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AN EFFECTIVE AND EFFICIENT METHOD TO ESTIMATE AVERAGE PADDY YIELD IN SRI LANKA OVERCOMING THE LIMITATIONS OF THE CURRENT METHOD

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ABSTRACT

The Department of Census and Statistics of Sri Lanka carries out an island-wide scheme of estimating paddy's average yield, called the Crop Cutting Survey. It was debated and argued about the accuracy of the data generated through a cumbersome approach which consumes a lot of labour and resources. The objective of this study was to develop an efficient method that would help overcome the limitations of the current method. This study was accomplished through a questionnaire survey and an experimental survey. The GIS technology has been applied to measure the cultivated area and yield measurements by weighting the harvested yield. The weight measurements were subjected to different scaling factors derived through the study. The sample survey of the new methodology was carried out in the Ampara district for 2 paddy varieties namely BG357 and AT362 for the 2017/2018 Maha season. A stratified Random sampling method was used for the selection of paddy parcels. Average paddy yield, scaling factors, and cost of production of these two paddy varieties were calculated separately. The district's estimated paddy yield through the proposed method is 4568.17 kg/ ha, which is not significantly different from the published average (4562 kg ha^{-1}) in paddy statistics of 2017/2018. This is obvious that the proposed method was more effective and efficient than the current method, which assists the government in planning and making policy decisions at the correct time.

Keywords: Crop cutting survey, Paddy, Scaling factor, Yield estimation

INTRODUCTION

Rice is known to be one of the topmost important staple food crops, mainly grown based on three irrigation regimes in Sri Lanka as major, minor, and rainfed irrigation schemes. Consequently, the estimation of paddy yield is one of the vital actions undertaken by government departments to screen the development of the sector and offer insurance on time to the sector. Therefore, reliable and timely estimates of rice crop areas and their production capacity are essential for providing information for decision-makers to formulate appropriate policies in the case of deficit or surplus. The accurate estimation of rice yield is important to assure food security and promote the country's sustainable development. It is needed to import in case of setback or to send out in case of surpluses, especially in locales characterized by climatic vulnerabilities; therefore, determining crop yield before harvest is prominent (Sawasawa, 2003). The Sri Lankan Census and Statistics Department carries out an island-wide investigation at the district level to estimate the average yield of paddy, called the Crop Cutting Survey. It was debated and questioned the quality of the data generated by a cumbersome approach that consumes a lot of labour and resources. Based on crop-cutting sample data from the districts, the average yield per acre/hectare of paddy is calculated in the Colombo headquarters, and paddy production is calculated on a seasonal basis using a complete list of paddy crop cutting survey results based on hundred percent enumeration of paddy growing parcels during the particular season and area. Crop yield estimates in many countries are based on traditional techniques of crop selection. The yield estimation was based on field-based visits and reports and is often arbitrary, expensive, time-consuming and vulnerable to greater error due to inaccurate observations of soil, leading to a low assessment of crop yield and estimate of the crop area (Reynolds *et al*., 2000). However, such a technique has three major drawbacks, such as being time-consuming, subjective and prone to significant inconsistencies due to insufficient ground observations, which result in the poor evaluation of crop output. (Prasad *et al.*, 2006).

Many studies using modern techniques have been done to estimate rice yields. Some of those techniques are remote sensing, microwave imagery and crop modelling (Chang *et al*., 2005). Remote sensing techniques can provide quantitative and instantaneous information on crops in wide areas (Chang *et al*.,

2005). Additionally, NASS of the USDA (2009), stated that remote sensing-based sampling methods were good solutions for large-area crop acreage monitoring systems. The benefit of using remote sensing methods to predict rice production is it allows government planners and decision-makers to devise effective policies to calculate either how much to import in the event of a shortage or else, if possible, to sell in the event of a surplus and to buy rice at a comparatively lower price at the appropriate moment without any delay (Noureldin *et al*., 2013).

According to the census of agriculture, a bi-annual crop cutting survey collects paddy statistics in three main aspects of paddy extent, average yield and production but in the existing methodology of crop cutting survey has some limitations like; there are nearly 20-30 experimental plots are needed for one divisional secretary division, high non-sampling errors; because only 1/3 of sampling is done by the department of census and statistics (Saddhananda., 2022).

The initiation of paddy crop cutting experiments took place in 1952, overseen by the Department of Census and Statistics in Sri Lanka, which holds responsibility for this (Saddhananda., 2022). Subsequently, paddy production witnessed a notable increase due to the Mahaweli Accelerated Development Multipurpose Programme and the successful completion of various related agricultural projects (Saddhananda., 2022).

In agrarian services, approximately two-thirds of the paddy data collection work is completed. The over estimation of cultivated land is common as, a result of inaccurate information provided by farmers in hopes of receiving fertilizer subsidies. Additionally, there is no effective system in place to cross-check or monitor this data. Moreover, small-scale farmers often do not measure their yields due to the bulkiness of measuring tools, and field officers are hesitant to carry these tools to the fields because it is time-consuming and labor-intensive. Furthermore, practical difficulties, such as delayed estimations, fields that have already been harvested, and inaccessible fields, contribute to the unreliable nature of the data, as officers may not report accurate information in a timely manner.

This study revealed a new approach for crop-cutting surveys utilizing GIS technology, with the objective of developing an efficient and effective method for paddy yield estimation that will assist in overcoming the existing system's shortcomings. Additionally, to determine the scaling factors for the AT362 and BG357 rice varieties, as well as to quantify the cost of paddy production in the 2017/2018 Maha season in the Ampara district, and to compare those values to various statistical references.

METHODOLOGY

The selected study area was the Ampara district consisting of 19 divisional secretariats divisions. They are Damana, Mahaoya, Pothuwil, Lahugala, Alayadiwembu, Akkarapaththu, Eragama, Sammanthurei, Uhana, Dehiaththakandiya, Padiyathalawa, Kalmunai, Karthivu, Navinthanveli, Ninthavur, Ampara, Thirukkovil, Sainthamarathu, Addalachchenai (Figure 01). The selected study area belongs to DL_2 agro-ecological region which belongs to the Eastern province.

Fig. 1. 19 DS divisions of the Ampara district

The sample size was 76 paddy parcels, and a stratified and Random sampling technique was used to select paddy parcels**.** Paddy parcels are the rough sketch of the selected paddy field which the sample is ***drawn. The*** proposed protocol was tested with the varieties BG357 and AT362**.** Here, divisional secretariats divisions were used as a stratum and paddy variety was used as a substratum and from each substratum, two paddy parcels were selected randomly. GIS technology was applied to estimate the area under cultivation of selected paddy parcels. The harvested rice plants of the crop cutting experiment have to be collected to the gunny bag and wrapped gunny bag with rice plants should be brought to the threshing floor.

Paddy statistics and extent of cultivation data was collected through a questioner led in 19 DS divisions concerning the BG357 and AT362 paddy varieties.

The area of each paddy parcel was measured by using GIS technology and GPS coordinates of each paddy parcel were taken by using the Google Maps mobile phone app. At that point area of each paddy parcel, was measured by using the Google earth pro application. Then the yield of each paddy parcel was measured and the average weight of a filled yield bag and partially filled bags were weighted separately. Afterwards, total numbers of fully filed paddy yield bags and partially filled bags were counted. Total paddy yield for each

paddy parcel just after harvesting was calculated and then total dried stage paddy yield for each paddy parcel was calculated with the assistance of separate dried stage scaling factors according to the paddy varieties and the average paddy yield of each paddy parcel was calculated separately. At last, calculate the average paddy yield for two related varieties separately in the Ampara district. 20 number of 5kg paddy samples were taken (10 by each variety) at the time of just after harvesting. Paddy yield samples were dried for an optimum temperature level separately and weighed after drying. Then dried samples were milled and weighed. In the end, average scaling factors were calculated separately for two varieties at the drying and milling stages. The cost of production is composed of the cost of seed paddy, the cost of bed maintenance, the cost of land preparation, the cost of bund preparation and sawing, fertilizer application, other applications, and harvesting.

When estimating of average paddy yield, the assumed area of each paddy parcel was measured by using google earth pro application (A), total numbers of fully filed paddy yield bags were counted as (N), and an average weight of a filled yield bag was measured as (M) and therefore total weight of filled bags (M_1):

$$M_1 = M \times N \quad \text{---------- Equation [1]}$$

The height of a filled paddy yield bag (H) and the height of a partially filled bag (h), then the weight of a partially filled bag (m);

$$M = \frac{h}{H} \times M \quad \text{--------- Equation [2]}$$

The total paddy yield for each paddy parcel at the stage of just after harvesting(M_2) ;

$$M_2 = M_1 + m \quad \text{---------- Equation [3]}$$

The total dried stage paddy yield (Y) for each paddy parcel using dried stage scaling factors (x) according to the paddy varieties and average paddy yield (Q) of each paddy parcel was calculated separately using the equations;

$$Y = m^2 \times x \quad \text{---------- Equation [4]}$$

$$\frac{Y}{A} = Q \quad \text{---------- Equation [5]}$$

When calculating the average paddy yield for two relevant varieties (Q1 – Values for average paddy yield of variety 1, Q2- Values for average paddy yield of variety 2, n – Number of paddy parcel), the equations (Σ Q1 / Σn →for paddy variety 1) and (Σ Q2 / Σn →for paddy variety 2) were used.

Additionally for the calculation of the scaling factors;

$$SF_{After\ drying} = \frac{M_{Dried}}{M_{Harvesting}} \quad \text{---------- Equation [6]}$$

$$SF_{After\ milled} = \frac{M_{Milled}}{M_{Harvesting}}$$

In these two equations, the weight of 10 samples just after harvesting ($M_{Harvesting}$), the weight of dried samples (M_{Dried}) and the weight after milled (M_{Milled}) and scaling factors (SF). For calculating average Scaling factors:

$$ASF_{After\ drying} = \frac{SF_{After\ drying}}{n} \quad \text{--- Equation [7]}$$

$$ASF_{After\ milled} = \frac{SF_{After\ milled}}{n}$$

RESULTS AND DISCUSSION

The graph showed that the average paddy yield of AT362 (Figure 02) was 4490.50± 578 kg/ ha (1881kg/ac) whereas the average paddy yield of BG 357 was 4645.85± 432 kg/ ha (1818 kg/ ac) (Figure 03). There is no significant difference between the average paddy yield of AT362 and BG357 paddy varieties ($P<.01$). According to the rice varietal distribution in Sri Lanka (2017), Bg 94-1, AT362 and BG357 are the main three varieties spread in the Ampara district (Figure 04).

The average yield of these two varieties was 4,568.17 kg/ha which was estimated using this study formulated methodology. According to Paddy statistics (2017/2018), district average yields were 4,562 kg/ha which was estimated by using the existing crop-cutting survey method. STD error of district average was 105 kg/ha according to paddy statistics, whereas according to the study calculation, it was 81 kg/ha. The lower confidence level of the district average was reported as 4,357 Kg/ha, whereas the upper confidence level was 4,767 kg/ha in paddy statistics while it was 4,409 Kg/ha and 4,728 kg/ha respectively, which was calculated by the formulated methodology.

According to the paddy statistics (2017/2018), the average yield of paddy estimated for the 2017/2018 Maha season was 4,302 kg/ha. The highest average yield of 6,355 kg / ha was reported during this season from Udawalawe special area. The second-highest average yield of 5,773 kg/ha was reported from the Hambantota district. The estimated paddy production for the (2017/2018) Maha season was 2,396,926 MT. This is about a 63% increase compared with the previous Maha season.

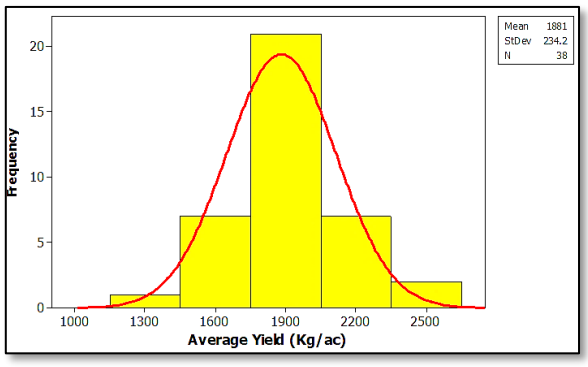

Fig. 2. Shows the average yield of AT362 (kg/ac)

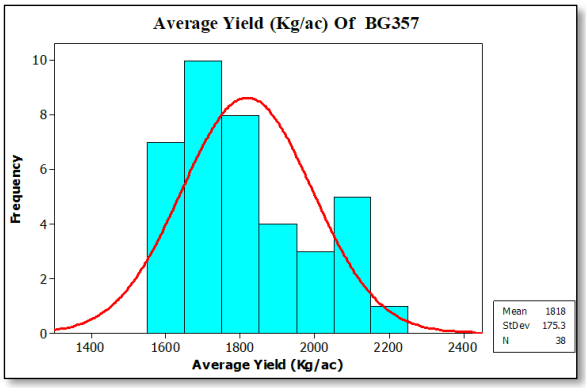

Fig. 3. Shows the average yield of BG357 (kg/Ac)

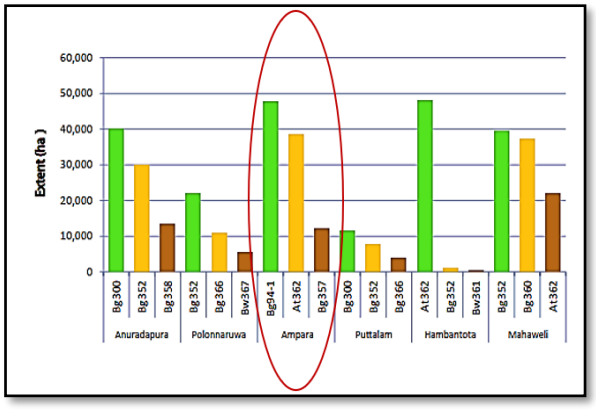

Fig. 4. Shows the spread of three major rice varieties in Ampara district

The highest production of 332,767 MT of paddy was valued from the Ampara district and it was responsible for about 14% of paddy production of the country. Only 61,028 ha was sown in Ampara district (2017/2018) Maha season (major schemes) whereas 60,974 ha was harvested and the average yield of Ampara district (major scheme) was 4,803 kg/ha.

The scaling factor after the drying stage of AT362 is 0.93516 ± 0.007 and for the BG 357 is 0.91260 ± 0.01. There is no significant difference between AT362 and BG 357 (P<.01). The scaling factor after the milling stage of AT 362 is 0.671 ± 0.01 and for the BG 357 is 0.6445 ± 0.02. There is no significant difference in Scaling factors after the drying stage and milling stage between AT362 and BG 357(P<.01).

The cost of production of AT362 is 32.67 ± 5.7 Rs/kg, whereas BG357 is 34.44 ± 4.85 Rs/kg for milled rice. Hence the study discloses that the cost of production of BG357 was a little higher than the AT362. Therefore, it is profitable to cultivate AT362 than the BG357. However, the Central Bank report (2017), reported that the cost of production in the 2015/2016 Maha season was 18.82 Rs kg^{-1} in the Ampara district. Obviously, the calculated figures from this study are closer to the figures reported by different statistics reports. Therefore, this study suggested a suitable alternative application that overcomes the difficulties of the current crop-cutting survey, and this method is effective and more efficient.

CONCLUSIONS

The average yield of AT 362 and BG 357 varieties were 4,568.17 Kg/ha which was estimated using this study formulated methodology. According to Paddy statistics (2017/2018), district average yields were 4,562 Kg/ha which was estimated by using the existing crop cutting survey method. Hence it was obvious that calculated average paddy yield through the existing methodology and proposed methodology was quite similar and there was an assurance for the accuracy of the proposed methodology. According to the results, BG362 has a higher scaling factor than BG357. Hence, the technical efficiency of the paddy variety is higher in AT362. when considering the scaling factor, it was profitable cultivating the AT362 variety than BG357. Thus, the study discloses that the cost of production of BG357 is much higher than the AT362. When considering only the cost of production it is profitable of cultivating AT362 than the BG357.

The methodology introduced through this study was a suitable application to overcome the limitations of the conventional crop-cutting survey method. This effective and efficient method enables the government to make timely policy decisions and planning. Ultimately, it greatly contributes to regional development and the national economy.

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RECENT ADVANCEMENT IN AGARWOOD INDUCTION TECHNOLOGY: A COMPREHENSIVE REVIEW FOR THE TRANSFORMATION OF ARTIFICIAL AGAR RESIN INDUCTION METHODS

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ABSTRACT

Agarwood is a high valued resinous wood containing aromatic constituents of sesquiterpenes and phenyl-ethylchromones. They are typically found in the wood tissue of Thymalaeaceae family trees such as Aquilaria and Gyrinops species once they have been physically or chemically damaged or diseased by microbial pathogens. However, the natural occurrence based agarwood production is inadequate to fulfill the worldwide market demand as it never reach to the potential yield. As a result, recent advancements in artificial agarwood induction technology have led to the efficient production of agarwood resin, surpassing conventional methods. These technologies include mechanical, biological, and chemical methods, as well as combinations of these techniques such as fungal inoculation, nailing, drilling, partly trunk pruning, aeration method, agar-wit, C-A kits, Agar-sit, Bottle dipping, ChemJet, Pinhole infusion, Automated transfusion, Agar-Bit and Bamboo stick method. While interdisciplinary approaches have pros and cons, chemical inducers have shown rapid invasion inside particular tree species trunks to produce superior agarwood resin at consistent rate. Therefore, diverse forms of inducers can be utilized to develop this finest fragrance resinous wood in cultivated trees within a short period of time compared to the natural approach and almost similar in the quality compared to the natural agarwood formations. The objective of this review paper was to presents a comprehensive collection of agarwood resin inducing methods and their potential to enhance the total production and the quality of agarwood in the final harvest. The application of these techniques has significant implications to the agarwood industry as it seeks to meet the growing global demand for this highly sought-after and valuable product leading to a high profitable business. Therefore, this review article serves as a valuable collection of resource for the researchers and industry professionals who are ambitious to develop their agarwood industry to a new level.

Keywords: Agarwood, Aquilaria species, Artificial methods, Fragrance industry, Resin induction

INTRODUCTION

Out of the forest products in the world, Agarwood is one of the most luxurious and expensive commodities that has a higher market demand via ranked as the most valuable number one product in the world out of the available plant-based aromatics (Kanazawa, 2017; López-Sampson and Page, 2018). This fragrance resin is formed due to a self-defense mechanism in the genera Aquilaria and Gyrinops, that in the family Thymalaeaceae. However not each species of this genus produces high-quality agarwood, and not all release the desired aromatic odor. In the natural habitat, around 10% of the agarwood producing Aquilaria plants may contain this resinous wood (Ngadiran *et al.,* 2023). Agarwood formation will be initiated when the self-defense mechanism activates inside the tree and the sickness of the trees are responsible for this resin formation. (Zhang *et al.,* 2012). The trees are particularly responsive to physical damage, chemicals, and microbial infections and produce agarwood. There are natural methods and artificial methods for agarwood induction. Agarwood resinous develops naturally only when trees are exposed to particular environmental conditions. For example, wildfires, grazing, attacks stem boring insects, lightning strikes, or microbial activities (Liu *et al.,* 2019; Fitriasari *et al.,* 2021). Agarwood resin formation in a natural manner is a very rare phenomenon in the wild up to the expected severity and when considering commercial agarwood production, artificial inoculation methods should be practiced. Without the inoculation process, only 1-2% yields can be expected from the full potential, and the productivity of the trees is not enough for profit gaining. Furthermore, natural development takes time more than artificial methods. Generally, this takes around 25 to 30 years (Chowdhury *et al.,* 2016; Wu *et al.,* 2017).

In fact, the low rate of agarwood availability in the natural habitat leads to insufficient supply to reach the market demand (Chhipa and Kaushik, 2017). Therefore, these species are dangerously close to going extinct as a direct result of the illegal harvesting of trees in the wild and the limited availability of

agarwood development inside those plants (Lee and Mohamed, 2016). Therefore, cultivating the agarwood-producing trees became the trend and established the industry in a legal manner. These farmers use a different kind of techniques to initiate the formation of agarwood as well as get a productive harvest from those methods. Some of them are conventional methods and some are novel techniques that have been developed by going through scientific research. Indigenous methods such as placing nails, chopping the tree by using an axe, or making pinholes, burning, trunk splitting, and extensive removal of the outer bark were used commonly by small-scale farmers and wild agarwood hunters (Tian *et al.,* 2013; Yan *et al.,* 2019). There also some techniques that the farmers were used to develop the agarwood inside the trees by using beneficial insects and snails by providing a desirable environment for them in the trees. Such as drilling the main branches, trunk, and roots of older trees facilitate a desirable environment for snails and insects like ants within the trees (Akter *et al.,* 2013).

Conventional methods for agarwood development are more affordable, but those methods are labor-intensive and time-consuming. Furthermore, the amount of agarwood production is low and cannot predict the quality of the resinous wood and the extracted oil (Tan *et al.,* 2019). Also, as a result of some methods that under conventional methods badly influence the tree by which frequently inhibiting the tree's growth, in some cases the tree died after practicing these methods. (Liu *et al.,* 2013). Because of this, the agarwood industry always seeking novel and efficient methods to induce resinous production in order to protect wild resources from such declines. Many artificial methods for inducing agarwood resin have been developed to produce agarwood that has higher quality and is similar to agarwood that were in natural habitats.

Nowadays, the industry utilizes combination methods that are based on physical damages and use different kinds of chemical inducers as well as bioinoculants (Faizal *et al.,* 2020). The invention of a synthetic technique for agarwood production has a twofold objective: enhancing both the yield and the quality of both the raw agarwood resinous wood and the extracting essential oil. Nonetheless, these distinct strategies have their own set of pros and cons. In this article, we have endeavored to provide a detailed and up-to-date account of the various technologies employed for agarwood production. Our review encompasses a broad range of methods and techniques, including both traditional and modern approaches.

Agarwood Formation

Agarwood is formed after the wound infection of healthy plants, and better healthy sound trees never produce agarwood (Rasool and Mohamed, 2016). Initially, it remains in tylose or gel form after initiating the formation. Agarwood's antimicrobial and anti-disease properties are due to sesquiterpenes (Xu *et al.,* 2013). The development of Agarwood within trees is unrelated to the diameter and height of the plants, as well as the volume of timber. However, some resin characteristics and aroma properties can be influenced by species, environmental variations, genetic variation of species (Ngadiran *et al.,* 2023), and geographical distribution, as well as the inoculation method (Subasinghe and Hettiarachchi, 2015). Furthermore, seasonal changes and rainy weather can hasten this formation faster than other seasons (Chowdhury *et al.,* 2016). Previous studies show factors like soil fertility, temperature, light intensity, and humidity influence this formation process. When it comes to yield, plants grown in poor soil produce more agarwood than trees grown in rich soil. However, when soil conditions are in a good manner, trees develop well and produce resin for their self-defense throughout the inoculation period, contributing to high-quality agarwood (Hamdan *et al.,* 2021). Plants are more susceptible to infections that are cultivated at higher elevations (Turjaman *et al.,* 2016). Also, the ability to produce agarwood in juvenile trees is higher than in matured trees (Rasool and Mohamed, 2016).

Agarwood formation takes several years, and older trees respond to inoculation more slowly than younger trees, implying that agarwood formation in older trees is uncommon (Liu *et al.,* 2013). In contrast, even three-year-old cultivated trees can produce agarwood after artificial treatment (Rasool and Mohamed, 2016). It was confirmed through a chemical analysis by Rainforest Project (TRP) in Vietnam (Ngadiran *et al.,* 2023).

The agarwood formation is linked to starch and sugar metabolism and electron microscopic observations of *A.sinensis* confirmed this. The healthy wood contained a large number of starch granules, which were degraded after the wounds were placed and the Agarwood accumulated (Xu *et al.,* 2013). When it comes to agarwood formation, living parenchyma cells are the most valuable component because they perform the biosynthetic process of resinous substances (Rasool and Mohamed, 2016).

Inoculation methods

The inoculation process is critical for the formation of agarwood within the Thymelaeaceae family species. Because the tree's self-defense mechanism should be activated, and resins should be produced because of the tree's illness. The theory behind this inducing is that the trees are being intentionally damaged (Zhang *et al.,* 2012).

There are two ways to inoculate the trees. Those are natural and artificial. Inoculation of trees occurs rarely

naturally, and when comes to commercial agarwood production, artificial inoculation methods should be used. The productivity of the trees is insufficient for profit without a synthetic inoculation process. When all these methods and inducers are considered, the chemical composition and quality of the outcome can be varied (Lee and Mohamed, 2016).

Natural formation

Gnawing ants and other insect damages, as well as other natural damages to trunks or branches, are commonly responsible for these damages. Bacterial infections, physical damage from wind, and lightning strikes may also be factors in the formation of agarwood inside trees (Xu *et al.,* 2013). Some caterpillars also bore the trunk, causing agarwood to form (Turjaman *et al.,* 2016). It has been observed that these things have a very low probability of occurring and naturally inducing the agarwood (Subasinghe and Hettiarachchi, 2013). It around 7% of trees are produce agarwood in their natural habitat (Chowdhury *et al.,* 2016). When comes to *Aquilaria spp.*, it's about 10% (Liu *et al.*, 2013).

Artificial inoculation techniques

Artificial agarwood induction is a successful approach for obtaining a fruitful agarwood harvest from commercial agarwood plantations (Yin *et al.,* 2016). The traditional method of inducing agarwood is to make wounds in the tree with sharpened tools. Chemical methods are also used for this, particularly in large commercial-scale agarwood plantations. It is the most recent method used in this industry (Subasinghe and Hettiarachchi, 2013). Among these artificial methods, small-scale and large-scale farmers commonly use nailing, drilling, aeration, agar-wit, partially-trunk pruning, burning-chisel drilling, fungal inoculation, CA kits, agar-sit, agar-bit, bamboo stick method, etc. (Liu *et al.,* 2013). The chemical-inducing approaches are more effective and cost-effective, and they are also easier to practice than others. Moreover, results are also provided in a timely manner (Rasool and Mohamed, 2016). However, in some countries, this method has become unpopular due to the environmental impact of these chemicals leaking into the water and causing soil pollution (Turjaman *et al.*, 2016). Indigenous techniques, such as peeling off the bark and promoting infection, are also used in some countries to induce this process. There could be other reliable artificial methods for producing agarwood (Chowdhury *et al.,* 2016). The method of inoculation can have an impact on the agarwood's quality. For example, axe chopping, holing, and nailing yield low-grade agarwood. According to studies, the resin and oil content of chemically induced agarwood is higher than that of wild agarwood (Zhang *et al.*, 2012).

According to research, pure water cannot induce the formation of agarwood within trees (Liu *et al.*, 2013). If the trees are heavily attacked by insects such as *Heortia vitessoides*, the endophytic fungal inoculation process is not recommended because the trees are already sick and may die. (Turjaman *et al.*, 2016).

Fig. 1. Extracted agarwood A) Naturally formed resinous wood extracted from *G. walla* B) Artificially induced resinous wood extracted from *A. crassna*

Fungal inoculation technique

The basic principle of this method is the development of fragrance resin inside the tree by making wounds and inoculating the tree with beneficial fungi species (Ngadiran *et al.,* 2023).

To produce this artificial infection, pure or mixed fungus strains can be utilized in the fungal inoculation procedures. The wood gets a richer or dark crimson color after a fungal infection (Elias *et al.,* 2017). An infection may enhance resin synthesis when the host responds to the rise in infection induced by fungal growth. The variety of sesquiterpenes retrieved from mycological metabolites is related to these compounds' strategies for cooperating with other species and defending themselves, culminating in the creation of resinous wood (Rohlfs and Churchill, 2011).

The fungi are inoculated into 8 cm deep holes drilled in the tree trunk. 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 (Chowdhury *et al.,* 2016).

Fig. 2. Fungal inoculation by drilling and injecting inoculum

Generally, natural inoculation can be possible by *Aspergillus spp., Botryodyplodia spp., Diplodia spp., Fusarium bulbiferum, F.oxysporum, F.laterium, F.solani, Penicillium spp.,* and *Phythium spp*. like species. And these can benefit from the immunization process (Ngadiran *et al.,* 2023). *Cunninghamella, Curvularia, Lasiodiplodia*, and *Trichoderma* are also used for this purpose in some cases. However, *Fusaruim solani, Cunninghamella bainieri,* and *Lasiodiplodia theobromae* are commonly used in this Inoculation process (Rasool and Mohamed, 2016). *Fusarium solani* is the most effective agarwood-forming agent (Turjaman *et al.,* 2016). Tunstall first used this fungi inoculation technique in 1929. (Liu *et al.,* 2013).

In addition, to use a single fungus, some studies used a combination of species to inoculate *Aquilaria spp*. and examined formulations (Justin *et al.,* 2020; Ma *et al.,* 2021). In addition to *Aquilaria spp.,* and *Gyrinops versteegii,* Domke has been infected with a variety of fungal inoculants, including *F. solani, Rhizopus spp*., and *Trichoderma spp* (Mega and Nuarsa, 2019). *F. solani* strains, Gorontalo and Jambi were inoculated into *G. versteegii* trees in another study and resulted in agarwood formation (Faizal *et al.,* 2020)

When practicing this method high humidity, as well as the carbon sources availability and energy sources, can promote fungal growth. However, not all fungi can initiate the agarwood induction process. When using this method, triangle factors play an important role in agarwood formation in these trees. In that case, the tree as the host, Endophytic fungi as the inoculating agent, and the Environmental are the three triangle factors (Turjaman *et al.,* 2016).

Drilling method

An electrical hand drill is typically used to place drill holes in the tree trunk, limbs, and main branches (Akter *et al.,* 2013; Faizal *et al.,* 2017). The drilled pores were spirally placed from the ground up to the crown. Drill holes were spaced 3 to 5 cm apart and then infected with agarwood-inducing powder or remaining open to allow natural agents easier access (Chowdhury *et al.,* 2016). This is a strategy for attracting insects to the tree to infect it. To speed up the infection, the liquid syrup can be added to pores and attract natural agents such as beneficial insects to it. Every 2-3 months, pores are examined and wounded again (Ngadiran *et al.,* 2023).

Nailing method

In the past, the most common method was nailing. Hammering nails into the trunk is used in this method. However, the quality of the agarwood produced by this method is not as desired, and it has a low market value (Persoon, 2007). In some cases, when the nails were installed, they were partially inserted into the trunk (Chowdhury *et al.,* 2016). When using this method, a grown tree required an average of 20 kg of nails, which were placed every 10 cm along the tree's length (Turjaman *et al.,* 2016).

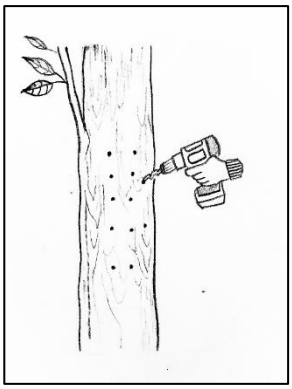

Fig. 3. Drilling method

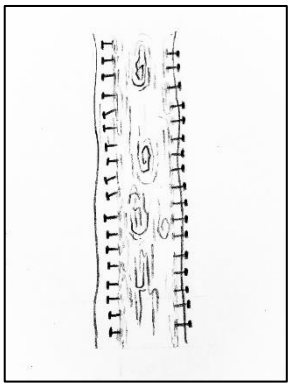

Fig. 4. Nailing method

Burning chisel drilling technique

The Burning-Chisel Drilling (BCD) method is also based on drilling the tree trunk at a higher temperature. The basic principle of this also creates wounds in the tree and activates its self-defense mechanism. The iron chisel or a drill bit used here is heated to Red-hot around 600^0C and is 1.2 cm in diameter. The holes created by the drilling will be approximately 20cm apart. Following that, the holes should be instantly sealed by using sterilized paraffin wax and it avoids contamination by detrimental microbes (Chowdhury *et al.,* 2016). It is best to avoid making holes up to 50 cm from the ground (Liu *et al.,* 2013).

Partly trunk pruning technique

Partly trunk pruning is practiced by sawing along a side of the tree trunk of the tree and placing cuts that are wide around 2-4 cm and 3-5 cm in depth. The lowest cut should be approximately 50 cm above the ground level. The distance between each pair of cuts should be about 20 cm. It is comparable to axing (Liu *et al.,* 2013). A study shows that the characterization of *A. sinensis* wounded tree trunks induced by BCD and the Agar-Wit method is similar to that of formed resinous wood by PTP. Moreover, the chemical composition, as well as vessel-occlusion formation, are similar to those methods (Zhang *et al.,* 2014). Chinese farmers have increasingly used both burning chisel drilling and partial trunk pruning in recent decades (Liu *et al.,* 2013)

In some areas, indigenous agarwood hunters remove the bark by peeling it to stimulate infection and harvest wood chips from live trees seasonally (Pojanagaroon and Kaewrak, 2003). Agarwood hunters in Papua New Guinea wounded agarwood inducing tree species and initiate agarwood production inside them, and around three years later hunters were able to harvest resinous wood of average quality (Gunn *et al.,* 2003).

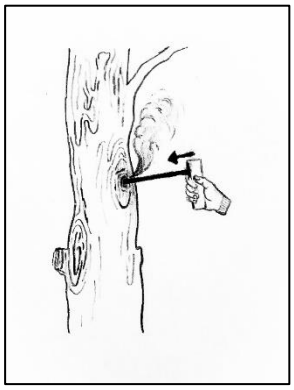

Fig. 5. Burning-Chisel Drilling method

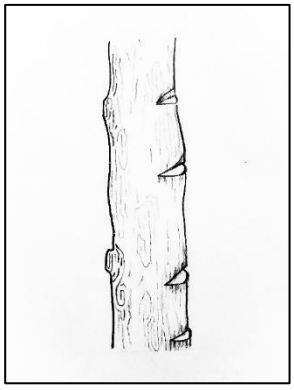

Fig. 6. Partly trunk pruning technique

Aeration method

The basic principle of this method is introducing a foreign material into a wound that is created artificially and prevents pores from healing and maintaining a long-term infection (Liu *et al.,* 2013). This therapy induces the tree's self-defense mechanisms to respond. The introduced aeration device may have holes in it or grooves on its outside surface or both. Introduce device could be made of plastic, bamboo, proffered wood, or another organic substance, or it could be made of metal, such as iron. Mostly this device is with 2cm diameter. When implanting this, it may extend 2 to 15 cm from the tree's exterior.

A resin-inducing substance may also be applied to the cells around the wound in this approach. It has the potential to kill the parenchyma cells in the vicinity of the xylem's injured region. It could be sodium bisulfate, iron powder, NaCl, chitin, ferrous chloride, ferric chloride, cellobiose, or yeast extract, for example. Also, sodium bisulfate solution, Difco yeast extract, or iron powder can use as a 1:1:3 ratio (Ngadiran *et al.,* 2023). Also, the inducing agent for agarwood formation could be an organism, such as an insect, microbe, or a fungus like *Deuteromyota sp*., *Ascomycota sp*., or *Basidiomycota sp*. This method has the ability to produce agarwood ten times faster than natural agarwood production (Chowdhury *et al.,* 2016).

Agar-wit method

Whole tree agarwood induction (Agar-wit) is a high-quality agarwood-inducing method that has been available since 2013. (Liu *et al.,* 2013). This is a chemical-producing method used in industry (Rasool and Mohamed, 2016). This method can produce agarwood within 20 months of applying the treatment to the plant (Zhang *et al.,* 2012). The inoculation process will involve drilling holes in the trunks of the trees up to the xylem and injecting inducers into these holes using a transfusion set. The 5 mm holes will be placed in the tree by avoiding 50 cm of trunk from the ground level. Electrical drills are typically used for drilling.

The benefit of this method is that the natural flow of water in the xylem transports the inducer throughout the tree due to transpiration. As a result, Agar-wit can produce systemic agarwood from the stem of the entire tree. Within a few months, the resin will be produced around the wound (Liu *et al.,* 2013). Low-pH substances such as formic acid, as well as high-pH substances, can be used in this method to induce agarwood inside the trees by killing the live cells (Chowdhury *et al.,* 2016). This method produces superior-quality agarwood with a high resin content as well as ethanol-soluble extract content. When compared to other methods, this method produces a consistent agarwood yield (Liu *et al.,* 2013) in a short

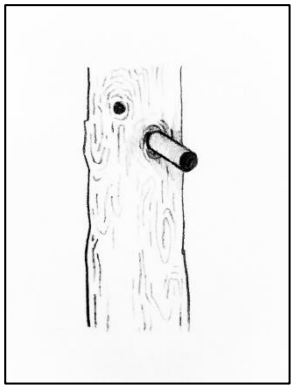

Fig. 7. Aeration method

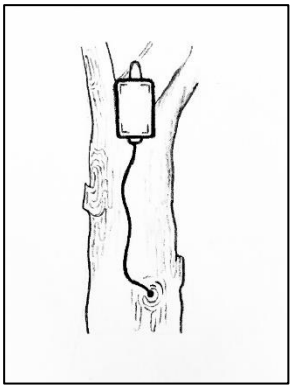

Fig. 8. Agar-wit method

period of time (Zhang *et al.,* 2012).

Cultivated agarwood kits

The technology comprises a Cultivated Agarwood Kit (CA Kit) for each tree treatment, which contains specific tubes and capsules containing a non-hazardous chemical substance (Blanchette and Chowdhury, 2009). Blanchette from the University of Minnesota in Vietnam invented CA-Kits. To establish CA-Kits in trees, numerous holes should be placed in tree trunks, placing the holes from the base of the stem to the upper end of the stem. As a result, using the CA-Kit, agarwood can be induced and produced near the holes. The basic theory behind this method is to keep the incision open with a small plastic pipe, and then inoculate it with different chemical media into the prepared wound. This chemical therapy is far superior to previous physical wounding treatments since it allows for easy examination of the discolored region (Rasool and Mohamed, 2016).

Agar-sit method

To obtain agarwood development meanwhile avoiding the cutting down of a whole tree for harvesting agarwood and using fungal inoculant for agarwood production, an effective approach known as the “Trunk Surface Agarwood-Inducing Technique”

or “Surface Inducement Technique” (Agar-sit) was developed. This technique First, roughly 50 cm of bark was removed to reveal a rectangular xylem surface. The bark remained attached to the tree trunk for future coverage. Using a knife, grids (2cm×2cm) 1.5±2.0 cm deep were created. One tree yielded several rectangular barks. between the upper and lower rectangular surface, the distance will be maintained at 20 cm, while the distance between opposite ones on opposite sides of the tree trunk was maintained at 5 cm. The inoculant liquid that contains beneficial fungus was sprayed into the grids by using a watering container or applying with a brush. The infected xylem was afterward enclosed by the original bark. Then six months later, the surface degradation layer was removed, and the agarwood created was severed, leaving the xylem open for another round of agarwood induction.

This strategy protects trees while also making agarwood collection more convenient. Meanwhile, there was a large output and high quality of agarwood available, as well as the possibility of recurrent agarwood creation from the same tree, and this method is well-known among farmers who cultivate agarwood for oil production. This strategy, however, is strongly recommended for trees older than 10 years (Chen *et al.*, 2018).

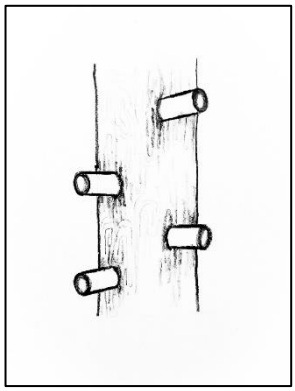

Fig. 9. CA Kit method

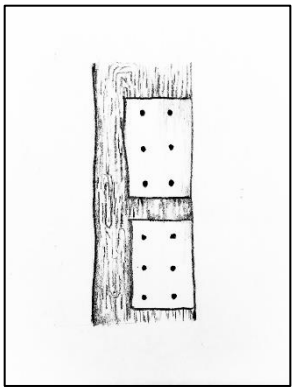

Fig. 10. Agar-sit method

Bamboo stick method

In general, the bamboo stick method is also known as the knocking method (KM), and it is practiced alone on agarwood producing trees as well as in combination with the injection method. In this method mainly bamboo sticks or culms are utilized to convey a chemical agarwood inducer as well as a part of the maintaining the aeration process. Because of the microfibrils that surround the epidermis, parenchyma cells, also vascular bundles, it has outstanding mechanical capabilities for this work (Habibi and Lu, 2014). When bamboo sticks or culms are introduced into a tree's trunk, it slowly releases the agarwood inducer into the surrounding tissues of the tree trunk, resulting in a protracted period of fighting between the tree's defense mechanism and then the stress created by the inoculant. The dynamic interplay of trees and constant stress results in the formation of agarwood. A layer of resinous wood is gradually created, and if the ideal atmosphere is fulfilled, this agarwood creation thickens. If the tree is in good health and the tree's defensive mechanism wins, the treated bark will most likely end up in white tissue, where the wounded bark has recovered. An external force from a bamboo stick, on the other hand, can cause major harm, even death, if the treated tree is weak. Farmers can often gain about 5-10 grams of agarwood per hole after about a year and a half of successful treatment using this method. However, a longer time period is preferable for a healthy yield. Furthermore, the harvest quality is comparable to natural Agarwood.

Independently performing the knocking method (KM) as follows, an electric impact drill bit was used to drill two holes per 10 cm2 region of stem, 5 cm deep into the phloem or xylem, in a spiral pattern from the ground of the tree trunk. Bamboo sticks are soaked overnight in the agarwood inducement solution and were placed into each hole. Those sticks are 6 cm long and 1 cm in diameter (Peng et al. 2021). Agarwood inducement solution will be made by using acetic acid, sodium chloride, as well as fruit enzymes (Ngadiran *et al.*, 2023). After that, the bamboo sticks will be placed into each hole.

The knocking method, which is combined with the injecting method, is accomplished in the same manner as the previous. After that Using the injecting and knocking methods alternately, the agarwood inducement solution was applied to each hole.

In terms of quality, the procedure effectively produced agarwood that nearly resembled natural agarwood. 2021 (Peng et al. 2021).

Bottle dipping method

Bottle dipping is a procedure that involves placing the bottle to gradually drip the agarwood inducer into the holes for inoculating the trees. Parafilm will be used to prevent inoculant leaking and connect the bottle to the wound via the hose, and close it by using clay (Justin *et al.*, 2020). By using this method of bottle dripping, approximately 10 mL to 20 mL inoculants can be delivered in each hole, and a total of 1 to 3 L of inoculant delivered per tree (Mustapa *et al.*, 2022).

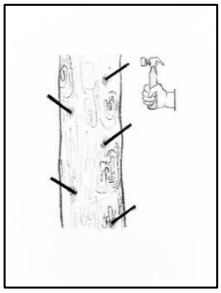

Fig. 11. Bamboo stick method

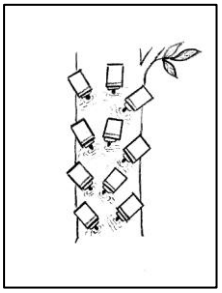

Fig. 11. Bottle dipping method

ChemJet method

The ChemJet approach is similar to the bottle dipping in that the pressurized injection is placed upside down into the hole and dripped gently into the lesion. In this case, also, the same amount of discharge can be delivered to the holes of the tree and a total of 1-3 liters of inoculum will be delivered into a single tree (Mustapa *et al.*, 2022).

Pinhole infusion method

To practice the pinhole-infusion technique, deep holes of 4 to 5 cm with a width of 0.5 cm are drilled into the trunk. For softening the tissues of the wood formic acid treatment is introduced to the drill holes. This procedure was conducted to promote the absorption and dissemination of the fungus solution into the near cell, and the inoculated hole was then covered with tape (Tian *et al.*, 2013).

Moreover, Agar-Sit is a similar procedure that has been attempted with very slight variations from the pinhole-infusion method (Chen *et al.*, 2018).

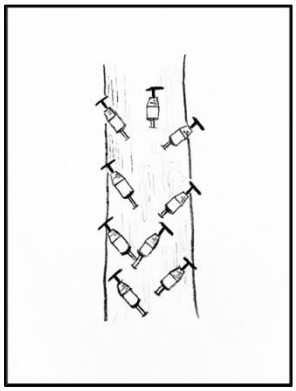

Fig. 12. Bottle dipping method

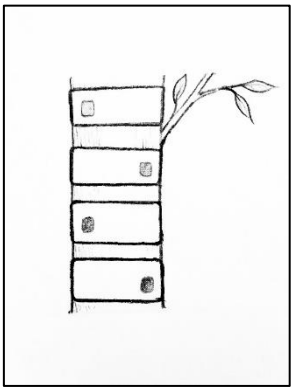

Fig. 13. Pinhole infusion method

Automated transfusion set method

An automated transfusion set is a sophisticated transfusion method that introduces to the agarwood industry. In this system, a magnetic sensor container, a DC water pump, and a transfusion outlet are all included. This system was based on an Arduino, so the Arduino controller was used to control all the modules of the kit. The setup program is developed to determine the discharge volume of the inoculant solution with maintaining consistent pressure during the operation. As a result, both operating time and labor costs are reduced by this application (Roslee *et al.*,2018). The inoculum is very similar to other methods, such as the agar-wit technique. However, the application technique is at the next level (Ngadiran *et al.*, 2023).

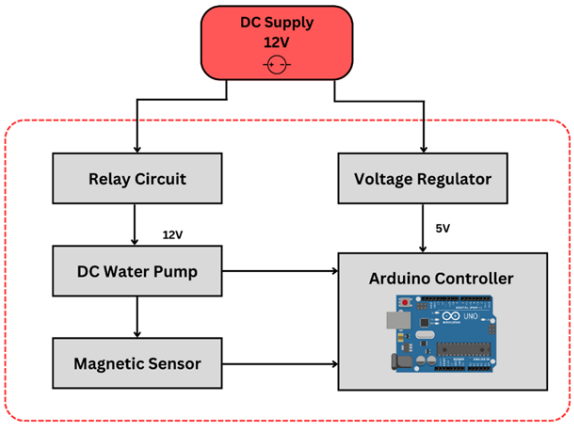

Fig. 14. Diagram of the hardware arrangement for the automated transfusion set

Biological method

Biological inoculation also practices introducing fungi to plants. It is done by vectors like weevils and termites but controlling the insect population should be strictly caring and preventing an outbreak is a challenge. If not, it negatively affects the trees and other species (Turjaman *et al.*, 2016).

Agar-Bit method

This method contains both chemical and biological inducers. Biologically agarwood-inducing technology (Agar-Bit) in which a combination of plant hormones, and bacteria were injected into the stems 300 cm from the ground. Moreover, a penetration enhancer is used for successful inoculation. Approaching six months after inoculation, it was observed that this method produced high-quality agarwood with a promising output of essential oil and chromone content compared with the burning nails and BCD methods (Wu *et al.*, 2017, 2020). Another study shows, within three months, the combination of nitrogen fertilizer and *F. solani* enhanced the production of agarwood in *A. malaccensis* compared to the use of these treatments alone (Wahyuni *et al.*, 2018). In addition, using a mixture of formic acid and *Fusarium spp*., resulted in the content of accumulated chromones increasing significantly as in other related studies and this has resulted after 12 months of inoculation (Chen *et al.*, 2017).

Challenges in the Agarwood Industry and the Agarwood Inoculation Process

The agarwood industry encounters various challenges in both the agarwood inoculation process and the identification of agarwood-producing trees. In the inoculation process, factors such as the age of the tree, the inoculation method, and environmental conditions contribute to the alteration of resin quality and composition. This inconsistency poses difficulties in meeting market demands and maintaining product standards. Additionally, accurately identifying agarwood-producing trees is crucial for sustainable

production practices and resource preservation. These challenges require focused efforts to ensure consistent resin quality, meet market demands, and promote responsible practices in the agarwood industry.

Agarwood trees are an integral part of forest ecosystems, supporting biodiversity and environmental balance. However, the agarwood industry must address the potential environmental impacts associated with unsustainable practices and the cultivation of agarwood plantations (Persoon and van Beek, 2008). Uncontrolled or excessive harvesting of agarwood trees from natural forests can lead to deforestation and habitat degradation. This can disrupt ecosystems, endanger wildlife, and reduce biodiversity. Unsustainable practices can also result in soil erosion, water pollution, and the depletion of natural resources (Xu *et al.,* 2013). To mitigate these environmental impacts, sustainable cultivation methods, such as agroforestry systems or plantation establishment, can be implemented. These approaches allow for the cultivation of agarwood trees while maintaining forest cover (Desa *et al.,* 2021).

Rapid depletion resulting from overharvesting poses a significant problem for agarwood production (Persoon and van Beek, 2008). The misidentification of agarwood formation and lack of knowledge about the subject contribute to this issue, leading to illegal and unsustainable harvesting practices that negatively impact natural agarwood sources worldwide. The current production is insufficient to meet the demand due to the low natural growth rate of agarwood species (Yin *et al.,* 2016). Unfortunately, the majority of Aquilaria and Gyrinops forests have already vanished (Xu *et al.,* 2013).

Deforestation is also a major concern for the depletion of agarwood sources. Many traders and consumers do not prioritize sustainable production practices, engaging in the industry solely for quick profits. This has led to a phenomenon known as "gold fever," where the news about agarwood spreads rapidly within society, driving further unsustainable harvesting. To address these issues, the cultivation of agarwood should be emphasized for production purposes, while harvesting from the wild should be avoided (Persoon and van Beek, 2008). Consequently, agarwood trading is now subject to strict controls by authorities worldwide.

In terms of commercial cultivation, farmers often lack the necessary knowledge to select the appropriate species for cultivation on their lands. Their choice of species is typically influenced by the providers of planting materials (Lee and Mohamed, 2016). As a result, farmers may invest 6-7 years in maintaining plantations only to face setbacks due to ineffective techniques. Additionally, certain internal secrets and patented knowledge remain inaccessible to the public, limiting the dissemination of comprehensive information. Workshops, seminars, and meetings often provide only a fraction of the patented findings (Turjaman *et al.,* 2016)

The agarwood industry encounters challenges related to the uncertainty of resin quality after the inoculation process. The quality of agarwood resin can vary in terms of aroma, color, and potency. Several factors contribute to this inconsistency, including the age of the tree, the inoculation method used, and environmental conditions (Najib *et al.,* 2011).

The age of the tree plays a crucial role in determining resin quality. Younger trees tend to produce resin of lesser quality compared to mature trees. Consequently, achieving consistent resin quality becomes challenging as the age of the trees used for inoculation varies (Hidayat *et al.,* 2010). Additionally, the method of inoculation, whether through drilling holes or making incisions, can influence the resin's quality. Variations in the depth, size, and location of the incisions or holes can lead to inconsistent resin formation (Chowdhury *et al.,* 2016). Environmental conditions also impact resin quality. Factors such as temperature, humidity, soil quality, and sunlight exposure influence the resin's chemical composition. Agarwood trees grown in different regions or under varying climatic conditions may produce resin with different qualities, posing a challenge for maintaining consistent standards (Najib *et al.,* 2011).

To address these challenges, researchers and producers are exploring innovative methods such as controlled environment cultivation and optimized inoculation techniques. These approaches aim to create more favorable conditions for resin formation and enhance the overall quality of agarwood.

Agarwood resin is valued for its unique chemical composition, which contributes to its distinct fragrance and therapeutic properties. However, the inoculation process can alter the composition of the resin, leading to variations in its chemical profile. This challenge raises concerns regarding the authenticity and reliability of agarwood products (Tajuddin *et al.,* 2016).

During the inoculation process, the introduction of fungal inoculum triggers a response in the tree, resulting in resin formation. However, this response can lead to changes in the resin's chemical composition (Lee and Mohamed, 2016). The presence of different fungal species and their interactions with the tree's defense mechanisms can influence the production of specific compounds responsible for the resin's fragrance and therapeutic properties (Yin *et al.,* 2016).

The alteration in resin composition poses challenges for producers who strive to maintain consistent product quality and meet market demands. Consumers expect agarwood products to exhibit specific aroma

profiles and therapeutic benefits. Any significant deviations in the chemical composition may affect the desired properties and reduce consumer confidence in the product's authenticity (Ngadiran *et al.,* 2023).

The agarwood industry heavily relies on market demand and consumer perception. Any changes in resin quality or composition can significantly impact consumer preferences and purchasing decisions, presenting a challenge for the industry. Inconsistent resin quality resulting from variations in the inoculation process can lead to reduced market demand. Consumers seeking high-quality agarwood products expect consistent sensory experiences, including specific aromas and therapeutic properties (Zich and Compton, 2001). When resin quality varies significantly, it becomes difficult to meet these expectations consistently, resulting in a loss of consumer trust and reduced demand (Mohamed and Lee, 2016).

Moreover, consumer perception plays a vital role in determining market demand. If consumers perceive that the agarwood industry lacks transparency or if authenticity concerns arise due to altered resin composition, it can have a negative impact on market demand. Educating consumers about the complexities of the agarwood inoculation process, the factors influencing resin quality, and efforts to ensure consistency can help alleviate these concerns and maintain market demand.

To address these challenges, producers and stakeholders in the agarwood industry must prioritize quality control and adopt standardized practices throughout the inoculation process. Implementing certification systems and promoting transparency in sourcing, production, and processing can help build consumer trust and support market growth (Adhikari *et al.,* 2021).

The agarwood inoculation process requires careful consideration of environmental factors and completion of the disease triangle, which involves the interaction of a susceptible host, a virulent pathogen, and a conducive environment. Failure to manage these factors properly can result in ineffective inoculation, decreased resin production, and increased susceptibility to diseases (Rohlfs and Churchill, 2011).

Environmental factors, such as temperature, humidity, and soil conditions, play a crucial role in the success of agarwood inoculation. Deviations from optimal conditions can hinder resin formation and negatively impact the overall yield. Adequate moisture levels in the soil, appropriate temperature ranges, and proper sunlight exposure are essential to promote healthy tree growth and resin production (Rabgay *et al.,* 2020). Additionally, the completion of the disease triangle is critical in fungal inoculations. Agarwood trees are vulnerable to various diseases caused by pathogenic fungi. When making incisions or drilling holes for inoculation, there is a risk of introducing pathogens to the tree, which can lead to infections. These infections can weaken the tree's immune system, reduce resin production, and even cause tree mortality (Rohlfs and Churchill, 2011).

To mitigate the risk of disease outbreaks, producers should implement effective disease management strategies. This includes using disease-resistant agarwood tree varieties, maintaining optimal environmental conditions, employing proper hygiene practices during the inoculation process, and monitoring tree health regularly. Integrated pest management techniques and timely interventions can help minimize the risk of disease and maintain a healthy agarwood plantation (Syazwan *et al.,* 2019).

Inoculating agarwood trees by physical interventions to introduce the fungal inoculum, these procedures can potentially damage the trees if not performed with precision and care. Improper inoculation techniques or inadequate post-inoculation care may lead to irreversible harm, including infections, stunted growth, or even tree mortality. Drilling holes or making incisions in the wrong locations or at incorrect depths can cause unnecessary damage to the tree. Careful consideration of tree anatomy, growth patterns, and proper techniques are crucial to minimize harm. Additionally, inadequate post-inoculation care, such as failure to prevent infections or provide suitable environmental conditions, can further exacerbate plant damage (Akter *et al.,* 2013; Faizal *et al.,* 2017).

Proper training and education of agarwood producers, along with the dissemination of best practices, can help reduce the risk of plant damage. Emphasizing precision, hygiene, and monitoring tree health after inoculation are essential steps in preventing long-term harm to agarwood trees (Ngadiran *et al.,* 2023).

The challenges encountered in the agarwood industry, including the agarwood inoculation process and the identification of agarwood-producing trees, have far-reaching implications for market demand, sustainability, and the preservation of natural resources. However, recent advancements in agarwood induction technology offer promising solutions to overcome these obstacles and transform artificial agar resin induction methods. These advancements encompass a range of innovative techniques, including controlled environment cultivation, optimized inoculation methods, disease management strategies, and precision-based practices. By embracing and implementing these advancements, the agarwood industry can significantly enhance resin quality, effectively meet market demands, promote sustainable production practices, and ensure the long-term viability of this invaluable resource. It is of utmost importance for stakeholders to remain updated

on the latest advancements in agarwood induction technology, as these developments hold the key to the continued growth and development of the industry while addressing its inherent challenges.

CONCLUSIONS

The present global demand for agarwood, the high-valued resinous fragrant of the wood, cannot be met by natural harvests from the forests alone. The natural causes of invasions present in the environment is limited and inefficient to induce the production of this precious resource inside trees up to its full potential. Fortunately, recent advancements in artificial agarwood induction technology have led the production of agarwood resin be efficient by surpassing conventional methods. These technologies include mechanical, biological, and chemical methods, as well as combinations of these techniques. Chemical inducers, in particular, have shown rapid invasion inside particular tree species trunks to produce superior agarwood resin at highly efficient and consistent rate. However, all such methods are being practiced in the industry or in the research level, have their own merits and limitations.

Therefore, to get the utmost benefit from this industry, the most appropriate induction method should be selected by the stakeholders by considering their own specific circumstances. In such instances, the feasibility, safety, replicability, targeted market, cost-effectiveness and the financial stability of each method should among the most priorities if the industry is to be transformed in to a high profit oriented business.

Furthermore, it is crucial to acknowledge and address the multitude of challenges encountered in the agarwood industry. These challenges include potential alterations in resin quality and composition, market demand fluctuations, completion of the disease triangle in fungal inoculations, risk of permanent plant damage, environmental impacts, and increased vulnerability to pest and disease outbreaks. By proactively addressing these challenges through sustainable practices, responsible sourcing, and effective regulations, the agarwood industry can achieve thriving growth while upholding the delicate equilibrium between economic prosperity and environmental preservation.

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FIRST REPORT OF VIRAL NERVOUS NECROSIS IN ASIAN SEA BASS, LATES CALCARIFER CULTURED IN SRI LANKA

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ABSTRACT

Viral Nervous Necrosis (VNN) caused by Betanodavirus is a devastating disease in aquatic animals. The virus infects both marine and freshwater fish worldwide. Vacuolating necrosis of neural cells of the brain, the retina of the eye, and the spinal cord of the infected fish are the primary histological lesions of the condition. It causes up to 100% mortality in larvae and juvenile fish and can cause significant death in adult fish. The present study detected viral nervous necrosis in larvae and fry of Asian sea bass (Lates calcarifer) with progressive mortality of up to 95% in one week during the Northeast monsoon when the mean water temperature was 27 to 29°C. Histopathological examination of the moribund fish revealed extensive vacuolation and gliosis in the olfactory bulb, the optic lobe of the forebrain, and the inner and outer layer of the retina. Furthermore, tissues of the brain and the retina had intracellular inclusion bodies suggesting viral etiology, further justified by the negative results of the bacterial and parasitic examinations. The Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) test additionally confirms the etiology diagnosis using specific primers designed previously. The histopathology and RT-PCR results suggest that the mortalities of Asian sea bass were due to the VNN. The present finding is the first report of the VNN associated with mass mortalities in Asian seabass cultured in Sri Lanka. These crucial findings emphasize the need for quarantine and control strategies to prevent the spread of the virus and outbreak of the disease.

Keywords: Asian sea bass, RT - PCR, Sri Lanka, Vacuolating necrosis of neural cells, Viral Nervous Necrosis,

INTRODUCTION

Viral Nervous Necrosis (VNN), caused by Betanodavirus, is known as viral encephalopathy, and retinopathy (VER) is a devastating disease in Asian sea bass, especially in larvae and juveniles (Munday *et al*., 1997; Yothikoshi and Inoue, 1990). The virus damages and destroys the fish's central nervous system, causing up to 100% mortality in marine (Bigarre *et al*., 2009; Zorriehzahra *et al*., 2019a), freshwater (Hedge *et al*., 2003; Binesh *et al*.,2013; Praveenraj *et al*., 2018) aquaculture. Betanodavirus is non-enveloped and icosahedral with a diameter of 20–30 nm, with two positive-sense RNA strands known as RNA1 and RNA2. RNA1 encodes RNA-dependent RNA polymerase (RdRp), a mitochondrial enzyme responsible for viral replication (Nopadon *et al*., 2009), and RNA2 encodes the capsid protein. The first nodavirus infection was detected in Japanese parrotfish (*Oplegnathus fasciatus*) (Yoshikoshi and Inoue, 1990). Then the disease was reported in barramundi (*Lates calcarifer*) farmed in Australia (Glazebrook *et al*., 1990). The VNN has been found in up to 120 marine and freshwater fish species worldwide, including Asian seabass (*Lates calcarifer*) (Vela-Avitúa *et al*., 2022).

The transmission of the VNN can be horizontal and vertical. Horizontal transmission happens from diseased fish, virus-carrier animals, contaminated water, trash fish, and cannibalistic fish (Cherif *et al*., 2009; Mannin and Ransangan, 2011). On the other hand, vertical transmission from broodstock gonads and sperm where the betanodavirus can be detected. Similarly, the virus also presents in fertilized eggs and passes to the next generation (Kuo *et al*., 2012). VNN can survive without a host for over a month and transmit to fish from water or other animals (Gomez *et al*., 2011). The virus can infect fish between the temperature ranges of 16 to 30°C (Zorriehzahra *et al*., 2019a). A temperature closer to 16 °C will result in the sub-acute form of sickness characterized by necrosis on the upper jaw and head areas. The acute phase of the disease has been linked to the elevated sea temperature up to 30°C when nervous indications arise (Le Breton *et al*., 1997). The high-water temperature of more than 30°C inhibits viral proliferation in fish (Yuasa *et al*., 2007). The water temperature and viral strains strongly influence the brain's viral load (Toffan *et al*., 2016).

Several steps are involved in diagnosing the VNN in fish, including clinical signs, necropsy findings, molecular methods, and histopathology (Munday *et al*., 2002; Hegde *et al*.,2003; Yuasa *et al*., 2007). The affected fish swim rapidly, spiralling, whirling, lying down at the bottom, and becoming dark-skinned (Yoshikoshi and Inoue, 1990). Further, the swim bladder contains severe hyperinflation in diseased juveniles (Zorriehzahra *et al*., 2019b). Hemorrhages are found in the brain, liver, and spleen tissues (Yang *et al*., 2022). Many reports showed the numerous molecular tools for detecting VNN in fish, which have become an effective and precise method for virus detection. The primary molecular method for diagnosing VNN is the laboratory's reverse transcription polymerase chain reaction (RT -PCR) (Grotmol *et al*., 2000). The World Organization for Animal Health (OIE) consents to amplify RNA2 fragments as a routine diagnostic of VNN disease. However, due to low sensitivity, the nested RT - PCR could only detect the virus if the sample has a high viral load. Real-time quantitative RT-PCR assay (qRT-PCR) is a precise and powerful tool for the detection and quantification of betanodavirus (Dalla Valle *et al*., 2005; Kuo *et al*.,2012; Liu *et al*., 2012). The characteristic histopathological lesions of VNN are severe degeneration, pyknosis, shrinkage, and basophilic cells in affected tissues and vacuolation of the central nervous system and retina of the affected larvae and juvenile fishes showing abnormal swimming behavior (Fukuda *et al*., 1996; Bigarré *et al*., 2009; Liu *et al*., 2012). Zorriehzahra *et al*. (2019a) reviewed that the vacuolated cells and vacuoles are mainly present in the bipolar and ganglionic nuclear layer of the retina in the eyes. Gliosis in the central nervous system is another typical histological lesion associated with VNN in fish (Costa and Thompson, 2016). Histopathological examination of the tissues is a vital diagnostic tool for identifying infectious diseases, as conventional methods often fail to identify the organism for various reasons (Gupta *et al*., 2009).

Mass mortality of Asian sea bass fries in hatcheries in Sri Lanka is suspected to be caused by Viral Nervous Necrosis (VNN), which can have significant ecological, economic, and scientific implications. This can result in substantial financial losses for hatcheries and aquaculture operations, impacting hatchery owners and the broader aquaculture industry. Identifying and mitigating VNN's causes can help safeguard the economic sustainability of the aquaculture sector and contribute to our understanding of aquatic diseases and their transmission. Controlling VNN in Asian sea bass fries can prevent the virus from spreading to other aquatic species, reducing the risk of disease outbreaks in the broader aquatic ecosystem. Addressing this complex issue is essential for the sustainability of aquaculture, fish well-being, and the availability of Asian sea bass as a food source. Therefore, the investigation was carried out to identify the causative agents for mass mortality of Asian sea bass fry in the Sri Lankan hatchery and underlying factors influencing mortality.

METHODOLOGY

Mortalities of similar clinical signs were observed in the Asian sea bass hatchery located on the East coast of Sri Lanka from the first week of November 2018 to February 2019. There were two incidences of the outbreak during the sampling period. The first outbreak was observed in Mid November 2018, when the larvae on 14 days old. Twenty larvae with an average body size of (3-5 mm in length) were collected during the first outbreak, with significant progressive mortalities starting from 20 to 95% in one week. During the second outbreak in early January 2019, we collected another 20 Asian sea bass fries (28 days old) with an average body weight of 1.8±1.6 g. Wet mount of the whole body of larvae and fries from both outbreaks were examined by light microscopy for the presence of parasites. The bacterial examination was also performed in larvae and fries by plating tissue samples on TSA (Oxoid, England) supplemental with 1.5% (w/v) NaCl and TCBS (Himedia, India) medium and incubated at room temperature and read every 24 hours for five days as described by Frerichs (1989). The larvae and fries suspected of VNN were brought to the laboratory in Liquid nitrogen. The Committee of Ethical Clearance of the Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Sri Lanka, granted approval for the study. The ethical clearance's approval number is VER 15-008.

Histopathological examination

The samples of moribund larvae and fry were fixed in 10% phosphate-buffered saline (PBS), and tissues were prepared according to standard procedures described by Gupta et al (2009) for paraffin embedding and sectioned at 5 µm before being stained with Haematoxylin and Eosin (H&E), particularly on the retina of the eye and the brain.

Reverse Transcriptase Polymerase Chain Reaction (RT -PCR)

Total RNA was extracted from whole Asian seabass larvae and fries using the Trizol reagent (Invitrogen, Carlsbad, USA). First-strand cDNA was synthesized using a SuperScript™ VILO™ cDNA Synthesis Kit (Invitrogen, USA). We used the primers (VNN -F1 : 5’GGATTTGGACGTGCGACCAA 3’ and VNN -R1 : 5’CTGAATTTCGAACTCCAGTG 3’) published by Intamaso *et al*. (2018). One microliter of reverse-transcribed cDNA was used as a template

in the polymerase chain reaction amplification. An aliquot of 25 µl of the overall reaction mixture was made up of 0.1 pg - 1 mg of RNA template, 0.4 M forward and reverse primer, 0.4 mM MgSO4, 12.5 µl of reaction mix, and 1µlL of Super Script® III One-Step RT-PCR System with Platinum® Taq DNA polymerase (Invitrogen, USA) as per the manufacturer's instructions. The PCR amplification reaction started with a denaturation stage at 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min respectively, with the final extension step took place at 72 °C for 5 min. The PCR product was visualized by using 2% agarose gel electrophoresis stained with ethidium bromide. Materials from already confirmed VNN isolates, and healthy Asian sea bass fish served as the positive and negative controls.

RESULTS AND DISCUSSION

The catastrophic deaths of Asian sea bass larvae and fry at a hatchery in Sri Lanka were attributed to viral nervous necrosis because of the disease's clinical symptoms, histological findings, and PCR results. The clinical signs of the first outbreak on the 14days old larvae were characterized by becoming transparent with the chromatophores retracted and floating on their side at the surface with a hyperinflated swim bladder (Figure 1 A), followed by progressive mortality from 20 to 95% in one week. In addition to these findings, the affected fish showed abnormal swimming backward, an enlarged swim bladder, uneven skin colour (Figure 1 B), and abdominal distension in another outbreak. These findings are similar to reports in the literature (Fukuda *et al*., 1996; Bigarré *et al*., 2009; Yang *et al*., 2022). Also, mortalities were recorded during the Northeast monsoon in Sri Lanka, where the seawater temperature ranged from 27 to 29°C. The VNN virus can thrive in this temperature range and produce clinical illness (Zorrihzahra *et al*., 2019). However, the water temperature became more than 30°C during the dry season, and the virus could have been inactive to cause disease. Therefore, we did not observe any outbreaks when temperature exceed 29°C. However, Toffan *et al*. (2016) showed that nodavirus replication in vivo is a composite process regulated by both the genetic features of the viral strain and water temperatures. Based on similarities in the partial RNA2 sequences, betanodaviruses can be classified into four genotypes: striped jack nervous necrosis virus (SJNNV), red-spotted grouper nervous necrosis virus (RGNNV), tiger puffer nervous necrosis virus (TPNNV), and barfin flounder nervous necrosis virus (BFNNV), and the optimal temperatures for the growth of these viruses are 20–25°C (SJNNV), 25–30°C (RGNNV), 20°C (TPNNV), and 15–20°C (BFNNV) (Nakai *et al*., 2009; Hata *et al*., 2010). Generally, Asian sea bass, European sea bass, red spotted groupers, and other groupers are more susceptible to RGNNV (Nakai *et al*., 2009). Although we could not do the sequencing of the PCR isolates of our samples due to various limitations, anyone can come to the idea that the strain of betanodaviruses associated with mass mortalities in Sri Lanka could be an RGNNV as we found the outbreak when the temperature is 27 to 29 °C and the PCR product of 198 bp (Intamaso *et al*., 2018) based on the recommendations by the OIE's (2019) for the confirmatory diagnosis of VNN. We used RT-PCR to extract total RNA from afflicted larval tissues and fries with clinical symptoms attributed to VNN. The amplification of tissues from 10 fries and ten larvae produced a target cDNA band on agarose gel electrophoresis of about 198 bp. The previously positive samples that produced 198 bp were used as a positive control for the subsequent PCR reaction (Figure 2). We used the previously published primers to identify RGNNV yielding 198 bp (Intamaso *et al*., 2018).

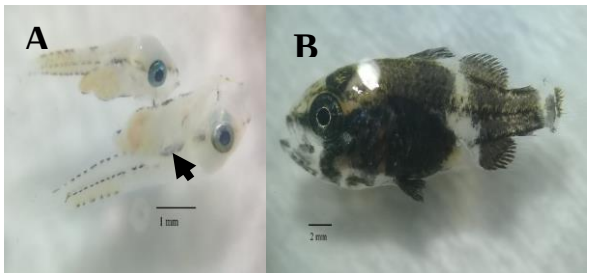

Fig. 1. The 14 days old Asian seabass larvae attributed the clinical signs suspected for VNN with transparent bodies floating on their side at the surface with a hyperinflated swim bladder (A) and 28 days old fry with uneven skin colour (B).

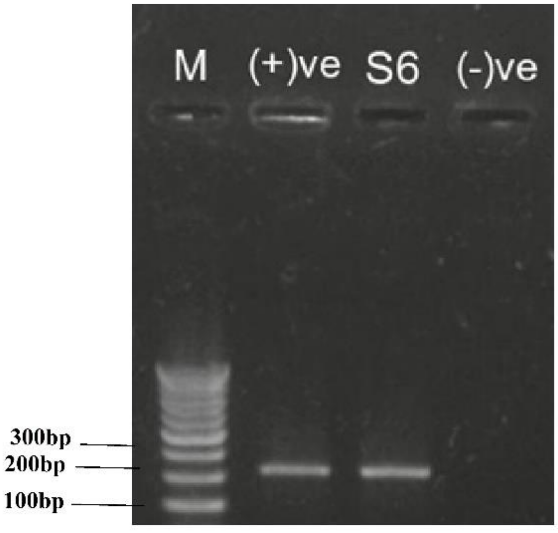

Fig. 2. Gel electrophoresis (2% agarose) for screening of VNN in affected Asian sea bass in hatchery by RT-PCR. Lane M- 100bp marker, Lane (+)ve: cDNA positive control, Lane S6: tissue from VNN infected fish, and Lane (-)ve: Negative control.

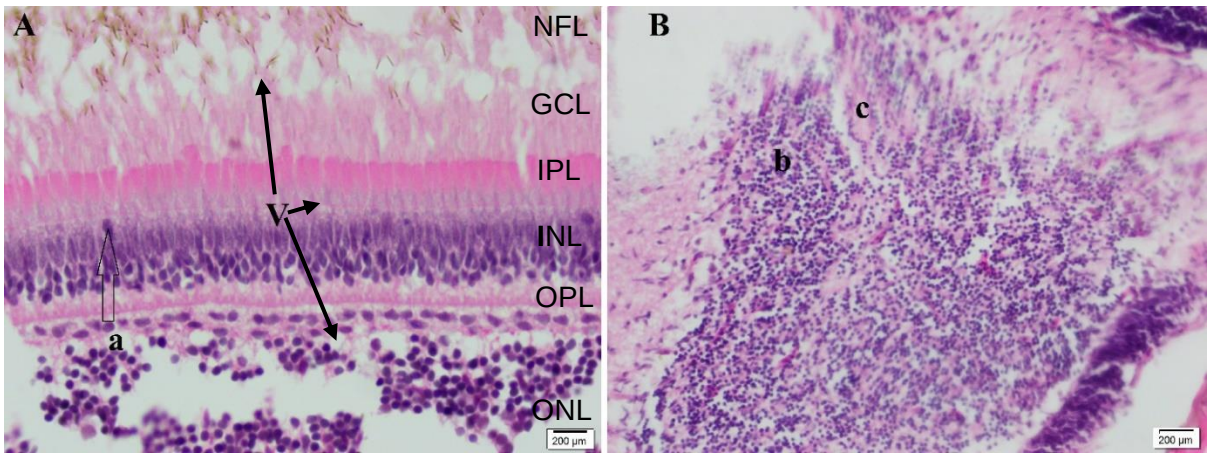

Fig. 3. Histopathological changes induced by viral nervous necrosis (VNN) in Asian sea bass (*Lates calcarifer*). A- shows the vacuoles (v) in the retina's nuclear, plexiform, and ganglion cell layers, and basophilic inclusion bodies in the outer nuclear layer (a). B- shows the presence of gliosis (b) and neuronophagia (c) in the brain. NFL – Nerve fiber layer, GCL- Ganglion cell layer, IPL- Inner plexiform layer, INL- Inner nuclear layer, OPL- Outer plexiform layer, ONL- Outer nuclear layer.

Furthermore, we only identified these viruses in the larvae and fries below five weeks of age, with a similar finding to Jaramillo *et al*. (2017). As the virus is age dependent, the mortality occurs up to 100% only in the larvae and fries when their age is up to five weeks of age. Afterward, the diseases remain subclinical, or the viruses in lower doses in juvenile and adult fish (Ariff *et al*., 2019).

The histopathological examination of the present study revealed extensive vacuolation and neuronal degeneration of the retina of the eye and the olfactory lobe of the forebrain in all affected fish (Figure 3), as indicated by Azad *et al*. (2006). Interestingly, no parasites or bacteria associated with the disease were found in any of our samples. The lesions caused by betanodavirus can be seen in the histopathological section of different cells, including nerve cells, epithelial cells and myocardial cells and blood cells of juvenile and larval fish (Grotmol *et al*., 1997; Tanaka *et al*., 2001). However, the present study document the histopathological lesions in the eye and the brain as the primary target tissue of the fish is the nerve cells in the brain and eye (OIE, 2019). The retina of some infected fish showed marked necrosis in the inner nuclear layer, outer and inner plexiform layers, and ganglion cell layer in the retina (Fig 3-A). The outer and inner nuclear layers, outer and inner plexiform layers, and ganglion cell layers of the retina had numerous large vacuoles formed by fragmentation and degeneration of infected cells. The outer nuclear layers of the retina had characteristic homogenous basophilic intracytoplasmic inclusion bodies (Figure 3 A -a). Further to this finding of the inclusion bodies in eye tissues in the present study, Yuwanita *et al*. (2013) found characteristic inclusion bodies in the liver, kidney, and eye tissues. The brain histopathological section showed clear gliosis in the olfactory lobe, a common finding in the CNS of fish affected by VNN (Shetty *et al*., 2012; Costa and Thomson, 2016). Vacuolar degeneration was severely detectable in dendritic cells extending into the outer molecular layer of the cerebellum. Gliosis and neuronophagia, characterized by the accumulation of microglia around degenerated or necrotic neurons, were seen in the olfactory lobes of the forebrain, as mentioned by Zorriehzahra *et al*. (2019b). We observed the necrosis of the brain at 14 DPH (Fig 3-B), which is supported by the finding of Azad *et al*. (2006), where the first signs of brain necrosis can be observed from 6dph. In the present study, the retina of the eye of affected fish had a large number of vacuoles in the inner and outer layers of nuclear and plexiform, and ganglion cell layers, as identified by Zorriehzahra *et al*. (2019a) and Azad *et al*. (2006).

To our knowledge, this is Sri Lanka's first report of Asian sea bass VNN infection. Control strategies such as vaccination quarantine measures, biosecurity, and getting fry and fingerlings from disease-free stock must be adapted to control and eradicate the disease (Jeney, 2017; Hegde *et al*., 2003). Therefore, there is a possibility that VNN can be spread to ornamental fish, and it is essential to take necessary control measures to eradicate the disease in other fish species.

CONCLUSIONS

The findings of histopathology, RT-PCR, and history and clinical signs confirm that VNN is associated with mass mortality of the Asian sea bass larvae and the fry on the Eastern coast of Sri Lanka. In the present study, diseased larvae and fries collected during mass mortalities were characterized by degeneration of nervous tissues (necrosis, vacuolation, and gliosis) and the presence of inclusion bodies in the histopathological examination of whole larvae and fries. However, the occurrence is highly related to the water temperature. The findings are emphasized that the Asian sea bass hatcheries should be aware of this disease, especially during the Monsoon in Sri Lanka.

It is necessary to conduct further research, including the other freshwater and marine species, and should be related to detailed molecular characterization.

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DEVELOPMENT OF SNACK USING CASSAVA (*Manihot esculenta*) AND WHEAT (*Triticum aestivum*) FLOUR MIXTURE

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ABSTRACT

This research work aimed to assess the suitability of incorporating cassava flour as a supplementary ingredient alongside wheat flour for snacks. In addition, this investigation explored the advantages combining cassava and wheat flours, considered the unique flavor and mixture characteristics imparted by cassava flour. Five different treatments were tested based on the ratios of cassava to wheat flour by dry weight. The aroma, taste, appearance, color, mouthfeel, and overall acceptability of developed snacks were evaluated using sensory tests. The 50 trained panelists trained in 5 -point hedonic scale of 1 (dislike very much) to 5 (like very much). The best snacks preparation composition identified by sensory tests were evaluated for its moisture, ash, color, and texture. Results revealed that the mixture containing a ratio of cassava flour to wheat flour; 25:75 was the most favorable sample across all sensory attributes. Selected best snakes contain moisture content 7.84%, total ash 3.37%. Colour values (L, a* and b*) were 51.61, 6.80 and 28.78 respectively. Hence, it can be recommended that the snack mixture of 25% cassava flour with 75% wheat flour can be selected as the best snack mixture. The findings may lead to the development of a snack while expanding the understanding of cassava flour's versatility in food production.*

Keywords: *Cassava, Manihot esculenta, Snack, Triticum aestivum, Wheat flour*

INTRODUCTION

Snack products become popular in Sri Lanka and wheat used as the major ingredient to of snack product industry. Substitution of wheat flour by different kinds of flour in snack making is economically essential in Sri Lanka as wheat is imported by other countries. There is novel trend of using mixture of flour for snack products producing industry to be economical and viable.

Cassava (*Manihot esculenta*) is the perennial woody plant cultivated mostly as an annual crop. This drought tolerant crop is an important source of energy for people in the tropical and sub-tropical regions (Cockand Howeler, 2012). Cassava is a valuable food crop emerging as a staple food for most of the African countries. The roots and leaves are good sources of nutrients such as carbohydrates, protein, and vitamins (Bayata, 2019). The majority of production is used for human consumption while less amount is used for animal feed. The crop has a high yield potential under good conditions and compared to other crops it stands well in the marginal land (Montero, 2003). Therefore, this is one of the potential crops to fulfill the food demand of increasing population (Hidayat *et al*., 2023).

The mass production of cassava flour represents a significant in the agricultural and food processing sectors. However, despite the abundance of cassava flour, its full potential remains largely untapped in the food industry. Currently, cassava flour is predominantly relegated to the role of a thickening agent or additive, mainly finding its place in the baking process, where it contributes texture and consistency (Abidinand Devi, 2013). Unfortunately, its utilization as a primary or leading ingredient in food products remains limited. To identify the full potential of cassava flour in the food industry, it is imperative to study its compatibility with other ingredients, particularly wheat flour, which is a staple in many food preparations worldwide. Therefore, this study was conducted with the aim of evaluating the applicability of cassava flour as an alternative to wheat flour for the development of snacks.

METHODOLOGY

Selection of Raw materials

Well-matured cassava (variety: kirikawadi) tubers were collected from cassava cultivation in Dambadeniya, Kurunegala District in Sri Lanka located at 7.3697° N, 80.1512° in low country intermediate zone with an average maximum temperature range from 22 to 30 °C and with average annual precipitation 20000 mm.

Preparation of snack

This study was performed on a laboratory scale. The collected cassava tubers were peeled and thoroughly washed. After that the cleaned tubers were cut into small pieces (2mm size). These small pieces were then subjected to oven drying at a temperature of 55°C for a duration of 16-18 hours until the moisture content reduced up to 8%, and they were subsequently ground using an electric grinder. The resulting cassava flour was sifted through a mesh with a pore size of 250 µm. A combination of wheat flour and cassava flour was prepared according to treatment specifications. In accordance with existing literature, ingredients such as turmeric powder, curry powder, salt, pepper, garlic, ginger, water, and vegetable oil (in equal proportions across all treatments) were added to the mixtures of cassava and wheat flour in appropriate quantities. Preliminary investigations were carried out to determine the appropriate ratios of other raw materials to incorporate into the snack. Five distinct treatments were employed based on the cassava-to-wheat flour ratios by dry weight: T1 (100% cassava, 0% wheat), T2 (75% cassava, 25% wheat), T3 (50% cassava, 50% wheat), T4 (25% cassava, 75% wheat), and T5 (0% cassava, 100% wheat, serving as the control)

Sensory Evaluation of the snack

Sensory evaluation was carried out to determine the suitable ratio of cassava flour and wheat flour to develop the snack. The acceptability of the five treatments was tested by using sensory analysis (appearance, smell, taste, mouthfeel, and overall acceptability) of a five-point hedonic scale of 1 (dislike very much) to 5 (like very much) and a sensory panel consisting of 50 trained panelists (Head *et al*., 1977).

Physiochemical characterization of the snack

The best mixture was selected according to the sensory characteristics and physiochemical properties such as moisture, ash, color, and texture of selected best snacks were evaluated by following methods.

Moisture content

The moisture content was determined by drying at 105°C to constant weight using the AOAC (1990) method.

Texture and colour of the snack

The Instron, TA, XT2 texture analyzer has been adapted to perform a texture analysis of the snacks. A compression test was carried out to measure snack texture and force at rupture was considered as the texture of the snacks (Gunathilake, 2018).

The process of determining color involved measuring three parameters specified by the CIELAB system. These color-related parameters, namely L*, a*, and b*, were determined using a colorimeter that was equipped with a diffuse reflectance setup known as the Color Quest II Sphere. These measurements were conducted through reflection, utilizing an observation angle of 10 degrees, the standard D65 illuminant, and the exclusion of specular reflection (abbreviated as RSEN). The L* value corresponds to the perception of lightness, where L* = 0 represents black and L* = 100 signifies white. In contrast, a* and b* are indicators of chromaticity, with +a* representing the color red and -a* indicating green. Similarly, +b* signifies yellow, and -b* represents blue.

Total Ash

Total ash content was performed by gravimetry after incineration in a muffle at 550°C using the AOAC (1990) method.

Statistical Analysis

Data gathered from the sensory evaluation was analyzed according to Friedman non-parametric test at 95 % level of significance using Mini tab statistical software.

RESULTS AND DISCUSSION

Sensory characteristics of the snack

The results of the sensory analysis of treatments show that there was a significant ($P \leq 0.05$) difference between the five treatments with respect to appearance, smell, taste, mouthfeel, and overall acceptability. Among the five treatments, treatment 04, that combination of cassava and wheat flour with the ratio of 25: 75 has shown the highest median values of 4.7, 4.8, 5.0, 4.9, 5.0 for appearance, smell, taste, mouthfeel, and overall acceptability respectively. In addition, the highest sum of ranks was recorded as 233.0, 233.5, 232.5, 234.0, 232.5 for appearance, smell, taste, mouthfeel, and overall acceptability respectively. Accordingly, treatment 04 was the most preferred snack mixture in comparison to the other 4 snack mixtures for its sensory qualities (Table 1, Figure 1). Indeed, previous research conducted by other scientists has identified an important factor in achieving desirable snack structure, namely, the presence of gluten. Gluten, a protein found in wheat, plays a crucial role in providing elasticity and structure to many baked goods, including snacks. Consequently, to attain optimal snack texture and quality when combining wheat and cassava flours, it is typically necessary to incorporate a substantial amount of wheat flour into the mixture. (Abidinand Devi, 2013) snacks (Maziya-Dixon *et al*., 2017), bread (Udofia *et al*., 2013), biscuits (Oluwamukomi *et al*., 2011) etc shown acceptance in different combinations.

Physiochemical characteristics of the snack

Selected best treatment (treatment 04) that used cassava and wheat flour with the ratio of 25: 75 was

Table 1. Friedman test results for sensory evaluation

Variable	Cassava and Wheat flour 100:0	Cassava and Wheat flour 75:25	Cassava and Wheat flour 50:50	Cassava and Wheat flour 25:75	Cassava and Wheat flour 0:100	Highest Sum of Rank	P – value
Appearance	1.00	4.20	2.10	4.70	1.00	233.0	0.000*
Smell	2.00	4.40	1.60	4.80	1.20	233.5	0.000*
Taste	1.80	4.80	1.80	5.00	1.60	232.5	0.000*
Mouth feel	1.10	4.40	2.00	4.90	1.10	234.0	0.000*
Overall acceptability	2.00	5.00	2.00	5.00	2.00	232.5	0.000*

*Value are medians of each response *value are significantly differs at 0.05 significance level according to the Friedman test*

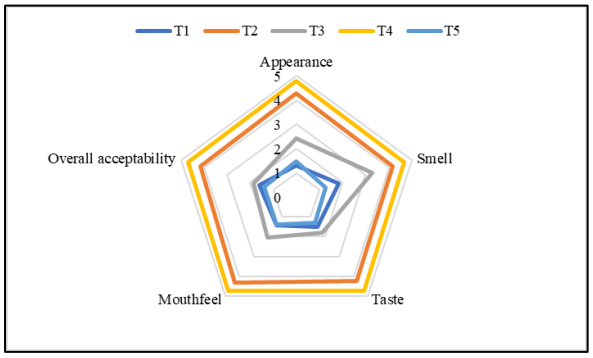

Figure 01. Radar plot of hedonic sensory rating of developed snacks

tested for its physiochemical characteristics such as moisture content, ash, color, and texture. The results revealed that, the moisture content of 7.48 g/100g. This moisture level indicates that the snack maintains a desirable crispiness while retaining a sufficient amount of moisture to enhance palatability. According to Awolu *et al*., (2015) the mixture of rice flour (58g/100g), cassava flour (33g/100g) and groundnut flour (9g/100g) give 20g/100g as the optimum moisture content for the extraction snack production process.

The total ash content, indicative of mineral composition, was found to be 3.37 g/100g. This measurement underscores the potential nutritional value of the snack, with its contribution to dietary minerals such as calcium, magnesium, and phosphorus. According to Obadina *et al.*, (2013) ash content of extruded cassava-based snacks ranged from 2.00 ± 0.35 to 2.90 ± 0.00. while moisture content ranged from 2.90 ± 0.14 to 8.60 ± 0.00.

Texture and colour of the snack

Texture analysis was used to determine a textural parameter of snacks. Snack hardness was taken as its texture value that can be defined as maximum tolerable force snack can withstand without breaking (force at rupture). Maximum hardness value of the selected best snack was 7.50 N. According to the previous studies, this value implies that the snack product offers a satisfying textural experience, combining a pleasing crunchiness with a balance that enhances consumer enjoyment (Luo *et al*., 2020).

Color properties were assessed through L*, a*, and b* values, which respectively represent lightness, red-green color, and yellow-blue color. The best snack selected by sensory test exhibited color values of L* = 51.61, a* = 48.47 and b* = 46.61 suggesting a visually appealing product with a vibrant and inviting appearance. Previous literatures are also proved it (Han e*t al*., 2010)

CONCLUSIONS

It can be concluded that according to the sensory test, snack made from the mixture of cassava flour with wheat flour; 25: 75 (T4) showed higher significant acceptance values. Further this treatment snacks were tested for moisture, ash, color, and texture. They showed acceptable values, the mixture of cassava flour can be used as an supplementary for wheat flour to produce good quality snacks.

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DEVELOPMENT OF CUTTING PROPAGATION TECHNIQUE FOR ORNAMENTAL PLANT *Allamanda cathartica* (RUKKATHANA)

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ABSTRACT

Allamanda cathartica is an indigenous, widely distributed plant in Sri Lanka and is identified as Rukkathana. It is cultivated for large yellow flowers which have numerous medicinal and ornamental values. The propagation of Allamanda cathartica through conventional cuttings has limitations since the sprouting process is very slow and the success rate is remarkably low. Therefore, with the objective of developing a suitable cutting propagation technique for Allamanda cathartica plants an experiment was carried out in a two factor factorial manner at the University of Colombo Institute for Agro-Technology and Rural Sciences, Hambantota, Sri Lanka. Different types of stem cuttings: hardwood, semi-hardwood, softwood and shoot tips were tested in different potting media of pure sand, pure coir dust and sand and coir dust at the ratio of parts by volume 1:1 under controlled environmental conditions. There were twelve treatment combinations and four replications each containing three units and those were arranged in a Complete Randomized Design manner. Survival, rooting, shoot length, number of roots and root weight were recorded and statistical analysis was done by SAS 9.1.3 package. The study revealed that shoot tips exhibited better survival in a mixture of sand:coir dust and sand only media, as well as in coir dust and a mixture of sand:coir dust for hardwood cuttings. However, the lowest survival was in semi hardwood planted in sand only compared to all other treatments. Shoot tips showed superior rooting and shoot length in pure coir dust and a mixture of sand:coir dust media, while other cuttings showed the lowest rooting when planted in sand only media. The highest root weight was observed in shoot tips planted in sand:coir dust media, followed by shoot tips in pure coir dust, while the lowest root weight was recorded in softwood, semi hardwood, and hardwood planted in sand only, as well as in softwood cuttings planted in sand:coir dust media. The results suggest that the choice of growth media should be based on the type of cutting to improve rooting and growth performance. Higher aeration in growth media was found to be crucial for improving root initiation and stimulating metabolic processes. It can be concluded that, shoot tips planted in sand with coir dust can be recommended for mass multiplication of Allamanda cathartica.

Keywords: Allamanda cathartica, Cutting, Media, Propagation, Rukkathana

INTRODUCTION

Vegetative or asexual propagation has been established for the reproduction of plants that exhibit desirable characteristics. Asexual methods of propagation used different plant parts such as roots, stems, or leaves of stock plants for grafting, tissue culture, division or cutting propagation. Generally, propagation by stem cutting has numerous advantages, many plants can be grown in high density trays from a limited amount of stock plants as compared to other sexual means of reproduction such as via seeds. It is typically less expensive, quicker and relatively simple (Hartmann *et al*., 2002). Stem cutting production involves removing the shoot tip from the mother plant and planting it in the growing substrate to root. This method allows for the retention of foliar flowering habits and characteristics that may not be carried over via seed production. Successful propagation of ornamental plants by rooting vegetative stem cuttings depends on several factors including the physiological status of stem cuttings, the propagation environment, fertility management, and growth regulator treatments, whether applied to the stock plant prior to harvesting or exogenously applied rooting hormones to stem cutting (Hartmann *et al*., 2002).

The capacity of each species to propagate vegetatively through cuttings is influenced by both internal and external factors. Internal factors comprise the physiological state, age, phytosanitary status, hormonal equilibrium, presence of anatomical barriers to rooting, presence of leaves and buds, and the season of the mother plant. External factors, as reported by Owusu *et al.* (2014), Amri *et al.* (2010),

and Dias *et al.* (2012), relate to the environmental conditions that occur during rooting, such as humidity, temperature and water availability.

Propagation of some ornamental plants is feasible through vegetative means, especially in plants that produce no seeds. The propagation of *Allamanda cathartica* in the vegetative mean is difficult, possibly due to the existence of latex within the plant parts that might suppress the root formation. Generally, this plant is propagated through stem cuttings using hardwoods or semi - hardwoods but with limited success. Hence, it is worth identifying an appropriate propagation technique having higher success. Hence, the present study was conducted to identify the most suitable propagation material and an appropriate potting medium for propagating the ornamental plant *Allamanda cathartica.*

METHODOLOGY

The research was carried out at the University of Colombo Institute for Agro-Technology and Rural Sciences in Hambantota, Sri Lanka. The area falls under the Low Country Dry Zone Agroecological region in Sri Lanka DL 5 where the mean annual temperature range is between 29ºC - 33ºC. The typical soil type of the area is Reddish Brown Earth.

Collection of planting materials
Healthy branches of *A. cathartica* were detached at early in the morning from a pest free healthy mother plants. Soon after detaching, the cut end of the branch was dipped in a bucket containing clean water to prevent it from drying through moisture losses during transport.

Preparation of planting material
Those branches were cut and separated as hardwood (fully matured brownish and woody parts), semi hardwood (partially matured and slightly woody), softwood (soft and succulent parts just below the shoot tip) and shoot tips (topmost three nodes with shoot tip) with three nodes. Those were separated under water to prevent air trapping in vascular systems that otherwise restrict the root formation. A slant cut was made at the abaxial end using a sharp blade to increase the surface area that facilitates rooting.

Treatment structure
The experiment involved a two factor factorial experiment with a total of 12 treatment combinations, each replicated four times with three experimental units. The experimental design was completely randomized.

The treatments were as follows;
T1 – Shoot Tip + Sand
T2 – Softwood + Sand
T3 – Semi hardwood + Sand
T4 – Hardwood + Sand
T5 – Shoot Tip + Coir dust
T6 – Softwood + Coir dust
T7 – Semi hardwood + Coir dust
T8 – Hardwood + Coir dust
T9 – Shoot Tip + Sand, Coir dust 1:1 mixture
T10 – Softwood + Sand, Coir dust 1:1 mixture
T11 – Semi hardwood + Sand, Coir dust 1:1 mixture
T12 – Hardwood + Sand, Coir dust 1:1mixture

Preparation of potting media
Pure coir dust, pure sand and a mixture of sand and coir dust 1 : 1 (v/v) were used as growing media for *A. cathartica*. Pure sand and coir dust were sieved to remove unwanted materials and to get fine particles to facilitate rooting.

Preparation of pots
Pots of 10cm x 15cm were prepared with black polyethylene (250 gauge) and holes at the bottom to facilitate the drainage of excess water. The pots were filled with the potting mixtures treated with a Captan fungicide to sterilize the media.

Planting of cuttings
Just before planting, the basal cut surfaces of all stem cuttings were garnished with a rooting hormone containing 0.03% Indole Butyric Acid to promote rooting. Each cutting type was carefully planted in polyethylene pots inserting at least a node completely to be inside the media.

Maintenance of cuttings
Planted cuttings were maintained under completely sealed propagator, covered using 500 gauge transparent polyethylene sheet. The structure was maintained under 50% shade condition throughout the period of four weeks. Watering of cuttings was not practiced during the period they were kept under the propagator.

Data collection
In the fourth week after the establishment following data were collected.

Survival percentage
Survival percentage was calculated using following equation;

$$\text{Survival percentage} = \frac{Survived\ cuttings}{No.of\ cuttings\ planted}\ x\ 100$$

Percentage of roote cuttings
Rooting percentage was calculated using following equation;

$$\text{Rooting percentage} = \frac{Rooted\ cuttings}{No.of\ cuttings\ survived}\ x\ 100$$

Number of leaves
Number of leaves were manually counted and recorded after two weeks.

Shoot length
Shoot length was measured in cm using a ruler after two weeks.

Number of roots per cutting
Newly emerged roots were counted manually and recorded.

Root weight
Roots were carefully separated and fresh weight was measured (g) using an analytical balance.

Data analysis
Collected data were statistically analyzed using ANOVA procedures and the difference between the treatments means was compared using Duncan's Multiple Range Test (DMRT) at 5% significance level by SAS 9.1.3 package.

RESULTS AND DISCUSSION

Survival percentage of cuttings
Significant interactions ($P<0.05$) were found between the growth media and type of cutting on survival percentage of cuttings of *A. cathartica* (Table 1). Higher survival rates were found in shoot tips planted in mixture of sand:coir dust media (CD) and sand only media, hard wood cuttings planted in coir dust media and mixture of sand:coir dust media when compared to other treatment combinations except shoot tips, soft wood cuttings and semihard wood cuttings planted in coir dust media. The lowest survival percentage was observed where semi hardwood cuttings planted in sand only media, compared to all other treatment combinations. Sand can function as a growth medium whether it is employed alone or in combination with other substances. It appears likely that sand possesses the characteristics of an ideal growth medium, including a sufficient amount of gas-filled pore space and an oxygen diffusion rate for healthy respiration to maintain root uptake (Fonteno and Nelson, 1990). High aeration in a growth medium is especially crucial for improving root initiation and stimulating metabolic processes (Yeboah and Amoah 2009). Further, adequate drainage in the propagation media ensures that excess water can drain away, allowing air to fill the spaces between particles. Oxygen is vital for root development as it facilitates cellular respiration, which provides energy for root growth. While cuttings require sufficient moisture to initiate root formation, excessively water-retentive media can be harmful (Eed *et al.*, 2015).
In order to prevent cuttings from rotting, the medium must have adequate drainage. When considering the drainage qualities, sand is a good medium. According to Meerow (2007), coir dust offers exceptional structural stability, water absorption, drainage, and cation exchange capability. Therefore, it is crucial to mix these media types to create a medium with the best drainage and aeration.

Percentage of rooted cuttings
It was revealed that, there was a significant interaction ($P<0.05$) effect between the tested factors in rooting percentage of cuttings of *A. cathartica* (Table 1). Shoot tips planted in pure coir dust and mixture of sand with coir dust media showed significantly highest rooting percentage while softwood, semi hardwood and hardwood cuttings planted in sand only media showed lowest rooting percentage. Similar results were reported by Waziri *et al*. (2015), the favorable effects of the coir dust based media on sprouting, the number of leaves, plant height, diameter, and leaf size seen in this study were probably caused by the availability of sufficient moisture content to induce rooting and root growth.
Eed *et al.*, (2015) mentioned that, the effect of interaction between the two factors of growing media and type of stem cutting on *Bougainvillea spectabilis* plants proved that, the basal stem cutting gained significantly the highest rooting percentage at all growing media used, compared with the middle and terminal cuttings. According to Hartmann *et al.* (1990), variations in the stems degree of juvenility may have an impact on nodal position and, consequently, root production. In vertically growing stock plants, alterations in environmental conditions, such as radiation intercepted, may also have an impact on the rooting response. However, changes in the rate of maturation along the shoot could influence root production (Jensen, 1967). In general, the capacity to initiate roots rises with distance from the apex (Hansen, 1986).

Shoot length
There was a significant ($P<0.05$) interaction between growth media and cutting type on shoot length of *A. cathartica* (Table 1). Shoot tips planted in a mixture of sand with coir dust showed highest shoot length (Plate 1). It was followed by all the other cutting types planted in all the tested media. This result might be due to the fact that shoot tip cutting generally produces a new plant faster since a well-developed shoot is already present as reported by Gary (1982). The results of this study agreed with the results of Hartmann and Kester (1975) who reported that the presence of leaves on cutting stimulates the influence on root. Similarly, Ismail (2011) found that the shoot tip cutting of *Dieffenbachia* species gave high percentage of rooted compared to other cutting. An experiment on *Bougainvillea* by Eed *et al.*, (2015) indicated that, highest plant height was significantly recorded with medium containing soil + sand (1:1), whereas the lowest value for this character was observed with medium composed of soil only, in comparisons with the other media studied.

Number of roots
Significant interaction ($P<0.05$) was found between growth media and cutting types on number of roots of *A. cathartica* (Table 1). Shoot tips planted in pure

Table 1. Survival %, percentages of cuttings produced roots, shoot length, root number and root weight of different cutting types in different potting media

Media types (M)	Cutting Type (C)	Survival (%)	Percentage of cuttings produced roots (%)	Shoot length (cm)	Root number	Root weight (g)
Sand	Tip	91.67^{a}	91.67^{ab}	2.30^{b}	10.50^{b}	0.30^{c}
Sand	Softwood	58.33^{bc}	8.33^{d}	1.63^{b}	1.67^{e}	0.06^{d}
Sand	Semi hardwood	16.67^{d}	16.67^{d}	1.25^{b}	2.00^{e}	0.05^{d}
Sand	Hardwood	33.33^{cd}	11.11^{d}	2.19^{b}	2.00^{e}	0.06^{d}
CD	Tip	75.00^{ab}	100.00^{a}	3.28^{b}	16.23^{a}	1.33^{b}
CD	Softwood	75.00^{ab}	66.67^{bc}	2.15^{b}	3.50^{de}	0.10^{cd}
CD	Semi hardwood	83.34^{ab}	83.34^{ab}	3.25^{b}	4.71^{d}	0.23^{cd}
CD	Hardwood	91.67^{a}	91.67^{ab}	3.33^{b}	5.29^{d}	0.27^{cd}
Sand + CD	Tip	100.00^{a}	100.00^{a}	7.35^{a}	17.94^{a}	1.57^{a}
Sand + CD	Softwood	66.67^{bc}	75.00^{abc}	1.77^{b}	7.75^{c}	0.05^{d}
Sand + CD	Semi hardwood	50.00^{bc}	50.00^{c}	2.83^{b}	10.50^{b}	0.09^{cd}
Sand + CD	Hardwood	91.67^{a}	83.34^{ab}	3.12^{b}	7.75^{c}	0.19^{cd}
	M × C	*	*	*	*	*

CD – Coir Dust; Means followed by the different superscripts in a same column are significantly different at DMRT 5%. '' represents significant at 5% and 'ns' represents not significant.*

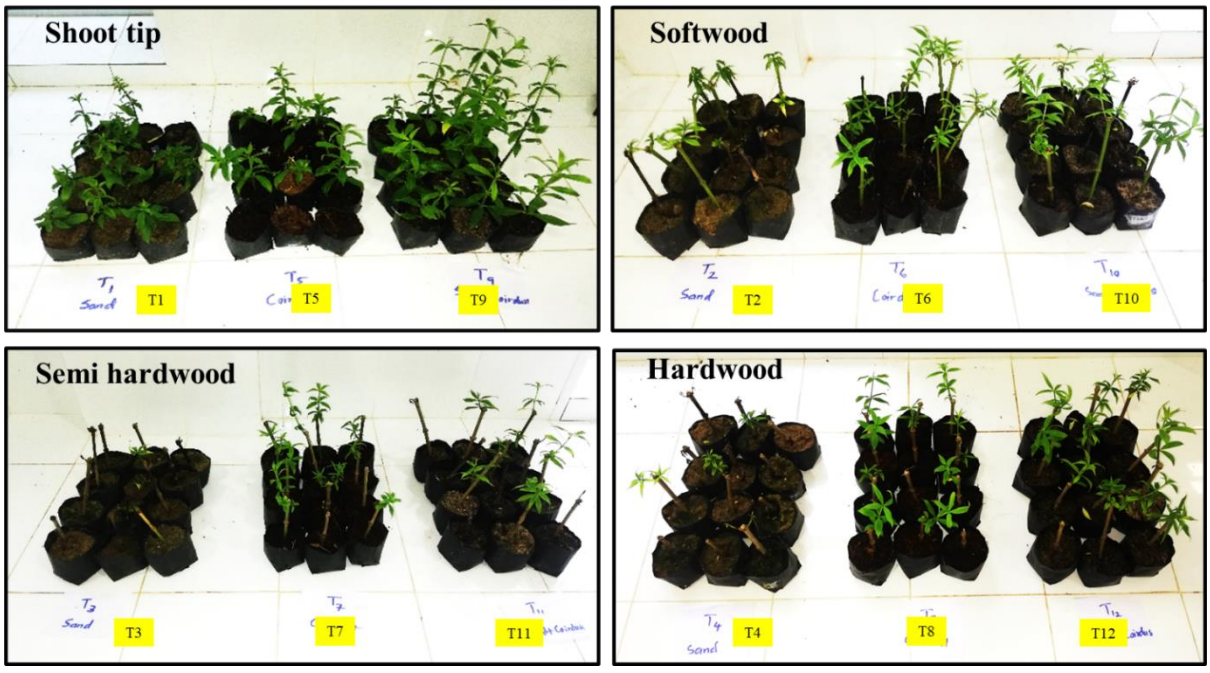

Plate 1. Overview of the *A. cathartica* plants in propagation experiment

coir dust and a mixture of coir dust and sand produced significantly higher number of roots. Lower values were recorded in softwood, semi hardwood and hardwood cuttings planted in sand only media. The variability in responses are likely related to differences in physical properties of the growth media (Khayyat *et al.,* 2007) and the supply of air and water to the growing plant (Baiyeri, 2003). Water can present a major barrier to the diffusion of oxygen so that excess water may result in anoxia at the base of the cutting (Loach, 1985). A similar study done using sawdust and sand as growth media exhibited that the sawdust showed lower need for irrigation than sand or sand:sawdust throughout the experimental period suggesting that it retained most of the water that was supplied to it. The potential for this growth medium to retain large amounts of water at the expense of plant growth and survival has been demonstrated by Ofodile *et al.* (2013) and Caspa *et al.* (2014). This can be applied for coir dust also having similar properties.

Root weight

A significant interaction (P<0.05) was observed on root weight between the tested factors; growth media and cutting types of *A. cathartica* (Table 1). It was recorded that, highest root weight found on the shoot tips planted in a mixture of coir dust: sand (1:1) media followed by a shoot tip planted in a coir dust only media. The lowest values were recorded in softwood, semi hardwood and hardwood cuttings planted in sand only media and soft wood cuttings planted in mixture of sand:coir dust (1:1) media. Enhanced aeration potential and drainage capacity/porosity, which promote root development and spreading, are responsible for better root growth (Hartmann and Kester, 1975; Olabunde and Fawusi, 2003; Puri and Thompson, 2003). Yeboah and Amoah (2009) found that increased aeration in rooting media is responsible for boosting metabolic processes and enhancing root initiation. Their research focused on the rooting performance of *Vitellaria paradoxa*. According to the result, the rooting potential of cuttings can be greatly influenced by the type of rooting media utilized. The study done by Muhammad Farooq *et al.* (2018) showed that the potting medium had a substantial impact on the fresh weight of roots per cutting. Also, the study done by Haile Abebe (2017) discovered that the rooting media had a substantial impact on root fresh weight and showed that least root mass was discovered in stem cuttings rooted in agricultural soil. Significant impacts of rooting material on the root fresh weight of rooted cuttings were also found by Shah *et al.* in 2006.

CONCLUSIONS

The study found that the survival and rooting percentages of the cuttings were significantly affected by the interaction of the media and cutting type. Shoot tips generally had higher survival rates and produced longer shoots, while semi-hardwood and hardwood cuttings planted in sand media showed lower values. The study highlights the importance of proper growth media selection, particularly in terms of drainage, porosity, and aeration, in enhancing root development and overall plant growth. The findings can be useful for plant propagators and farmers to select appropriate media and cutting for successful propagation of different plant types.

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