Chapter-1
INTRODUCTION

The understanding and knowledge about medicinal plants must have been accumulated in the course of past many centuries. It was much to the credit of the people of India that had they made acquaintance with a much larger number of medicinal plants than the natives of any other country in the world. Though at present synthetic and microbial agents have been employed in the synthesis of modern medicine, a major contribution to the pharmaceutical industry, comprising 25% of the prescribed drugs, is from plant sources (Principe, 1991). The World Health Organization (WHO) estimated that approximately 40% of the developing world's population meet their primary health care needs through traditional know-how.

According to the survey of All India Ethnobotanica of Ministry of Environment (1985-1990), there are at least 6000 species of medicinal plants in India, which were classified
and used by traditional practitioners in tribal areas and by other village communities. More than 87% of medicinal plants are used in different combinations in the preparation of more than 33-patented herbal formulations at present. As a result developing countries are showing contemporary interest in the cultivation of medicinal plant-herbs, shrubs and trees aiming at their national and regional economic development.

According to the oldest holy book 'Rig Veda' many plants were used to cure diseases and the medicine used to cure was 'aushadhi', or 'heat producer'. It is worth mentioning that 'Ramayana' (Maharishi Valmiki) has narrated 'Sanjivini bodhi' as a curative of fatal diseases / injuries. In the ayurvedic system of medicine, the use of medicinal plants was disseminated in the field of medicinal science of Charaka and Shushruta. 'Kurma Purana' recommends regular drinking of fruit juice for long span of life and golden colour of the skin and brushing the teeth with tender twigs.
'Matsya Purana' reports that the paste of plants is a good sterilizer for clothings, beds, furniture, coat of armour, ornaments, canopy and fans made of wool and furs.

There is reference in 'Brahmavaivarta Purana' that the consumption of ripe fruits helped to eliminate the ailment caused by excessive bile. 'Agni purana' states that the inhaling of the oil extracted from medicinal plants ensures longevity, increases the poetic acumen and helps treatment of gastroenteritis.

Many trees are sacred to the goddess of riches and are cultivated everywhere in Hindu gardens. Various plants and their parts were used to cure diseases by Chinese, Egyptians, Greeks, Romans, Persians and Arabians.

The leaves when fresh are astringent, digestive, laxative and febrifuge when fresh to remove 'Vata' (Wind) and 'Kapha' (Phlegm). They are used in opthalmia, deafness and inflammations. In native practice, leaves are applied to the head in the delirium of fever and to the chest in acute bronchitis. The decoction
of the leaves is also given for asthma. The fresh juice of the leaves is given with other combinations for costiveness and jaundice. The extracted juice of leaves is used in opthalmia and other eye affections. The leaf poultice is smeared on inflamed parts. The flowers allay thirst and vomiting and hence used in dysentery. The unripe fruit is oily, bitter, acrid, sour and tasty. It is an appetiser, dysentery cure and removes pain. The oil is used to cure 'Vata'. A decoction of unripe and half ripe fruits, baked for 6 hours, is used as astringent, digestive and stomachic medicine, and is administered in diarrhoea and dysentery. So also fruits powdered is taken with water for dysentery.

The ripe fruit is acrid, bitter and sweet. It is used as a tonic for 'tridosha' and good for the heart and brain. The ripe fruit is nutritious, delicious, aromatic, alternative and laxative. It is given with sugar candy to prevent the growth of piles and to remove habitual constipation. Syrup of ripe fruit is
used in dyspepsia and is used as household remedy for diarrhoea and dysentery. The fruit is also used as sherbath drink and its daily use is known to prevent epidemic of Cholera. There is also reference to the use of seeds, which, when crushed and extracted with petroleum ether gives a light yellow oil which has been found to possess very good purgative effect. The bark decoction of about 20ml if taken thrice a day for one week is a good remedy for intermittent fever. The juice of the bark is given with other combinations, as a remedy for poverty of seminal fluid.

The root is used to cure pain in abdomen, palpitations of the heart, urinary troubles, hypochondriasis, melancholia and it removes 'Vata', 'Pitta' and 'Kapha'. The root is one of the ingredients of the decoction 'Bruhat-Panchmula' and 'Dasamula', in Ayurveda. The root bark is refrigerant and is given in fevers, asthma with palpitation of the heart. Leaves, fruits and roots have antibiotic properties.
In Allopathic system of medicines, the sherbath prepared with the ripe fruit and sugar candy is administered to the patient of dysentery. To treat the complaints of mother after childbirth, a plaster prepared from the top of the fresh unripe fruit with other combinations was used. A thick mixture of the twin fruit with other combinations is used as a smear to check the flow of milk due to breast pain or suppuration. The entire content of a ripe fruit prepared as a liquid drink with other combinations is used for patients of puerperal fever. The old fruit with other combinations of certain medicinal plant parts is used as medicine to cure cholera.

In Unani system of medicines, the plant parts are used as an ingredient for the preparation of 'Belgiri'.

In Homoeopathic system of medicines, the plant parts are used to destroy phlegm and they are a good remedy in fevers associated with catarrhal symptoms and are also good for dropsy,
bleeding piles, dysentery, diarrhoea, bowel complaints and are a good laxative.

In the modern system, the fruits of Bael are chiefly used in chronic diarrhoea and dysentery. They are a good laxative. The sherbath prepared from the fruit pulp is good for bacillary dysentery. The half ripe fruit is used as astringent and digestive tonic. It is also antidiuretic, anthelmintic, antipyretic, carminative and is used as a tonic.

This work is to elucidate the possibility of cultivation of medicinal plants. The human adventure is concerned with different kinds of parasite and diseases. The mortality of human life is the force that helps to enlighten the aspects of disease control. The primitive man adopted some control measures to get rid of diseases. They comprise the use of different parts of plant species. There is an authentic record of the use of medicine by primitive man. The Eastern and Western Himalayas and Nilgiris are well-known for many varieties of medicinal
plants. Now, cultivation of medicinal plants is wide-spread in several states of Indian Union. Appreciable research work is being carried out in the Central Institute for Medicinal and Aromatic Plants in Lucknow. The experimental cultivation of medicinal plants is in collaboration with the Medicinal Plants Committee of the Government of West Bengal. The Uttar Pradesh Government has initiated the programme of cultivation of some medicinal plants in various districts on a co-operative basis. Many of the medicinal plants are used to extract medicines in Ayurveda, Unani, Homoeopathy and Allopathy in India and abroad.

In chemical analysis, the leaves, bark and roots are found to contain reducing-sugars and tannin mainly. The fresh leaves, on distillation yield an oil of a yellowish-green colour and of neutral reaction of an aromatic odour and bitter taste; soluble in alcohol and miscible with carbon bisulphide. Leaves yield γ-sitosterol, aegeline, aegelenine and lupeol. In addition, they yield sitosterol, rutin, marmesinin, β-
sitosterol, glucoside and essential oil. Heartwood yields a ferro-quinoline alkaloid, dictamine, marmasin and β-sitosterol. The wood ash contains potassium and sodium compound, phosphates of lime and iron, calcium carbonate, magnesium carbonate, silica, sand and carbon. Ripe fruits contain xanthotoxol, scoparone, scopeletin, umbelliferone, marmesin, skimmin and β-sitosterol glucoside. The fruit pulp contain mucelage, pectin, sugar, tannin (tannic acid), volatile oil, bitter principle, ash-2% and balsamic principle. The bark of the root and stem contain umbelliferone, other coumarins, β-sitosterol, aurapten, marmin, lupeol, two unidentified alkaloids and two unknown compounds- their proportion varies with the age of the bark.

The leaves are used for the preparation of medicines and it is also used as cattle feed. Often villagers at Calcutta, in Calcutta market its various parts and products and they fetch about Rs. 5 to 6 per day, but its leaves, bark and gum are marketed only on a minor scale. The
fruit shell is used to prepare yellow dye of rare occurrence. The stem yields a good gum. It is used as adhesive. The bark is used as medicine as narcotic. Its wood, small sliced pieces of stem, is a good fuel and can be burnt without much smoke. The sliced pieces are prized in the market and sold as a very costly fuel wood for religious purposes in India and bring about Rs. 5 to 600 per tree/year revenue to the local populace. The plant is an important component in the forest management to provide economic uplift to villagers. The cultivation of this medicinal plant species at commercial level can definitely help to earn foreign exchange through export. So, there is every need to promote the cultivation of this medicinal plant.

Our present national flora, possess a wide range of medicinal plants in varied agroclimatic conditions. Many of them are not fully exploited for their economic use. The pharmaceutical industry all over the country is making consistent effort to search out newer, potent and cheap source of raw materials and their
derivative chemicals to widen the product range and enhance the trade potential of their pharmaceutical preparation. The cultivation of medicinal plants offers considerable scope for rural employment and earning of foreign exchange through export, considerable efforts to standardize seed production and necessary action to spread the cultivation of medicinal plants. The most critical factor in productivity is non-availability of quality seeds and less application of seed germination technologies. As there is no regular demand for seed, no agency has come forward to produce seeds regularly. At least in future there is a need to establish seed production programme and seed technologies so that the interested farmers can produce the seedlings whenever they require.

Even now we are used to collecting medicinal plants from natural in-situ flora. The increasing demand of these herbals is progressively depleting available forest. Overexploitation of herbals from natural flora causes extreme shortage year after year. The
existing Cultural practices are perhaps a hindrance to the cultivation of this rare medicinal plant through seed propagation and micro propagation. Therefore the most pertinent problem is need-based and scientific research in seed germination.

The cultivation of medicinal plants provides opportunities for genetic improvement. Both in-situ and ex-situ cultivation programmes could be promoted especially to protect those rare, endangered and vulnerable species. They are most threatened in their natural cultivation. These medicinal plants can in principle be an environmental benefit. According to the Red Data Book of the Botanical Survey of India, over 425-species of plants have been listed as rare and threatened. Of these 109-species, which include many medicinal plants, are facing extinction. There is a growing need for medicinal plants because of their efficacy, safety and lesser side effects and hence a conservation of these, which are rare and endangered on account of indiscriminate
exploitation; has become the need of the hour. Due to indiscriminate collection from the wild, many plants are facing the risk of becoming endangered and it is high time to conserve them. It is estimated that 70% of the indigenous demand is not met from domestic production at an estimated value of Rs. 1,225 crores in 1994-95. In the export arena, there has been a substantial increase in the export of medicinal plants and their products from 86-crores (1989-90) to an anticipated 200-crores in 2000AD (Thamburaj and Irulappan, 1992).

Kerala is endowed with varied agro climatic conditions for growing a wide range of medicinal plants but they are not fully exploited. The efforts taken to popularize the cultivation of these crops are far from satisfactory. On the other hand, seed production in medicinal plants is an area not yet been adequately explored. The field environments, cultural practices, genetic influences, harvest time, biotic and abiotic factors also form important considerations. A good quality seed is a pre-requisite for
obtaining a healthy plant, but its production is a specialized job requiring skill, care and training. But no information is available on these lines regarding any of the seed propagated medicinal plants. In medicinal plants, seed production is very much influenced by the environmental conditions particularly the photoperiod and temperature in the matter of flowering and seed set. The occurrence of hard seed is a common phenomenon in many of the species. Time of harvest may affect seed size and embryo maturity. Immature seeds are usually smaller and lead to lower viability resulting in poor planting value.

The selected bibliography of the thesis also throws much light on source information on the work done on different aspects of such plants as cultivated crops, vegetables, herbs and shrubs. But similar research and cultural study on medicinal plants have to gain momentum at an accelerated rate. It is in view of these that the study has been undertaken.
The classical work of Crocker (1953), Toole et al. (1956) and other pioneers has no doubt contributed to the knowledge regarding several aspects of seed germination and seed research. In the last two decades sophisticated aspects of research in the field have thrown more light on the dormancy factor and viability in seed germination. Germination of the seeds depends on both external and internal factors. Water availability, temperature and light intensity are important external factors. Similarly seed moisture content, viability of the embryo, dormancy duration—seed coat, food reserves are important internal factors. Germination is in effect re-animation of the quiescent but viable embryo seed during the physiological and biochemical changes.

In medicinal plants, the influence of photoperiod and temperature during seed setting is an important factor. Development of hard seed is also a common phenomenon in many of the medicinal plants. In leguminous and medicinal
plants seed dormancy and hard seed coats have been recorded (Amen, 1968). The hard seed coat, which is impermeable to water and gases causes mechanical restriction for the growth of the embryo. It has been recorded that accumulation of growth inhibitors in seed inhibits its viability. The rate of seed germination in plants, juvenile seedling growth and seedling vigour differs among different plant species and among different genera depending on factors available during germination.

Growth is related to the sum total of all metabolic activities. It is well-known that the passage of plant through various phases of the life cycle, viz., vegetative, reproductive and maturity is accompanied by marked morphological changes including a change in the development pattern, metabolic drifts, enzymic patterns, endogenous levels in growth regulators and so on.

The treatments of presowing seeds were originally developed in Russia to overcome
stress situation especially drought (Henckel and Kolotova, 1934). Chinoy and his co-workers modified this pretreatment method to improve germinability, seedling growth and yield of plants (Chinoy, 1967; Chiony, et al., 1969). The presowing treatments of seeds with growth regulators and other substances have been proved to stimulate many physiological and biochemical processes ultimately leading to an increased growth and yield (Chinoy, 1968; Saxena, 1989; Singh and Saxena, 1991). It is agreed that the presowing treatments lead to an increase in tissue hydration, respiratory activity, redistribution of nutrient reserves and enhancement of seedling growth (Korneev, 1963; Dawson, 1965).

The presowing treatment with growth regulators have resulted in a very positive response in the height of the plant, dry weight of the plant parts and in yield characteristics (Chinoy, 1962; Padma, 1980; Pakeeraiah et al., 1987; Naidu et al., 1991; Singh and Saxena, 1991). It is of interest to observe that these
presowing seed treatments are beneficial in influencing the metabolic changes in plants. Synthesis and utilization of macromolecules like carbohydrates, proteins and nucleic acid from the period of germination till the growth and development in a number of plants have been reported (Cherry, 1963; Chinoy et al., 1969).

A slight damage or mechanical injury to the seed coat would release the seeds from their dormancy to induce germination. There are reports that high temperatures cause a change in the structure of the seed coat, thus causing a change in permeability (Marbach and Mayer, 1975). The seed with hard seed coat has been found to achieve high germination by being soaked in boiling water (Mayer and Shain, 1974).

Scarification of hard-coated seeds has achieved high germination by immersion in concentrated H₂SO₄ (Phipps, 1973). The chemical treatments would release the seed from their dormancy to induce germination and it would help
to reduce the barrier due to inhibitory substances present in the seed coat.

The plant-growth regulators are known to play a major role in the process of growth and development leading to enhanced crop productivity. Thus the study of growth control mechanisms in growth substances which regulate plant growth is one of the active fields of plant physiology. The inductive effect of seed treatments with known concentrations of hormone solutions is reflected on post inductive stages of plant such as seedling growth, vegetative plant growth and development. Cholodny (1936) called such treatments as “Seed hormonisation”.

The exogenous application of growth substances has a marked influence on the germination of seeds. Much attention has been paid by various researchers on the application of growth substances on seed germination and seedling growth. Most of the present evidence of the roles of plant hormones in the generative processes is based on the effects of external
application of these compounds on the seed (Tillberg, 1977). However, there are only a few reports on the seed germination in medicinally important plants like Cassia (Bhatia, 1976; Bhatia and Chawan, 1983; Shirai and Kagei, 1985; Kohli and Kumari, 1986; Singh and Murty, 1987-a, 1987-b; Singh, 1989; Thakur, 1989), especially in relation to the hormonal control. Organic solvent infusion of bioactive chemicals into the seed was demonstrated by Khan (1977). By using growth regulators such as IAA, GA₃, Kinetin and others, useful results have been achieved in crop plants like rice and ground nut. Practical use of these growth regulators has been largely adopted in potato, wheat and other important crops.

Reserve mobilization and metabolic changes associated with seed germination have been the objectives of several studies and this subject has been revived by many (Bewley and Black, 1983, 1985, 1994; Khan, 1978; Mayer and Poljakoff-Mayber, 1989). Germination is characterized by the hydrolysis of reserves, including lipids,
proteins and carbohydrates of the storage tissues. The products such as sugars and amino acids are subsequently translocated in the embryonic axis for synthesizing cellular constituents required for growth and differentiation. Studies on reserve mobilization (Mayer and Poljakoff-Mayber, 1989) have shown that various substances such as soluble sugars, insoluble polysaccharides, soluble protein and nitrogen as well as nucleic acid, phosphorus move out of the cotyledon and are transferred to other part of the growing embryo.

A net loss of metabolites and nutrients during seedling establishment has been explained and interpreted in terms of hormonal regulation and sink-source metabolism of reserves (Bewley and Black, 1983, 1994; Copeland and McDonald, 1995) and some ecological explanations are also available in many references cited by Mayer and Poljakoff-Mayber (1989). But the pattern and control of mobilization in dicotyledonous plants are not well-known.
The rate of intake of water and utilization of seed reserves are some of the important physiological and biochemical processes associated with germination. In maintaining the level of seed reserves carbohydrates, proteins, fats and nutrients as well as growth hormones are some of the internal factors controlling the germinability of seeds.

In the annual crops, extensive work has been carried out on the physiology and biochemistry of seed germination, whereas the tree crops have not received much attention.

The changes in biochemical activity involve respiratory rate, activity of enzymes engaged in carbohydrate, fat and protein metabolism and changes in cellular membrane and other essential bio-organelles (Anderson, 1970; Berjak and Villiers, 1972; Sen and Osborne, 1977). Studies have revealed that reserve mobilization during seed germination is very slow and even after 10 days of seedling growth, more than 70-80% of the dry matter is retained in the cotyledons.
The most interesting factor and advancement is that some of the growth regulators either promote or retard the activities of the enzymes as well as the other related factors. The changes in storage materials during germination are the result of the activity of many hydrolytic enzymes. Either these enzymes are present in the dry seed or they very rapidly become active as the seed imbibes water and many are synthesized de novo.

Generally enzymes breaking down starch, proteins, hemicellulose, polyphosphates, lipids and other storage materials become rapidly active as germination proceeds. Such enzymes are not necessarily produced in the same cells in which the storage materials are located. Moreover there exists a signalling system which regulates the production of enzymes and the interaction between different parts of the seed such as embryonic axis and cotyledons, endosperm, embryo and aleurone layer - depending on the seed.
Generally enzymes break down starch, sugar, protein, hemicellulose, polyphosphates, lipids and other storage materials when germination proceeds (Mayer and Poljakoff-Mayber, 1989). They further suggest that when the seeds become hydrated, very marked changes are observed in their chemical composition.

There are three main types of such chemical changes generally observed in various parts of the seed namely; 1) the breaking down of certain reserve materials in the seed; 2) the transmission of broken-down products from one part of the seed to another (especially from endosperm to embryo or from cotyledons to the growing parts) and 3) the synthesis of new materials from the broken-down products; and the initiation of protein synthesis. The relation between enzyme activity and loss of viability has been extensively studied.
Objectives of the study

Keeping the foregoing account in view, the present investigation has been planned on the following aspects:

1. To establish a most suitable method for promoting seed germination.

2. To establish an efficient method of breaking seed dormancy.

3. To study the effect of pre-germination treatments on seed germination and seedling growth.

4. To study the effect of pre-germination treatments and growth substances like IAA, NAA, GA₃, and Kinetin on the metabolic status.

5. The project undertaken to study the various aspects of metabolic drift during seedling growth and utilization of breakdown products.

6. A humble attempt has been made to observe the relationship of cell
constituents and enzymes are included in this study to elucidate the quantitative changes of these contents during seed germination and seedling growth.

The data and the observations are reported in the subsequent pages, supported by relevant literature on the subject and other possible interpretations.