *Pestalotiopsis*, *Rhizopus*, *Rigidoporus*, *Trichoderma* and *Xylaria* (Budi, Santoso & Wahyudi, 2010; Chen et al., 2017; Chong et al., 2015; Cui et al., 2013; Faizal et al., 2017; Lisdayani, Anna & Siregar, 2015; Monggoot et al., 2017; Santoso et al., 2011; Sen et al., 2017; Subasinghe, Hitihamu & Fernando, 2019; Tamuli et al., 2005; Tian et al., 2013; Zhang et al., 2012, 2014). Based on available literature, *Xylaria* sp., *Lasiodiplodia* sp., *Colletotrichum* sp. and *Botryosphaeria* sp. have been suggested as promising inoculants (Chhipa, Chowdhary & Kaushik, 2017). *Fusarium* has also been recommended as a good candidate for agarwood induction (Du et al., 2022).

Studies on artificial induction of agarwood in India

In India, there have been several attempts at isolation and identification of the microbes associated with the species *Aquilaria malaccensis*, and their subsequent use in artificial induction. Tamuli et al. (2000) isolated *Fusarium oxysporum* and *Chaetomium globosum* from infected trees. Mitra and Gogoi (2000) isolated *Botryodiplodia theobromae*, *Fusarium solani*, *Mucor hiemalis* and *Rosellina necatrix* from the diseased agarwood caused by insect boring. Over and above the said fungi, they also isolated *Aspergillus candidus*, *A. chevalieri*, *A. flavus*, *A. tamari*, *Epicoccum granulatum*, *Penicillium citrinum* and *Penicillium* sp. from the agarwood induced by mechanical injuries. Puzari and Saikia (2000) studied the inner woody tissues after artificial inoculation with four different fungi, *viz*., *Fusarium* sp., *F. oxysporum* (Isolate No 1 and 2), *Trichoderma* sp. and one unidentified fungus, isolated from infected agar plants, and found oleoresin deposition. Barthakur, Mitra and Gogoi (2000) isolated 15 fungal species from the rhizosphere soil of trees growing naturally in undisturbed area and 9 fungal species in disturbed area, of which *Mucor hiemalis*, *M. spinosus*, *Aspergillus ochraceous*, *Trichoderma album*, *T. koningii* were common. Tamuli et al. (2005) successfully used *Chaetomium globosum* and *Fusarium oxysporum* for production of agarwood in *Aquilaria malaccensis*. Premalatha and Kalra (2013) found genera of endophytic fungi such as *Cladosporium*, *Curvularia*, *Fusarium*, *Phaeoacremonium* and *Trichoderma* as members of the agarwood community of *Aquilaria malaccensis* whereas only two taxa, i.e., *Alternaria* sp. and *Trichoderma* sp. were isolated from healthy wood, indicating that generally resinous wood contained high

diversity of microflora in comparison to healthy wood. Nagajothi et al. (2016) found colonization by *Aspergillus*, *Penicillium*, *Fusarium*, *Lasiodiplodia* and *Chaetomium* resulting in formation of agarwood. They analyzed cellulolytic activity, lignin degradation and laccase production by 17 fungal isolates among which *Aspergillus niger* showed highest activity for all three parameters. They suggested that enzymes produced by the pathogenic or saprophytic fungi could be playing a major role in defense mechanism which ultimately becomes responsible for agarwood production, and thus there is possibility to develop *Aspergillus niger* as an inoculant for agarwood production. Chhipa and Kaushik (2017) isolated the fungal and bacterial community from inside the stem of *Aquilaria malaccensis* and the surrounding soil from 21 different sites in Assam and explored their potential in inducing Agarospirol production by artificial infection. In total 340 fungi and 131 bacteria were isolated from 50 stem samples, and 188 fungi and 148 bacteria from 50 soil samples. The dominant fungal genus in stem was *Trichoderma* while in soil *Aspergillus* was dominant. In bacteria, *Bacillus* genus showed dominance in both stem and soil samples. Forty fungal and bacterial isolates were assessed for their potential to induce agarwood formation in *Aquilaria malaccensis* and only 31% of bacterial and 23% of fungal isolates showed their ability in inducing Agarospirol production. Among bacteria *Pantoea dispersa* and among fungi *Penicillium polonicum* showed the highest production compared to other isolates. Chhipa, Chowdhary and Kaushik (2017) reviewed the genus wise occurrence of endophytic fungi and reported that *Fusarium* sp. showed highest presence in *Aquilaria* spp. The other fungal endophytes included *Alternaria* sp., *Botryosphaeria* sp., *Cephalosporium* sp., *Chaetomium globosum*, *Cladophialophora* sp., *Cochliobolus lunatus*, *Colletotrichum gloeosporioides*, *Colletotrichum* sp., *Coniothyrium nitidae*, *Cunninghamella bainieri*, *Curvularia* sp., *Cylindrocladium* sp., *Epicoccum* sp., *Fimetariella rabenhorstii* A20, *Geotrichum* sp., *Glomerularia* sp., *Gonytrichum* sp., *Guignardia mangiferae*, *Hypocrea lixii*, *Lasiodiplodia theobromae*, *Leptosphaerulina chartarum*, *Monilia* sp., *Mortierella* sp., *Mycelia sterilia* sp., *Mycosphaerella*, *Nigrospora oryzae*, *Nodulisporium* sp., *Ovulariopsis* sp., *Paraconiothyrium variabile*, *Penicillium* sp., *Phaeoacremonium*, *Phoma* sp., *Phomopsis* sp., *Pleospora* sp.,

Table 1. Inoculant formulations for artificial induction of agarwood

Inoculant formulation	Producer and Address	Country
Gaharu Resin Essence	Tanali Esteem, No. 45, Jalan PUJ 8/7, Taman Puncak Jalil, Bandar Putra Permai, 43300 Seri Kembagan, Selangor Darul Ehsan	Malaysia
Agarwit-100, Agarwit-400, Agarwit-500, Oudzene, Garamone A10, Oud LB1, Oud MIT 18, Oud MIT 20, Kristozen W-2, Biozen- WMF, Chemosach-P, Oleton B3, HPFCI-M10	Vanadurgi Agarwood India Ltd., S-816, 8th Floor, Front wing, South Block, manipal Centre, Bengaluru- 560042	India
Wood nails with fungus	Lao Agar International Development Co. Ltd., 7th Floor, Ammata Commercial Office Tower Khamphengmeuang Road, Ban Nonghai, Hadsaiyfong District, Vientiane Capital, Lao PDR.	Lao PDR
Agarwood Organic Inoculation Syrup	AWK Research India, Pvt Ltd., Thrissur-Kuttippuram Road, Nadakkave, Malappuram District, Kuttippuram, Kerala, India, Pin – 679582	India
Ahi Resin Gold	Avadi Herbs India, C/O Anwar Hussain, Padam Pukhuri Natun Bazar, Hojai, Assam, India	India
Agarwood inoculant	Tailormade Tea, Kathoni Bagan Morongi Block 2 No., Purajanghal Gaon, Golaghat-785613, Assam, India.	India
Oudino (Nano-inoculant)	Universiti Malaysia Pahang, 26600 Pekan Pahang, Malaysia	Malaysia
Sasi inoculant (Paste and liquid)	Rain Forest Research Institute, Sotai Ali, Chenijan P.O., Jorhat- 785110, Assam, India	India
GSL Inoculum	School of Life Sciences and Technology, Institut Teknologi Bandung, Jalan Ganeca 10, Bandung 40132, Indonesia	Indonesia
Bio serum Gaharu Lampung	Universitas Lampung, No.1 Bandar, Lampung 35145, Indonesia	Indonesia
Ant-processed inducer (ApI)	Secoin Applied Biotech Center	Vietnam
GAS de-Denai Agarwood Inducer	Gaharu Anugerah Sarawak	Malaysia
Witsawa's Agarwood Inducer	Agarwood Learning Center, 471/1, M.1, T.Wiangchai A. Wiangchai, Chiangrai, Thailand 57210	Thailand
Vaksin PLT, Vaksin NVI and Vaksin Kunig	Telupid Gaharu Enterprise	Malaysia
Inoculan SGB	Gaharu Sarawak	Malaysia

Ramichloridium sp., *Rhinocladiella* sp., *Rhizomucor variabilis*, *Sagenomella* sp. and *Trichoderma* sp. Pandey et al. (2017) found *Phaeoacremonium parasiticum* to be a promising candidate for agarwood induction through inoculation. Mochahari et al. (2020) isolated *Alternaria* sp., *Curvularia* sp., *Rhizopus* sp. and *Sterilia* sp. from one-year old *Aquilaria malaccensis* seedlings, and *Penicillium* sp., *Fusarium* sp. and one unidentified fungus putatively *Cladosporium* from agarwood chips. Kalra and Kaushik (2017) while reviewing the chemistry and quality of agarwood observed that most of the investigations on the chemical composition of agarwood have been with wild samples, and very little with resin produced using artificial methods, and also stated that the artificial induction methods have not ensured sustainable and quality supply of agarwood, calling for more extensive and streamlined research.

Role of Fusarium species

Analysis of the various studies on isolation of microbes and their use in induction of agarwood, indicates that *Fusarium* is the most common microbe. *Fusarium* sp. is the most widely isolated followed by *Cladophialophora* sp. from *Aquilaria* sp. (Chhipa & Kaushik, 2017). Isolates of *Fusarium* sp., have been evaluated for their potential in induction of agarwood (Mucharromah & Santoso, 2008). Various species of *Fusarium* were isolated from parts of Indonesia, *viz*., *Fusarium solani*, *F. tricinctum*, *F. sambucinum* and *F. moniliformae*, and these were successfully used to induce agarwood formation in *Aquilaria malaccensis* and *A. microcarpa* (Budi, Santoso & Wahyudi, 2010; Santoso et al., 2011). *Fusarium* species have been successfully used to induce agarwood in *Aquilaria beccariana* (Iskandar & Suhendra, 2012). Study on several isolates of *Fusarium* from North Kalimantan showed the presence of *Fusarium solani*, *Fusarium* sp., *F. fujikuroi*, *F. oxysporum* and *F. ambrosium* (Nurbaya et al., 2014). A study to determine the presence of *Fusarium* sp. in *Aquilaria malaccensis* that had undergone fungal inoculation revealed presence of five isolates of *Fusarium* sp., besides others and the consistency of *Fusarium* sp. was higher on top stem and could be found in all the parts of the stem. (Lisdayani, Anna & Siregar, 2015). Similarly, *Fusasium* sp. showed highest presence in endophytic fungi isolated from *Aquilaria* spp. (Chhipa, Chowdhary & Kaushik, 2017). Turjaman, Hidayat and Santoso (2016) successfully induced agarwood in *A. microcarpa*, *A. crassna* and *A. beccariana* within 75 days of inoculation with *Fusarium solani*. *Fusarium solani* has been found highly effective in agarwood initiation in *Aquilaria*, and is considered the most potent essential oil inducer as it can colonize phloem boundaries within xylem tissues to induce agarwood compounds (Faizal et al., 2017). A combination of treatment with *Fusarium solani* and nitrogen fertilization has also been suggested to boost agarwood production (Wahyuni, Triadiati & Falah, 2018). *Fusarium solani* and *Fusarium oxysporum* have been found responsible for agarwood induction in Indonesia (Zulfendi, Idroes & Khairan, 2019). *Fusarium solani* has been found to be the most dominant isolate in all artificially inoculated and naturally infected agarwood of *Aquilaria malaccensis*, and possibility of developing inoculant using *Fusarium* sp., *Polyporales* sp. and *Schizophyllum commune* has been suggested (Ramli et al., 2022). A literature survey showed that 59 endophytic fungal strains of 16 genera induce agarwood production, most of which belong to *Fusarium* (28 identified strains). Hence, *Fusarium* was recommended as a good candidate for further studies on fungal induced agarwood production (Du et al., 2022).

CONCLUSION AND WAY FORWARD

Natural agarwood production takes several years and sometimes host-microbe interaction does not take place in the plants, which becomes major problem for the cultivators. With the increasing demand for agar in international market and the decrease in plant population in the wild, many people have started planting it in farmlands in the last two decades. However, in many areas the natural infection does not occur, which necessitates artificial induction of agarwood. Commercial cultivation is already in practice or is recommended in many countries, as plantations or agroforestry systems, as revealed by some of the recent literature from India (Ahmed & Bhagabati, 2021), Bangladesh (Hossain et al., 2021), Malaysia (Elias, Ibrahim & Mahamod, 2017), Indonesia (Insusanty, Ikhawan & Sadjati, 2018), Pakistan (Ullah, 2020), Nepal (Adhikari, Pokhrel & Baral, 2021), Thailand (Jha, 2014), and Vietnam (Persoon & van Beek, 2008). Commercial cultivation needs the support of artificial induction of agarwood, failing which it may not be economically viable. All over the world in areas where agarwood is taken up for commercial cultivation, inoculum is also produced and distributed under various trade names. Some of the commercial formulations in India and abroad are listed in Table 1.

The commercial cultivation of agarwood needs the support of a reliable inoculation programme. The major areas of research and development related to artificial induction of agarwood are as follows:

(a) In view of large number of endophytes and other pathogens involved in inducing the formation of agarwood, the most virulent ones need to be identified.

(b) Variation in strains within the identified microbes needs to be identified, in terms of their virulence, and the capacity to induce agarwood formation quickly and in large quantity.

(c) Ideal combination of methods-physical, chemical and biological need to be devised for increasing the production of agarwood. It has been suggested that induction of agarwood using a combination of chemical and biological methods may be the optimal technique (Zhuang et al, 2022).

(d) The quality of the product-agarwood chips or oil in terms of the chemical composition and desirable properties of the

trade, need to be assessed for various methods of inoculation as well as various inocula used in trade.

(e) The availability of inoculum has to be increased by increasing the facilities for inoculum production, in both the public and private sectors.

(f) The method of inoculation needs to be simplified, so that the farmer can inoculate the trees himself, without any need to depend on any expert agency.

(g) The inoculum which is now mostly in a liquid form, needs to be converted to forms that can be easily transported or stored, *viz*., powders, tablets, creams and pastes.

(h) The technology of production now confined to labs, needs to be transferred to commercial producers of inoculum through Material Transfer Agreements, so that the supply of inoculum is decentralized. This has just started in India where the Rain Forest Research Institute has taken the initiative for transfer of microbial strains to commercial labs, but this needs to be expanded on a large scale, to the labs in all those areas where agarwood is planted but natural induction does not take place, as in the case of Kerala and Karnataka, which have about 5 million trees on farmlands.

(i) There may be ideal combinations of host and the pathogen, due to genetic variations in both of them, and these combinations need to be identified for the commercial plantation programmes combining the provenances or genotypes of the host with the pathogen that can lead to maximum production of agarwood.

(j) The search for new pathogens and new strains of already known pathogens, which are more potent should continue.

(k) The multiplication protocol for the pathogens used in inoculum needs to be standardized, as they may lose virulence after repeated multiplication. The mother cultures need to be maintained in a repository for getting back to the original material.

(l) The identified pathogens need to be categorized based on molecular genetics and samples maintained at National repositories, to settle disputes that may arise on the identity of the pathogen and its strain, between the research organizations and the commercial labs.

(m) Quality control of the inoculum is also essential to ensure that sufficient pathogen load is available. The shelf life of the various inocula needs to be standardized.

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