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SHORT COMMUNICATION

Insect infested agarwood: A newly prized product of agarwood market in Bangladesh

Md Najmol Hoque¹, Mohammad Mehedi Hasan Khan¹, Md Fuad Mondal^{2*}

¹Department of Biochemistry and Chemistry, Sylhet Agricultural University, Sylhet 3100, Bangladesh

²Department of Entomology, Sylhet Agricultural University, Sylhet 3100, Bangladesh

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M Harun Rashid

*Corresponding Author

Md Fuad Mondal

mondalfm.entom@sau.ac.bd



ABSTRACT

Agarwood is a highly prized product in perfumery world. Insect infested wood demands higher price than other exported agarwoods. A comparative analysis was made among three categories of agarwood *viz.* white wood, screw injected agarwood and insect infested agarwood to evaluate the ether extract and total phenolic contents. The wood samples were collected from Sylhet region of Bangladesh. The ether extract oil contents were 1.80%, 20.49% and 11.08% for white wood, screw injected wood and insect infested wood, respectively. Total phenolic contents were 2.55 mg g⁻¹, 3.6 mg g⁻¹ and 2.97 mg g⁻¹ from white wood, screw injected wood and insect infested wood, respectively. Fungal attack following the insect infestation may have a positive effect on the quality of agar products. Further study is recommended to understand how insect infestation improves the quality of agar product.

Keywords: Agarwood, insect Infestation, ether extract, phenol content, market price

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1 Introduction

Agarwood is highly valuable and prized product that is extracted from agar tree (*Aquilaria malaccensis*) under Thymelaeaceae family. It is the heartwood of agar tree and the resultant substance of plant defense against external attacks and impacts such as pathological product, insects, and bacteria, mechanical injury inserted by human being or outer factors (Dinh, 2010). Agar farming in private sector has changed the lifestyles of many people in the Sylhet region of Bangladesh (Siddiquee, 2011). It is gradually changing the economic status of the area and at least 30,000 people are earning their livelihood from this silently rising sectors, where about 121 agar processing factories have been already established in different regions of Moulvibazar under Sylhet, Bangladesh (Abdin, 2014). Being a profitable form of farming, it has a

great contribution in reducing poverty. Agar trees are now being cultivated on commercial basis and these factories are totally export oriented and highly labour intensive in different regions of Moulvibazar district Sylhet, Bangladesh (Abdin, 2014). These industries contribution to GDP is about USD 3750000 per year in Bangladesh (Siddiquee, 2011).

Agarwood exporters of this country export their products both as chips and oils. Generally two categories of agarwood (*Aquilaria malaccensis*) are exported by the agar workers of Bangladesh. Screw injected method for agarwood cultivation is common in Bangladesh (Akter et al., 2013) and the other is naturally insect infested agarwood. Naturally agar plant (*Aquilaria malaccensis*) is infested by insect borer *Zeuzera conferta* Walker (Kalita, 2015). International market price for Agar wood chips ranges from USD 20 to USD 6000 kg⁻¹ based on its quality (Akter et al.,

2013). According to our survey to local exporters, they get more prices from insect infested agarwood chips than that from screw injected wood. The local exporters export screw injected agarwood chips at USD 65 to USD 95 kg⁻¹ based on its quality, on the other hand insect infested agarwood chips are exported at USD 800 to USD 1100 kg⁻¹. Therefore our research was aimed to evaluate the quality of different categories of agarwood sold by the local exporters of Moulvibazar district of Sylhet region in Bangladesh.

2 Materials and Methods

2.1 Plant material

Three categories of agarwoods were collected (Fig. 1) from several reputed factories of Moulvibazar district of Sylhet region in Bangladesh in 2016. These were naturally insect infested agarwood and others were artificially screwing injected wood and white wood. The woods were grinded with mechanical grinder machine (Chilli powder grinding machine). The grinded wood were then passed through a mesh (0.3 mm mesh) to get similar sized grinded wood.

2.2 Evaluation of ether extract

Ether extract (EE, Oil) was extracted with soxhlet extraction apparatus. At first, boiling flask was dried in oven at 105 °C for 1.5 h and cooled on desiccators. The flask was then weighed and labeled. Then the condenser was made ready to use by fixing it with the water inlet and outlet pipes. Five gram (5 g) of each sample was taken in to a thimble. A piece of absorbent cotton was placed and lightly pushed on the thimble. The thimble was transferred to the soxhlet extractor and the extractor was connected with the boiling flask. Diethyl ether (~180 mL) was poured inside the extractor through its upper opening. The boiling flask was loaded on the heating unit and by the mean time previously ready condenser was fixed with the upper opening of the extractor. The whole set was then fixed with a stand to avoid movement/fall down. Heater and water switches were turned on. The heater regulator was adjusted so that 2-3 drops per second of diethyl ether dribbled from the condenser to the boiling flask (40 °C). It was allowed to heat till the sample solution (with diethyl ether) on the extractor was clear (2.5 to 3 h). Diethyl ether was cycling throughout the set several times. The boiling flask was separated from the extractor. The ether inside the flask was allowed to evaporate. Then the flask was allowed to cool in desiccators and weighed. EE on a wood sample was calculated and expressed as percent (%).

2.3 Determination of total phenolics

For the determination of total phenolics, the Folin-Ciocalteu method (Singleton and Rossi, 1965) as described by Khan and Chaudhry (2010) was followed. Extracts for the determination of total phenolics were prepared by suspending 0.1 g samples (wood samples) in 20 mL of 75% acetone in a 50 mL falcon tubes. The contents of the falcon tube were vortexed for 30 min using a vortex mixture (Whirlimix, Fison Limited). An aliquots (0.1 mL) of each extract were mixed with 0.4 mL of water, 0.25 mL of 2N Folin-Ciocalteu reagent and 1.25 mL of sodium carbonate solutions (20%). The same procedure was repeated at least nine times for the each sample. After standing for 40 min at room temperature, the absorbance was read at wavelength of 660 nm using colorimeter (Digital Photo Colorimeter). Tannic acid was used as a standard and the results obtained were expressed as mg tannic acid equivalent/ g of sample, on dry matter basis.

3 Results and Discussion

The three categories of agarwood were evaluated by the ether extract. Ether extraction is a method for determining oils/fats (Lumley and Colwell, 1991). Agarwood samples showed great performance in ether extract result. The results are shown in Table 1. The results showed that screw injected wood produced highest contents of agarwood essential oils (20.49%). Insect infested woody samples produced 11.07% agar oils. Insect injuries can produce more oils (Hare, 2011), phenolic contents and their secondary metabolites which may contribute to the long oil shelf-life, color and several organoleptic characteristics, including taste (bitter, astringent, pungent, throat-catching (Bulotta et al., 2014). On the other hand white agarwood, which was the lowest in wood quality, can produce oils content 1.80%. Generally insect infestation is not uniform (Mitra et al., 2000) in the agarwood tree which is present in screw injected method, a vital factor for oil content in Agarwood. Plants secrete different chemicals called secondary metabolites to protect themselves from external attack (Mazid et al., 2011). These metabolites are monoterpenoids, sesquiterpenoids, homoterpenoids and Phenolics Akter et al. (2013); Adeyemi (2012). Sesquiterpenes are considered to be less volatile than other terpenes, have a greater potential for stronger odors or contribute to the fragrance (essence) of plants that generate them.



Figure 1. Agarwoods collected from Moulvibazar district of Sylhet region in Bangladesh

Table 1. Ether extract and phenolic contents of agarwood collected from Moulvibazar district of Sylhet region in Bangladesh

Agarwood type	Ether extract (%)	Phenolics (mg g ⁻¹)
White wood	1.80±0.03	2.55±0.05
Screw injected	20.49±0.04	3.6±0.052
Insect infested	11.08±0.94	2.97±0.07

Oil is the most determining factor in agarwood industry which leads its marketing value (Akter et al., 2013). Though amount of oil from insect infested wood are less, but its quality and fragrance durability was found the highest. In this study total phenolic content was determined from three categories of agarwood samples. Researchers have found positive relationship between beneficial insect attack and phenol production (Zhao et al., 2018). Total phenolic contents were found highest in case of screw injected wood samples and lowest for white wood (Table 1).

Folin-Ciocalteu colorimetric method determined total phenol in mg g⁻¹ which were 3.6±0.05, 2.975±0.07, 2.55±0.05 mg g⁻¹ from screw injected, insect infested and white agarwood, respectively. These results indicated that insect infested and screw injected agarwood contain higher phenolics compound. In recent year researchers found that, fungi infection in agarwood plants are observed when the tree is bored and infested by the trunk borer *Zeuzera conferta* Wlaker (Kalita, 2015). In case of screw injection, microbes grow around the screw which ultimately helps produce black resinous oils (Kalita, 2015; Zhang et al., 2010). The percentage of natural microbes attack in screw injected agarwood tree is very low (Kalita, 2015) whereas borer insect (*Zeuzera conferta*) has important role in the growth of microbes that helps produce different fragrance resin depending on fungal infection (Hare, 2011).

Screw is metal which is unable to secrete any chemical during its injection in agar tree. On the other

hand both insects and plants can secrete secondary metabolites that might have influence on fragrance durability. In our another part of research we found that, insect infested agarwood oil emit the fragrance until 60 days but the other categories of agarwood oils emit fragrance until 30 days (data not shown here). The larvae of *Z. conferta* make vertical tunnels in the tree which is the initial site of infection of fungi and gradually moves upward (Kalita, 2015). In both cases, microbes grow which may produce different secondary metabolites. There might be a good insect-fungal relationship that makes agarwood high quality fragrance, long shelf life and durability which have made it more preferable and pricer to the buyers.

4 Conclusions

Insect infestation can be a better alternative to screw injected method of agarwood preparation in terms of quality of the product. However, before representing clear information about the insect infested wood, it would be important to perform the chemical profiling of the oils from insect infested wood. In future, we will do chemical profile analysis of insect infested agarwood for further confirmation of the secondary metabolites.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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