Chapter I

**INTRODUCTION**

1.1: Introduction:

The World Health Organization (WHO) estimated that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs. Demand for medicinal plants is increasing in both developing and developed countries due to growing acceptance of herbal products in human health care as they have no side-effects. Medicinal plants comprise approximately 8000 species and account for about 50% of all the higher flowering plant species of India. Over one and a half million medicinal practitioners use medicinal plants in preventive, promotive and curative applications. In recent years, the growing demand for herbal product has led to a quantum jump in volume of plant materials traded within and across the countries.

According to the estimate of Export Import Bank the international market of medicinal plants related trade is US$ 60 billion per year and growing at the rate of 7%. According to the World Bank study, the herbal drug market is supposed to grow to US$ 5 trillion by 2050 (Malik et al. 2011). Though India has a rich biodiversity, the growing demand is putting a heavy strain on the existing resources. For meeting the future needs cultivation of medicinal plants has to be encouraged. The higher production can be achieved easily through advanced cultivation practices, application of agrochemicals like growth hormones, proper and balanced supply of essential mineral nutrients and by managing the biotic and abiotic stress conditions.

1.2: Status of medicinal and aromatic plants:

1.2.1: Global Scenario:

Globally 77000 plants (18.2% of total plant species) are currently put to use for medicinal purpose. According to United Nations Comtrade database world export and import (in million US$) of perfumery and pharmaceutical plants and their parts has increased over 50% during the year 2005-2010 (Schippmann et al. 2006). Global import and export (2005-2010) of medicinal plants is in the tune of US$ 2.09 and 2.08 billion/year, respectively (Rajeswara Rao et al. 2012).

1.2.2: Indian Scenario:

India has 15 agro-climatic zones and 17000-18000 species of flowering plants of which 6000-7000 are estimated to have medicinal usage in folk and documented systems of medicine, like Ayurveda, Siddha, Unani and Homoeopathy (http://www.nmpb.nic.in/ dated 14/5/13) (Fig 1.1). The Indian herbal market is rising sharply and is expected to hit ₹ 14,500 Crores with exports reaching ₹ 9,000 Crores by the year 2012. The herbal market has an annual compounded growth rate of 20% and 25%, respectively (http://www.gits4u.com dated 14/5/13).

The aroma and fragrance industries represent a market of US $18 billion annually and the international trade of essential oil presents an average rise of 10% per year (Schwab et al. 2008). Essential oils are the most important raw materials in the food and pharmaceutical industries due to their therapeutic, antimicrobial and antioxidant activities. They also have biological activities that make them able to be used as herbicides, pesticides and anticancer compounds (Mahmoud and Croteau 2002).

1.3: Ajowan - Source of essential oil and Thymol:

The essential oil of Ajowan is extracted from the mature seeds and the oil yield is about 2.5-5% which is a major source of Thymol having medicinal value. The oil obtained from different localities varies in composition and concentrations of Thymol, p-Cymene, γ-Terpinene, α-Pinene, β-Pinene, Myrcene and other minor constituents.
1.4: Ajowan as a commercial seed spice crop:

Out of the 20 seed spices grown in India, coriander, cumin, fenugreek are major ones. Whereas, ajowan, dill, nigella, celery and anise are the minor ones. Seed spices are used as whole and processed form for flavoring, seasoning and imparting aroma to variety of food items and beverages. Besides this they have medicinal properties and hence used in various pharmaceutical and cosmetic industries. In 2008-09, the export of seed spices from India was 4,71,000 Tonnes and this helped to earn ₹ 54,915 billion worth of foreign exchange (Aishwath et al. 2011). The use of spices like Ajowan is increasing worldwide as they are natural, rather than artificial additives for seasoning and flavoring of foods. Therefore an increasing trend in export of seed spices has been observed in the last decade particularly in Asian, Latin American and Middle Eastern developing countries.

1.5: Trade of seed spices:

There has been ever increasing demand of seed spices and the global demand is 1,50,000 Tonnes, of which India contributes 83,550 Tonnes annually, accounting for 55.7 % of the total world trade (Peter and Abraham 2007). Keeping this in view, seed spices are considered not only cash crops but they are also "dynamic crop commodities", particularly because of their great export potential and hence if their quality and yield is improved by using special agro-techniques the export will increase beyond our expectation. The export of seed spices together accounts for 28% of volume and 18% in value of total export of spices from India which has shown substantial increase in both quantity and value during 2008-09 as compared to last year. Although the export of seed spices is increasing year by year but it is unstable.

1.6: Production and market of Ajowan in India:

During the year 2010-11 Ajowan, occupied an area of about 27,257 ha and produced about 19,327 Tonnes of seeds (Indian Spice Board). The market price of seeds ranges between ₹ 9,500-11,500 per Quintal and that of oil is about ₹ 2,000-5,000 per Kg. India despite being the largest producer of seed spices could not exploit the value addition potential, as major export is in the form of raw produces but there is a great potential for increasing export of Indian spices in the form of value added
products and for this there is need to enhance the quality as per international standards.

**1.7: Need for the cultivation of aromatic plants:**

Demand for medicinal and aromatic plants is ever increasing at an alarming rate throughout the world as they are safe to use, comparatively cheaper, easily available and superior in all aspects as compared to synthetic chemicals (Thomas et al. 2000). Though India has a rich heritage and biodiversity, the growing demand is putting a heavy pressure on the existing aromatic plants and as a result of over exploitation some species have become rare. To increase India’s contribution in the export market, systematic cultivation on large scale is an urgent need of the time.

At present, the medicinal and aromatic plants are mainly collected from forests, which is adulterated and of very low quality. To maintain the purity, quality and guaranteed supply their systematic cultivation is the best option to capture the foreign market. At the same time attempts should be made to improve the yield and therapeutic principles in them. For meeting the future needs cultivation of aromatic plants has become necessary and for this desirable agro-techniques should be developed.

But unfortunately well established cultivation practices are not yet developed for medicinal and aromatic plants. Cropping pattern, fertilizer requirement, irrigation, plant protection, harvesting techniques, storage, post harvest technology etc are the areas requiring prime attention and systematic research.

**1.8: Factors affecting growth, yield and production of secondary metabolites in medicinal and aromatic plants:**

Although the growth, yield and production of secondary metabolites in medicinal and aromatic plants are controlled by their genetic make-up, but the biosynthesis and accumulation of secondary metabolites is strongly influenced by environmental factors such as light, temperature, water, biotic and abiotic stress conditions, soil types and application of agrochemicals like plant hormones and fertilizers (Yazdani et al. 2002).
PGRs:

The growth regulators include both growth promoters and retardants which have been shown to modify the canopy structure and yield attributes. The natural phytohormones like auxins, cytokinins, gibberellins, ethylene and abscisic acid as well as synthetic PGRs play an important role in regulation of seed germination, vegetative growth, flowering, fruiting, yield and physiology of treated plants. The foliar application of various PGRs is highly popular and useful for improving crop production in agriculture and horticulture.

Micronutrients:

Different types of micronutrients such as B, Mo, Cu, Mn, Mg, Zn, Fe, and Cl, are recognized as essential for normal growth and reproduction of most the crops. Application of micronutrients plays a key role(s) in the production of quality material and high yield of agricultural crops as they are involved in physiological and biochemical processes like photosynthesis, N\textsubscript{2}-fixation, respiration, activity of enzymes, protein synthesis, saccharide metabolism and functional, structural or regulatory co-factors in various metabolic processes of the plants (Taiz and Zeiger 2006). The foliar applications of micronutrients provide a means of supplying nutrients to crops more rapidly than soil applications. Micronutrients like Fe, Mn and Zn are very commonly applied to crops for their improvement and hence, more emphasis is given on them.

Abiotic stress conditions:

Abiotic stress conditions like salt and drought mainly affect the metabolic processes directly and consequently seed germination, growth, yield and production of secondary metabolites in various types of crops including medicinal and aromatic plants (Selmar 2008).

Salt stress:

Salinity limits agricultural production throughout the world and is becoming an increasingly global problem which affects about 20% of global irrigated land (Munns and Tester 2008) Screening of salt tolerant crop species and the level of
impact of salinity stress on yield and other attributes require more attention so that we can utilize saline land for cultivation to cope up with food sufficiency. According to (Selmar 2008) low level of salinity may act as the elicitor to improve the production of secondary metabolites in medicinal and aromatic plants, because stimulated production of secondary metabolites help the plants for tolerating the stress. Role of secondary metabolites as protection against stress is well documented.

**Water stress:**

Water stress is one of the most serious abiotic stress prevalent worldwide and has become the major constrain in agriculture, hampering crop production. It has negative effect on plant growth, development, yield and also impairs almost all metabolic processes. However, mild water stress for a short span of time may act as an elicitor for the biosynthesis and accumulation of biologically active compounds in medicinal and aromatic plants (Selmar 2008). Rigorous efforts are required for the screening of drought tolerant species of medicinal and aromatic plants.

**1.9: Importance of physiological studies on medicinal and aromatic plants:**

Physiological, biochemical and enzymological understanding is the basis for planning the strategies of improvement in growth, yield and secondary metabolites in medicinal and aromatic plants. Many primary metabolites such as different types of photo assimilates (reducing sugars, starch, total carbohydrates and proteins) form the carbon chain of secondary metabolites and very often they are the precursors in metabolic pathways involved in biosynthesis of secondary metabolites. The rate of production of photo-assimilates, their translocation and utilization at active growing points governs the growth and yield attributes. In plant cells the biomolecules like sugars, proteins, amino acids etc are the source of energy for growth, development, flowering, fruiting etc.

Hence, understanding the status of primary metabolites (rate of synthesis, accumulation/storage and utilization) is highly useful for predicting the growth, yield and secondary metabolite production. The investigations on antioxidants and activities of antioxidant enzymes serve as the indicator of stress tolerance ability of these plants. Understanding of mineral nutrient scenario in soil and in different plant parts is also helpful to know the growth, yield and secondary metabolite contents.
Almost all the secondary metabolites synthesized in medicinal and aromatic plants are derived from primary metabolites. The various pathways leading to synthesis of secondary metabolites and the precursor compounds are shown in (Fig 1.2).

1.10: Information on Ajowan:

Ajowan is native to India and cultivated in the states of Madhya Pradesh, Andhra Pradesh, Gujarat, Bihar, Maharashtra, Uttar Pradesh and Rajasthan. It is also cultivated in Iran, Egypt, Pakistan, Afghanistan, etc.

Ajowan (vernacular name ova) *Trachyspermum ammi* L. Sprague belongs to family Apiaceae. It is an erect, branched, annual herb, growing up to 90 cm in height. Stem is 7-9 mm thick near the base and is striated and hollow throughout its length and branching profusely (Plate I A). The leaves are glabrous or pubescent, tender, pinnately divided, 24 cm long and 14 cm across at the maximum spread and possesses clasping leaf bases. The laminar portion consists of one terminal segment and 7 pairs of lateral segments. The ultimate segments of the leaf are linear, and measure 1.0-2.5 cm long. The first pair of leaf segments arises within 3 cm of the base while the second arises at a distance of 8 cm (Plate I B).

The inflorescence appear to be latent, since axillary branches push them to the side and grow above them. As a result of repeated branching, inflorescences of different ages appear at different heights, the inflorescence stalk is about 9.5 cm long. The inflorescence is compound umbel consisting about 16 umbellets and each umbellet has about 16 flowers. An involucre of segmented bracts lies at the base of the umbellet stalk and each of the peripheral flowers of the umbellet is associated with the bract. The umbellets radiate around a central point on 2.2 cm long stalks. The peripheral flowers have long stalk (4mm), while the inner ones have short stalk (Plate I C and D).

The flowers are actinomorphic, white or white with a brown centre, showing mixture of hermaphroditic and functionally male flowers. The inferior ovary of the hermaphroditic flowers is greenish-white and is 1.3 mm long and 1.2 mm broad. The corolla consists of 5 bi-lobed white petals that are 1 mm long and 1.5 mm wide (including both lobes). The five stamens alternate with petals and each stamen is 1 mm long. The stigma is knob like. Dome shaped papillae occur all over the ovary.
wall (Plate I E). The fruits are ovoid, aromatic, cremocarps, 2-3 mm long, grayish-brown in colour, mericarps compressed, with distinct ridges and tubular surface. The fruit easily divide into two one-seeded mericarps. The term fruit and seed appear to be used interchangeably in the literature (Plate I F).

1.11: Medicinal importance of Ajowan:

Fruits are antidiarrhoeal, antiseptic, antispasmodic, carminative, stimulant, stomachic and tonic, beneficial in bronchitis, atonic dyspepsia and flatulence, dipsomania, hysteria, sore throat, plaster or poultice applied to abdomen in colic, an important ingredient in various Ayurvedic formulations prescribed for cough, digestive disorders, tonsillitis, urticaria, and infections with worms. Oil is antiseptic, carminative and expectorant, efficacious in bronchial pneumonia and other respiratory disorders. The aqueous extract of fruits (Ajwan-ka-arak) is a popular remedy for diarrhea. The leaf juice is anthelmintic, roots are carminative, diuretic, febrifuge, useful in stomach troubles (Asima et al. 1995).

Ajowan seeds also possess antiseptic, antimicrobial, antifungal, antibacterial, nematicidal, anti-inflammatory, anthelmintic, digestive, germicidal and abortifacient (Pathak et al. 2010). Ajowan seeds are employed alone or in combination with other spices and condiments in pickles, confectionery and beverages, having good remedy for indigestion. A paste of the crushed fruit is applied externally for relieving colic pains, used in lotions and ointments.

1.12: Processed products of Ajowan:

The major processed products are ajowan oil, thymol crystals, oleoresin, dethymolized oil (thymene) and fatty oils (Malhotra and Vijay 2004).

Ajowan oil:

The characteristic odour of ajowan oil is due to the high content of Thymol (35-60%), which crystallizes out from the oil and sold in Indian markets as ajowan ka phool, or sat-ajowan and is much valued in medicines (Plate III). Ajowan oil is aromatic, stimulant and carminative consisting of γ-Terpinene, p-Cymene, α-Pinene, β-Pinene, Myrcene, Limonene, Terpinene-4-ol, α-Terpineol, α-Thujene, α-Terpinene etc.
Thymol crystals:

Ajowan oil is treated with aqueous alkaline solution to obtain Thymol crystals (Plate III) which are used as an ingredient of deodorants, mouthwashes, toothpastes and gargles because of its bactericidal action against the oral bacteria. It is also used in many skin ointments/powders.

Oleoresin, fatty oils and dethymolized oil (thymene):

Ajowan oleoresin prepared from seeds gives a warm, aromatic and pleasing flavour to food products and is used in processed foods, snacks, sauces and various vegetable preparations (Pruthi 2001). Fatty oil extracted from Ajowan seeds is used in various pharmaceutical and cosmetic industries and also in making disinfectant soaps and insecticides. The Thymol-free fraction of the oil, known as ‘thymene’ because of its close similarity with corresponding portion of thyme finds application in soap perfumes (Malhotra and Vijay 2004).

1.13: Improved varieties:

Continuous efforts are made to release improved and elite varieties of Ajowan. The improved varieties under cultivation are Gujarat Ajowan 1, Pant Ruchika, Ajmer Ajowan 1, Ajmer Ajowan 2, Lam Sel. 1, Lam Sel. 2 and Rajendra Mani (Johnykalluprackal and Ravindran 2006).

1.14: Agronomy:

Soil and climate:

It is mainly grown as a winter crop in subtropical and temperate climate. It can grow well on any soil type, but performs best in humus rich loamy soil. It is grown as a rainfed crop in heavy soils, whereas it requires irrigation in light textured soils.

Seed sowing:

It is generally propagated by seeds and the seed rate is 3-4 Kg/ha. The field is ploughed repeatedly during September-October, incorporating organic manures and
the seeds are broadcasted or drilled in rows (45 cm apart) in November which germinate in 7-14 days after sowing.

**Irrigation and manuring:**

Light irrigation is given immediately after sowing and later on at 7-10 days interval, the weeding is generally done as and when required. Farm yard manures or organic manures are applied at the rate of 10-15 Tonnes/ha. N, P, K and S are applied at 80: 30:30:50 Kg/ha respectively for obtaining best yield. Half dose of N, full of P, K and S should be applied as a basal dose, whereas remaining dose of N should be applied in equal parts at an interval of 30 and 60 days of sowing (Malhotra and Vashishta 2004).

**Plant protection measures and harvesting:**

Ajowan is not affected by any type of diseases and pests but collar rot and powdery mildew are observed in some pockets which may be controlled by spraying 0.2% Mancozeb and wettable sulphur (0.2%) respectively. The crop is harvested when mature (umbels and fruits turn brown), air dried for 3-4 days, and then threshed or rubbed to release the seeds. Seeds are separated from extraneous plant material by winnowing and stored in cool, dry place.

**1.15: Chemical composition of seeds:**

Seeds are used in small quantities for flavouring foods as anti-oxidants, as preservatives or in medicines or for the manufacture of essential oils. The physico-chemical composition is: Moisture 9-9%, Protein 15.4%, Fat (ether extract) 18.1 %, Crude fibre 11.9%, Carbohydrates 38.6%, Mineral matter (Total ash) 7.1%, Calcium 1.42%, Phosphorus 0.30%, Iron 14.6 mg./gm, Calorific value 379.4 per 100gm (Qasim and Khan 2001).

**1.16: Biosynthesis of Ajowan oil:**

The Ajowan oil is synthesized in secretory cells associated with oil glands present in seeds and is stored in it in the form of micro droplets. The oil is mainly composed of monoterpenes with major constituent as Thymol. The essential oil and
triglycerides are located in separate, well-defined compartments, mericarp channels and seed endosperm respectively (Svoboda and Svoboda 2000).

1.17: Biosynthesis of Thymol:

Within the secretory cells, Terpenes are formed from the universal five-carbon building blocks, isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP), which are synthesized in plastidic methylerythritol pathway (MEP) and cytosolic mevalonate (MVA) pathway (Sallaud et al. 2009) (Fig. 1.3). The DMAPP and IPP are condensed together to form geranyl diphosphate (C10), the usual precursor of the monoterpenes, and DMAPP and two units of IPP are combined to form farnesyl diphosphate (C15) the precursor of most sesquiterpenes.

Thymol synthesis takes place by the aromatization of γ-Terpinene to p-Cymene through its hydroxylation, which starts with γ-Terpinene as initial monoterpene substrate and proceeds via the aromatic p-Cymene as an intermediate compound (Fig. 1.4a). Thus, both γ-Terpinene and p-Cymene are its precursors and γ-Terpinene synthetase is the key enzyme in biosynthesis of Thymol (Poulose and Croteau 1978a and b). The recent view proposed by Crocoll et al. (2010) indicated that, the first step from GPP to γ-Terpinene is catalyzed by monoterpane synthases (TPS) which is then converted into Thymol by the action of single cytochrome P450s, which is the second step of oxidation in which p-Cymene is a side product (Fig 1.4b).

1.18: Extraction of essential oil:

The dried seeds are crushed and distilled to obtain the essential oil and which on an average contain 2.5-5% oil.

The average seed yield is about 12.83 Q/ha under irrigated conditions and 5.2 Q/ha under rainfed condition. Considering the major constrains in cultivation of Ajowan present investigation was undertaken with the following objectives.

1.19: Objectives:

- To analyze the effect of different plant growth regulators, micronutrients, salt and water stress on seed germination, seedling growth and physiology of Ajowan.
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- To evaluate the effect of PGRs, micronutrients, salt and water stress on physiological, biochemical and enzymological aspects of Ajowan during growth and development in field conditions.

- To determine the improvement in growth, yield and thymol content of Ajowan by using PGRs, micronutrients and induction of salt and water stress.