

**STUDY OF AGAR PRODUCTION IN TWO SELECTED
AREAS OF BANGLADESH**

A THESIS

By

FAISAL AHMED

Examination Roll No. 09 FPM JD-02 M

Semester: July –December 2010

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**MASTER OF SCIENCE (MS)
IN
FARM POWER AND MACHINERY**

**DEPARTMENT OF FARM POWER AND MACHINERY
BANGLADESH AGRICULTURAL UNIVERSITY
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ABSTRACT

In this study an attempt is made to find out the prospects of agar oil production from agar tree. This survey was carried out during the periods of July'09 to November'10 at the selected eight small scale agar based enterprises in Haluaghat and Barlekha upazillas.

During this survey plant growth, number of fruits, agar cultivation process, and agar production were studied. From the canopy structure the plant density in one hectare was calculated. If intercropping is done 2.5 cm distance between each plant, approximately 1600 plants can be planted in one hectare and total cultivation expenditure approximately, stood at Tk.432700. The yield of the final harvesting tree at 15-20 years were found almost 35-50 gm agar oil and 15-80 kg agar wood per tree. During the first 7th year the farmer could get a negative return. But cultivation after 15th year the estimated net income of the farmers would be around Tk.25,30400 per hectare. The estimated agar oil content per hectare is found to be 40 kg and the estimated 1 gm distillable agar oil price is Tk. 80. From an established plantation thus, a net income of approximately, Tk. 25-30 lakhs after 15 years per hectare may be generated. The average annual net revenue of the agar based enterprises was found to be Tk 103282. So, it indicates agar production is a profitable business.

Agar is a new economical tree in our country. So, necessary steps should be taken immediately to make it popular among farmers and small entrepreneurs. Thus, we can save a substantial amount of foreign currency which is being spend to import perfumery materials.

CHAPTER-1

INTRODUCTION

Agar oil and agaru or agarwood are the most exalted perfumery raw material obtained from the infected wood of agar tree. This transformed wood due to fungal infection yields agar oil on distillation that has unique fragrance and high export value. The agar oil known in the East as Agar-attar, is one of the perfumery's oldest materials. It is considered one of the costliest perfumery raw materials used in high-class perfumery and as a fixative, imparting a lasting balsamic odor to the product. Its traditional use in India, Middle Eastern and Far Eastern perfumery, both as skin daub and as a fixative, rivals its former usage holy incense by the Hebrews. Agar tree (*Aquilaria agallocha*) is distributed in all the Northeastern States and widely cultivated. It is now rarely found in wild state. *Aquilaria agallocha* Roxb (Family Thymelaeaceae), locally known as agar, is a moderately tall and erect evergreen tree. It may grow to a height of 20 m. The species is distributed naturally in India, Bhutan, Bengal and Myanmar. It is particularly found in Assam on hill forest of Khasia, Garo, Naga, Cachar and Sylhet . The wood is soft and light. The main value of the species is the formation of dark color, resinous and aromatic substance in word called agar.

1.1 Definition of Agar:

According to the US Pharmacopeia, agar can be defined as a hydrophilic colloid extracted from certain seaweeds of the Rhodophyceae class. It is insoluble in cold water but soluble in boiling water. A 1.5% solution is clear and when it is cooled to 34-43°C it forms a firm gel which does not melt again below 85°C. It is a mixture of polysaccharides whose basic monomer is galactose. These polysaccharides can be sulphated in very variable degrees but to a lesser degree than in carrageenan. For this reason the ash content is below those of carrageenan, furcelleran (Danish agar) and others. A 5% maximum ash content is acceptable for agar although it is normally maintained between 2.5-4%. Agar is the phycocolloid of most ancient origin. In Japan, agar is considered to have been discovered by Minoya Tarozaemon in 1658 and a monument in Shimizu-mura commemorates the first time it was manufactured. Originally, and even in the present times, it is made and sold as an extract in solution (hot) or in gel form (cold), to be used promptly in areas near the factories; the product was then known as tokoroten.

Its industrialization as a dry and stable product started at the beginning of the 18th century and it has since been called kanten. The word "agar-agar", however, has a Malayan origin and agar is the most commonly accepted term, although in French- and Portuguese-speaking countries it is also called gelosa. Agar in the form of a sweetened and flavored gel has been known in the Orient for ages. It is known in Japan as “Kanten” meaning “cold weather,” in China it is “Dongfen” or “frozen powder.” The word “agar” is Malayan and is used in the double form agar-agar, originally referred to jellies of certain seaweeds especially *Eucheuma muricatum* of the East Indies. It was said that Chinese migrants to the East Indies imported the Japanese kanten for their own use. They also called it agar-agar. The Europeans in the East Indies learned to use this Japanese product for making fruit jellies, and subsequently introduced it to Europe. Thus, a Malayan term became attached to a Japanese product.

Agar production by modern industrial freezing techniques was initiated in 1921 in California, U.S.A. by Japanese named Matsuoka. Now the biggest agar factory in the U.S.A. is the American Agar Company in San Diego, California. During the second world war the production of agar commenced in Portugal and Spain as well. In Japan, some two-thirds of the agar makers still rely on the natural winter weather to produce strip agar and square agar. The rest have modern equipped factories using the mechanical freeze-thaw process. Even in today's modern agar factories the fundamental principle of extraction and purification (freeze-thaw) of agar is similar to that found by Tarozaemon more than three hundred years ago.

In 1945 Dr. Tseng defined agar as “the dried amorphous, gelatin-like, non-nitrogenous extract from Gelidium and other agarophytes, being the sulfuric acid ester of a linear galactan, insoluble in cold but soluble in hot water, a one per cent neutral solution of which sets at 35°C to 50°C to a firm gel, melting at 80°C to 100°C.”

Araki (1966) referred to agar as a gel-forming substance obtainable from certain species of red seaweeds called “agarophytes” composed of neutral gelling molecules, agarose, and to a lesser extent acidic non-gelling molecules, agaropectin. He also noted that agaropectin is closely related to agarose as it has a similar backbone structure. Araki's definition of agar involved the chemical structure of the polymers, but it was an over-simplification of the complex continuum between neutral and highly charged polymers existing in algae.

The American Society for Microbiology (1981) in the “Manual of Methods for General Bacteriology” defined agar according to Araki as “an extract from certain red marine algae consisting of two polysaccharides, agarose and agaropectin, with the former comprising about 70% of the mixture”. It is not enzymatically degraded by most bacterial species: agar gels are stable up to 65°C or higher, yet molten agar does not gel until cooled to about 40°C, and agar gels have a high degree of transparency. Rees (1969), from chemical structure, defined the agar family of polysaccharides as the polymers sharing a common backbone structure: 1,4-linked- 3,6 -anhydro- α -L-galactopyranose alternating with 1,3-linked-B-D-galactopyranose , which may be “masked” to a varying extent by different sugar residues. He pointed out that agar belongs to the family of polysaccharides and that the component having the greatest gelling tendency is agarose.

1.2 Uses of Agar

Agar was the first phycocolloid to be used in the human food industry. In the beginning it was only used in the Far East, but the applications have been extending all over the world for more than a century. The increasing range of applications is due to the particular gelling characteristics which are not present in any other phycocolloid, gum or gelatin. As a result the price for food grade agar is higher than that of other phycocolloids with gelling properties which are also permitted as food additives. In addition, these characteristics allow agar to be used successfully and even exclusively in certain scientific and industrial applications.

Agar applications in the food industry are based on its special characteristics and the most important applications are the following.

- In confectionery, to prepare jellies, marshmallows and candies or candy fillers. In marmalade production, agar is used as a thickening and gelling agent.
- Mitsumame production in Japan is very important; this is a fruit salad mixed with agar gel cubes, duly coloured, salted and flavoured with fruit flavour. The agar used for this kind of fruit salad must allow the cans to be sterilized without the cubes melting or losing their corners or edges. For this purpose certain types of Gelidium agar are used.

- In bakery, agar is used to cover cakes, in icing doughnuts, and when it is applied to chocolate it allows a good adherence to the base without cracking. In general agar is utilized to prevent dehydration of these confectionery products.

- Agar is also important in fruit jelly preparations. When compared with pectin, agar has the advantage of not needing high sugar concentrations to form a gel. Its application in yoghurt is also very important especially when consumers started to require less acid products and, therefore, casein cannot contribute to the maintenance of the product consistency, as it previously did. In the meat industry, and especially in the preparation of soft boiled sausages, its use has permitted the reduction of fat content that acted before as bonding. Today the industry is trying to limit fat content in order to reduce cholesterol.

- Agar is also used on a large scale in canned products like "scatola" meat (beef blocks in gelatine) - very popular in Italy, or chicken in gelatine - very common in Canada, cow tongue in gelatine -selling well in Denmark, lamb tongue in Australia, or other different types of meat and fish aspics. In dressings and extracts it is used as a thickener and stabilizer. In smaller quantities, agar is used to increase the viscosity of some alcoholic liquers.

- The important gel-forming properties of agar have permitted the expansion of its use to applications other than the human food industry. To prepare casting moulds, an aqueous solution of high concentration (8% or more) is used with the addition of glycerin or glycols, as well as a preservative to avoid gel surface contamination by moulds; bacterial contamination is impossible because a gel with such a high concentration has very little free water left where bacteria can grow (it has a very low A_w). These kinds of gels are also used in sculpture, archaeology, and in other works in which a perfect or precise reproduction is essential. For this reason it is also used in dental moulds as it is possible to make better and more precise reproductions, in spite of the fact that casting moulds prepared with agar are more expensive than those prepared with alginate paste. The purpose of adding glycerin in these uses is to avoid cast dehydration since a balance with the outside humidity can be achieved and therefore stable gels can also be obtained which do not appreciably change the gelling and melting points. Glycerin also expedites heat

transfer permitting a faster gel melting in a boiling water-bath. The perfect reversibility of an agar gel permits it to be repeatedly used in this application and its low gel point makes it possible to be used as a dental mould. Another rare application is the preparation of food to feed insects during their larval stages.

- The breeding of certain insects on a large scale, such as the Mediterranean fruit fly that attacks fruit trees or Pectinopora glosipeii that attacks cotton plantations, has made plague control possible through a type of biological warfare. Large numbers of insects are grown and sexually sterilized by gamma rays. When released they drastically reduce the reproduction of the overall population.
- The application of agar in pharmacy as a smooth laxative is well known. Lately it has been used as an excipient in pharmaceutical preparations. In some Western countries agar is used as an antirheumatic since a prolonged treatment has permitted important improvements in patients' health. Agar has been used to stabilize cholesterol solutions. Due to its low tendency to precipitate in alcoholic media, it is possible to prepare agar gels with alcohol concentrations that will burn when a match is applied to them.
- Agar has been used in orchid nurseries for a long time. Improvement in cellular cultivation know-how has brought another important application of agar mainly in the techniques that, starting from meristems, produce perfect and virus-free clones of plants.

CHAPTER-2

OBJECTIVE

Based on reference on the work of agar production the following objectives are selected:

Main objective: To study the extraction process of agar oil from agar tree at selected small agar based Enterprises.

Specific objectives are:

- To familiar with the agar tree & study the physical properties of the agar plant.
- To study the cultivation method.
- To study the process of agar oil extraction.
- To study the possible agar oil and agar wood production in one hectare of land and its estimated cost analysis.
- To study the financial performance of the study site.

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CHAPTER-3

REVIEW OF LITERATURE

The main purpose of this chapter is presented up-to-date information regarding the research topic addressed here. Important information related to the present study is represented below.

3.1 Agar oil formation Methods

Yanagawa (1938) showed that agar is either extracted directly from the agarophyte (native agar) in conventional agar production or is extracted after chemical treatment of the agarophyte in industrial agar production. In general, native agars obtained from *Gracilaria* species have low gel strength. However, this characteristic is improved by alkali treatment. Optimal alkali treatment conditions vary with agarophyte species & harvest site.

Rees (1961) described that alkali treatment converts the biological precursor of agar, L-galactose-6-sulphate into 3,6-anhydro-L-galactose, which results in higher gel strength. It has also been shown that the agarose content of agar increases after alkali treatment.

Izumi (1970) stated that the enzymatic hydrolysis of its agar occurs spontaneously even at relatively low moisture contents, but at variable rates depending on the Gracilaria species and its origin. Gracilaria harvested in India, Sri Lanka, Venezuela, Brazil, and generally in warm waters, has an agar (agarose) less resistant to enzymatic hydrolysis than the Chilean Gracilaria which is the most stable. Nevertheless, the stability of agar contained in Gracilaria is less than that of Gelidium; Gelidium agar can be preserved in seaweeds indefinitely provided they have been well treated.

Matsushashi and Takahashi (1971) surveyed the agar production in Nagano Prefecture, Japan, showed that native agars from the *Gelidium* species (refined by mechanical freezing) have gel strengths from 500-600 g/cm² representing the best quality of bar and string agar. He also showed alkali –treated *Gracilaria* extracted by boiling with water in open vats then frozen naturally (conventional extraction method) generally have gel strengths above 600g/cm².

Arnott, S., et al. (1974) stated that in agar gels, helicoidal structures have been verified by X-ray diffraction, similar to those found in carrageenan. However because agar contains

3,6-anhydro-L-galactose, the helices are left-handed whereas in kappa and iota carrageenan, which contains 3,6-anhydro-D-galactose, they are right-handed (dextrogyres). Also the helix pitch is shorter than the 26Å of carrageenan. This is explained by several authors as being due to the lower content of sulfate groups that possibly cause a tighter and more compact net. Molecular configuration changes and agarose interaction in sol-gel transitions have been well studied through ultra vacuum circular dichroism (u.c.v.d).

Xaixaso and A.L.B (1987) described that the production of agarophytes from natural stocks is very much influenced both by seasonal factors and by harvest pressures exerted on them during the preceding cropping season. Because their growth cycles are greatly influenced by environmental conditions and by man's exploitive activities, their production is unreliable. They are prone to over-exploitation, and the need to manage and conserve their stocks is of prime importance in order to sustain or further enhance their productivity and prevent over-exploitation.

Poblete et al. (1987) mentioned that the application of a management scheme to natural stocks has been shown to significantly improve the total production of the beds. In Chile, the annual production of *Gracilaria* in Lenga Cove located in San Vicente Bay increased from 80 tonnes to 600 tonnes after the application of a management programme. It is apparent from their studies that the strict application of a management scheme had improved the production ecology of the bed, resulting in an increase of seven and a half times its normal production

Trono et al (1988) informed that the production of agarophytes from natural stocks is very much influenced both by seasonal factors and by harvest pressures exerted on them during the preceding cropping season. Because their growth cycles are greatly influenced by environmental conditions and by man's exploitive activities, their production is unreliable. They are prone to over-exploitation, and the need to manage and conserve their stocks is of prime importance in order to sustain or further enhance their productivity and prevent over-exploitation.

Koshy (1988) informed that Information on the abundance and distribution of the resource in space and time may be gathered through biomass samplings of the stocks. The

application of available methodologies may differ slightly, depending on the behavior of the resource. However, the transect quadrat method is widely used especially in situations where stocks are not homogeneously distributed in space. The size of the bed is first delineated and permanent transects are marked. The orientation of the transects is generally related to certain ecological gradients, such as depth and wave exposure. Biomass samplings are generally done on a monthly basis along the transects; the size of the quadrat varies from 0.25-1.0 m².

Madgwick (1990) said that practical use for seaweeds, especially now, in the field of biotechnology are more diversified than have been hitherto anticipated. Now available are the possibilities of enzymatic post-harvest modification of alginic acid, extraction and purification of non-protein amino acids with bio-inhibitory effects and the use of seaweed biomass as a sole nutrient source to heterotrophic microbes of mineral biodegradation .

Amerisia et al (1990) described that in an experimental level, a feed technologist has boiled *Eucheuma* to extract the colloid, mixed the resulting colloid proportionally in a molasses mixer and incorporated it into fish feeds. It is claimed to work well as a binder. China is the world's leading seaweed producer. It uses seaweeds primarily for domestic markets and human consumption. Lately, China is more interested in the use of Carrageenan for improving food product, and so could be a potential for a large volume market. They also culture about 5000 MT of *Gracilaria* per annum around Hainan. With promising results, China expects a substantial development of *Gracilaria* culture to meet the increased demand for agar.

Orosco and Suito (1991) analysis of *G. chorda* agar and shown that 80% of its sulfate content was alkali-labile & was converted to 3,6-anhydro-L-galactose upon alkali treatment.

Nukaya and Kusunose (1992) stated that *G. verrucosa* has a higher yield than the species imported from Taiwan & processed under the same conditions. The Taiwan species has an agar yield of 10.6±1.2% with a gel strength of 660±45 g/cm².

Truus (1994) mentioned that the composition and structure of agar from *A. tobuchiensis* were produced by various methods including 13C-NMR spectroscopy. He was shown that the polysaccharide has only slight deviations from the structure of agarose and usually

contains 0.6-0.9% sulfate groups, depending on the technology (especially on the duration of washing of agar gel with water). A content of sulfates of 0.6% serves as a criterion for discrimination of polysaccharide fractions into agar and agarose. Purification of agar by chromatography on DEAE-Sephadex and QAE-Sephadex is used for agarose production. The goal of this work was to produce agarose from *A. tobuchiensis* without the expensive step of chromatographic purification.

Azmi (1997) stated that Agar use as a perfume has been recorded in the Old Testament. Agar wood incense has been burned to produce a pleasant aroma for centuries, on important religious ceremonies, by Buddhists, Hindus and Muslims.

Sukhoverkhov, Kadnikova and Podkorytova (2000) described that Red algae (Rhodophyta) are used as raw material for the production of agar and similar products. In the Far East of Russia, food and microbiological grade agars are produced from the red alga *Ahnfeltia tobuchiensis* (Kanno et Matsubara) Mak. obtained in Peter the Great and Izmena bays, Kunashir Island.

Chang et al. (2001) described that Agar, eaglewood, gaharu and aloeswood are alternative names for the resinous, fragrant and highly valuable heartwood produced by *Aquilaria agallocha* Roxb. (Thymelaceae) and other species of the Indomalasian tree genus *Aquilaria*. The species, which attains a height of about 40 m, is tropical evergreen in nature and sometimes is also named as *A. malaccensis*. It occurs widely in south and south-east Asia, including in Bhutan, Nepal, India, Myanmar, Malaysia, Indonesia, Thailand, Vietnam and Papua New Guinea.

Chowdury et al. (2003) explained that agar, eaglewood, gaharu and aloeswood are alternative names for the resinous, fragrant and highly valuable heartwood produced by *Aquilaria agallocha* Roxb. (Thymelaceae) and other species of the Indomalasian tree genus *Aquilaria*. The species, which attains a height of about 40 m, is tropical evergreen in nature and sometimes is also named as *A. malaccensi*.

Venkataramanan et al. (2003) described that among different fungal species reported to be associated with agar zones, few could exhibit pathogenesis with the development of disease symptoms while others seem to be of saprophytic nature in different eco-geographical conditions.

Buriyo *et al.* (2003) stated that tanzania has large quantities of natural stocks of the commercially exploitable agarophyte algae, such as species of *Gracilaria*. The most abundant species, *G. debilis* (previously known as *G. crassa*, *G. fergusonii* and *G. salicornia* have been reported to have a potential for agar production, however, these are not currently exploited. This is partly due to lack of information on their suitability (and demand) for the production of good quality native agar.

Maheshwari *et al.* (2003) observed that the characteristic components of agarwood oil were found to be lower in the oils obtained from healthy samples. The oils obtained from artificially inoculated agarwood have no such differences with the oils of healthy wood though little changes were observed. This may indicate that naturally infected type of agarwood would not be achieved by artificially screws injected plants oil. The observations made by us showed that the microflorais of great importance in production of specialized type of agarwood for best quality agar oil. However, there may exist variants or eco-types within the agarwood plant species. If natural variant or type exists within the plant species, the fungal pathogens might be host type specific or variant specific. If it is so, there may exist specific host variant pathogen/host type-pathogen relationship, which determines the success of artificial inoculation. Therefore, identification of natural variant or eco-type and the specific host-pathogen relationship under different ecological conditions is expected to give clue for unraveling the secret of agar formation. Then only artificial supplement of inoculum to the specific host might give positive result for induction of disease in the plant. On the basis of above fact it may be concluded that *A. agallocha*, may be utilized as a source of natural octacosane, cycloheptane, 4-methylene-1- methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl- and diisooctyl phthalate respectively.

Alam (2004) stated that topographically, the upazila consists of small to medium hillocks locally termed as tillah. Soils in the area are sandy-loam to clay-loam, and are slightly acidic. The average annual rainfall ranges between 600 and 2,000 mm. November–February (winter) are relatively dry months, with hot weather in March and April. So, it is very fertile climate for agar plants.

Tamuli *et al.*, (2005) investigate the difference in composition of oils obtained from healthy, naturally infected and artifically inoculated eaglewood using GC and GC-MS analyses. This investigation shows a marked difference in the oil compositions among the treatments with regards to their quality. Valerianol (3.0%) and tetradecanoic acid (7.1%)

contents were recorded higher in the oils of naturally infected plants than in that of healthy ones (0.1% and 6.9% respectively). Pentadecanoic acid was totally absent in the oils of healthy plants, whereas it was found in a greater amount (6.8%) in the oil of naturally infected plants. In contrast dodecanoic acid (3.1%), pentadecanoic acid (6.2%), hexadecanoic acid (31.5%) and octadecanoic acid were found in the oils of healthy plants, while the oils obtained from naturally infected plants contained lower amounts of these components (2.3%, 4.8%, 20.0% and 1.0% respectively).

Bhattacharyya et al. (2005) isolated a new sesquiterpene, agarol and a coumarinolignan, aquillochin, respectively, from the oil of agarwood. He further characterized the presence of two more sesquiterpene alcohols, jinkohol II and jinkoheremol, from the Indonesia agarwood oil. Nakanishi et al. (1984) again reported that a benzene extract of an Indonesian sample of 'Jinkoh' agarwood was found to contain α -agarofuran, 10-epi- γ -eudesmol and oxoagarospirol.

Semesi (2005) examined that the algae were collected during the wet and dry seasons, from Oyster Bay in Dar es Salaam and Chwaka Bay in Zanzibar. In the laboratory, algal samples were sorted, identified using Jaasund and other relevant taxonomic literature and pooled in respective taxa. Plants for agar extraction were rinsed with tap water, placed in plastic trays and dried in the sun. Sundried samples were further dried at 60 degree Celsius in the oven until a constant weight was reached before agar extraction.

Hayder et al. (2005) mentioned that Agar is one of the most promising non-timber forest products (NTFPs) of Bangladesh, and earned Tk.1, 300M through exports of attar (agar oil) in 2004. About 25,000 workers were engaged in cultivation, collection, processing and marketing of agar and agar-based products in that year. Despite the huge demand in local and international markets, no major extension program has so far conducted by governments or other agencies in Bangladesh. The Forest Department (FD) recently raised some agar plantations in denuded and encroached forest areas of the Chittagong and Sylhet districts. Agar is found irregularly in the forests of Sylhet, Chittagong and the Chittagong Hill Tracts (CHTs). There are also some privately owned agar plantations in the north-east, particularly in Maulvibazar district where many families have been engaged in production and marketing of agar and agar-based secondary products for several decades. Of the 121 registered agar-based factories nationally, 111 are located

within this region, making a major contribution to regional employment and gross domestic product.

Nakanishi et al. (2006) examined that The three types of essential oil in different types of woods from *A. agallocha* were analyzed by GC-MS electron impact ionization (EI) method on GC-17A gas chromatograph (Shimadzu) coupled to a GC-MS QP 5050A mass spectrometer (Shimadzu); fused silica capillary column (30 m x 2.5 mm; 0.25 mm film thickness), coated with DB-5 ms (J&W); column temperature 100°C (2 min) to 250°C at the rate of 30°C/min; carrier gas, helium at constant pressure of 90 Kpa. Acquisition parameters full scan; scan range 40-350 amu.

Yarish et al. (2007) examined that agar is extracted from species of the red algal genera *Pterocladia*, *Gelidium*, *Gracilaria*, *Phyllophora*, *Ahnfeltia*, *Campylaeophora*, *Acanthopeltis*, *Gelidiella* and *Gracilariopsis*. About 10,000 tonnes of agar are currently produced worldwide. The major producing countries are Japan, Spain, Chile, Mexico, China and the Republic of Korea. Prices of the product depend on gel strength specifications and on the agar application.

Gavino et al. (2007) stated that the production of agar-producing seaweeds comes from three sources, namely gathering of drift materials, direct harvest from natural stocks and cultivation. At present, the bulk of the seaweeds used for agar manufacture still come from harvested natural stocks. There are no available and accurate data on the contribution of agarophytes produced through culture, but judging from the genera presently produced in different countries, which were imported by Japan in 1984. About 50% of the raw seaweeds presently processed into agar still come from natural stocks. The genera presently utilized in the international market for agar production are *Gracilaria*, *Gelidium*, *Pterocladia*, *Gelidiella*, *Ahnfeltia* and *Ceramium*.

ASB (2008) surveyed that Haluaghat upazila is located at 25.1250° N latitude and 90.3500° E latitude (Fig-1). It has 49520 units of house hold and total area 356.07 km². The Barlekha upazila covers an area of about 430 km² located between 24° 50'N latitude and 92° 18' E latitude.

3.2 Gel formation and structure

Armisen et al (1978) stated that the agar gels, helicoidal structures have been verified by X-ray diffraction, similar to those found in carrageenan. However because agar contains 3,6-anhydro-L-galactose, the helices are left-handed whereas in kappa and iota carrageenan, which contains 3,6-anhydro-D-galactose, they are right-handed (dextrogyres). Also the helix pitch is shorter than the 26A° of carrageenan. This is explained by several authors (Arnott et al., 1974) as being due to the lower content of sulfate groups that possibly cause a tighter and more compact net. Molecular configuration changes and agarose interaction in sol-gel transitions have been well studied through ultra vacuum circular dichroism (u.c.v.d). The gelation process from solution (colloidal sol) happens as per the scheme shown in Appendix- B. which shows the different steps starting from random coil, through the left-handed dual helix formed by hydrogen bond formation that then will be the base for the macro grid that will give the gel rigidity. The hydrogen bond formation can be obstructed and even prevented if a proton-catching agent (like urea or guanidine) is added to the sol to be gelled. Such additions prevent agar or agarose gelation and result in a solution, similar to glycerin, which when cooled does not gel. By removing the proton catcher, the hydrogen bonds will form and therefore the gel-forming ability will be restored. In the same way, considering that dry agar, be it in powder, flake, square or strip form, is really a dry gel (xerogel), its solubility in the cold is not possible as it maintains the hydrogen bonds formed during the gelation prior to its dehydration.

A fundamental characteristic of an agar and agarose gel is what can be called "gelation hysteresis". An agar or agarose gel, when cooled, forms a gel at temperatures between 32° and 43°C depending on the seaweeds used, as that will determine the presence of a variable quantity of methyl groups. However when the well formed gel is heated, a temperature of 85°C must be reached to get the gel to melt and to become a sol. Such a big difference between gelling and melting temperatures is exceptional when compared with the rest of the phycocolloids. It is explained by a greater number of hydrogen bonds and

the lack of sulfate groups, which produce a gel with helix pitches much shorter than those of carrageenans, and that, in contrast, does not show cation reactivity.

The characteristic of "viscosity hysteresis" is also remarkable. This can be demonstrated by a solution or colloidal sol prepared at boiling point and held in a thermostatic bath, for example at 80°C, and then its viscosity measured. Afterwards it is held at a lower temperature, at 50°C for example (above the gelling temperature of the sol) keeping it there for a few hours. Subsequently it is held again at 80°C and once this temperature is reached its viscosity is determined again, and it gives values higher than those initially measured. When this temperature is maintained, viscosity values decline slowly almost down to the values measured the first time. Therefore the viscosity values obtained for a solution of agar could depend on its previous history. Agar has the ability to form gels upon cooling of a hot solution to 30 – 40°C and to melt to sols upon heating to 90 – 95°C. At temperatures above the melting point of the gel, thermal agitation overcomes the tendency to form helices and the polymer exists in solution as a random coil. On cooling, a three-dimensional network builds up in which double helices form the junction points of the polymer chains.

3.3 Properties of Agar

Amoott et al. (1974) identified several properties of agar. The most important characteristics of agar are the following.

1. Its great gelling power in an aqueous environment allows it to form gels which are more resistant (stronger) than those of any other gel-forming agent, assuming the use of equal concentrations.
2. The simple water solution has that gelling power. There is no need to add reagents to produce gelation, such as potassium (or proteins as is necessary with carrageenans), calcium (or other divalent cations as is necessary with alginates). High sugar concentrations or an acid environment (as is necessary with pectins) are not needed.
3. It can be used over a wide range of pH, from 5 to 8, and in some cases beyond these limits.

4. It withstands thermal treatments very well, even above 100°C which allows good sterilization.
5. A 1.5% aqueous solution gels between 32°C-43°C and does not melt below 85°C. This is a unique property of agar, compared to other gelling agents.
6. Agar gives gels without flavour and does not need the additions of cations with strong flavours (potassium or calcium), it can be used without problems to gel food products with soft flavours.
7. It assimilates and enhances flavours of products mixed with it and acts as a fragrance-fixer permitting their long term fixation.
8. Its gel has an excellent reversibility allowing it to be repeatedly gelled and melted without losing any of the original properties.
9. Transparent gels that are easily coloured can be obtained whose refractive index can also be easily increased by adding sugar, glucose, glycerine, etc., given them an attractive brightness.
10. The gel is very stable, not causing precipitates in the presence of certain cations as happens to alginates with calcium.

3.4 Variation of species and origins of Agar

In Bangladesh two types of *A. agallocha* could be identified as '***Jati Sanchi***' and '***Bhola sanchi***' in the population. '***Bhola sanchi***' is comparatively of quick growing but yield is less than that of *Jati sanchi*. It is the *Jati sanchi* that is preferred for commercial cultivation.

- *Aquilaria apiculina*, found in phillippines
- *Aquilaria agallocha* , found in Bangladesh
- *Aquilaria baillonil*, found in Thailand and Cambodia
- *Aquilaria baneonsis*, found in Vietnam
- *Aquilaria beccarain*, found in Indonesia
- *Aquilaria brachyantha*, found in Malaysia

- *Aquilaria crassna*, found in Malaysia, Thailand and Cambodia
- *Aquilaria cumingiana*, found in Indonesia and Malaysia
- *Aquilaria filarial*, found in China

(Source: Wikipedia, 2009)

3.5 World agar production

Bhattacharyya et al (2005) calculated the agar oil and agar production in different countries all around the world. They found that the Asian country Japan is the height producer of agar production and the total 6683 (MT) agar produces around the world.(Table-3.1)

Table 3.1 Agar production in different countries, 2005

<u>Country</u>	<u>Total (MT)</u>
Japan	2 440
Spain	890
Chile	820
Korea Republic	600
Morocco	550
Portugal	320
Taiwan	275
Argentina	197
Indonesia	150

PROChina	140
Mexico	80
USA	70
France	65
Brazil	60
New Zealand	26
Total	6 683

Source: Bhattacharyya et al (2005)

3.6 World major agar Exporters

The world famous website Wikipedia (2009) published the major agar and agar products exporter all around the world. They described Chile is the highest agar exporter country all around the world in 2008-2009.(Table-3.2)

Table 3.2. Major Agar Exporters,(2008 & 2009)

<u>Country</u>	<u>2008</u>	2009
	(MT)	(MT)
Chile	976	873
Korea Rep.	682	749
Spain	555	715
Morocco	510	615
Japan	529	430
Portugal	331	347
Total	3 583	3 729

Source: (Wikipedia ,2009)

3.7 The artificial cultivation of *Gracilaria* agar

With the development of the agar-agar industry, the need for Gracilaria increased further. Experiments on artificial cultivation have been carried out in many countries in recent years. The stake-net culture of G. asiatica in the tidal zone was carried out by Sukhoverkhov and others (Institute of Oceanology, Academia Sinica,(2006) in Qingdao; culture of G. sjoestedtii was carried out by Nakanishi and others (2000) . They reported that China is the foremost country in Gracilaria culture. Remarkable achievements, especially along the southern coast of mainland and the western coast of Taiwan, have been reported. Two methods are chiefly used for artificial cultivation of Gracilaria in China. One is pond-scattering culture and the other is raft culture of stake-rope culture. The first method is most suitable for species from which sporelings are easy to obtain. G. tenuistipitata v. liui is an example, it propagates vegetatively and can be harvested from time to time during the whole growing period. G. parvaspora and G. blodgettii are other examples. They propagate through spores released now and then in spring, summer and autumn. In this method, sporelings are cultured in seawater ponds, saline lakes or wasted salt pans. The procedures are easy. The second method is used to culture species which have good quality and high agar content, such as G. asiatica, G. tenuistipitata, G. gigas and G. sjoestedtii, etc. They propagate only through spores.

Artificial cultivation of Gracilaria is carried out by taking into account its biological characteristics. Suitable sites must be selected and effective measures must be adopted in order to get good results.

3.7.1 Selection of culture sites

A basic requisite in the selection of a site for the cultivation of Gracilaria is an understanding of the ecological factors required and culture method chosen. Generally, three types of cultivation sites exist, namely, areas inside bays, offshore regions, and ponds.

a. Criteria for selecting inside bay sites

- i. The shoal is flat and wide. Water remains during ebb tide so that the fronds will soak and grow there and not dehydrate and die due to exposure to air.
- ii. Hard, sandy clay bottoms are good for the growth of fronds. Unsuitable sites are muddy shores where the water becomes turbid and the growth bases will be buried easily.
- iii. Areas exposed to frequent typhoons, floods and strong waves should be avoided. Seawater should be clean and unpolluted. The rate of water exchange should be good.
- iv. A certain amount of freshwater should be let in. The seawater should be rich in nutrient salts and its nitrogen content should be above 100 mg per cubic meter of water.
- v. The specific gravity range of the seawater should be 1.010 to 1.025.
- vi. The temperature should be above 0°C in winter and below 35°C in summer.

If the raft culture method is used on shoals inside a bay, the first two requisites mentioned above are of great importance, but the water depth during ebb tide should be maintained at 1.5 meters deep. If raft culture is carried out in the tidal zone, the first two requisites are very important.

b. Criteria for selecting ponds as farm sites

At present, ponds are used to culture G. tenuistipitata v. liui along the coast of South China and the western coast of Taiwan.

- i. Some enclosed fields too salty for growing rice and too shallow for raising fish can be used to culture Gracilaria. Renovated salt pans and pools can also be used.
- ii. The water is 0.3–0.5 meters deep.
- iii. The optimum specific gravity is about 1.010. The suitable range is from 1.005 to 1.015. Growth will be hindered if it is above 1.020.
- iv. The optimum temperature ranges from 20°C to 30°C Gracilaria will stop growing if it is over 30°C or below 10°C and will rot and die if it is above 35°C.
- v. The desirable pH value is about 8.0. If it is below 7, fronds die.
- vi. Sandy clay bottom is suitable. Muddy shores should be avoided because turbidity due to the high organic load of mud and silt will hinder the growth of the species cultured.

Production experience has proved that the criteria considered for selection of sites are not very strict. Areas from inside bays to offshore can be used for Gracilaria culture in most cases, but local geographical and sea conditions should be considered and suitable measures be taken accordingly.

3.7.2. Spore collection and sporeling culture

Spore collection and sporeling culture methods were derived gradually with the development of production. Two methods are used, one is used in the open sea, the other indoor.

a. Collecting spores and culturing sporelings in the sea

This method is simple and cheap but requires a great deal of labor. Various kinds of growth bases (attachments) and a certain amount of Gracilaria fronds are scattered on the shoal. Spores will be released naturally and germination follows. Then sporelings will be seen attaching on the growth bases. Sometimes, spore-filled water is used. The detailed procedure is as follows:

(i) Site selection

Farming sites are always selected in flat areas inside bays or offshore areas with a hard bottom, clean water and a good water current exchange. Specific gravity of the seawater should be from 1.010 to 1.025. At ebb tide, there should still be some remaining water. It is good if there are lots of shells, small stones and broken corallina on the bottom as they can be used as growth bases. Pools and crab ponds can also be used as sites to collect spores and culture sporeling because water depth and water exchange can be controlled in these ponds.

(ii) Preparation of growth bases

The kind of growth bases used depends on local conditions. Small stones, shells and broken corallina can be used. The surface of the growth bases should be clean and easy to be attached to by spores. About 500–600 tons of multi-angular stones weighing not more than 0.5 kg each are used per hectare. Shells of oysters, scallops, and clams, can be used; 180–210 tons of these shells are scattered over one hectare. The outer surface of the shell

is turned upward if possible because it is rough and suitable for the spores to attach to. Broken corallina is also a good growth base and can be found in some places along the coast of South China. Sixty-strand (3×20) vinylon ropes and 33-strand (3×11) PVC ropes have been used since the 1960's; 15×15 cm mesh nets woven with these ropes are also used as growth bases.

b. Preparation of mature fronds of *Gracilaria*

Fronds are selected before spore collection. They must be strong, with luxuriant branches, intact, without damage, and with numerous sporangia on them. Cystocarps project outside the frond and are easy to be recognized. Numerous tetrasporia formed are seen as tiny red specks faintly visible inside the tetrasporophyte if observed against the sun. One must make sure only mature spores are collected. They can be identified from the characteristics of the sporangium.

- i. Characteristics of the mature carposporangium. When carpospores are mature, cystocarps are seen projecting highly upwards. The apical part of the cystocarp is round and smooth like a steamed bun. The pore of the cystocarp is transparent and somewhat white in color. A big white spot with pores indicates that carpospores have already been discharged and the fronds can not be used. The plumpness of the spores can also be checked through a microscope.
- ii. Characteristics of the mature tetrasporangium. When the tetraspores are mature, they can be clearly seen as evenly distributed big red spots when viewed against the sun. The sporangium has a very clear and crossed groove when observed through a microscope.

c. Management of the mature fronds and collection of spores

Different methods used are as follows:

- i. A certain amount of fronds is left during harvest for the seaweeds to flourish again. This method can be used in the place where Gracilaria is growing naturally. Growth bases are scattered. Spores discharged attach on the growth bases and germinate.

- ii. The fronds are dried and stimulated to release spores for collection. Strong and mature fronds are selected and washed clean with seawater and then dried in the shade or under the sun. If dried in the shade, fronds can be arranged on a bamboo mat or hung on a rack in bundles for 2 to 4 hours. Drying time varies according to the temperature, moisture, and air ventilation. When the fronds surface is dry and some irregular wrinkles start to appear, the treatment can be stopped. If dried under the sun, the fronds must be turned over now and then. Only a short time is needed. After the drying treatment, the fronds are cut into 2 to 3 long pieces. Generally, 15 to 20 kg fresh fronds of Gracilaria are needed per mu (1/15 or 0.066 ha). Fronds scattered in the farm site absorb water. This results in the rupture of the sporangium and subsequent release of spores. It has been reported that about 60,000 tetraspores or more than 40,000 carpospores are released from a Gracilaria plant (Mathieson, 1975). Spores set free sink slowly and attach on some stones and shells as growth bases. They subsequently germinate. A small disc forms in a week.

This method is suitable to be used in an area where not so many mature fronds of Gracilaria can be obtained. The work is carried out on a fine day and in the evening when the temperature is steady.

d. The germination of spores

Spores are elliptical in shape immediately after being set free. After 10 minutes, they become round by absorbing water. The diameter is about 30 microns but it varies among species. For example, the carpospore of G. tenuistipitata is about 23–40 microns in diameter and the tetraspore is 24–56 microns. The shape of these two is exactly the same. There is a nucleus in the center of the spore and the chromoblast is stellate in shape and red in color, and lighter in the periphery.

Usually a spore after being set free from the parent will quickly attach on the growth base and then germinate. The spore is cleaved through the center into two equal parts. This is the first cleavage. If the spore is spherical, the cleavage takes place on the projected part first and two unequal parts are formed. The second cleavage will take place before long

and is perpendicular to the first cleavage; four cells are formed. In some species, the second cleavage is parallel to the first one so that 3–4 parallel cells are formed. After that, each cell divides irregularly and many small cells are formed.

In March 1978, an observation on the germination of G. tenuistipitata spore was made in Zhanjiang coast. It showed that the germination period of each spore is not the same; 24 hours after the attachment of the spores, some of them remain unchanged, some reached the two-cell stage, some the four-cell stage and some even reached the multicellular stages.

At the beginning, germination takes place inside the spore and spore diameter is nearly unchanged. Several days later, a small multicellular disc is formed. It attaches firmly on the growth base and gradually grows bigger. The diameter reaches 80–90 microns, and differentiation also begins to take place among cells. The pigment of the cells in the central part of the disc becomes darker than that of the cells in the peripheral part. The cells in the central part project upward. An erect body is formed. Generally an erect sporeling is seen a month later.

e. Collecting spores and culturing sporelings indoor

To find a more scientific way of collecting spores and culturing sporelings, experiments have been carried out indoor since 1959.

Features of this method are as follows:

- i. It is necessary to wash and handle the mature fronds carefully. Harmful organisms like diatoms, Protozoa and Annelida which attach on the fronds of Gracilaria will hinder the germination of spores and so must be kept away before spore collection is carried out.
- ii. The growth bases must be cleaned and disinfected. It is usually small and the water quality is not easy to be controlled, therefore growth bases must be brushed clean and the vinylon ropes must be soaked in seawater before hand.
- iii. The sea water used has been settled and filtered of diatoms, Protozoa, and other organisms that hinder germination of the spores.

- iv. Factors like temperature (20–25°C), light intensity (about 5000 Lux), content of nutrient salts (nitrogenous fertilizers about 1 ppm) and specific gravity (about 1.020) of the seawater can be controlled optimally indoor.

3.7.3 *Gracilaria* cultivation methods

In order to raise the per unit area output of Gracilaria, the sporelings should be scattered for culturing when they grow up to a certain length. The starting time of scattering culture is different in various sea areas. Generally, the sporelings can be scattered for culturing when they grow to about 10 cm long. The scattering culture usually begins in mid November in Fujian, December in South China, and September-October in North China. At present, the following are some of the effective measures for Gracilaria culture in China:

a. Scattering sporeling culture:

When the temperature decreases in the later part of autumn, the spores grow rapidly. This is the time to scatter the sporelings for cultivation in the shoal. In this method the shoal is harrowed and levelled, and the seabed is weeded. Then the spores with their original growing bases such as little stones, shells or grists are scattered in a good order on the shoal. During scattering, the growing bases are arranged in rectangles separated by ridges, so that the interval between growing bases are about 30 cm. This is a simpler way to culture the seaweed. Normally, the fronds will reach 50–100 cm in length after 3–4 months of cultivation. In the fertile shoal, and with good management, the production may be as high as 750 kg per hectare in one production season.

b. Pond scattering culture:

Some Gracilaria such as G. tenuistipitata which multiply by vegetative propagation can be cultured with this method. After the sporelings of G. tenuis-tipitata are scattered into a pond, the fronds will grow naturally. When the growth fills up the pond, most of them may be harvested for sundrying or processing. Some are left in the pond to continue to grow so that the fronds can be harvested continuously. This method has been used for more than 10 years in the southwestern part of Taiwan. The area used for culturing Gracilaria in Taiwan

is about 300 hectares. The yield is over 1,000 tons. The same method had been used for culturing G. asiatica, G. chorda or G. parvaspora, however, the culture of these Gracilaria are less efficient than culturing G. tenuis-tipitata. Now let us describe the method in detail:

The sporelings are transported to the site in spring (April-May) and scattered evenly into pond. 4,500 kg of sporelings are scattered in a hectare. Generally, this is carried out in the morning or evening or during a cloudy day, so that the sporelings will not be dried by direct sunlight. In order to prevent the fronds from being blown down by wind, some little bamboo poles should be stuck in the pond to support them. The water depth should be kept at 20–30 cm or so. In June, when temperature rises, the pond water depth should be increased to 60–80 cm. Some fertilizer should be spread in the pond as the fronds are growing. In Tainan country, 3 kg of urea is spread in a hectare every week. The effect is considerable. In some places, manure is put in the pond periodically. This is quite efficient too. The water should be changed every 2–3 days, and kept in good quality and be clear. Harmful seaweeds such as Enteromorpha, Ulothrix, Acanthophora, etc. should be removed from the pond.

Generally, 30–40 days after scattering the sporelings, the fronds will be ripe for the first harvest, some fronds should be left to flourish and continue the culture. The period of growing and harvesting could last for quite a long time. The harvesting can be from June to November. Normally, the fronds stop growing when the water temperature drops to below 8°C. December-March (because the temperature is too low then) is a low production period. However, in some places such as Pingdong Country, Gracilaria is cultured during December-July when the water temperature is favorable.

Production (with the use of advanced technology) in Taiwan Province reaches to 12 t/hectare per year in average. In the polyculture pond, in which shrimps, crabs and Gracilaria are cultured, the yield will be much higher.

The pH value of seawater may seriously affect the growth of Gracilaria. A stronger light intensity is needed for the growth of G. tenuistipitata. Our experiment showed that the compensation point of photosynthesis of this seaweed is 20.3 $\mu\text{EM S}$. The saturation point of photosynthesis is 340 $\mu\text{EM S}$ in its flourishing seasons such as autumn, winter or spring, and 95.9 $\mu\text{EM S}$ in its decrepit season summer. The pure photosynthesis of the fronds is 0.72 mg $\text{O}_2/\text{g fresh wt. per hr.}$ and the latter is 0.30 mg $\text{O}_2/\text{g fresh wt. per hr.}$ The

seawater should be kept clear and maintained at a depth of 20–30 cm in winter and spring so that there is enough sunlight for photosynthesis and the growth can be accelerated. In summer, the depth of the seawater should be kept above 50 cm. In this way, the temperature of the water will not rise rapidly, and the Gracilaria will be able to tide over the summer safely. The yield of Gracilaria will increase when the seawater is changed regularly and supplement of manure is provided. It is desirable to change the seawater and apply fertilizers such as $(\text{NH}_4)_2\text{SO}_4$ or urea 1 g per cubic meter weekly. The sandy-muddy seabed is chosen for pond culture.

3.7.4 The approach for increasing the yield of *Gracilaria*

The methods for cultivating Gracilaria have been described above. However, the unit output of Gracilaria is much less than that of the kelp (Laminaria) because the fronds are smaller and its growth cycle is shorter. More studies on increasing the output of Gracilaria are needed. At present, the effective increment approach is adopted or is being studied, by some units in regard to four aspects:

a. Cutting method:

In the natural seawater, some broken fronds of Gracilaria can continue to grow. This reveals that Gracilaria possess a strong regenerative ability. In the spring of 1973, scientists of the South China Sea Oceanographic Institution, Chinese Academy of Science, conducted a cutting trial with 40 of over 10 cm long Gracilaria tenuistipitata fronds in Zhanjiang port. In the experiment, only the 10 cm long base of the fronds was cultured. About one month after cutting the fronds grew to cover 10 cm again. The average growth rate was about 0.6 cm/day. The result was remarkable. From the winter of 1973 to the spring of 1974, a further experiment on cutting increment was conducted in our mariculture farm showed that if the base of the fronds for cultivation is too short after cutting, the growth of the seaweed will be retarded. The growth of the fronds with 1/4–1/2 been cut off was compared with that of the controls not cut. Cutting off 2/3 resulted in obviously slow growth. On the whole, the yield of Gracilaria can be increased by cutting. The seaweed was cultured in rafts in shallow sea. The polyethylene sporeling ropes were tied on the floating bamboo. The tufts of fronds were gripped every 10 cm on the rope. We cut the fronds when they were growing, and measured the weight, and calculated the ratio of the increment. Raju and Thomas of India reported an experiment of the culture of G.

edulis carried out in the east shore of Krusadia Island from January, 1967-May, 1968. Usually this seaweed is found under the lowest tidal line. When they grow up, their fronds will reach to 50 cm. Their sporelings appear during June-July and December - February. Their mature period is from May-June. At the beginning of the trial, the top part (1 cm long) of the frond was gripped in a nylon threads at 5 cm intervals. Then the threads were twisted on a coir rope. The rope with sporelings was kept about 30 cm under the surface of the water and fixed on stakes stuck on the seabed. In the later experiment, the sporelings were gripped on coir ropes directly. The harmful seaweeds were cleared away every 1/2–1 month.

The result showed that, in the first month, the length of the fronds ranged between 4.4–6.0 cm, the fresh weight ranged between 0.5–0.7 g, and dry weight ranged between 0.025–0.030 g. From the second to the fifth month the fronds grew faster. At last, the length ranged between 35–40 cm, fresh weight ranged between 25.5–30 g and dry weight between 1.5–30 g and dry weight between 1.5–2.0 g. After five months of culturing, the fronds could be reaped. When reaping, the base of the fronds should be reserved for replanting. The second harvest was in 30 months after the first reaping. During the period of the second and the third harvest, the seaweeds grew more flourishingly and the yield was increased. The unit output in a 1 meter long rope could reach to about 3.5 Kg fresh weight. This Gracilaria can grow throughout one year in India. The gripping sporeling culture and periodical cutting method are certainly meaningful approaches.

b. Increase yield by fertilizing:

Gracilaria grow well in the natural seabed where the fresh fertile seawater comes in. If the seaweed is cultured on barren seawater, application of fertilizers is a useful approach for increasing growth. In South China, some units considered the seawater is fertile, so fertilization is unnecessary.

The experiment showed that the growth rate of the fertilized groups, in which the sporeling ropes were dipped in fertilizer for 24 hours, was higher than that of the controls. The group fertilized with urine and manure grew most rapidly. The average growth rate reached to 2.34 cm/day. While the growth rate of the group fertilized with urine was 2.26 cm/day, that of the control was only 1.13 cm/day. The total increment in length of the fronds in the group fertilized with urine and manure was 82.84 cm total, 0.97 cm/day in

average. The length of the group fertilized with urine increased by 58.3 cm, 0.68 cm/day in average. The controls increased by 43.88 cm in total, 0.51 cm/day in average. This showed that by dipping the sporeling rope into organic manure before gripping, the growth of Gracilaria may be increased obviously. By dipping the ropes into fermented urine and manure, the effect of the fertilizer lasted for a longer of time and the result was much better. This was a preliminary trial. In this approach, less labor is needed, the organic manure is easy to obtain, and the cost is low, and is a simple and effective way to increase the yield of Gracilaria in some regions.

This approach is similar to fertilizing with barnyard manure in agriculture. The effect of the fertilizer can last for a long time. Since the concentration of the manure is very high, the gripped sporelings should be transferred to seawater as soon as possible. Otherwise, if the sporelings were kept on shore for some time the water in the epidermal cells of the fronds will be easily lost; the fronds will dehydrate, and die soon after culturing in the seawater. This is worth paying attention to.

The effect of fertilizing is also proved by the experiment conducted by the Shandong Mariculture Farm in 1959. After culturing for more than four months, the length of the fronds fertilized was 14% more than that of the controls. The results are shown in Table 5 (on page 35).

This experiment clearly showed that supplementary fertilization of Gracilaria in fertile seawater is still effective. The only job in this fertilizing method is to fertilize the sporeling ropes before gripping. Little labor is needed. Therefore this method is easy to spread for mass production.

c. Increasing the yield of gracilaria with growth hormone:

Growth hormone is a material which actively influences growth of plant. Its function is to promote the extension of the plant cells. It is reported that the hormone increases the respiration of the plant cell first, then increases the asparagine fiber and other material forming the cell wall. These rapidly increases the volume and respirator intensity of the cell and the soluble material. Osmotic pressure causes the cell to absorb a certain quantity of water so that the cell length and volume become large. As a consequence, the plant's

growth is accelerated. Growth hormones have such aforementioned effects on Gracilaria growth.

d. Removing harmful organisms: Harmful organisms (mainly Enteromorpha, Ectocarpus, Calothrix, Cladophora, Polysiphonia etc.), they establish a foothold, they multiply in large numbers and attach to the Gracilaria and hinder nutrient assimilation and the photosynthesis of Gracilaria so that the growth, yield, and quality are very adversely affected. The preventive approach is to keep harmful seaweeds away from the growing fronds. The culture pond should first be drained of water, after which the bottom should be dried by sunlight and ploughed. Slaked lime should be scattered on the ploughed bottom. Fresh seawater is induced to the culture pond only after harmful organism have been removed. As Gracilaria growth is hampered by attaching mud, the fronds should be flushed frequently. Some fishes and shellfish may bite and break the fronds and cause a certain loss.

Clearing away harmful seaweeds is complicated work. According to our experiment, Ectocarpus and Polysiphonia separate themselves from Gracilaria automatically as temperature rises and so has little effect on the culture Gracilaria. Tightly attaching diatoms on the fronds of Gracilaria are hard to remove. The quality of harvested fronds is influenced badly by these organism. These attaching organism can be flushed away after the fronds are dipped into 1/20,000 formalin solution. And the Gracilaria can continue to grow. To clear away Enteromorpha, a solution of 3–6% paraquat is sprayed on the seaweeds after ebb tide. When flood tide comes Enteromorpha, will become white gradually and die and separate themselves from the net. In pond culture, the seaweeds should be drained dry, then paraquat solution can be sprayed on the seaweeds. In this way the harmful seaweeds can be cleared away.

The history of Gracilaria culture is only several decades. With the development of science and the industry and agriculture, the culture of Gracilaria will continue to develop as well.

3.7.5 Harvest and simple processing methods.

Some species of Gracilaria such as Gracilaria tenuis-tipitata v. liui, G. parvapura, etc. culture in ponds can be reaped while they are growing. It is desirable to harvest on a clear day so that the fronds can be dried by sunlight. A part of the fronds should be left in the

pond for them to flourish and develop in to a new crop for harvest. After reaping, the fresh fronds be washed clean and then put on a grass lawn for drying by sunlight. In moderate condition, the ratio of dry weight to fresh weight is 10.0%–12.5%.

Gracilaria Asiatica, G. tenuistipitata, G. gigas and G. sjoestedtii etc. can be harvested after 3–5 month cultivation. In South China, harvest starts from March. The last harvest should not be later than mid April. The harvest season in Fujian is one month later than in Guangdong. In North China harvest takes place in June-July. When harvesting, the fronds are just picked up by hand, washed clean of mud and weeds and then sundried. In the past, it was necessary to wash and dry the algae several times and bleach the algae till they become yellowish in sunlight. Normally, the ratio of dry weight of fresh weight is 6–7%.

At present, Gracilaria is used to make agar. The agar content in various species are different. The agar content in G. tenuistipitata, G. asiatica, G. gigas and G. sjoestedtii are more than 25%, that in G. parvaspora and G. blodgettii are about 20%, while that in G. tenuistipitata v. liui is only or so. According to analysis the agar content in the tender part of the fronds is lower than that in the mature part of the fronds. When the dry algae are stored, attention should be paid to keep the storehouse dry and well ventilated. The dry algae should not be exposed to humid air otherwise they become mildewed, or the agar content will be affected.

CHAPTER-4

METHODOLOGY

4.1 The study Site

The study was conducted at Haluaghat upazila (Sub-district) of Mymensingh district and Barlekha upazila of Maulvibazar during July, 2009 to November, 2010. The majority of agar garden and agar-based small cottage Enterprises of the country are located in these areas. Haluaghat upazila is located at 25.1250° N latitude and 90.3500° E longitude (Fig-4.1). It has 49520 units of house hold and total area 356.07 km^2 . The Barlekha upazila covers an area of about 430 km^2 located between $24^{\circ} 50'$ latitude and $92^{\circ} 18'$ longitude (Fig-4.2).



Fig. 4.1: Map of the Haluaghat upazila

Topographically, these two upazila consists of small to medium hillocks locally termed as tillah. Soil in the study area are sandy –loam to clay loam and slightly acidic. The average rainfall ranges between 600 and 2000 mm (Hyder 2005). November- February (winter) are relatively dry months with hot weather in March and April.



Fig. 4.2: Map of the Barlekha Upazila

4.2 Survey Method

Eight small-scale agar- based enterprises were chosen at random from within two upazillas, namely Haluaghat (Fig.4.3) and Balekha. A semi- structured questionnaire was developed for interviewing agar garden and agar-based small enterprises owners. Interviews were performed during daytime with an average duration 45 min. Data were sought on production of agar, cultivation process, agronomy of agar plant, and their age with respect to agar oil production, market value and economics per hectare. On each survey topic, respondents were encouraged to express their views and provide additional information regarding agar husbandry in those areas.



Fig. 4.3: Agar garden at Haluaghat

4.3 Agar cultivation in the survey area

4.3.1 Soil and Climate

Agar plant prefers high humid, sub-tropical climate with rainfall 1800-3500 mm per annum. It grows from sea level up to 500-m altitudes. It is a sun-loving plant and requires lot of sunshine. It prefers well-drained deep sandy loam-to loam rich in organic matter but can profitably be grown in marginal soils and also in shallow soils over rocky beds with cracks and crevices. It grows well in hill slopes and forest environment. The traditional agar growing areas show that it prefers acidic soil reaction. The mycorrhiza and other beneficial fungi which seems to be responsible for oil formation in the agar tree being soil borne requires acid soil for their population build up.

4.3.2 Propagation

Agar is propagated by seeds, which are available in the month of June – July. The germination of practices should be adopted. After planting young seedlings in poly bags, they are arranged in bed supported by bamboo poles around. At monthly interval the shifting of bags should be done to prevent the penetration of roots into the soil. Shifting of

seedlings should be followed by light watering to avoid wilting due to disturbances in the root system. Root trainer may be used successfully. Seed is epigeal, therefore, special care should be taken in nursery management. They are first germinated in sand beds and then transferred to poly bags. Seed has short viability period (7-10 days).

4.3.3 Transplanted in poly bags

From 25 days onward when the cotyledons just drops down the seedlings are transplanted carefully to poly bags arranged under temporary shade. Normal management short cycle plantation yields only essential oil or 'agar attar' of low quality (*Boya* oil). The plantation may be planned in two ways: (a) planting at wider spacing along with some suitable intercrops and harvesting at the end of the crop cycle. (b) Planting at comparatively closure spacing and harvested at 2-3 phases. In the second approach about 8-10 years of planting about 40 % selected trees may be harvested with a view to thin out the plantation for better growth and development of the remaining trees and also to get a substantial income.

4.3.4 Field layout

Agar is a long-term plantation crop. A profitable plantation may be of 15 years cycle or more. The seed is epigeal, therefore, special care should be taken in nursery management. They are first germinated in sand beds and then transferred to poly bags. Seed has short viability period (7-10 days).

4.3.5 Planting time

Planting should be done when the plants have the greatest chances of survival. The best time is during the rainy season (May-September).

4.3.6 Pit for Planting

Under average condition spacing ranges between 2.5-5 m, (initially accommodating about 1700 plants per hectare) which at later stages i.e., after 8-10 years of growth maintained at 4-5 m by harvesting in phase manner. When the planting is raised with some other forest species the spacing may be given accordingly. The distance for avenues and public places depends upon the situations and purpose of planting which may range between 3-4 m.

Planting of the saplings is done in well-prepared pits of size 50 x 50 x 50 cm made in advance (Fig.4.4) and preferably in the evening time or during the cloudy weather. After planting staking should be done to keep the seedling in upright position and the soil around the plant should be firmly consolidated. Immediately after planting watering is necessary..



Fig. 4.4: Pit for planting agar tree

4.3.7 Agar in Agro-forestry

Agar tree is suitable for growing on field boundaries and for dividing whole plot into sub-plots. Not only this, agar tree is also grown on borders of gardens, school compounds, office compounds, parks and residential sites. The good capacity for pollarding and coppicing has made it suitable to fit in agro-forestry. The canopy of Agar tree is such that it allows sunshine penetration partly. Thus, it can be planted in field boundaries, bunds etc., without affecting the field crops. Besides, agar tree has been successfully grown for strip planting along banks of ponds, tanks, canals and roads. In hilly areas / *tillas* as in Barak valley it can be planted on poor soils on hill slopes, *tilla* tops. They help in reducing soil erosion and land sliding caused by rushing water during rainy season.

4.4 Cultural operation

Soil working to a radius of 50 cm is to be done once in 3-4 months. Fertilizer application should also be followed by these operations preferably twice in a year, before and after monsoon from second year onwards. Agar seedlings are foraged by goats or cattle. To protect plantation, fencing is necessary. Initial 4-5 years period should be protected from farm animals. Trenching around the plantation has also given good success. All the

replacements of casualties should be done in the same planting season and if necessary second replacement may be done during the second year using large size seedlings.

4.4.1 Intercropping

Vegetables/pulses or aromatic crops like Patchouli (*Pogostemon cablin*), Sugandhmantri may be cultivated as short season/short term intercrops during first three to five years of plantation. In the later stages shade loving medicinal plants like Sarpagandha (*Rouvolfia serpentina*), long pepper (*Piper longum*) may also be grown for another few years depending on plant population and land type. Ginger/Turmeric may also be planted leaving about 50 cm around plant base. Both the crops are exhaustive in nature for which some special care has to be taken. This type of crops should not be taken more than two seasons.

4.4.2 Manuring

It is not necessary to apply inorganic fertilizers at the time of planting. Fertilizers should be applied after complete establishment and only from second year of planting. Well-decomposed cowdung/FYM @ 10-15 kg/pit of size 50 cm³ may be applied in pit and well mixed with soil prior to planting

4.4.3 Coppicing ability of the tree

Agar tree regenerates freely. This characteristics facilitates (1) harvesting of infected tree leaving the tree trunk for quick regeneration for a second crop and (2) seed production from the coppiced tree once identified as a good mother plant from quality and production point of view. Coppicing during 10-15 years age the growth of new shoots is at a faster rate and attain harvestable within next 10-15 years with comparatively higher yield of distillable wood. A second coppicing depends on the condition of the growing environment and root system.

4.4.4 Fertilizer application in the survey area

The fertilizers should be applied along with decomposed cowdung/compost @10 -15 kg/tree. In the virgin forestland initially no fertilization is required. Later depending on crop growth fertilization may be resumed accordingly. From 6-7 years of growth nitrogenous fertilizer @ 400-500 g/tree per year may be applied in two splits during pre

and post monsoon period (Table-4.1). This may help in keeping the tree wood soft, with higher content of cell sap enabling easy insect boring followed by fungal infection and spread of infected area over a larger wood volume i.e., higher rate of bioconversion.

Table 4.1 Fertilizer requirement in the survey area

Fertilizers per plant		N;P ₂ O ₅ & K ₂ O at 10 :10 : 4 ratio	
Applied		When P is in the form of SSP When DAP is	
2nd year @ 200 g/plant	Urea	182 g	110 g
	S S P	518 g	--
	MOP	55 g	55 g
	DAP	----	182 g
3rd year @ 300 g/plant	Urea	275 g	166 g
	S S P	781 g	---
	MOP	83 g	83 g
	DAP	---	275 g
4th year onwards till 10th year @ 500 g /plant	Urea	458 g	277 g
	S S P	1300 g	--
	MOP	138 g	138 g
	DAP	--	138 g

(SSP-Single super phosphate containing P₂O₅ -16 %, DAP- Di-ammonium phosphate containing N 18 %and P₂O₅-45 %, and MOP- Muriate of potash containing K₂O -60 %)

4.4.5 Plant protection measure

In agar plantation no such serious pests and diseases have been observed. However, *Heortia vitessoides* a leaf-eating caterpillar is considered to be the most destructive pest causing damage by complete defoliation of agar plantations and has become a real menace to the plantations in this region. The intensity of attack is more in the trees grown in open than under shade and during drier season (March/April) the infestation is comparatively higher than rainy months (July/August).

4.4.6 Control

- 1) Hand collection and destruction of the young caterpillars while in clusters.
- 2) At severe attack spraying with Ekalux EC 25, Endosalfan 35 EC Thiodan), Fenitrothion 50 EC (Sumition) or uvacron 40 EC is done twice at 10-15 days interval. While plant protection measures by pesticide application is resorted to, it is to be remembered that the beneficial insect borer associated with agar formation is not affected particularly in the later stages of growth.
- 3) Severe infected tree should be treated with an extra dose of nitrogen.

4.4.7 Harvesting

The physical age, growth rates and / or wood volume or physiological maturity do not govern the harvesting age of agar tree for commercial purpose. It is the infected tree and whose further growth is arrested due to physiological imbalance is harvested and yields agarwood and oil. Generally, the bad and deformed trees attain harvestable first unlike other forest species. The healthy trees are left to undergo stresses or subject to infection either naturally or artificially to induce oil formation. The harvesting is done on selection and continues for a longer period from a plantation raised at the same time.

4.4.8 Harvesting time

Although the collection of agar trees for oil extraction as well as for agaru is done almost throughout the year, the best time is during February-May, the dry season when the plants remain almost dormant or less active. During this period maximum concentration of oil with less waxy substances is obtained.

4.4.9 Yield

The yield of commercial products of agar tree is not uniform in all productive trees. It varies greatly and is almost unpredictable. After 10 years of planting with intensive management each infected tree may yield about 30-40 kg 'Dum type' to *Kolagachi* product for oil extraction, depending on infection intensity. Therefore, quality of oil varies depending on types of wood used for distillation.

4.4.10 Agar processing

Two types of commercial products are obtained from a harvested agar tree (a) agaru or agarwood that is used as incense and (b) Essential oil or agar oil or agar attar. Agar is obtained from older trees while oil is distilled from old as well as younger trees. After

felling a tree, the leaves and smaller branches are removed. Then the tree is cut into logs (pieces of 2-2.5 ft.). Thereafter, the logs are splitted to separate out the infected and non-infected woods. The agarwood of any grade if detected is first separated out with the help of indigenous tools like hacksaw blade and '*Batali*' and graded them based on the oleoresin impregnation, colour density, specific gravity and finally the odour.. Agar oil is obtained by steam distillation of harvested wood chips or coarse powder in special type of distillation unit. Distillation is continued for 5-10 days or more using firewood as the energy source.

4.5 Planting Height

Plant height of the 10 sample plants were measured from the ground level to the tip of the longest stem after 30 days of plantation. Data was taken each 30 days interval up to ten months when the plant height reach about 150-300 cm height which is the favorable height for collecting data. Average growth of the plants was calculated.

4.6 Perimeter of the plants

Perimeter of the sample plants were measured at the height of 60, 90 and 120 cm from the ground level and also mean vale was calculated.

4.7 Number of Fruits per plant

Number of fruits in each sample plant wee counted and total number of fruits was calculated after the 10 months plantation. Their average was taken as the number fruits per plant (Fig.4.5).



Fig. 4.5: Fruits of agar plant

4.8 Process of agar oil extraction

Figure-4.5 is a flow chart showing the steps used in both of the dehydration processes used to produce agar. Treatment and reagents used in each case will be very variable depending on the species of seaweed used, its origin and even the time of the year when it was harvested.

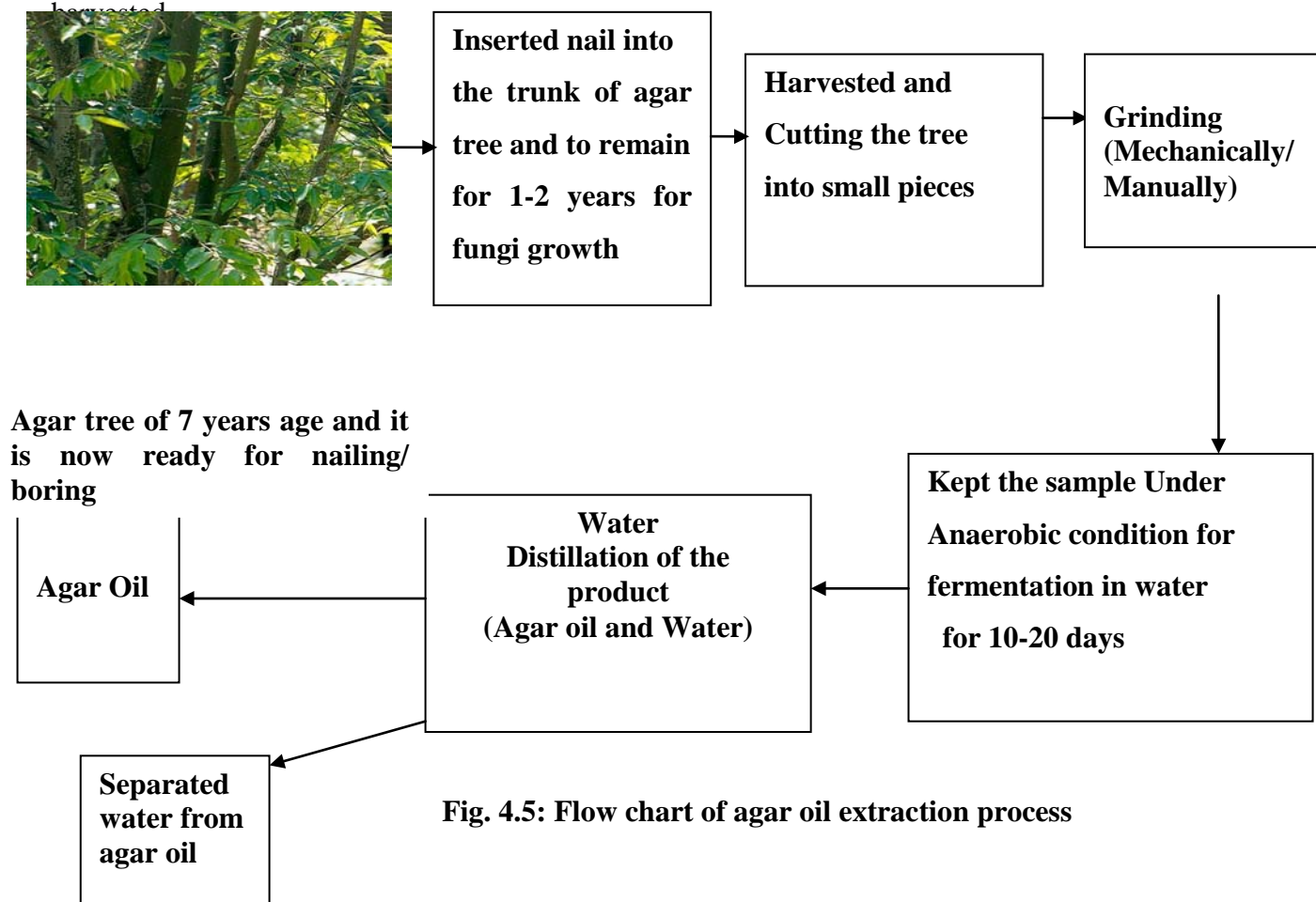


Fig. 4.5: Flow chart of agar oil extraction process

4.9 Distillation process of essential agar oil

Distillation accounts for the major share of essential oils being produced today. As most of the essential oils of commerce are steam volatile, reasonably stable to action of heat and practically insoluble in water hence are suitable for processing by distillation.

1. Water or hydro distillation
2. Steam cum water distillation
3. Steam distillation

4.9.1 Water / Hydro distillation for agar oil extraction

Water or hydro distillation is one of the oldest and easiest methods being used for the extraction of essential oils. In this method the conical flask which contained sample of agar oil is placed in a burner for heating sample.

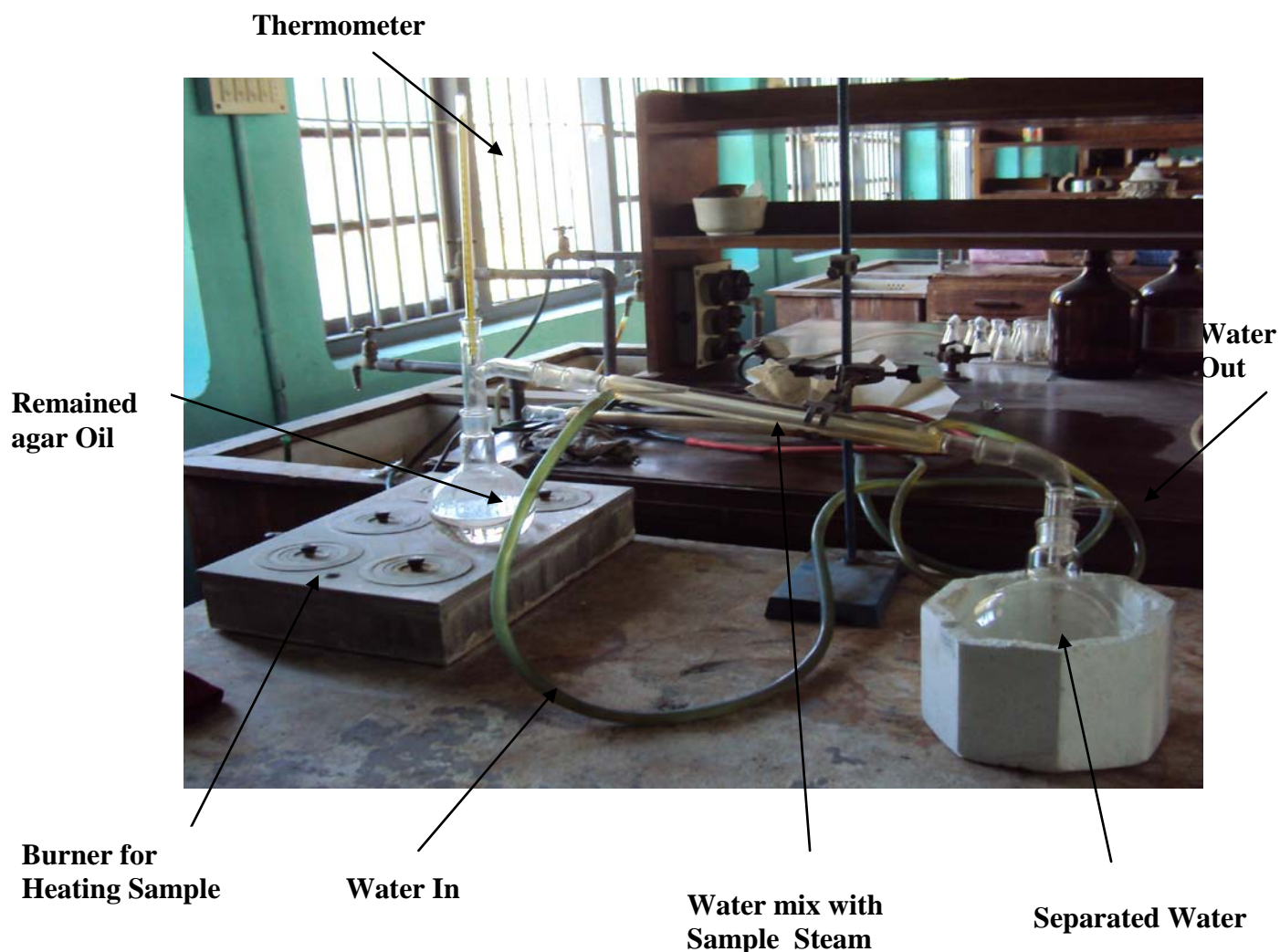


Figure: Hydro distillation plant

The sample is heated and the thermometer records temperature. One pipe supply 'Water in' and other pipe is used to 'Water out'. The sample water is vaporized and its water vapor mixes with incoming water. Then, vapor forms water. Thus, the sample free from water and forms essential agar oil. These types of units are still being used for the preparation of 'Rooh' and 'Itr' of Gulab, Khus, Rajanigandha, Bela, Sewali phool, etc.

4.10 Diameter and projected area of the plants

From the perimeter, diameter and projected area of the plants are height of 60, 90 and 120 cm from the ground was calculated. Mean value, standard deviation and coefficient of

variance were also calculated. From the area covered per plant the maximum number of plants that can be planted in one hectare of land was calculated.

4.11 Estimated yield

From figure -5. 5 it is shown that the production rate of agarwood varies with different ages. The agarwood production rate increases with increasing trees ages. It is economical and profitable to agar processing after 15-20 years. The high ages agar tree gives very valuable argu oil after treatment.

4.12 Economic analysis

The economic analysis was done by estimating the cost of agar cultivation in one hectare of land and by estimating the yield and income from one hectare.

4.13 Estimated Financial performance of the small-scale agar based enterprise

The average annual expenditure of the surveyed small agar based enterprises were calculated and net revenue of those enterprises were also estimated.

CHAPTER-5

RESULT AND DISCUSSION

5.1 Plant growth

Like all perennial plants, agar tree displays vigorous growth in the youth that tails off gradually towards maturity. Plant height is one of the important parameters which are positively correlated with the yield of fruit. For better understanding the trends of plant height of each month up to ten months after plantation has been presented in Table 5.1. The average growth has also been shown in the bar in Fig.5.1. It has been found that after 300 days or 10 months of plantation the average height of the plants reached around 170 cm. It has also been observed that in the first six months the plant growth rate was higher than that in the rest of the four months.

Table 5.1 Periodic growth of agar plant

	Plant height (cm)									
Survey Plant No	After 30 days	After 60 days	After 90 days	After 120 days	After 150 days	After 180 days	After 210 days	After 240 days	After 270 days	After 300 days
1	18	25	45	63	94	121	140	147	157	162
2	23	40	53	74	96	125	135	156	163	173
3	16	29	43	61	88	130	143	157	162	178
4	18	35	50	72	98	114	129	137	153	165
5	24	44	62	80	102	112	127	140	160	171
6	26	48	65	85	108	115	134	148	156	169
7	21	38	51	80	101	126	130	145	159	176
8	20	36	47	69	93	121	137	151	163	170
9	16	32	48	67	91	115	139	152	156	176
10	25	33	49	60	101	114	127	135	146	161
Average	20.7	36	51.3	71.1	97.2	119.3	134.1	146.8	157.5	170.1
STD	3.494	6.511	6.708	8.203	5.671	5.866	5.393	7.21	4.96	5.629
CV (%)	16.88	18.09	13.07	11.54	5.83	4.92	4.02	4.91	3.15	3.31

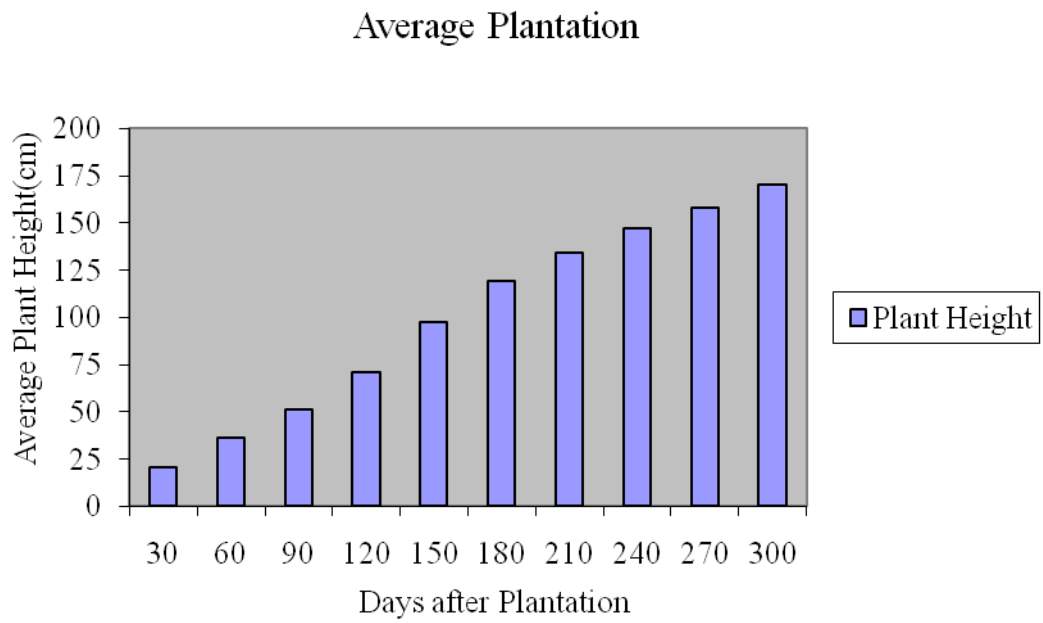


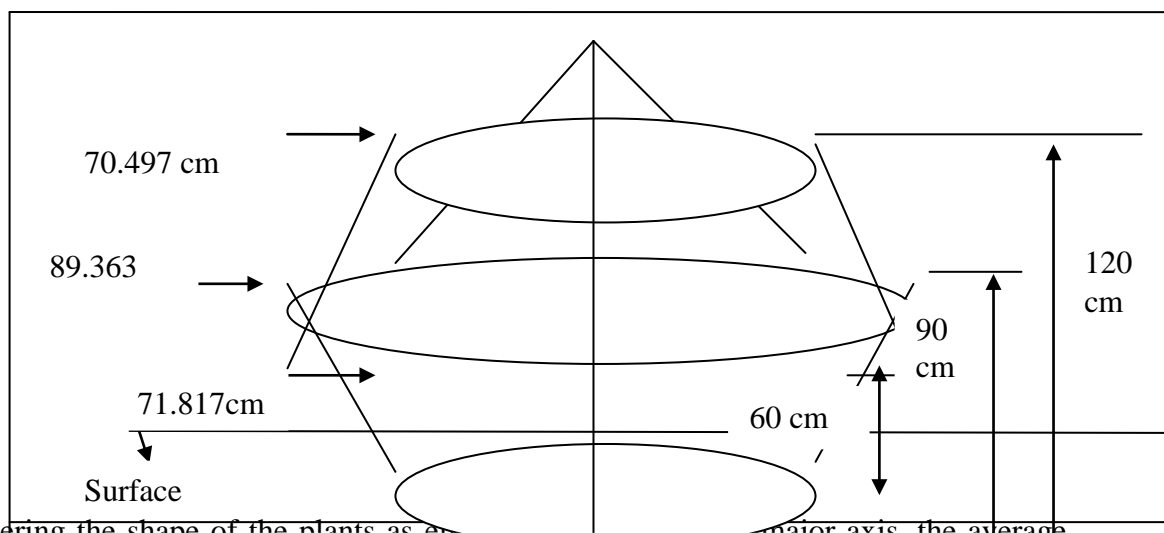
Figure 5.1: Average agar plant growth

5.2 Plant density

Total number of branches of ten months old plant has been counted and it has been found that the average number of branches of a plant was around 21. At different height from the ground level, perimeter of the plants with branches was measured and was observed that the maximum perimeter of the plants was at 90 cm height (Table 5.2). Using the average plant diameter at different height from Table 5.2 the canopy structure of a agar plant has been shown in Fig. 5.2.

Table 5.2 Area covered by the individual plant at the age of 10 month

Survey plant no	No. of branches	Perimeter (πd), cm			Diameter (d),cm			Projected area (πd^2) , cm ²		
		at 60 cm	at 90 cm	at 120 cm	at 60 cm	at 90 cm	at 120 cm	at 60 cm	at 90 cm	at 120 cm
1	19	180.330	279.397	181.649	57.43	88.98	57.85	2589.09	6215.19	2627.10
2	14	179.294	223.882	177.85	57.11	71.36	56.64	2559.42	3990.75	2518.35
3	12	135.334	159.198	142.085	43.16	50.70	45.25	1458.22	2017.83	1607.34
4	25	271.672	299.713	230.319	86.52	95.45	73.35	5876.28	7151.90	4223.47
5	29	259.053	269.098	257.009	82.50	85.75	81.85	5342.91	5765.42	5259.05
6	22	261.593	355.448	284.547	83.31	113.2	90.62	5448.34	10059.2	6446.41
7	24	227.656	325.304	215.561	72.50	103.6	68.65	4126.16	8425.37	3699.57
8	21	198.102	284.173	206.298	63.09	90.50	65.70	3124.57	6429.35	3388.44
9	20	264.136	300.184	258.139	84.12	95.65	82.21	5554.80	7174.40	5305.41
10	17	277.893	309.604	260.149	88.56	98.60	82.85	6148.32	7631.74	5388.34
Avg.	20.3	225.505	280.603	221.361	71.817	89.363	70.497	4222.81	6486.11	4046.35
STD	4.86	46.75	52.24	42.68	14.89	16.63	13.59	1589.54	2134.15	1689.28
CV (%)	23.94	20.73	18.62	19.28	20.73	18.61	19.28	37.640	32.90	41.75

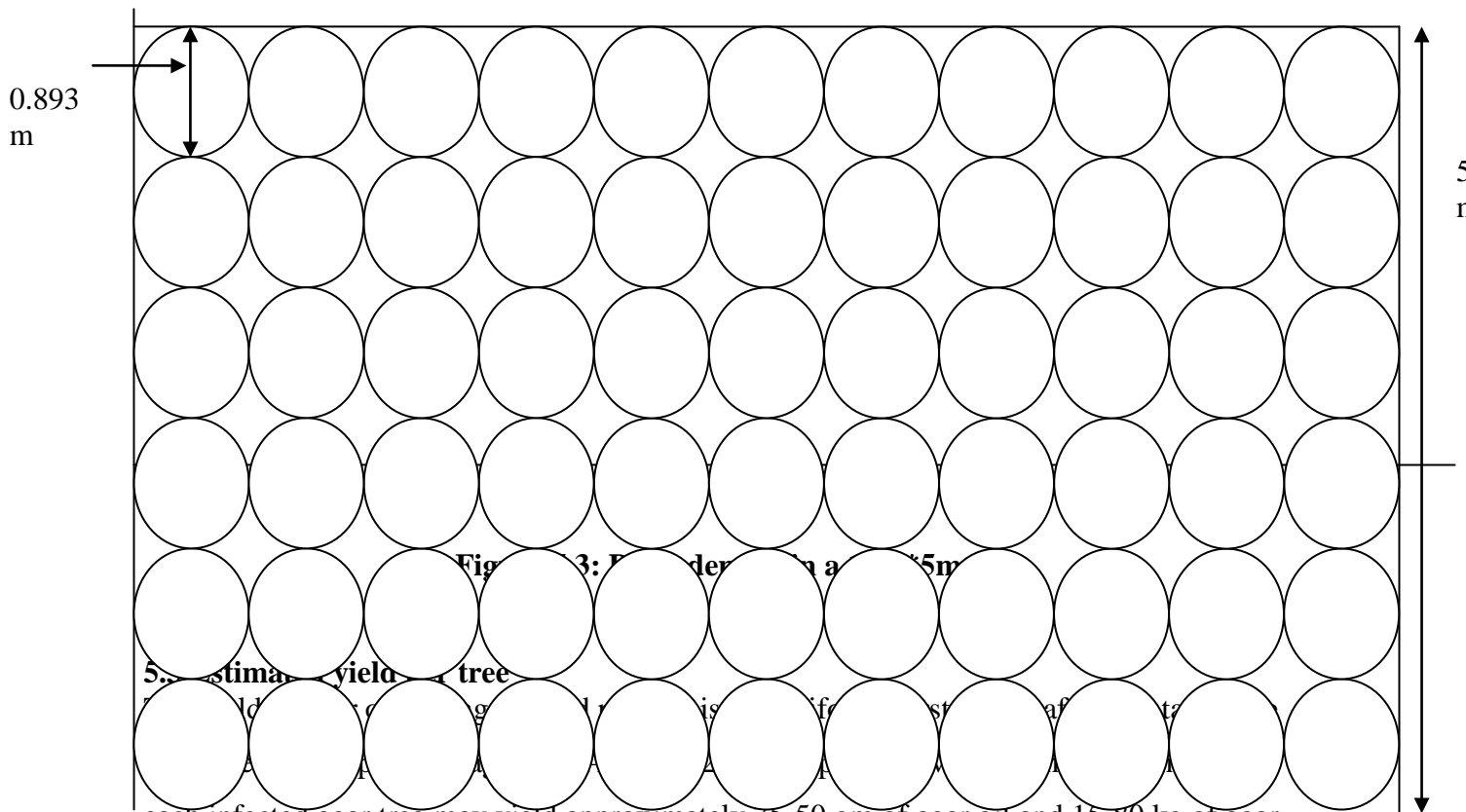


Considering the shape of the plants as ellipsoids, the major axis, the average

Figure 5.2: Plant canopy structure

diameter at 90 cm height was found to be 89.363 cm (Table 5.2). If the length of the plot is 10m and width is 5m then the total number of plant can be accommodated in a row is $10 \text{ m} / 0.89363 = 11.19$ or 11 and the number of row will be $5 \text{ m} / 0.89363 = 5.59$ or 6. So in a $10 \text{ m} * 5 \text{ m}$ or 50 m^2 plot the number of the plant can be planted is $11 * 6 = 66$ as shown in fig.5.4. According to this in one hectare land the approximate number of plants can be planted is 13,200 . If intercropping is done 2.5 cm distance between each plant should be kept. Based on this approximately 1600 plants can be accommodated in one hectare of land.

10 m



5.5. Estimated yield of tree

Each infected agar tree may yield approximately 35-50 gm of agar oil and 15-80 kg of agar wood in Fig.5.4 and in Fig.5.5. The fungal infection takes long time to mature and trees about 50 years old have the highest concentration (2.5 - 5.0 kg/tree). Sometimes all the tissues under the bark of the tree may be found synthesizing oil and also agarwood. True agarwood is heavier than water.

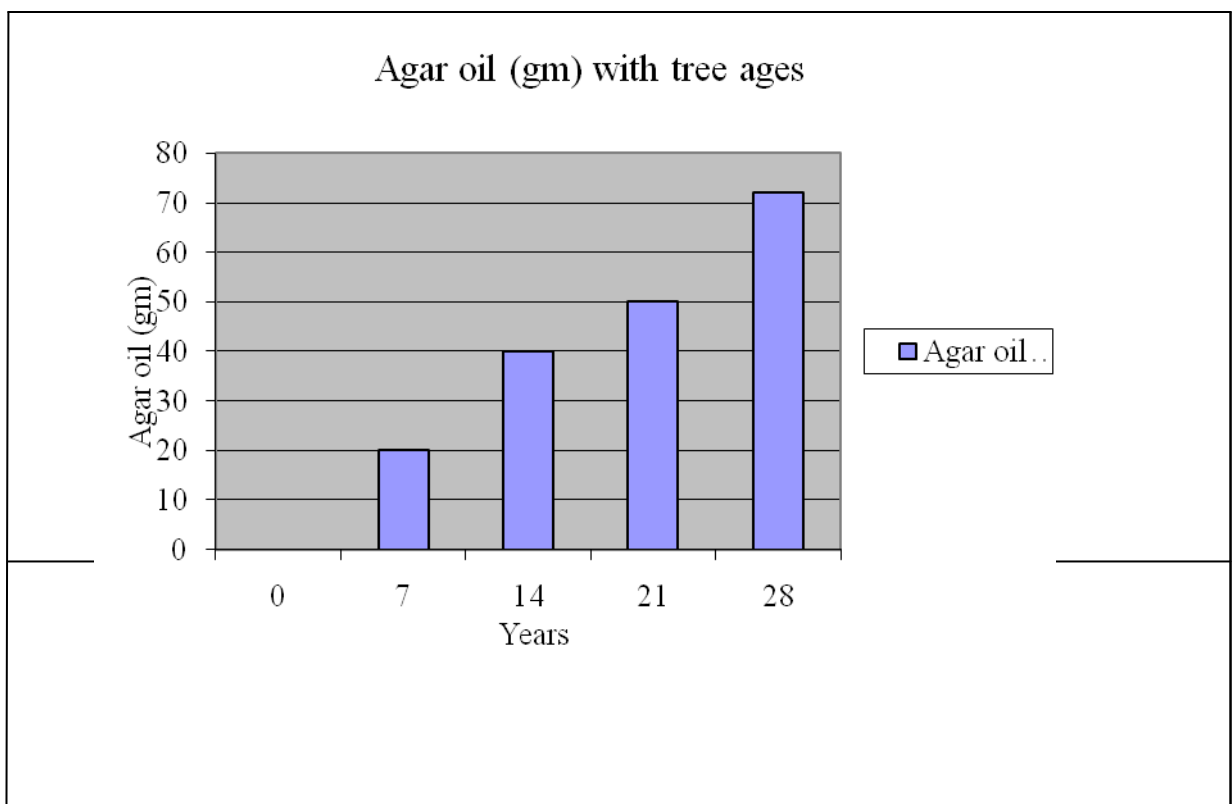


Fig. 5.4: Yield of agar oil with tree ages

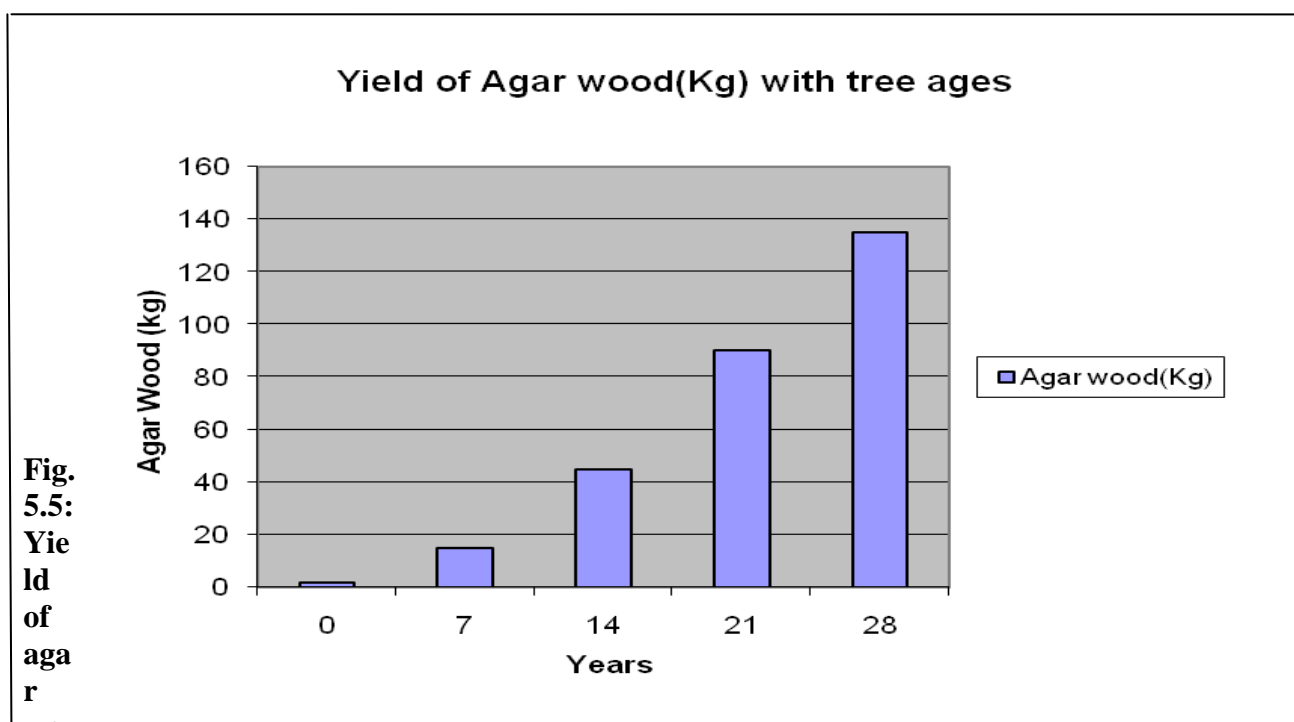


Fig. 5.5: Yield of agar wood

od with tree ages

5.4 Number of fruits per plant

The fruit is capsule, 3.5-5cm long, ovoid and brown when mature. The pericarp is coreacious, hard, woody and thinly velvety. A fruit contains 3-4 seeds (Alam, 2004). The average number of fruit produced from a plant was around 30 as shown in Table 5.3.

Table 5.3 Calculated Fruits Number

Survey plant no	Number of fruits
1	25
2	45
3	36
4	23
5	24
6	55
7	18
8	16
9	21
10	28
Average	29.1

5.5 Cost Analysis of agar production

The total expenditure of agar cultivation up to 15 years rises to Tk.432700 (Table-5.4) and agar oil processing expenditure Tk.365900 (Table-5.5). So, grand total expenditure approximately, Tk.789600. Gross return after processing agarwood, argu oil and agar based products approximately Tk.33 lakhs 20 thousands. After 15 years net income approximately, Tk.25-30 lakhs per hectare. Attar was the main primary products of the local factories and attar production was the profitable business in the sub-district. Besides attar, the agar chips residue was used by bakers and agar sticks were used to produce scented flame, small branches of agar woods were used as firewood.

Table 5.4 Estimated expenditure for agar tree cultivation per hectare

				Age in Year			
Heads of Expenditure	1 st yr	2 nd yr	3 rd yr	4 th yr	5 th yr	6-8 th yr	9-15 th yr
Cost of fencing & repair L.S	1500	-	3000	-	5000	5000	7000
Land Preparation	5000	-	-	-	-	-	-
Pit (30*30*30 cm) making							
1600*tk.2/pit	3200	-	-	-	-	-	-
Cost of saplings 1600* tk.5.00	8000	-	-	-	-	-	-
Planting cost@tk.4.00/plant	6400	-	-	-	-	-	-
Compost	9000	8000	8000	8000	8000	-	-
Fertilizers	-	5000	6000	8000	9000	-	-
Application cost tk.2.00/plant	3200	3200	3200	3200	3200	-	-
After care/year	5000	5000	5000	5000	6000	20000	30000
Inoculation@ tk.100/tree * 1600 trees	-	-	-	-	-	160000	50000
Miscellaneous exp.	1300	1600	1600	1600	1500	3000	7000
Total Expenditure	42600	22800	26800	25800	32700	188000	94000
Total Expenditure up to 8th year	338700						
Next 7 years	94000						
Total expenditure (Tk)	432700						

5.6 Estimated agar oil processing expenditure per hectare

The expenditure of agar oil formation including the fuel cost, labour cost, fermentation cost and distillation plant cost .The total agar oil processing cost 3 lacs 65 thousands 9 hundreds taka.

Table 5.5 shows the estimation expenditure of agar oil processing per hectare.

Item Sl. No	Item	Amount	Amount (Tk)
1	Cutting and grinding cost of tree@ Tk.300/tree	800 nos. of tree	240000
2	Fermentation cost		15000
3	Distillation plant cost (Capacity 20 Kg)	1	100000
4	Fuel cost for distillation		1 900
5	Labour cost @ Tk.150/day up to 15 years	100 labour/Year	225000
Total			Tk. 365900

Grand total expenditure of agar oil extraction for one hectare = Total cultivation expenditure + Total processing expenditure of agar oil formation

$$= \text{Tk. } (432700 + 365900)$$

$$= \text{Tk.789600}$$

5.7 Estimated yield and income

As mentioned in the section 5.2, if intercropping is done 2.5 cm distance between each plant 1600 plants can be accommodated in one hectare of land. Assuming 50% survival rate after 15 years almost 800 plants can be established for harvesting in one hectare.

$$\text{Final Harvested agar tree Number} = 800$$

Assume, One agar tree of 15 years gives distillable agar oil = 50 gm and 15 kg agar wood

$$\text{So, total production of agar oil of 800 nos. agar tree for 1 ha} = 40000 \text{ gm}$$

$$= 40 \text{ kg agar oil}$$

$$\text{And Total production of agar wood} = 15 \text{ kg} \times 800 \text{ nos. tree} = 12000 \text{ kg}$$

Price of distillable agar oil = Tk. 80/gm

Price of agar wood = Tk.10/kg

So, Total price of agar oil = 40000×80
= Tk.3200000

And total price of agar wood = 12000×10
= Tk. 120000

So, Gross return = Tk. (3200000+120000)
= Tk. 3320000

Net income = Tk. (2060000 – 789600)
= **Tk.25, 30400 /15 years/ha**

From an established plantation thus a net income of approximately, Tk. 25-30 lakhs after 15 years per hectare may be generated. Intercropping in the early stages of growth can generate extra income.

5.8 Production rate of agar based small enterprises

Attar, agarbati and agar chip residues were the main agar-based products in the study area that are traded both in local and international markets. Attar was the main or primary product of the local factories, and attar production was the most profitable business in the sub-district. Yearly production of attar varied between 11.28 and 37.5 kg per factory. Besides attar, the agar chip residue was used by bakers and agarbati (agar sticks) were used to produce scented flame; small unused branches of agar trees were used as firewood. Table-5.5 reports the financial analyses of the average annual expenditure of the surveyed factories was calculated as Tk 94030 (compared with the current average capital investment required to establish an agar-based factory of about Tk 560,000), and the average annual income from both primary and secondary products was about Tk187750. Incomes were higher in factories that produced attar of higher grades and exported this to foreign countries. The average annual net revenue of the agar based enterprises was found to be Tk 103282 . So, it indicates agar production is a profitable business.

.Table 5.6 Financial Performance of the surveyed small agar based Enterprises ^a

Notes: ^a In the financial analysis, the cost of raw materials was not included (except for Factories 3–5 which also collected a proportion of total raw materials from outside sources) because most of the farmers of the area collected raw materials from their own or self-managed sources without keeping any reliable records. The respondents however argued that the cost should be an additional 60–75% over the total expenditure

^b Each value under expenditure and income represent the mean two year (i.e. 2007-2008) adjusted to the 2009 price.

Enterprises No.	Expenditure ^b (Tk)				Revenue (Tk)			
	Labour ^c	Fuel	Other costs ^d	Total	Attar sales	Sales by product ^e	total	Net Revenue ^f (Tk)
1	20000	9500	12170	41670	56000	700	56700	15030
2	15000	7600	83735	106335	200000	2500	202500	96165
3	15000	9500	108500	13300	224000	3000	227000	94000
4	24000	28500	63170	115670	216000	2000	21800	102330
5	75000	31350	108320	214670	400000	6000	406000	191330
6	36000	28500	71500	136000	250000	5250	255250	119250
7	17600	11400	15600	44600	180000	1400	181400	136800
8	35000	28500	16500	80000	150000	1350	151350	71350
Avg.	33412.5	19356.3	59936.9	94030.6	209500	2775	187750	103282

^c This cost includes an allowance for unpaid labour of the operator and their family estimated at the present wage rate (wage rate/day; male, Tk 160; female, Tk 90 and children, Tk 60)

^d Other costs include an allowance for fixed costs (e.g. permanent structures and processing tools, over a rotation length of 15 years), depreciation, other unplanned costs and cost of raw materials from outside sources, where necessary

^e The by-products include agarbati and other forms of agar-based products, but excluding attar

^f Net revenue is estimated as total revenue less total expenditure

CONCLUSION AND RECOMENDATIONS

The survey clearly revealed that the agar-based enterprises makes a great contribution to the country's economy and has further potential for socio-economic up-liftment in the rural areas through providing job opportunities for poor and semi-skilled workers. The enterprises generate substantial revenue locally, after allowing for costs, and are an important source for foreign currency earnings. Globally, the demand of agar-based products (mainly agar oil) for manufacture of perfumes, cosmetics and pharmaceuticals is high and increasing, and there is scope for government to promote expanded production. If Bangladeshi agar-based industry is supported and marketing facilities are improved for selling products abroad to attract high prices, this has much potential to generate foreign exchange along with creation of employment opportunities for thousands of workers. The main problems facing the industry include financial and technical limitations, lack of skilled labour and raw materials, market insecurity (with large price fluctuations) and lack of research to increase productivity. Both government and non-government organizations can play a role in overcoming these constraints. The Forest Department has recently successfully established approximately 785 ha of agar tree plantation in denuded forest areas of Sylhet, Chittagong and CHTs. The FD can encourage planting on many other denuded and degraded areas, including fallow land (i.e. unused government land) and other public land (e.g. along roads and railway tracks, canals and embankments) by allowing landless and marginal farmers to plant agar on a participatory (i.e. benefit sharing) basis. The existing transit rules for transportation of forest products should also be revised to facilitate easy transportation of agar trees to processing units. The production cost of agar-based products is highly sensitive to fuel costs, hence expanding the supply of natural gas in all factory areas is desirable. In recent years, some dishonest traders have disrupted the country's long achieved goodwill on agar-based products to foreign countries by exporting adulterated attar. A high standard of monitoring and quality assurance is needed to overcome this unforeseen situation.

In one hectare of land the maximum number of plants can be established are 13,200 and from that number of plants approximately, 18 kg agar oil could be produced after 15 year plantation.

The economic study shows that during the first year the net benefit of the farmer would be negative, but from the seven years of plantation could receive a net benefit around Tk.3 core approximately.

Bangladesh is highly populated country. To find out for agar tree cultivation might not be easy. As agar tree yield on infertile soil and a good agar oil can be produced with little efforts, it can be planted on alongside the canals, water streams, roads, railway line boundaries specially hilly areas. Huge amount of foreign currency can be saved if these land areas can bring under agar cultivation.

RECOMMENDATIONS

- (1) Verification of the present state of the art on the use of agar oil and agar products should be done before adapting the agar cultivation in the country.
- (2) Proper propagation method (seeding by traditional or tissue culture method) are introduced through research for maximum production.
- (3) Estimated yield through interviewing of the agar based enterprises owners.
- (4) Further research on agar oil, agar products value of agar tree is to be conducted.
- (5) Methods of performance study of agar oil formation should be developed and simultaneously its economic analysis performed.