

**Standardization of cultivation, harvesting and processing
techniques of *Artemisia absinthium***

Sajad Ahmed
(2015-FOR-56-M)

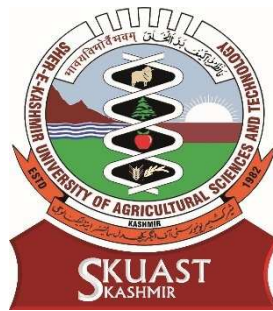


Faculty of Forestry
**Sher-e-Kashmir University of Agricultural Sciences &
Technology of Kashmir**

2018

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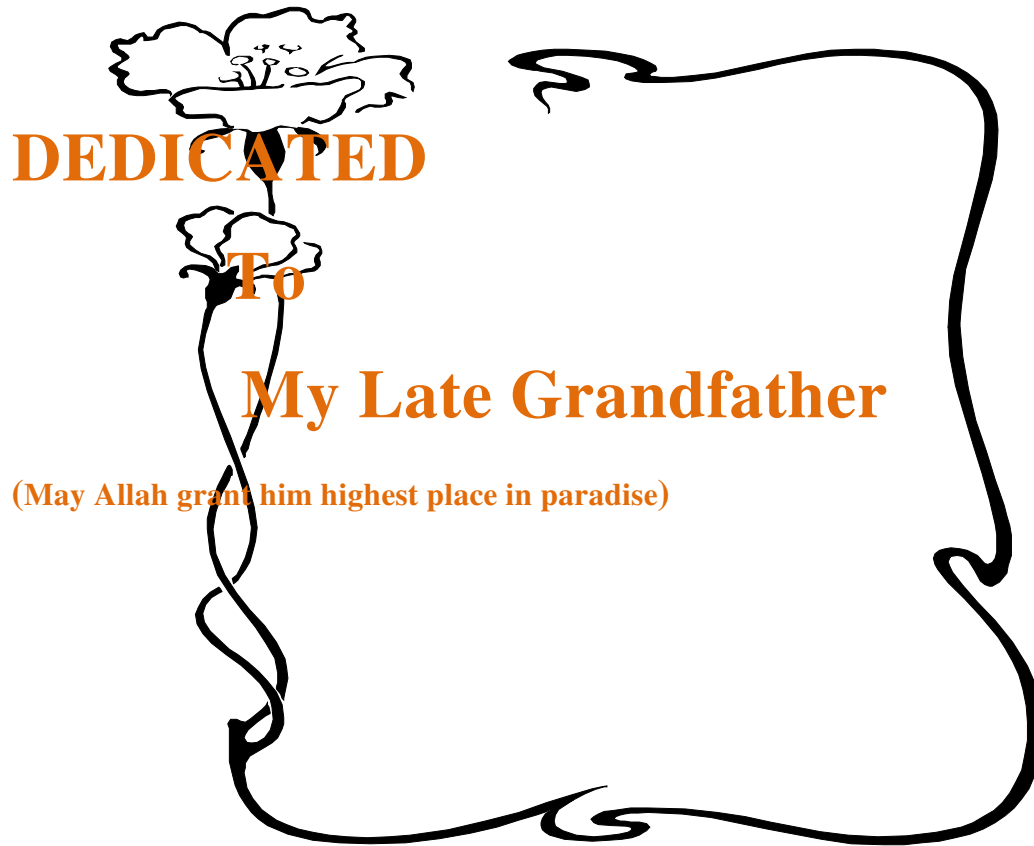
Thesis

Submitted to

**The Faculty of Forestry
Sher-e-Kashmir University of Agricultural Sciences &
Technology of Kashmir
in partial fulfillment of requirements for the award of the degree of**

Master of Science in Forestry

2018



“I WILL ALWAYS LOVE YOU”

“DADA JI”

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Faculty of Forestry

Certificate – I

This is to certify that the thesis entitled, “**Standardization of cultivation, harvesting and processing techniques of *Artemisia absinthium***” submitted in partial fulfillment of the requirements for the award of the degree of **Master of Science in Forestry, to the Faculty of Forestry, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir** is a record of bonafide research work carried out by **Mr. Sajad Ahmed (Regd. No. 2015-FOR-56-M)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that any help or information received during the course of investigation has duly been acknowledged.

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Title of the Thesis : **Standardization of cultivation, harvesting and processing techniques of *Artemisia absinthium***

ABSTRACT

Artemisia absinthium commonly known as wormwood or “Tethwan” is a perennial herb, traditionally used for antihelminthic, insecticidal, antiseptic and febrifuge properties. The plant has been used as herbal medicine throughout Europe, Middle East, North Africa and Asia and at least nine different chemotypes have been recognized based on essential oil composition. The present study was carried out at Faculty of forestry, Benhama, Ganderbal, Jammu and Kashmir to standardize the cultivation, harvesting and processing methods of *Artemisia absinthium*. Under the present study *Artemisia absinthium* was cultivated from seeds and cuttings collected from three different sources (Ganderbal, Bandipora and Budgam) and stem cuttings were subjected to different IBA concentrations and oil was extracted by hydro distillation. The experiment on stem cuttings was conducted with five treatments viz., Control, IBA@250ppm, IBA@500ppm, IBA@750ppm and IBA@1000ppm to study the effect on rooting percentage, number of roots per cutting and root length of *Artemisia absinthium* cuttings of three sources (Ganderbal, Budgam and Bandipora). Maximum values for rooting percentage, number of roots per cuttings (5.49) root

length (13.33 cm) were observed when *Artemisia absinthium* cuttings when treated with IBA@750ppm. Likewise maximum values for rooting percentage (68.40 %), number of roots per cutting (4.72) and root length (9.90 cm) were recorded for Ganderbal source.

In the other experiment to standardize optimum growing medium. Potting media of Fine sand, Soil and Soil + Sand + FYM (1:1:1) to evaluate their respective effects on germination percentage, survival percentage, Number of plants that survived out of germinated seeds, root length (cm), shoot length (cm), root shoot ratio and biomass (g/plant) of *Artemisia absinthium* seedlings procured from three sources (Ganderbal, Budgam and Bandipora). Potting media of Soil + Sand + FYM (1:1:1) was found superior than fine sand and soil. Higher values for germination percentage (46.55%), survival percentage (68.34%), number of plants that survived out of germinated seeds (36.35), shoot length (2.93 cm), root length (9.93 cm), root/shoot ratio (3.39), biomass (45.32 g/plant) were recorded in seedlings raised in polybags containing potting media of soil, sand and FYM in equal proportions. Similarly seedlings from Ganderbal source were found superior than Budgam and Bandipora. Maximum values for germination percentage (48.66%), survival percentage (67.16%), number of plants that survived out of germinated seeds (32.99), shoot length (2.59 cm), root length (8.25 cm), root/shoot ratio (3.19), biomass (43.19 g/plant) were observed in seedlings procured from Ganderbal source.

The rooted plants were then transplanted in field at four different spacings(40 cm x 60 cm, 60 cm x 80 cm, 80 cm x 100 cm and 100cm x120 cm respectively). All the treatments significantly influenced the growth and yield parameters of *Artemisia absinthium* plants under field conditions. The herb was harvested in full bloom stage in the month of July. Maximum values for leaf fresh weight (9.25 tonnes/ha), Leaf dry weight (3.15 tonnes/ha), Above ground biomass (26.83 tonnes/ha) were observed when *Artemisia absinthium* seedlings procured from Ganderbal source were planted at a spacing of 40 cm x 60 cm. The essential oil was obtained by hydrodistillation in Clevenger apparatus from different parts viz. leaves, stem + branches, seeds and roots. The study revealed that leaves were economically most important part of the herb yielding maximum essential oil of 24.20 kg/ha when *Artemisia absinthium* plants procured from Ganderbal source raised from seedlings were planted at a spacing of 40 cm x 60 cm. Similarly stem + branches (4.03 kg/ha), seeds (1.44 kg/ha), roots (0.81 kg/ha) yielded the maximum essential oil in spacing of 40 cm x 60 cm in seedlings procured from Ganderbal source. Maximum essential oil (ml/kg) was also observed in leaves (6.47 ml/kg) followed by stem+branches (1.87 ml/kg), seeds (1.04 ml/kg) and roots (0.52 ml/kg) in *Artemisia absinthium* seedlings procured from Ganderbal source when planted at a spacing of 40 cm x 60 cm.

Keywords: *Artemisia absinthium*, Essential oil, IBA, Cuttings, ppm, Ganderbal, Spacing

Signature of Major Advisor

Signature of Student

Dated: _____ Dated: _____

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IN THE NAME OF ALLAH WHO IS THE MOST MERCIFUL, MOST COMPASSIONATE AND MOST GRACIOUS

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Place: Shalimar, Srinagar **Sajad Ahmed**

Dated:

CONTENTS

Chapter	Particular	Page No.
1.	INTRODUCTION	1-7
2.	REVIEW OF LITERATURE	8-20
3.	MATERIALS AND METHODS	21-26
3.1	Experimental site	21
3.2	Observations recorded	23
3.3	Estimation of oil content by hydrodistillation	25
3.4	Statistical analysis	26
4.	EXPERIMENTAL FINDINGS	27-39
4.1	Raising of <i>Artemisia absinthium</i> through seeds and cuttings	27
4.1.1	Rooting Percentage of <i>Artemisia Absinthium</i> under different IBA Treatments	27
4.1.2	Number of Roots per Cutting of <i>Artemisia absinthium</i> under different IBA Treatment	27
4.1.3	Length of Roots of <i>Artemisia Absinthium</i> under different IBA Treatments	28
4.1.4	Germination Percentage of seedlings under different potting media	28

4.1.5	Survival Percentage of seedlings under different potting media	29
4.1.6	Number of plants that survived after germination under different potting media	29
4.1.7	Shoot Length of germinated seedlings under different potting media	30
4.1.8	Root Length of germinated seedlings under different potting media	30
4.1.9	Root/Shoot ratio of germinated seedlings under different potting media	31
4.1.10	Biomass (g/plant) of germinated seedlings under different potting media	31
4.2	Out-planting performance in field plantation	32
4.2.1	Leaf Fresh Weight (tonnes/ha) under different spacing's after out planting	32
4.2.2	Leaf Dry Weight (tonnes/ha) under different spacing's after out planting	33
4.2.3	Above ground biomass (tonnes/ha) under different spacing's after out planting	34
4.3	Oil content estimation of <i>Artemisia absinthium</i>	34
4.3.1	Leaf oil yield (kg/ha) under different spacing's after out planting	34
4.3.2	Stem+branches oil yield (kg/ha) under different spacing's after out planting	35
4.3.3	Seed oil yield (kg/ha) under different spacing's after out planting	36

4.3.4	Root oil yield (kg/ha) under different spacing's after out planting	37
4.3.5	To find out oil yield/kg of leaves, separate experiment was conducted and the results are tabulated in table 19	38
4.3.6	To find out oil yield in the whole above ground biomass /kg separate experiment was conducted and the results are tabulated in table 20	38
4.3.7	To find out oil yield/kg separate experiment was conducted and the results are tabulated in table 21	38
4.3.8	To find out oil yield/kg separate experiment was conducted and the results are tabulated in table 22	39
5.	DISCUSSION	40-52
5.1	Raising of <i>Artemisia absinthium</i> through seeds and cuttings	40
5.1.1	Effect of different IBA treatments and potting media on growth and yield parameters of <i>Artemisia absinthium</i>	40
5.2	Out-planting performance of <i>Artemisia absinthium</i> in field plantation	47
5.3	Extraction of oil from <i>Artemisia absinthium</i>	48
5.3.1	Effect of source on growth and yield of <i>Artemisia absinthium</i>	50
6.	SUMMARY AND CONCLUSION	53-56
	LITERATURE CITED	I-XIX



Table No.	Particulars	After Page No.
1.	Taxonomical hierarchy of <i>Artemisia absinthium</i>	3
2.	Rooting Percentage of <i>Artemisia absinthium</i> under different IBA treatments	27
3.	Number of roots per cutting <i>Artemisia absinthium</i> under different IBA treatments	27
4.	Length of roots <i>Artemisia absinthium</i> under different IBA treatments	28
5.	Germination Percentage of seedlings under different potting media	28
6.	Survival Percentage of seedlings under different potting media	29
7.	Number of plants that survived out of germinated seeds	29
8.	Shoot Length of seedlings under different potting media	30
9.	Root Length of seedlings under different potting media	30
10.	Root/Shoot Ratio of seedlings under different potting media	31
11.	Biomass (g/plant) of seedlings under different potting media	31
12.	Leaf Fresh Weight (tonnes/ha) under different spacing's after out planting	32
13.	Leaf Dry Weight (tonnes/ha) under different spacing's after	33

Plate No.	Particulars	After Page No.
1.	Cuttings of <i>Artemisia absinthium</i> at different treatments	27
2.	Measurement of different parameters after harvesting	33
3.	Extraction of oil using Clevenger apparatus	34

Chapter-1

INTRODUCTION

Medicinal plants have been a part of human society for combating disease since time immemorial. Nature has been provided a worthy source of medicinal plants and impressive novel drugs have been isolated from them to treat various diseases. Various medicinal plants have been used for years in daily life around the world (Bhattacharya *et al.*, 2014). Traditional indigenous medicines plants are still used in developing countries and about 80% of the people are still dependent on plants for their basic healthcare (Anon., 2002; Malik *et al.*, 2010). Use of essential oils in therapeutics is becoming popular in India, China, Japan and European countries. Aromatherapy involves the use of essential oil aromatics derived from plants to cure diseases. The characteristic aroma is mainly due to variety of complex chemical compounds known as terpenoids. The term essential oil is concomitant to fragrances or perfumes because these fragrances are oily in nature as they represent the essence or the active constituents of the plants. They are called volatile or ethereal oils as they evaporate when exposed to air at ordinary temperatures. Essential oils are highly concentrated, low volume and high value products. Similar is the status of aromatic plants where essential oils/oleoresins derived from them form backbone of perfumery and flavor industry. Medicinal plants play a key role to understand the vibrant relationships between biodiversity and socio genic systems (Husain *et al.*, 2008; Mahmood *et al.*, 2011a).

Artemisia is a genus of small herbs and shrubs found in northern temperate regions. It belongs to family Asteraceae, one of the most numerous plant groupings, which comprises about 1,000 genera and over 20,000 species (Bora and Sharma, 2011). Artemisia species are one of the many traditional medicinal plants used for the treatment of infectious and non-infectious health

problems. *Artemisiaannua* is processed by pharmaceutical industry for the production of artemisin and artemisinin based combination therapies (ACT's) in the treatment of malaria (WAC, 2007). *Artemisia absinthium* L. is a highly medicinal plant and almost mentioned in all the books of herbal medicine, distributed mainly in the temperate zones of Asia, Europe and North America. *A. absinthium* is one of the important specie among the 45 members represented by genus Artemisiain the Indian flora, and is naturally distributed in the Himalayan region across Jammu and Kashmir at an altitude range of 1500-2700 m (Chopra *et al.*, 1956). *Artemisia absinthium* species grows from Pakistan to Kashmir, West Asia to Europe at the elevation 1500-2700 m. It grows wild in cultivated area forest areas.

A. absinthium L. is a hardy perennial sub-shrub, erect, woody stem (over 1 m) bears alternate, much divided, silvery ovate to obovate leaves with silky soft hairs on each side. Tiny, ray less yellowy-green flowers occur in late summer in loose panicles which arise from the woody stems. It has a distinctive fragrance, thrives in sunny positions in poor soils and can become very woody (Beigh and Ganai, 2017). It iscommonly known as wormwood or “vilayati afsanteen”, traditionally used because of its antihelminthic, insecticidal (Smith and Secoy, 1981), antiseptic and febrifuge properties (Nadkarni, 1976). Oil of *A. absinthium*has been found to repel the flies, fleas and mosquitoes and to kill house flies (Kaul *et al.*, 1978). In unani system of medicine, the plant is extensively used for fevers, swellings and inflammation of viscera *Artemisia absinthium* L. is known by different vernacular names like Afsanteen (Urdu); green ginger, absinthe, absinthium, wormwood (English); Apsinthion (Greek); *A. absinthium* (Latin); Afsanteen, Damseeah (Arabic); Ku ai, Yang ai (Chinese); Vermouth, Genepi (French); Wermut, Absinth (German); Afsantine (Iran); Anjenjo (Mexican); Majri, Karmala, Majtari, Mastiyarah (Hindi); Afsanteen (Unani), Absinthium (Hemopathy), and locally known as ‘Tethwen’ in Kashmir (Abdullah *et al.*, 2010); Beigh and Ganai, 2017).

The taxonomical hierarchy of *Artemisia absinthium* has been shown in Table-1.

Table-1: Taxonomical hierarchy of *Artemisia absinthium*

Taxonomy	
Kingdom	Plantae – Plants
Sub-kingdom	Tracheobionta - Vascular plants
Super-division	Spermatophyta - Seed plants
Division	Magnoliophyta - Flowering plants
Class	Magnoliopsida - Dicotyledons
Sub-class	Asteridae
Order	Asterales
Family	Asteraceae - Aster family
Genus	<i>Artemisia</i> L.
Species	<i>Artemisia absinthium</i> L.

1.1 Propagation of *Artemisia absinthium*

The conventional propagation of *Artemisia* species by seeds is a constraint for its production due to its tiny seeds that require a symbiotic association within its own microflora for germination most of the seedlings raised from such seeds would have phenotypic variation making uniformity of growth and form difficult to attain. Thus propagation of *Artemisia* species using cuttings supplied with Indole-3-Butyric Acid

(IBA) can be one of the ways of overcoming the problems outlined. Vegetative propagation using stem cuttings is an effective alternative means of raising planting stock for species with irregular seed supply or for clonal propagation. Auxins have been known to be involved in the process of adventitious root formation for a long time (Haissig and Davis, 1994) and the interdependent physiological phases comprising the rooting process are associated with changes in endogenous auxin concentrations (Heloir *et al.*, 1996). Auxins often hasten root initiation, increase the number and percentage of cuttings rooted as well as quality of roots produced per cutting (Leonardi *et al.*, 2001). Larsen and Guse (1997) also reported that the most reliable rooting hormone is IBA although others such as Naphthalene Acetic Acid (NAA) can also be used. Although there are reports that it may also be toxic to young/ succulent cuttings of certain species, IBA is still probably the best hormone for general use because of being non toxic to plants over a wide range of concentration levels (Kester *et al.*, 1990).

Potting media plays a key role in quality production of medicinal and ornamental plants. Natural soil, sand, vermicompost and peat are the most used growing substrates for the container production of annual and perennial ornamental plants (Venkatesan *et al.*, 2010). Potting medium is one of the important factors that can influence the growth and vigour of the cuttings to a greater extent. Cuttings raised in a good medium can ensure better establishment and growth when transplanted in the main field. Rooting performance depends on the type of medium used in the propagating structure. This is so because the various materials and mixes of materials that can be used in rooting of cuttings provide physical support, oxygen and water (Larsen and Guse, 1997). A cheap and successful medium will enable the farmers and nurserymen to produce good seedlings of medicinal plants for commercial cultivation (Bali *et al.*, 2013).

Agronomic characteristics, biomass and essential oil yield of aromatic and medicinal plants are influenced by many factors. Among these plant spacing deserves special attention (Yasin *et al.*, 2003). Singh and Singh (2002) also explained that establishment of optimum population per unit area of the field is essential to get maximum yield. Under conditions of sufficient soil moisture and nutrients, higher population is necessary to utilize all the growth factors efficiently. The level of plant population should be such that maximum solar radiation is utilized. The full yield potential of an individual plant is fully exploited when sown at wider spacing (Al-Ramamneh, 2009). Yield per plant decreases gradually as plant population per unit area increases. However, the yield per unit area is increased due to efficient utilization of growth factors. Good agricultural and collection practices for medicinal plants have been a concern all over the world (FAO, 2010). The growing demand of herbal products has also led to overharvesting from the wild causing concern over the long term environmental impact and availability of certain medicinal plant species. As a result of these concerns the herbal industry has come under ever-increasing pressure to provide consumers with assurance that herbal products in the market are safe to use and do not have a negative impact on the environment. In 2009 the National Medicinal Plants Board (NMPB) of India developed a set of standards known as good agricultural practices (GAP's) and good field collection practices (GFCP's) for medicinal plants. The key concern addressed by the guidelines are hygiene and cleanness, prevention of contamination, identification, efficiency productions and income, sustainability, documentation and traceability, social and legal concern.

1.2 *Artemisia absinthium* essential oil

Chemically, essential oils are very complex natural mixtures which can contain about 50-1,000 components at different concentrations. They are characterized by 2-3 major components at fairly high concentrations (20–70%), compared to other components present in trace amounts. Generally, these major components determine the biological properties of the essential oil. The components include two groups with different biosynthetic origins; the main group is composed of terpenes, and the other of aromatic and aliphatic constituents, all characterized by their low molecular weight (Abad *et al.*, 2012). The strong and aromatic smell of some species of *Artemisia* genus is mainly due to high concentrations of volatile terpenoids compounds present in their essential oils, especially in leaves and flowers. The chemical composition of essential oils from the *Artemisia* species has been extensively studied around the world. Many studies have shown that *Artemisia* species display significant intraspecific variations in the terpenic constituents of their essential oils. In some cases, the variation in the volatile components of these plants may occur during plant ontogeny or growth at different altitudes. The quality and yield of essential oils from *Artemisia* species is influenced by the harvesting season, fertilizer and pH of soils, the choice and stage of drying conditions, the geographic location, chemotype or subspecies, choice of plant part or genotype, or extraction method (Abad *et al.*, 2012).

The active constituents present in *A. absinthium* L. are the bitter substances, essential oils and other compounds. Bitter constituents (0.15-0.4%) consist of sesquiterpene lactones, a class of natural compounds with several proved medicinal properties and includes guanolide dimmers as absinthin (0.2-0.28%) and its isomers anabsinthin, anabsin, artabsin (0.04-0.16%), and absintholide germacrene type sesquiterpines artabin; matricin; beta-santonin and ketopenolid-A (Beauhaire *et al.*, 1982); (Akhmedov *et al.*, 1970) (Perez-Souto *et al.*, 1992). Essential oil isolated by steam distillation from *A. absinthium* varies qualitatively and quantitatively

according to geographical location and environmental conditions (Bakkali *et al.*, 2008). The yield of essential oil (0.2-1.5%) increases from arid to humid climate (Riahi *et al.*, 2015). The four main components described are: α -thujone, (Z)-epoxy-ocimene, trans-abinylacetat and chrysanthenyl-acetate. α -thujone is typical for plants grown in areas below 1,000 m above mean sea level and is present at levels of approximately 40-70% of the oil (Hagar *et al.*, 2006). (Z)-epoxy-ocimene is the main component in plants grown in Europe at altitudes higher 1,000 m above mean sea level. *A. absinthium*oil is mainly composed of beta-thujonecis-sabinyl acetate (chamazulene, nuciferol butanoate, nuciferol propionate, caryophyllene oxide, beta-ocimene, (Z)-anethole and limonene. Because of this, strong inhibitory effects have been observed on the growth of bacteria and fungi. The oil also revealed antioxidant activities (Cavar *et al.*, 2012; Bopitiya and Madhujith, 2013).

Most of the *Artemisia* species endemic to the Kashmir valley have been categorized as endangered. So keeping in view the importance of *Artemisia absinthium*, the present study was undertaken with the following objectives:

- Raising of *Artemisia absinthium* through seeds and cuttings collected from three different sources.
- Outpl anting performance in field plantation.
- Estimation of oil content from different parts of *Artemisia absinthium*.

Chapter-2

REVIEW OF LITERATURE

The outcome of previous studies forms the base for further scientific enquiry. Moreover review of related studies help us to conceptualize our research theme and to set research methodologies. Accordingly, the ensuing section gives a brief account of various studies conducted at national and international levels relating to the present theme. Keeping in view the multiple perspectives of the study entitled, **“Standardization of cultivation, harvesting and processing techniques of *Artemisia absinthium*”** an account of literature was reviewed under different headings as given below.

2.1 Cultivation of Artemisia species

Nguyen *et al.* (2017) propagated *Artemisia absinthium* through seeds at the experimental station of Szent Istvan University in Budapest. The seeds were sown in march 2016 and seedlings were raised in greenhouse for 2 months. The seedlings with 5-6 leaves were then transplanted into open field plots in june in three replications. Furthermore, the soil in the field was sandy loam with ph 7.8 having low humus content. Moreover, the plants were grown without additional fertilization; mechanical weed control and assured irrigation in dry periods. Measurements and sampling were carried out at the beginning of august at vegetative stage. 10 individuals from each accession were randomly chosen to evaluate harvest.

Nigussie *et al.* (2015) studied the effect of plant population density (four intra-rows viz. 40, 60, 80, 100 cm and four inter-row plant spacing's of 60, 80, 100, 120 cm) on growth and yield of *Artemisia annua* L. and found that maximum above ground biomass (72605 kg/ha), leaf fresh weight (9510 kg/ha), leaf dry weight (5392.7 kg/ha) and essential oil yield (23.39 kg/ha) were attained due to spacing combination 40 cm intra-row and 60 cm inter-row spacing.

ICAR (2014) has published an extension folder describing the cultivation of *Artemisia annua*. In the folder soil, climate, propagation, nursery raising of seedlings, land preparation, planting time, transplanting, crop nutrition, irrigation, intercultural operations, plant protection, insect, pest control, harvesting, processing, expected yield, major chemical constituents.

El-Naggar *et al.* (2013) cultivated *Artemisia annua* through seeds by sprinkling them uniformly onto the surface of soil and covering the seeds very lightly with compost (1-2 mm). The seeds germinated in a greenhouse at the beginning of October. The surface was kept moist and not let to dry out. Also, when the plantlets had about 10 true leaves and were about 10-14 cm high, approximately 8 weeks after sowing. They were pricked out, taking care not to damage the roots and then transplanted to the field. The plants were harvested just before flowering.

Kim *et al.* (2013) studied growth parameters of *Artemisia Montana* from Gyeongsangbukdo Ulleung-gun and planted in a test package at the Department of Herbal Crop Research of the Korean National Institute of Horticultural & Herbal Science on May 10, 2012. A randomized complete block design was used in triplicate. For planting density, spacing between furrows and rows was 90 and 30 cm, respectively. The planting intervals were 10 cm, 20 cm and 30 cm. After planting, 20 specimens were evaluated three times at 30 days interval. For extraction, block sampling was used. Leaf dry weight ranged from 32 to 79.3 g. The dry weight of the aerial plant organs was the highest in the 30 cm × 10 cm plots. However, no significant differences were observed between the result and planting distance of 30 cm × 20 cm. A higher number of aerial plant organs of *A. montana* were associated with a greater planting density. The dry weight of the aerial plant organs by harvest time was 2757 kg per acre, which was the highest in October. No significant differences were observed between the results collected in October, 2757 kg per acre,

and the dry weight of the aerial plant organs harvested in september, 2580 kg per acre.

Ellman (2010) on cultivation of *Artemisia annua* in Asia and Africa found out that the species maybe raised by seeds collected from wild plants or selected cultivars sown directly in the field, usually in rows or saucers at 0.5 m to 1.0 m intervals to facilitate irrigation and weeding. Additionally, the seedlings were transplanted from the nursery at a height of 10 cm to 12 cm, approximately 7 weeks after sowing. He also reported that F₁ hybrid seed planted at a spacing of 1 m × 1 m with 10,000 plants per ha had vigorous growth as compared to non-hybrid or F₂ hybrid seed which had less vigorous growth and were planted more densely with a population of 20,000 or even 30,000 plants per ha.

2.2 *Artemisia absinthium* essential oil

Nguyen *et al.* (2017) extracted essential oil in *Artemisia absinthium* in Hungary by distilling plant samples (g) in a Clevenger type apparatus. The essential oils were extracted from 50 g dried leaves of each accession by hydro-distillation (500 ml water) for 2.5 hours. The essential oil yield of the investigated 9 accessions (Belgien, Csor, English, Hungarian, Leipzig, Norwegen, Pakozd, Spanish and Wild Soroksar) was 1.107 ml/ 100 g as a mean, however, it varied on a large scale between 0.349 ml/100 g (Wild Soroksar) and 3.215 ml/100 g (Spanish). Also, all accessions from Hungary together with “English” and “Leipzig” were statistically equal, practically below 1%. Among them, highest mean value was reached by “Leipzig” and lowest level by “Wild Soroksar”. Furthermore, relatively high concentrations of 1.569 ml/100 g and 1.892 ml/ 100 g were determined from accessions “Norwegen” and “Belgien” respectively. The “Spanish” accession in our study presented an

exceptionally high level of volatile compounds which is significantly different from each of the other ones (3.215 ml/100 g).

Hodaj-Celikua *et al.* (2017) studied chemical composition of *Artemisia absinthium* in Albania. Air dried aerial parts were cut into small pieces and 50 g of sample was submitted to hydro-distillation for 2 hours using a Clevenger type apparatus and their analyses were performed by GC-MS. *Artemisia absinthium* essential oil was a pale yellow color and obtained in a yield of 0.4%. Twenty-nine constituents were identified, which constituted 97.3% of the total oil. The major constituents were neryl isovalerate (19.5%), geranyl isobutanoate (16.4%) and carvacrol (8.8%). The oil was rich in sesquiterpene hydrocarbons (23.5%), followed by oxygenated monoterpenes (13.8%) and oxygenated sesquiterpenes (3.5%).

Elliot (2017) analyzed essential oil of *Artemisia absinthium* and reported that herb contains at least 0.3% volatile oil and has a bitter value of not less than 15,000. He further reported that volatile oil contains thujone. Additionally, the herb contains bitter principles of the sesquiterpene lactone types absinthin, anabsinthin, artabsin, anabsin, also flavones, ascorbic acid, and tannins.

Abirami *et al.* (2014) conducted an experiment to work out the optimum spacing for obtaining maximum biomass, essential oil and artemisinin yields in antimalarial herb (*Artemisia annua* L.) in the semi-arid region of Gujarat. It was laid out in a randomized block design with 5 spacing treatments, viz. 30 cm × 45 cm, 30 cm × 60 cm, 45 cm × 45 cm, 45 cm × 60 cm and 60 cm × 60 cm. There were no significant differences in growth parameters like plant height and number of branches among different spacings. However, significant differences were observed in yield characteristics and was found that the mean fresh (30.79 t/ha) and dry (18.35 t/ha) herb yield and fresh (7.7 t/ha) and dry (3.91 t/ ha) leaf yields, were maximum under

spacing of 45 cm × 45 cm. The leaf harvest index and leaf: stem ratio were also significantly higher under optimum spacing of 45 cm × 45 cm. No significant differences were observed in essential oil content, their composition and artemisinin contents among the different spacing but the yields of essential oil (54.95 kg/ha) and artemisinin (8.88 kg/ha) were found maximum in the spacing of 45 cm × 45 cm.

Nurzynska-Wierdak and Zawislak(2014)studied the effect of tarragon (*Artemisia dracunculus*) planting density upon fresh and dry herb yield as well as the contents, yield and chemical composition of essential oil. The study was conducted in the year 2011 and 2012 at the experimental field of the University of Life Sciences in Lublin, Poland. Significant effect of tarragon planting density was demonstrated upon fresh and air-dry herb yield, grated herb yield and essential oil yield. Tarragon plants growing in higher density (40 cm × 40 cm) had higher yield of fresh, dry and de-stalked herb, as well as higher oil yield than the plants growing less densely (50 cm × 50 cm).

Dhen *et al.* (2014) have studied chemical composition and fumigant toxicity of *Artemesia absinthum* essential oil against *Rhizoptera dominica* and *Supodoptera littoralis*. Essential oil of the aerial parts obtained by hydro distillation and analysed by GC-MS in order to determine the chemical composition. The major chemicals identified were; camphor (24.81%), camazuliene (13.17%), bronylacete (5.89%) and myrecene (5.83%).

Choudhary and Choudhary (2013) studied effect of plant geometry on various growth parameters of *Artemisia annua*. The results of the study revealed that the effect of different spacing on plant height was significant at all the growth stages. At 60 days after sowing, the maximum plant height (46.00 cm) was recorded with 30 × 30 cm spacing, which was significantly higher as compared to 30 × 60 cm, 45 × 60

cm, 45 cm × 75 cm, 45 cm × 90 cm, 60 cm × 75 cm and 60 cm × 90 cm spacings. The effect of the spacing on dried leaf yield was pronounced in the planting geometry. At harvest highest leaf yield was recorded at 45 cm × 60 cm crop geometry. The study also revealed that the dried leaf yield showed an increase from (30 cm × 30 cm) to (45 cm × 60 cm) spacing.

Chauhan (2013) extracted essential oil from areal parts of three *Artemisia* species viz., *A. abyssinica* (collected from African Laboratory from Natural Products (ALNAP) garden), *A. absinthium* and *A. annua* (collected from local area of Ethiopia). Fresh aerial parts of the three plants were crushed by liquid nitrogen. Each of the three powdered aerial parts of the plant material were subjected to hydro-distillation for 1 ½ hour using a Clevenger type apparatus. The study further revealed that 45 g of *A. abyssinica* yielded 400 mg (0.9%) of oil, 100 g of *A. absinthium* yielded 460 mg (0.5%) of oil and 300 g of *A. annua* yielded 144 mg (0.05%) of oil respectively.

Zigene *et al.* (2012) studied growth parameters of *Artemisia annua* under varying growth ages at Wondo Genet Agricultural Research centre, Shashemene, Ethiopia. The experiment consisted of 6 growth stages (2, 3, 4, 5, 6 and 7 months after transplanting) which were arranged in randomized complete block design with 3 replications. The results showed that highest values were recorded at 5 months of transplanting for plant height (194 cm), fresh leaf weight per plant (382.87 g), and dry leaf weight per plant (98.36 g) and at 2 months after transplanting for fresh leaf/stem ratio (0.69) and dry leaf/stem ratio (0.64) respectively.

Farukh *et al.* (2012) collected three samples of *Artemisia absinthium* from two different locations in the central south of Tajikistan and obtained essential oils by hydrodistillation. They identified 41 compounds representing 72-94% of total oil

compositions and the major components of *A. absinthium* oil were myrcene (8.6-22.7%), *cis*-chrysanthenyl acetate (7.7-17.9%), a dihydrochamazulene isomer (5.5-11.6%), germacrene D (2.4-8.0%), thujone (0.4-7.3%), linalool acetate (trace-7.0%), phellandrene (1.0-5.3%), and linalool (5.3-7.0%). They further observed that the chemical compositions of *A. Absinthium* from Tajikistan was markedly different from those from European, Middle Eastern and other Asian locations.

Damtew *et al.* (2011) conducted an experiment to determine the effects of harvesting age and plant population density on agronomic and chemical characters of *Artemisia annua* L. during 2009. Four levels of harvesting age and five levels of population density were arranged in split plot design with three replications. Harvesting age was assigned to main plots and plant population density to sub plots. The effects of harvesting age and plant population density were assessed by analyzing data on growth, yield and yield components of *A. annua*. Interaction effects of harvesting age and plant population density was highly significant ($p < 0.01$) on plant height, leaf area index, dry leaf yield haG1 and significant ($p < 0.05$) on artemisinin yield. Dry leaf yield plant and artemisinin content were affected significantly ($p < 0.05$) by harvesting age and plant population density. Branch numbers/plant, essential oil (EO) content and EO yield were affected significantly ($p < 0.05$) by plant population density. In this study, the maximum dry leaf yield (3.15 t/ha), EO yield (19.2 kg/ha) and artemisinin yield (10.9 kg/ha) were attained at plant population density of 27, 777 plants per ha and harvested at 5 months after transplanting (MAT), 4 to 7 MAT and 7 MAT for dry leaf yield, EO yield an artemisinin yield, respectively.

Padalia *et al.* (2011) analyzed and compared by capillary GC and GC-MS the essential oil yield and composition of the aerial parts of *A. annua* growing in Uttarakhand, India, at different stages of development. The analyses led to the

identification of 81 constituents, forming 91-97.1% of the essential oil composition. However, the essential oil content was found to vary from 0.3% to 0.7% at different stages of growth. The major constituents were camphor (22.8-42.6%), 1,8-cineole (3.7-8.4%), linalool (0.1-11.9%), β -caryophyllene (2-9.2%), (*E*)- β -farnes (1.3-8.5%), germacrene D (0.5–7.3%) and 1-*epi*-cubenol (0.7-5.2%).

Rather *et al.*, (2011) studied mono and sesquiterpenoid composition in the leaves and stem of *Artemisia amygdalina* Decne obtained by hydrodistillation using a combination of capillary GC-FID and GC-MS analytical techniques. The analysis led to the identification of 25 components in the leaf and 32 components in the stem essential oils. The leaf essential oil was dominated by monoterpene hydrocarbons (38.2%) and oxygenated monoterpenes (43.8%) together constituting 82.0% of the total oil composition. The principal components were sabinene (10.2%), p-cymene (14.7%), 1,8 cineole (17.5%) and borneol (19.8%). The stem essential oil is dominated by monoterpene hydrocarbons (66.1%), oxygenated monoterpenes (12.8%) and sesquiterpene hydrocarbons (11.2%). The major constituents were α -pinene (6.0%), camphene (10.4%), β -pinene (40.2%) and borneol (5.7%).

Tzenkova *et al.* (2010) obtained essential oil from *Artemisia annua* by hydrodistillation of species growing wild in Bulgaria and identified thirty six chemical compounds like α -caryophyllene (24.73%), α -cuvabene (13.53%), α -copaene (7.42%), α -selinene (8.21%) artemisia ketone (8.45%) and camphor (3.61%). Moreover, they also found that the composition of the essential oil varied among different species growing across other geographic locations.

Nezhadali and Parsa (2010) studied volatile compounds in *Artemisia absinthium* from Iran. For this, the aerial parts of the plant *Artemisia absinthium* were collected in July 2010 from Binaloud mountain (Khorasan Razavi Province of Iran)

and subjected to air drying in a shadow place. A headspace solid-phase micro-extraction (HS-SPME) method followed by gas chromatography–mass spectrometry (GC-MS) was used for the analysis of volatile compounds in the leaves of *Artemisia absinthium*. The dried plant was powdered and the aroma compounds of a 0.05 g were isolated by HS-SPME fiber in 15 min. The chemical compounds of the plant were investigated by gas chromatography mass spectrometry (GC-MS). A total of 72 constituents, representing more than 97% of the volatiles were identified.

Haider *et al.*, 2010 analyzed oil of *Artemisia nilagirica*, a plant growing at different altitudes in Himachal Pradesh, India, by gas chromatography (GC) and gas chromatography/mass spectrometry. The main constituents of the oil show variation with changes in altitude. At lower, middle and higher altitudes, the major constituents were caryophyllene oxide (28.6%), borneol (35.8%) and camphor (46.9%), respectively. The characteristic compounds observed in plants from lower altitudes were 2-hexene-1-ol, β -thujone, thujanol, myrtenol and linalyl acetate, while the higher altitude plants were characterized by the presence of α -pinene, β -pinene, limonene, linalool, γ -gurijunene, germacrane D and farnesol. Camphor (19.3%), 1,8-cineole (19.2%) borneol (18.9%), camphene (4.6%) and β -caryophyllene (3.4%) were found to be the major components.

Verma *et al.* (2010) analysed essential oils from two distinct chemotypes of *Artemisia dracuncululus* L. (Tarragon) in Kashmir valley. The oils were analyzed using GC and GC-MS techniques. The chemical constituents of local variety were found out to be α -pinene (0.7%), myrcene (1.2 %), limonene (3.5 %), Z-(β)-ocimene (12.7 %), E- (β)-ocimene (4.6 %), α -terpinolene (2.7 %), 5-phenyl-1,3-Pentadiyne (5.1 %), methyl eugenol (0.7 %), capillene (60.2 %), elemicin (2.3 %), bicyclogermacrene (0.5 %), iso-elemicin (2.1 %) and germacrene- B (0.6 %). Furthermore, the major chemical constituents found in exotic variety were α -pinene (1.4 %), β -pinene (0.3

%), myrecene (0.1 %), p-cymene (0.2 %), limonene (2.8 %), Z-(β)-ocimene (1.6 %), E-(β)-ocimene (1.0 %), γ -terpinene (0.3 %) linalool (1.2 %), β -thujone (0.4 %), camphor (0.4 %), methyl chavicol (71.3 %), iso-menthol (0.1 %), bornyl acetate (0.7 %), carvacraol (7.7 %), α -terpinyl acetate (0.2 %), hexyl hexanoate (0.5 %), α -copaene (2.2 %), β -caryophyllene (0.2 %). The chemical constituents of the volatile oil were found out to be similar to French Tarragon.

Shafaghat *et al.* (2009) investigated the composition of the essential oil from *Artemisia fragans* from Iran. Hydro-distillation of leaves and roots of this species yielded 0.9% and 0.1% essential oils, respectively. GC-MS analysis allowed identification of 19 components, comprising 91.1% of the total oil from the leaves, while only nine compounds (93.8%) were identified in the roots. The main components of the leaf were chrysanthenone (23.8%), 1,8-cineole (23.7%), β -caryophyllene (9.6%), p-cymene (7.7%), filifolide A (5.7%) and filifolone (5–7%). In the root oil, the main constituents were camphor (67%) and camphen (16.9%).

Rezaeinodehl and Khangholi (2008) investigated the chemical composition of the essential oil of *A. absinthium* growing wild in Iran. The aerial parts were harvested at full bloom from an area between the villages of Deylamon and Asiabar, in Guilau province. Results showed that 28 components were identified, representing 93.3% of the oil, most of which were monoterpenes. The main components were β -pinene (23.8%) and β -thujone (18.6%).

Lopez-Lutz *et al.* (2008) used GC-MS to investigate the chemical composition of essential oil isolated from aerial parts of seven wild sages from western Canada (*A. absinthium*, *A. biennis*, *A. cana*, *A. dracuncululus*, *A. frigida*, *Artemisia longifolia* Nutt. And *A. ludoviciana*). A total of 110 components were identified, accounting for 71–98.8% of the oil composition. High contents of 1,8-cineole (21.5–27.6%) and

camphor (15.9–37.3%) were found in *A. cana*, *A. frigida*, *A. longifolia* and *A. ludoviciana*. The oil of *A. ludoviciana* was also characterized by a high content of oxygenated sesquiterpenes with a 5-ethenyltetrahydro-5-methyl-2-furanyl moiety, of which davanone was the main component identified (11.5%). *A. absinthium* essential oil was characterized by high amounts of myrcene (10.8%), β -thujone (10.1%) and *trans*-sabinyl acetate (26.4%). *A. biennis* yielded an oil which is rich in *cis*- β -ocimene (34.7%), *trans*- β -farnesene (40%), and the acetylenes (*Z*)- and (*E*)-en-yndicycloethers (11%). *A. dracunculus* oil contained predominantly phenylpropanoids such as methyl chavicol (16.2%) and methyleugenol.

Burzo *et al.* (2008) studied chemical composition of the essential oils of *Artemisia* sp. L. growing in Romania and reported the presence of some major compounds *viz.* eucaliptol, camphor, *cis*-verbenone and borneol or iso-borneol in *Artemisia austriaca* Jack, *A. Lerchiana* Weber *ex. Stechm* and *A. santonica* L. They further observed that essential oil in *A. annua* L. contained 60.20% artemisia ketone, *A. dracunculus* L. contained 42.34% sabinene and 24.92% methyl eugenol, *A. vulgaris* L. contained 41.46 germacrene D and 11.19% caryophyllene, *A. dzevanovskyi* *leonova in Wulf.* 57.13% eucaliptol and 20.37% borneol, *A. abrotanum* L. contained 12.27 % eucaliptol and 9.23 % borneol whereas *A. absinthium* L. contained 41.65 % pinene and 12.77 % myrtenil acetate.

Orav *et al.* (2006) studied the variation in essential oil composition of *Artemisia absinthium* obtained from different geographic regions of Europe by using capillary gas chromatographic and mass spectrometric analysis methods. Four chemotypes were found to be characteristic of *Artemisia absinthium* growing in Europe, colon sebnen and myrcene rich oil, alpha and beta thujone rich oil, epoxyocimene rich oil, and (*E*)- sabinyl acetate rich oil.

Three species of the genus, *A. scoparia*, *Artemisia sieberi* Bess and *Artemisia aucheri* Boiss, widely distributed in the desert area of Iran, were screened for chemical composition (Farzaneh *et al.* 2006). The essential oils were obtained by hydro-distillation of air-dried samples and their chemical composition identified by GC/MS. Oxygenated monoterpenes were the major components of the oils of the three species: α -thujone (81.7%), β -thujone (14.5%) (Figure 2) and 1,8-cineole (1.9%) were the major compounds in the essential oil of *A. scoparia*; the essential oil of *A. aucheri* was rich in linalool (44.1%), geranyl acetate (10.7%), (*E*)-citral (9.7%) and (*Z*)-citral (7.7%); and the essential oil of *A. sieberi* was rich in β -thujone (19.8%), α -thujone (10.5%), camphor (19.5%), verbenol (9.7%), *p*-mentha-1,5-dien-8-ol (6.4%) and davanone (5.8%).

Essential oil of *A. argyi* obtained by supercritical carbon dioxide extraction and hydrodistillation was analyzed by GC-MS to characterize its components (Wenqiang *et al.*, 2006). A total of 61 compounds were identified, of which the most abundant were 1,8-cineole (4.4%), borneol (3.5%), terpinol (10.1%), spathulenol (10%) caryophyllene oxide (6.5%), juniper camphor (8.7%), chamazulene (2%) and camphor (3.4%).

Kordali *et al.* (2005) investigated the chemical composition of the essential oil from four Turkish *Artemisia* species, *A. dracuncululus*, *A. absinthium*, *Artemisia santonicum* L. and *A. spicigera*. The main components of these essential oils were camphor (1.4–34.9%), 1,8-cineole (1.5–9.5%), chamazulene (17.8%), nuciferol propionate (5.1%), nuciferol butanoate (8.2%), caryophyllene oxide (1.7–4.3%), borneol (0.6–5.1%), α -terpineol (1.6–4.1%), spathulenol (1.3–3.7%), cubenol (0.1–4.2%), β -eudesmol (0.6–7.2%) and terpinen-4-ol (0.1–4.2%) respectively.

Cha *et al.* (2005) used GC/MS to analyze the chemical composition of the essential oil obtained from *Artemisia scoparia* in South Korea. The essential oil was rich in camphor (11%), 1,8-cineole (21.5%) and β -caryophyllene (6.8%).

Judpentiene and Mockute (2004) studied chemical composition of essential oils from aerial parts of *Artemisia absinthium* L. collected from six habitats of Vilnius. *Artemisia absinthium* L. plants were dried at room temperature (20-25°C). The essential oils were prepared by hydro-distillation for 2 hours using a mixture of hexane and ether as a collecting organic solvent. Also, GC-MS analyses were performed using a chromatograph interfaced with an HP 5971 mass. The content of thujones varied from traces to 36.3% of the oil.

The chemical composition of the essential oil from *Artemisia iwayomogi* from South Korea was analyzed by means of GC and GC-MS, and 85 constituents were identified, representing 96.2% of the total oil (Yu *et al.*, 2003).

Chapter-3

MATERIAL AND METHODS

The research problem entitled, “Standardization of cultivation, harvesting and processing techniques of *Artemisia absinthium*” was carried out in the year 2016. The details of the experimental site, material and research methodology are as under:

3.1 Experimental site

3.1.1 Location

The present investigation was carried out at Centre for Medicinal plants, Faculty of Forestry **Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Benhama-Watlar, Ganderbal Kashmir**, which is located within the university campus at an altitude of 1783.08 m above mean sea level, between 34⁰16'N latitude and 74⁰46'E longitude.

3.1.2 Climate

The experimental site falls in a mid to high altitude characterized by mild summers and severe winters. The average precipitation is 690 mm and most of which is received from december to april in the form of snow and rains. The mean metrological data for the experimentation period (2016-17) obtained from the metrological observatory Ganderbal. The total rainfall received during the experimentation period was 281.20 mm (November-December) and 418.80 mm (January-October) during 2016 and 2017, respectively. The minimum temperature ranged from -1⁰ to -6⁰ C and 3.4⁰ to 14.1⁰ C and maximum temperature varied from 17.7⁰ to 24.4⁰ C and 21.7⁰ to 32.5⁰ C and average maximum relative humidity ranged from 74.5 to 95.1 percent and 70.4 to 90.8 percent during plantation season. The

mean minimum relative humidity ranged from 43.1 to 78.7 percent and 37.1 to 80.1 percent during plantation season.

TECHNICAL PROGRAMME

EXPIREMENT NO. 1

(A) Raising through stem cuttings collected from three sources viz:

1. Ganderbal
2. Budgam
3. Bandipora

Cuttings of *Artemisia absinthium* species were collected in the 1st week of july from actively growing plants from three sites as above and were treated with growth hormones as below.

Different IBA treatments (5) were given to cuttings of *Artemisia absinthium* in five replications as follows:

- A. Control (water)
- B. IBA @ 250ppm
- C. IBA @ 500ppm
- D. IBA @ 750ppm
- E. IBA @1000ppm

The experiment was laid under controlled atmosphere in polyhouse in polybags, with fine sand as potting media.

Experimental design: Completely Randomized Design (CRD)

(B) Raising through seeds collected from three sources: Seeds were collected from three sources in the valley as above and were sown in different potting media in the month of march

Potting media:

1. Fine sand
2. Soil
3. Soil, Sand and FYM (1:1:1)

No. of replications: 06

Experimental design: (CRD)

EXPERIMENT NO. 2: To study out planting performance of *Artemisia absinthium*

The seedlings and the rooted cuttings were out planted in the field with following spacing:

A. **SPACING**

1. 40 cm x 60 cm
2. 60 cm x 80 cm
3. 80 cm x 100 cm
4. 100 cm x 120 cm

B. **PLANTING MATERIAL**

Seedling

Rooted Cutting

C. **SOURCES**

I. Ganderbal

II. Budgam

III. Bandipora

Experimental design = Randomized Block Design (RBD)

No. of treatments = 24

No. of replications = 3

No. of plants per replications = 10

3.2 Observations recorded

Observations were recorded on 12 randomly selected plants per treatment (5 plants per replication) in the last week of October, 2016. The observations were recorded for the following parameters in both the experiments.

3.2.1 Rooting Percentage (%)

The rooting percentage was calculated using the formula

$$\text{Rooting percentage} = \frac{\text{Number of Cuttings Rooted}}{\text{Total Number of Cuttings Planted}} \times 100$$

3.2.2 Number of Roots per Cuttings

The number of roots per cutting was recorded in centimeters by counting all the lateral roots that emerged from the cutting.

3.2.3 Length of Roots (cm)

Length of roots was recorded in centimetres using measuring scale.

3.2.4 Germination Percentage (%)

The germination percentage was calculated using following formula

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total Number of seeds sown}} \times 100$$

3.2.5 Survival Percentage (%)

The survival percentage was calculated using following formula

$$\text{Survival Percentage} = \frac{\text{No. of seeds that develop into seedlings after one growing season}}{\text{Total No. of seeds germinated initially}} \times 100$$

3.2.6 Height of seedling (cm)

The height of seedlings was measured using measuring tape from bottom to tip of the seedling at the end of growing season.

3.2.7 Root/Shoot Ratio

Root/Shoot Ratio was calculated using simple formula

$$\text{Root/Shoot Ratio} = \frac{\text{Length of root in cm}}{\text{Length of shoot in cm}}$$

3.2.8 Above ground biomass (kg/ha)

After cutting, the above portion of plants was removed and fresh weight in grams using electronic balance was calculated and reported as mean.

3.2.9 Leaf Fresh weight (g)

After cutting, the leaves were washed first with water to remove adhering dust. The leaves were weighed in grams using electronic balance and reported as mean.

3.2.10 Leaf dry weight (g)

The leaves were subjected to drying in mist chamber for 4 to 5 days for complete drying after that the leaves were weighed in grams using electronic balance and reported as mean.

3.3 Estimation of oil content by hydrodistillation

A. Leaves

Leaves were collected from the experimental plots when the plants were in full bloom stage. These leaves were dried in shade and subjected to distillation in a Clevenger apparatus.

B. Stem + Branches

This includes all above ground part biomass in full bloom stage. After drying the samples were subjected to hydrodistillation in a Clevenger apparatus.

C. Seeds

Along with other floral parts, seeds were collected from experimental plots. After drying the samples under shade the samples were subjected to hydrodistillation.

D. Roots

Roots were excised when the plants were in shade full bloom stage. After drying the samples under shade the samples were subjected to hydrodistillation in Clevenger apparatus.

Thus essential oil was obtained from all the parts (leaves, stem+branches, seeds and roots).

3.4 Statistical analysis

In both the experiments, the statistical analysis for each character was carried out on mean values. The data was subjected to analysis of randomized block design and control randomized design. The analysis of data was performed through O. P. Stat statistical software.

Chapter-4

EXPERIMENTAL FINDINGS

4.1 Raising of *Artemisia absinthium* through seeds and cuttings

4.1.1 Rooting percentage of *Artemisia absinthium* under different IBA Treatments

Cutting of *Artemisia absinthium* were collected from actively growing plants from three different sites of valley and subjected to IBA treatments Interpretation of data from Table 2 reveals that rooting percentage was significantly influenced when *Artemisia absinthium* cuttings were treated with different IBA concentrations. Analysis of data revealed that maximum percentage of rooting (84.09) was recorded when *Artemisia absinthium* cuttings were treated with IBA @ 750ppm. Control with no IBA treatment recorded the lowest rooting percentage of 37.46.

Critical analysis of data also indicates significant effect of source on rooting percentage with maximum values recorded for Ganderbal source (68.40) and minimum for Bandipora (60.88). Furthermore, no significant interaction effect of IBA concentrations and source was found on rooting percentage of *Artemisia absinthium* cuttings.

4.1.2 Number of roots per cutting of *Artemisia absinthium* under different IBA treatments

After three months of planting the cuttings were uprooted and secondary roots developed were counted. Roots up to 1 mm were counted. Data presented in Table 3 indicated that number of roots per Cutting was significantly influenced by different IBA concentrations. Perusal of data reveals that maximum number of roots per cutting (5.49) was recorded in cuttings treated with IBA@750ppm and minimum was recorded in control (2.21).

As is evident from Table 3, statistically significant effect of different sources on number of roots per cutting was recorded with maximum (4.72) and minimum (3.69) values observed in Ganderbal and Bandipora source respectively. Moreover, no significant interaction effect of IBA concentrations and source was found on number of roots per cutting.

Table-2: Rooting percentage of *Artemisia absinthium* under different IBA treatments

Treatments	Source			Mean
	Ganderbal	Budgam	Bandipora	
Control	41.10	37.71	33.58	37.46
IBA@250ppm	66.78	63.39	59.26	63.14
IBA@500ppm	75.97	72.58	68.45	72.33
IBA@750ppm	87.72	84.33	80.20	84.09
IBA@100ppm	70.44	67.05	62.92	66.80
Mean	68.40	65.01	60.88	

Factors	CD _{0.05}	IBA	Source x IBA
Source	2.53	3.26	N/S
S.E _m	0.89	1.15	

Table-3: Number of roots per cutting *Artemisia absinthium* under different IBA treatments

Treatments	Source			Mean
	Ganderbal	Budgam	Bandipora	
Control	2.47	2.22	1.93	2.21
IBA@250ppm	4.58	4.13	3.59	4.10
IBA@500ppm	5.34	4.80	4.18	4.77
IBA@750ppm	6.14	5.53	4.81	5.49
IBA@100ppm	5.05	4.55	3.96	4.52
Mean	4.72	4.25	3.69	

Factors	Source	IBA	Source x IBA
CD_{0.05}	0.30	0.39	N/S
	0.10	0.14	

4.1.3 Length of roots of *Artemisia absinthium* under different IBA treatments

Exposition of data from Table 4 reveals that different IBA treatments had a significant influence on length of roots of *Artemisia absinthium* cutting. Observations from Table 3 reveals that maximum length of roots (13.33 cm) was recorded when *Artemisia absinthium* cuttings were treated with IBA@750ppm followed by IBA treatment @ 500ppm. Control recorded the minimum length of roots (4.16 cm).

Perusal of data also indicated that experimental material collected from different sources had significantly different length of roots. Maximum length of roots (9.90 cm) was recorded in cuttings of Ganderbal source and minimum (7.65 cm) in Bandipora source. Non significant interaction effect of different IBA concentrations and source was recorded on length of roots.

4.1.4 Germination percentage of seedlings under different potting media

Seeds collected at three places in the valley were tested for germination studies. It is evident from data presented in Table 5 that different potting media had a significant effect on germination percentage of seedlings of *Artemisia absinthium*. Maximum percentage of germination (46.55%) was recorded in polybags containing potting media of soil+sand+FYM which was statistically different from germination percentage of 43.58% observed in polybags filled with fine sand only whereas the minimum (39.98%) was recorded in polybags consisting of only soil as potting media.

Among the different sources, Ganderbal Source recorded maximum germination percentage of 48.66% followed by Budgam source which recorded a value of 43.80. The lowest value for germination percentage (7.58%) was observed in the Bandipora Source.

Perusal of data also revealed a non-significant interaction effect of potting media and source on germination percentage of seedlings of *Artemisia absinthium*.

Table-4: Length of roots *Artemisia absinthium* under different IBA treatments

Treatments	Source			Mean
	Ganderbal	Budgam	Bandipora	
Control	4.65	4.19	3.64	4.16
IBA@250ppm	8.67	7.80	6.79	7.75
IBA@500ppm	11.98	11.18	9.38	10.72
IBA@750ppm	14.90	13.41	11.27	13.33
IBA@100ppm	9.29	8.36	7.28	8.31
Mean	9.90	8.91	7.75	

Factors	Source	IBA	Source x IBA
CD_{0.05}	0.41	0.53	N/S
S.E_m	0.14	0.19	

Table-5: Germination percentage of seedlings under different potting media

Potting Media	Source			Mean
	Ganderbal	Budgam	Bandipora	
Fine Sand	48.90	44.01	37.85	43.58
Soil	44.86	40.37	34.72	39.98
Soil+Sand+FYM	52.22	47.00	40.42	46.55
Mean	48.66	43.80	37.66	

Factors	CD_{0.05}
Source	1.80
Potting Media	1.80
Source x Potting Media	N/S

4.1.5 Survival percentage of seedlings under different potting media

Data presented in Table 6 indicates a significant influence of different potting media on survival percentage of seedlings of *Artemisia absinthium*. Highest survival percentage (68.34%) was recorded in seedlings from polybags filled with Soil+Sand+FYM as potting media whereas the lowest survival percentage of 49.11% was observed in polybags consisting of only soil.

Source of the experimental material also had a significant influence on survival percentage of seedlings of *Artemisia absinthium*. Maximum Survival percentage of 67.16% was observed in seedlings of Ganderbal Source whereas, the lowest values of 52.00% was recorded in Bandipora Source. A non significant interaction effect of potting media and source on survival percentage of seedlings of *Artemisia absinthium* was also observed.

4.1.6 Number of plants that survived after germination under different potting media

Perusal of data from Table 7 reveals a significant influence of different potting media on number of plants that survived out of germinated seeds. Highest number of plants (36.35) survived in polybags filled with potting media of Soil+Sand+FYM whereas the minimum (22.46) was recorded in polybags consisting of only soil as potting media.

Source also had a significant influence on number of plants that survived out of germinated seeds. Among the different sources, maximum number of plants that survived (32.99) was recorded in Ganderbal source whereas Bandipora source registered a minimum plant survival of 26.42. Potting media and source had a non significant interaction effect on number of plants that survived out of germinated seeds.

Table-6: Survival percentage of seedlings under different potting media

Potting Media	Source			Mean
	Ganderbal	Budgam	Bandipora	
Fine Sand	69.22	61.60	53.59	61.47
Soil	55.30	49.22	42.82	49.11
Soil+Sand+FYM	76.95	68.49	59.58	68.34
Mean	67.16	59.77	52.00	

Factors	CD_{0.05}
Source	1.20
Potting Media	1.20
Source x Potting Media	N/S

Table-7: Number of plants that survived out of germinated seeds

Potting Media	Source			Mean
	Ganderbal	Budgam	Bandipora	
Fine Sand	33.90	30.85	27.15	30.63
Soil	24.86	22.62	19.91	22.46
Soil+Sand+FYM	40.22	36.60	32.21	36.35
Mean	32.99	30.02	26.42	

Factors	CD_{0.05}
Source	1.82
Potting Media	1.82
Source x Potting Media	N/S

4.1.7 Shoot length of germinated seedlings under different potting media

Data on shoot length from Table 8 reveals that maximum shoot length of 2.93 cm was observed in seedlings raised in polybags with sand+ soil +FYM as potting media whereas the seedling of *Artemisia absinthium* recorded smallest shoot of 2.05 cm in polybags containing soil as potting media.

Among the different sources, seedlings from Ganderbal source recorded maximum shoot length of 2.59 cm followed by Budgam source (2.44 cm). Minimum length of shoots (2.18 cm) was observed in seedlings from Bandipora source. Two way interactions between source and potting media had no significant effect on shoot length of *Artemisia* seedlings.

4.1.8 Root length of germinated seedlings under different potting media

Elucidation of data on root length from Table 9 indicates a significant influence of potting media on root length of *Artemisia absinthium* seedlings. Maximum root length of 9.93 cm was observed in seedlings raised in polybags with Sand+Soil+FYM as potting media whereas the seedling of *Artemisia absinthium* recorded lowest values for root length (5.50 cm) in polybags filled only with soil as potting media.

Perusal of data also reveals a significant effect of source of experimental material on root length. Among the different sources, maximum root length of 8.25 cm was observed in seedlings from Ganderbal source whereas minimum length of roots (6.32 cm) was recorded in seedlings from Bandipora source. A non significant interaction of source and potting media was recorded on root length of *Artemisia* seedlings; however, the highest value of 11.26 cm was recorded in the seedlings of Ganderbal source when raised in polybags containing soil, sand and FYM in equal proportions as potting media.

Table-8: Shoot length of seedlings under different potting media

Potting Media	Source			Mean
	Ganderbal	Budgam	Bandipora	
Fine Sand	2.34	2.23	2.12	2.23
Soil	2.21	2.11	1.81	2.05
Soil+Sand+FYM	3.22	2.97	2.61	2.93
Mean	2.59	2.44	2.18	

Factors	CD_{0.05}
Source	0.22
Potting Media	0.22
Source x Potting Media	N/S

Table-9: Root Length of seedlings under different potting media

Potting Media	Source			Mean
	Ganderbal	Budgam	Bandipora	
Fine Sand	7.25	6.95	5.98	6.73
Soil	6.24	5.76	4.50	5.50
Soil+Sand+FYM	11.26	10.08	8.47	9.93
Mean	8.25	7.59	6.32	

Factors	CD_{0.05}
Source	0.42
Potting Media	0.42
Source x Potting Media	N/S

4.1.9 Root/Shoot ratio of germinated seedlings under different potting media

Exposition of data presented in Table 10 reveals that different potting media had a significant effect on root/shoot ratio of *Artemisia absinthium* seedlings. Maximum values for root/shoot ratio (3.39) were recorded in polybags containing potting media consisting soil+sand+FYM (1:1:1) in equal proportions which was statistically different from root/shoot ratio of 2.75 observed in polybags filled exclusively with soil only.

Data also suggested that source had a non-significant influence on root/shoot ratio of *Artemisia absinthium* seedlings raised in polybags. Perusal of data also reveals a non-significant interaction effect of potting media and source on root/shoot ratio of seedlings of *Artemisia*.

4.1.10 Biomass (g/plant) of germinated seedlings under different potting media

Examination of data on biomass from Table 11 indicates a significant influence of potting media on biomass (g/plant) of *Artemisia absinthium* seedlings. Maximum biomass of 45.32 g/plant was observed in seedlings raised in polybags with sand+soil+FYM (1:1:1) as potting media whereas the seedlings of *Artemisia absinthium* recorded lowest values for biomass (32.27 g/plant) in polybags consisting of only soil as potting media.

Perusal of data also reveals a significant effect of source of experimental material on biomass. Among the different sources, maximum biomass of 43.91 g/plant was observed in seedlings from Ganderbal source whereas minimum biomass (33.61 g/plant) was recorded in seedlings from Bandipora source. Data also reveals that two way interactions between source and potting media had no significant effect on biomass of *Artemisia absinthium* seedlings.

Table-10: Root/Shoot Ratio of seedlings under different potting media

Potting Media	Source			Mean
	Ganderbal	Budgam	Bandipora	
Fine Sand	3.23	3.18	3.01	3.14
Soil	2.85	2.76	2.64	2.75
Soil+Sand+FYM	3.50	3.40	3.27	3.39
Mean	3.19	3.11	2.97	

Factors	CD_{0.05}
Source	N/S
Potting Media	0.40
Source x Potting Media	N/S

Table-11: Biomass (g/plant) of seedlings under different potting media

Potting Media	Source			Mean
	Ganderbal	Budgam	Bandipora	
Fine Sand	44.07	39.22	33.73	39.01
Soil	36.45	32.44	27.90	32.27
Soil+Sand+FYM	51.20	45.57	39.19	45.32
Mean	43.91	39.08	33.61	

Factors	CD_{0.05}
Source	1.54
Potting Media	1.54
Source x Potting Media	N/S

4.2 Out-planting performance in field plantation

4.2.1 Leaf fresh weight (tonnes/ha) under different spacing's after out planting

The seedlings as well as the rooted cuttings were out planted in the field under different spacing. The interpretation of data from Table 12 indicates that spacing had a significant effect on the leaf fresh weight of *Artemisia absinthium* seedlings. It was found that maximum leaf fresh weight of 10.16 tonnes/ha was observed when *Artemisia absinthium* seedlings were planted at a spacing of 40 cm x 60 cm whereas the minimum values for leaf fresh weight (8.73 tonnes/ha) were observed for 100 cm x 120 cm spacing.

Analysis of data showed a significant effect of source of experimental material on leaf fresh weight. Maximum fresh weight of 9.56 tonnes/ha was observed for seedlings procured from Ganderbal. The lowest leaf fresh weight (9.20 tonnes/ha) was observed for Bandipora source.

Planting material also had a significant influence on leaf fresh weight. The highest value for leaf fresh weight (9.78 tonnes/ha) was observed when *Artemisia absinthium* was raised from seedlings as compared to raising *Artemisia absinthium* through cuttings (8.98 tonnes/ha).

Interaction of spacing, source and planting material had a non-significant effect on leaf fresh weight with maximum leaf fresh weight of 10.75 tonnes/ha recorded at a spacing of 45 cm x 60 cm for Ganderbal source when raised from seedlings.

Table-12: Leaf Fresh Weight (tonnes/ha) under different spacing's after out planting

Source	Ganderbal			Budgam			Bandipora			Overall Mean	Factor Mean
	Seedling	Cutting	Sub Mean	Seedling	Cutting	Sub Mean	Seedling	Cutting	Sub Mean		
Planting Material											
Spacing											
40cm x 60cm	9.25	8.39	8.82	8.73	7.79	8.26	8.29	7.33	7.81	8.30	Seedling=6.16
60cm x 80 cm	7.54	6.84	7.19	6.35	5.54	5.95	6.04	5.26	5.65	6.26	Cutting=5.35
80cm x 100 cm	5.32	4.61	4.97	4.98	4.17	4.57	4.75	3.97	4.36	4.63	
100cm x 120 cm	4.43	3.73	4.08	4.26	3.45	3.85	3.95	3.17	3.56	3.83	
Sub Mean	6.64	5.89	6.26	6.08	5.24	5.66	5.76	4.93	5.35		

Factors	C.D.		C.D.
Source	0.47	Source x Planting material	N/S
Planting	0.32	Source x Spacing	N/S
Spacing	0.54	Planting material x Spacing	N/S
		Source x Planting smaterial x Spacing	N/S

4.2.2 Leaf dry weight (tonnes/ha) under different spacing's after out planting

Exposition of data from Table 13 indicates a significant effect of spacing on leaf dry weight of *Artemisia absinthium* seedlings. Maximum leaf dry weight of 2.67 tonnes/ha was observed when *Artemisia absinthium* seedlings were planted at a spacing of 40 cm x 60 cm whereas the minimum values for leaf dry weight (2.30 tonnes/ha) were observed at 100 cm x 120 cm spacing.

Perusal of data also reveals a significant effect of source of experimental material on leaf dry weight. Maximum dry weight of 2.52 tonnes/ha was observed for seedlings from Ganderbal source. The lowest leaf fresh weight (2.42 tonnes/ha) was observed for Bandipora source.

A significant influence of planting material on leaf dry weight was also observed. Leaf dry weight of 2.57 tonnes/ha was observed when *Artemisia absinthium* plants were raised from seedlings as compared to raising *Artemisia absinthium* plants from cuttings (2.36 tonnes/ha).

Interaction of spacing, source and planting material had a non significant effect on leaf dry weight of *Artemisia absinthium* plants with maximum leaf dry weight of 2.83 tonnes/ha recorded at a spacing of 45 cm x 60 cm for Ganderbal source when raised from seedlings.

Table-13: Leaf Dry Weight (tonnes/ha) under different spacing's after out planting

Source Planting Material Spacing	Ganderbal			Budgam			Bandipora			Overall Mean	Factor Mean
	Seedling	Cutting	Sub Mean	Seedling	Cutting	Sub Mean	Seedling	Cutting	Sub Mean		
40cm x 60cm	3.15	2.90	3.02	2.97	2.65	2.81	2.82	2.49	2.65	2.83	Seedling=2.11 Cutting=1.84S
60cm x 80 cm	2.56	2.34	2.45	2.22	1.94	2.08	2.05	1.79	1.92	2.15	
80cm x 100 cm	1.81	1.62	1.72	1.69	1.42	1.56	1.62	1.35	1.48	1.59	
100cm x 120 cm	1.55	1.27	1.41	1.45	1.17	1.31	1.39	1.11	1.25	1.32	
Sub Mean	2.27	2.03	2.15	2.08	1.79	1.94	1.97	1.69	1.83		

Factors	C.D.		C.D.
Source	0.16	Source x Planting material	N/S
Planting Material	0.13	Source x Spacing	N/S
Spacing	0.19	Planting material x Spacing	N/S
Source x Planting material x Spacing		N/S	

4.2.3 Above ground biomass (tonnes/ha) under different spacing's after out planting

A statistically significant effect of spacing on above ground biomass was recorded. Data from Table 14 reveals that maximum above ground biomass of 24.06 tonnes/ha was observed for *Artemisia absinthium* when planted at a spacing of 40 cm x 60 cm whereas the minimum values (11.11 tonnes/ha) was registered at 100 cm x 120 cm spacing.

Source of experimental material also induced a significant effect on above ground biomass. Maximum above ground biomass (18.16 tonnes/ha) was observed for seedlings from Ganderbal source whereas the lowest (15.50 tonnes/ha) was observed for Bandipora source.

A significant influence of planting material on above ground biomass was also observed. Maximum of 17.86 tonnes/ha above ground biomass was observed when *Artemisia absinthium* plants were raised from seedlings and minimum of 15.52 tonnes/ha was recorded for plants raised from cuttings.

A non-significant interaction of spacing, source and planting material on above ground biomass was also observed with maximum above ground biomass of 26.83 tonnes/ha recorded at a spacing of 40 cm x 60 cm for Ganderbal source when raised from seedlings.

Table-14: Above ground biomass (tonnes/ha) under different spacing's after out planting

Source Planting Material Spacing	Ganderbal		Budgam		Bandipora		Overall Mean	Factor Mean			
	Seedling	Cutting	Sub Mean	Seedling	Cutting	Sub Mean			Seedling	Cutting	Sub Mean
40cm x 60cm	26.83	24.34	25.58	25.31	22.59	23.95	24.03	21.25	22.64	24.06	Seedling=17.86 Cutting=15.52
60cm x 80 cm	21.87	19.83	20.85	18.43	16.06	17.24	17.52	15.25	16.39	18.16	
80cm x 100 cm	15.44	13.36	14.40	14.43	12.09	13.26	13.79	11.53	12.66	13.44	
100cm x 120 cm	12.84	10.81	11.82	12.35	10.01	11.18	11.46	9.19	10.33	11.11	
Sub Mean	19.24	17.08	18.16	17.63	15.19	16.41	16.70	14.31	15.50		

Factors	C.D.		C.D.	
Source	1.36	Source x Planting material		N/S
Planting Material	1.10	Source x Spacing		N/S
Spacing	1.57	Planting material x Spacing		N/S
		Source x Spacing x Planting		Material
				N/S

4.3 Oil content estimation of *Artemisia absinthium*

4.3.1 Leaf oil yield (kg/ha) under different spacing's after out planting

Leaves were collected from plants when they were in full bloom stage. At this stage the plant has the maximum number of leaves which have attained maximum dimensions. The collected leaves were dried under shade and then subjected to hydrodistillation in a Clevenger apparatus for estimation of essential oil.

Maximum leaf oil yield of 19.94 kg/ha was observed at a spacing of 40 cm x 60 cm and minimum leaf oil yield (11.59 kg/ha) was observed at 100 cm x 120 cm spacing, suggesting a significant effect of spacing on leaf oil yield (Table 15).

Perusal of data also reveals a significant effect of source of experimental material on leaf oil yield. Maximum leaf oil yield (16.44 kg/ha) was observed for seedlings from Ganderbal source, whereas the minimum was recorded in Bandipora source (13.86 kg/ha).

Planting material also significantly affected the oil yield with the maximum leaf oil yield (16.33 kg/ha) observed for seedling raised plants as compared to plants raised from cuttings (13.77 kg/ha).

Interaction of spacing, source and planting material had a non-significant effect on leaf oil yield with maximum leaf oil yield of 2.20 kg/ha recorded at a spacing of 40cm x 60 cm for Ganderbal source when raised from seedlings,

Table-15: Leaf oil yield (kg/ha) under different spacing's after out planting

Source	Ganderbal			Budgam			Bandipora			Overall Mean	Factor Mean
	Seedling	Sub Mean	Seedling	Cutting	Sub Mean	Seedling	Cutting	Sub Mean			
Planting Material											
Spacing											
40cm x 60cm	24.20	20.91	22.56	20.86	17.80	19.33	19.33	16.55	17.94	19.94	Seedling=16.33 Cutting=13.77
60cm x 80 cm	19.47	16.79	18.13	15.86	12.78	14.32	14.75	13.43	14.09	15.51	
80cm x 100 cm	14.06	12.85	13.45	15.23	11.97	13.60	13.28	11.49	12.39	13.15	
100cm x 120 cm	12.35	10.90	11.63	13.84	10.23	12.04	12.75	9.48	11.12	11.59	
Sub Mean	17.52	15.36	16.44	16.45	13.20	14.82	15.03	12.74	13.88		

Factors	C.D.			C.D.			
Source	1.29	Source x Planting material			N/S		
Planting Material	1.05	Source x Spacing			N/S		
Spacing	1.49	Planting material x Spacing			N/S		
Source x Planting material x Spacing	N/S						

4.3.2 To find out oil yield/kg of leaves, separate experiment was conducted and the results are tabulated in Table 16

Critical analysis of data from Table 16 reveals a significant effect of spacing on leaf oil yield (ml/kg). Maximum leaf oil yield of 5.61 ml/kg was observed for a spacing of 40 cm x 60 cm whereas the minimum values for leaf oil yield of 3.68 ml/kg was observed at 100 cm x 120 cm spacing.

Perusal of data also revealed a significant effect of source of experimental material on leaf oil yield (ml/kg). Maximum leaf oil yield of 5.30 ml/kg was observed for seedlings from Ganderbal source in contrast to minimum of leaf oil yield of 4.08 ml/kg observed for Bandipora source.

A significant influence of planting material on leaf oil yield (ml/kg) was also observed. Maximum oil yield of 5.10 ml/kg was observed when *Artemisia absinthium* plants were raised from seedlings as compared to raising *Artemisia absinthium* plants from cuttings (4.24 ml/kg).

Interaction of spacing, source and planting material had a non significant effect on leaf oil yield of *Artemisia absinthium* plants with maximum leaf oil yield of 6.24 ml/kg recorded at a spacing of 40 cm x 60 cm for Ganderbal source when raised from seedling.

Table-16: Leaf oil yield (ml/kg) under different spacing's after out planting

Source Planting Material	Ganderbal		Budgam		Bandipora		Overall Mean	Factor Mean			
	Seedling	Cutting	Sub Mean	Seedling	Cutting	Sub Mean			Seedling	Cutting	Sub Mean
Spacing											
40cm x 60cm	6.47	5.86	6.17	5.67	5.22	5.45	5.45	4.98	5.21	5.61	Seedling=5.10 Cutting=4.24
60cm x 80 cm	6.05	5.47	5.76	5.34	4.69	5.02	5.21	4.00	4.61	5.13	
80cm x 100 cm	5.06	4.91	4.99	4.85	3.59	4.22	4.45	2.71	3.58	4.26	
100cm x 120 cm	4.35	4.21	4.28	4.80	2.85	3.83	3.54	2.33	2.93	3.68	
Sub Mean	5.48	5.11	5.30	5.17	4.09	4.63	4.66	3.50	4.08		
Factors	C.D.					C.D.					
Source	0.466	Source x Planting material				N/S					
Planting	0.381	Source x Spacing				N/S					
Spacing	0.538	Planting material x Spacing				N/S					
Source x Planting material x Spacing											
N/S											

4.3.3 Stem+branches oil yield (kg/ha) under different spacing's after out planting

Whole above ground biomass which consists of leaves, branches and main stem was harvested in full bloom stage. The biomass were dried under shade and subjected to hydrodistillation in Clevenger apparatus. Exposition of data from Table 17 indicated a significant effect of spacing on stem+branches oil yield. Maximum stem+branches oil yield 3.80kg/ha was observed when *Artemisia absinthium* seedlings were planted at a spacing of 40 cm x 60 cm whereas the minimum values for stem+branches oil yield (3.26 kg/ha) were observed at 100 cm x 120 cm spacing.

Perusal of data also reveals a significant effect of source of experimental material on stem+branches oil yield. Maximum stem+branches oil yield (3.58 kg/ha) was observed for seedlings from Ganderbal source. The stem+branches oil yield (3.43 kg/ha) was observed for Bandipora source.

A significant influence of planting material was also observed from stem+branches on oil yield. Highest oil yield of 3.65 kg/ha was observed when *Artemisia absinthium* plants were raised from seedlings as compared to raising *Artemisia absinthium* plants from cuttings (3.36 kg/ha).

All the interaction effects had non-significant influence on oil yield obtained from stem+branches. Maximum leaf oil yield of 4.03 kg/ha was recorded at a spacing of 40 cm x 60 cm for Ganderbal source when raised from seedlings.

Table-17: Stem+branches oil yield (kg/ha) under different spacing's after out planting

Source Planting material Spacing	Ganderbal			Budgam			Bandipora			Sub Mean	Overall Mean	Factor Mean
	Seedling	Cutting	Sub Mean	Seedling	Cutting	Sub Mean	Seedling	Cutting	Sub Mean			
40cm x 60cm	4.03	3.72	3.87	3.94	3.59	3.76	3.88	3.62	3.75	3.80	Seedling= 3.65	
60cm x 80 cm	3.75	3.48	3.62	3.76	3.45	3.60	3.66	3.27	3.47	3.56	Cutting= 3.36	
80cm x 100 cm	3.63	3.36	3.49	3.53	3.24	3.39	3.44	3.23	3.34	3.41		
100cm x 120 cm	3.47	3.22	3.35	3.41	3.11	3.26	3.32	3.03	3.17	3.26		
Sub Mean	3.72	3.44	3.58	3.66	3.35	3.50	3.58	3.29	3.43			

Factors	C.D.		C.D.
Source	0.11	Source x Planting material	N/S
Planting Material	1.10	Source x Spacing	N/S
Spacing	0.13	Planting material x Spacing	N/S
Source x Planting material x Spacing N/S			

4.3.4 To find out oil yield in the whole above ground biomass/kg separate experiment was conducted and the results are tabulated in Table 18

Exposition of data from Table 18 indicated a significant effect of spacing on oil yield obtained from stem+branches. Maximum oil yield from stem+branches (1.65 ml/kg) was observed when *Artemisia absinthium* seedlings were planted at a spacing of 40 cm x 60 cm whereas the minimum of 1.17 ml/kg were observed at 100 cm x 120 cm spacing.

A significant influence of source of experimental material on stem+branches oil yield (ml/kg) was also observed. Maximum oil yield from stem+branches (1.58 ml/kg) was observed for seedlings from Ganderbal source whereas minimum (1.27 ml/kg) was observed for Bandipora source.

Planting material has a statistically significant influence on oil yield from stem+branches (ml/kg). Highest oil yield of 1.53 ml/kg was observed when artemesia plants were raised from seedlings in contrast to raising *Artemisia absinthium* plants from cuttings (1.31 ml/kg).

Interaction of spacing, source and planting material had a non-significant effect on stem+branches oil yield with maximum oil yield of 1.87 ml/kg recorded from stem+branches at a spacing of 40cm x 60 cm for Ganderbal source when raised from seedlings.

Table-18: Stem+branches oil yield (ml/kg) under different spacing's after out planting

Source		Ganderbal			Budgam			Bandipora			Overall Factor Mean	
Planting Material	Spacing	Seedling	Cutting	Sub Mean	Seedling	Cutting	Sub Mean	Seedling	Cutting	Sub Mean		
		40cm x 60cm		1.87	1.72	1.79	1.67	1.56	1.61	1.61	1.49	1.55
60cm x 80 cm		1.76	1.62	1.69	1.58	1.42	1.50	1.55	1.25	1.40		
80cm x 100 cm		1.51	1.48	1.50	1.46	1.15	1.30	1.36	0.93	1.15		
100cm x 120 cm		1.34	1.30	1.32	1.45	0.96	1.21	1.13	0.83	0.98		
Sub Mean		1.62	1.53	1.58	1.54	1.27	1.41	1.42	1.13	1.27		
Factors	C.D.						C.D.					
Source	0.11	Source x Planting material					N/S					
Planting	0.09	Source x Spacing					N/S					
Spacing	0.14	Planting material x spacing					N/S					
Source x Planting materialx spacingN/S												

4.3.5 Seed oil yield (kg/ha) under different spacing's after out planting

Seeds along with the other floral parts were harvested they were dried under shade and subjected to hydrodistillation in Clevenger apparatus.

Data from Table 19 reveals a non significant effect of spacing, source and planting material on seed oil yield. Interaction of spacing, source and planting material also had a non significant effect on seed oil yield of *Artemisia absinthium* plants with maximum oil yield of 1.44 kg/ha recorded at a spacing of 40cm x 60 cm for Ganderbal source when raised from seedlings

Table-19: Seed oil yield (kg/ha) under different spacing's after out planting

Source Planting Material Spacing	Ganderbal			Budgam			Bandipora			Overall Mean	Factor Mean
	Seedling	Cutting	Sub Mean	Seedling	Cutting	Sub Mean	Seedling	Cutting	Sub Mean		
40cm x 60cm	1.44	1.31	1.38	1.33	1.19	1.26	1.15	1.07	1.11	1.25	Seedling=1.21 Cutting=1.10
60cm x 80 cm	1.34	1.21	1.27	1.27	1.15	1.21	1.09	0.99	1.04	1.17	
80cm x 100 cm	1.30	1.19	1.25	1.19	1.08	1.13	1.02	0.95	0.98	1.12	
100cm x 120 cm	1.24	1.13	1.19	1.15	1.02	1.09	0.99	0.88	0.94	1.07	
Sub Mean	1.33	1.21	1.27	1.23	1.11	1.17	1.06	0.97	1.02		

Factors	C.D.					C.D.					
SourceN/S	Source x Planting material			N/S							
PlantingN/S	Source x Spacing		N/S								
SpacingN/S	Planting material x Spacing			N/S							
Source	x		Planting			material		x			SpacingN/S

4.3.6 To find out oil yield/kg of seed separate experiment was conducted and the results are tabulated in Table 20

The effect of spacing, source and planting material on seed oil yield was found to statistically non-significant (Table 20). Interaction of spacing, source and planting material also had a non-significant effect on seed oil yield of *Artemisia absinthium* plants with maximum oil yield of 1.04 ml/kg recorded at a spacing of 40 cm x 60 cm for Ganderbal source when raised from seedlings

Table-20: Seed oil yield (ml/kg) under different spacing's after out planting

Source Planting Material	Ganderbal		Budgam			Bandipora			Overall Mean	Factor Mean	
	Seedling	Cutting	Sub Mean	Seedling	Cutting	Sub Mean	Seedling	Cutting			Sub Mean
Spacing											
40cm x 60cm	1.04	0.95	1.00	0.93	0.86	0.90	0.89	0.83	0.86	0.92	Seedling=0.85
60cm x 80 cm	0.98	0.90	0.94	0.88	0.79	0.83	0.86	0.70	0.78	0.85	Cutting=0.73
80cm x 100 cm	0.84	0.82	0.83	0.81	0.64	0.72	0.76	0.52	0.64	0.73	
100cm x 120 cm	0.75	0.73	0.74	0.81	0.54	0.67	0.63	0.46	0.54	0.65	
Sub Mean	0.90	0.85	0.88	0.86	0.71	0.78	0.79	0.63	0.71		

Factors	C.D.			C.D.							
Source N/S	Source x Planting material			N/S							
Planting N/S	Source x Spacing		N/S								
Spacing N/S	Planting material x Spacing			N/S							
Source	x	Planting		material			x	Spacing N/S			

4.3.7 Root oil yield (kg/ha) under different spacing's after out planting

Below ground biomass was harvested by pulling out the whole root from soil. Perusal of data from Table 21 reveals a non significant influence of spacing, source and planting material on root oil yield. A non-significant interaction effect of spacing, source and planting material was also observed. Maximum root oil yield of 0.81 kg/ha was recorded at a spacing of 40 cm x 60 cm for Ganderbal source when raised from seedling. The soil was washed away by water, dried then subjected to hydrodistillation in Clevenger apparatus.

Table-21: Root oil yield (kg/ha) under different spacing's after out planting

Source Planting Material Spacing	Ganderbal		Budgam		Bandipora		Overall Mean	Factor Mean		
	Seedling	Cutting	Sub Mean	Seedling	Cutting	Sub Mean			Seedling	Cutting
40cm x 60cm	0.81	0.75	0.78	0.76	0.67	0.72	0.66	0.60	0.63	0.71
60cm x 80 cm	0.75	0.68	0.72	0.71	0.65	0.68	0.62	0.55	0.59	
80cm x 100 cm	0.73	0.67	0.70	0.67	0.61	0.64	0.58	0.54	0.56	
100cm x 120 cm	0.70	0.64	0.67	0.65	0.58	0.62	0.56	0.49	0.53	
Sub Mean	0.75	0.69	0.72	0.70	0.63	0.66	0.60	0.55	0.57	

Seedling=0.68
Cutting=0.62

Factors	C.D.	C.D.
Source	N/S	Source x Planting material
Planting	N/S	Source x Spacing
Spacing	N/S	Planting material x Spacing
Source x Planting material x Spacing	N/S	N/S

4.3.8 To find out oil yield/kg of root separate experiment was conducted and the results are tabulated in Table 22

Analysis of variance reveals a non- significant influence of spacing, source and planting material on root oil yield (Table 22). A non significant interaction effect of spacing, source and planting material was also observed. Maximum root oil yield of 0.52 ml/kg was recorded at a spacing of 40 cm x 60 cm for Ganderbal source when raised from seedling.

Table-22: Root oil yield (ml/kg) under different spacing's after out planting

Source Planting Material Spacing	Ganderbal			Budgam			Bandipora			Overall Factor Mean	
	Seedling	Cutting	Sub Mean	Seedling	Cutting	Sub Mean	Seedling	Cutting	Sub Mean		
40cm x 60cm	0.52	0.48	0.50	0.46	0.43	0.45	0.45	0.42	0.44	0.46	Seedling=0.43 Cutting=0.39
60cm x 80 cm	0.49	0.45	0.47	0.44	0.40	0.42	0.43	0.39	0.41	0.43	
80cm x 100 cm	0.42	0.41	0.42	0.41	0.38	0.40	0.38	0.33	0.36	0.39	
100cm x 120 cm	0.37	0.36	0.37	0.40	0.37	0.39	0.32	0.29	0.31	0.36	
Sub Mean	0.45	0.42	0.44	0.43	0.40	0.41	0.40	0.36	0.38		

Factors	C.D.	C.D.
SourceN/S	Source x Planting material	N/S
PlantingN/S	Source x Spacing	N/S
SpacingN/S	Planting material x Spacing	N/S
Source	x	Planting material
		x
		SpacingN/S

Chapter-5

DISCUSSION

5.1 Raising of *Artemisia absinthium* through seeds and cuttings

5.1.1 Effect of different IBA treatments and potting media on growth and yield parameters of *Artemisia absinthium*

Adventitious root formation is a key step in plant propagation. From a scientific point of view, rooting is a highly interesting developmental pathway. Rooting among the vegetative methods of propagation is undoubtedly the most evolved and expanded method (Manica *et al.*, 2000; Awan *et al.*, 2012). Efficient rooting treatment yields a high percentage of rooted shoots and a high quality root system.

Of the phytohormones used for stimulating rooting process, auxins are plant growth regulators that are used to induce root formation from cuttings. Various auxins have been shown to improve overall rooting percentages, hasten root initiation, increase the number and quality of roots and promote the development of uniform roots (Blythe *et al.*, 2007) (Boyer *et al.*, 2013). Auxin is widely used on the stem cuttings for accelerating the formation of adventitious roots (Galavi *et al.*, 2013). Auxin has an effect on speed and increases the percentage of rooting of the stem cuttings (Kasim and Rayya, 2009).

The present study ascertains the role of IBA in initiating and accelerating the root development of *Artemisia absinthium*. Cutting of *Artemisia absinthium* were collected from vigorously growing plants from three different geographical sources. Cuttings were collected in the first week of July. Nine to ten inch terminal cutting

were obtained and treated with IBA as quick. The hormone (IBA) used in this study has been found to be reliable in stimulating the production, elongation and uniformity of adventitious roots from *Artemisia absinthium* cuttings. Data presented in Tables 2-4 reveal that rooting percentage, number of roots per cutting and length of roots was significantly influenced when *Artemisia absinthium* cuttings were treated with different IBA concentrations. Analysis of data reveals that with the increase in IBA concentrations, higher values for aforementioned parameters were recorded, the best results being observed for IBA concentration @ 750ppm. Reports of Ferreira and Janick (2002) also suggest that with increase in IBA concentration from 150 ppm to 750ppm, rooting percentage of *Artemisia absinthium* increases and decreases after further increase in concentration. Higher rooting percentage obtained at 750ppm may be due to enhanced cell division and callusing which have been associated with potential rooting (Hartmann *et al.*, 2002).

Al-Zebari and Al-Brifkany (2015) also reported an increase in rooting percentage and length of roots in citron with increase in IBA concentration from 0 to 1000ppm and a decrease with further increase upto 2000ppm. Baul *et al.* (2008) also observed a similar trend in the vegetative propagation of *Stereospermum suaveolens* with cuttings treated with 0.2% IBA producing the longest root. The use of different concentrations of IBA in Bougainvillea cuttings had a significant effect on root number, root length, root diameter, root weight compared to control (Niaz and Muhammad, 2002). The number of the induced roots per cutting of rosemary was significantly increased by time of cutting, soaking and concentration of the IBA (Elhaak *et al.*, 2015). The increase in root number and length by IBA improves the success and survival of the cuttings after transplanting in the soil.

With the increase in IBA concentrations, rooting performance showed a decreasing trend. High IBA concentrations may have been toxic to the cuttings, as

was the case for *Holarrhena pubescens* cuttings (Baul *et al.*, 2010). The possible reason may be the antagonistic effect of IBA with endogenous gibberellin and with other factors that has promoting effect on root formation. Overall growth of *Artemisia annua* decreased as the concentration of phytohormones increased (Weatherset *al.*, 2005). Similar results have been reported by Bais *et al.* (2001) who reported that the growth and yield of some other hairy root species was also inversely proportional to the concentrations of hormones used. High concentrations IAA is also known to stimulate the production of ethylene (Crozier *et al.*, 2000). Kumari *et al.* (2010) reported that auxins can control cell enlargement, bud formation and root initiation and also promote the production of other hormones. Ethylene being a growth inhibiting hormone inhibits the cell division and elongation thereby reducing the rooting performance. The inhibition of root growth and development might partly be due to the ethylene production which is triggered by high auxin concentration (Hopkins and Huner, 2004; Hartman *et al.* 2009). Increasing the concentration of IAA gradually led to a decrease in the frequency of root regeneration in *Artemisia vulgaris* L. (Sujatha and Kumari, 2007).

In this study control with no IBA treatment recorded the lowest rooting percentage, lesser number of roots per cutting and lowest length of roots suggesting that endogenous auxin concentration is not sufficient for inducing rooting in *Artemisia absinthium*. Similar findings have been reported by Bharathy *et al.* (2004) in case of carnation and by Kazankaya *et al.* (2005) in case of other cultivars of rose.

Potting medium is one of the important factors that can influence the growth and vigour of the cuttings to a greater extent. Seedlings raised in a good medium can ensure better establishment and growth when planted out in the main field. A cheap and successful medium will enable the farmers and nurserymen to produce good seedlings of medicinal plants for extensive and commercial cultivation. Growing

media also plays an important role in seed germination, seedling growth and vigour. The plant growing medium must be porous for root aeration and drainage and also capable of water and nutrient retention. Oxygen, of course, is required for all living cells. Composition and nutritional status of the medium is considered to be helpful for the production of good quality plants with higher growth and yield.

The growth and development of *Artemisia absinthium* was studied in 3 potting media viz., soil, fine sand, soil+sand+FYM(1:1:1). The data presented in Tables 5-11 reveal that different potting media had a significant effect on growth characteristics viz., germination percentage, survival percentage, shoot length, root length, root/shoot ratio, biomass of seedlings of *Artemisia absinthium*. Maximum values for all the growth parameters studied were recorded in polybags containing potting media of soil+sand+FYM (1:1:1) whereas the minimum was recorded in polybags consisting of only soil as potting media. Our results are in close conformity with those of Bharadwaj, 2013; Ramteke *et al.*, 2015; Bharadwaj, 2014; and Sardoei *et al.*, 2014 wherein all the reports suggest a significant increase in growth parameters when plants are raised in combination of potting media as compared to soil or sand alone. The reason for the best performance of soil+sand+FYM (1:1:1) is high organic matter content, which increases the water and nutrient holding capacity of the medium, which improve water utilization capacity of the plant. The higher available well decomposed organic matter may preserve soil humidity, increase nutrient content and improve soil structure which increase water absorption and maintain the cell turgidity, cell elongation and increase respiration at optimum level, leading to favourable root initiation in cuttings subsequently enhancing their survival. Organic matter may also improve nutrient availability and improve phosphorus absorption (Singh *et al.*, 2015). The results of the present study are also akin to those of Singh *et al.*, 2015 wherein they reported a significant increase in growth parameters of *Stevia* grown in mixed combination of potting media as compared to soil alone. A similar trend has also been

observed by Venkatesan *et al.*, (2010) in *Gymnema sylvestre* cuttings. The current findings are in accordance with the recordings of Abirami *et al.* (2010) who reported the influence of potting media and source of experimental material on growth parameters of *Myristica fragrans* Houtt. seedlings. Similar trends have also been observed by Mehmood *et al.*, (2013) in *Antirrhinum majus* and Riaz *et al.*, (2008) in *Zinnia elegans*.

One of the most important approaches to increase the yield of official parts of any medicinal species lies with developing suitable strain by selecting genetically superior lines among the existing populations. Cultivation of superior genotypes/high yielding varieties will help in reducing pressure upon the natural resources besides meeting the need of pharmaceutical industries. *Artemisia absinthium* is one such cosmopolitan species used as a raw material in traditional drug industry. Because of the variation occurring in climatic and soil conditions at different ecological niches in different regions of the Jammu and Kashmir, there is a great deal of variation in growth and yield parameters *vis-a-vis* accumulation of active constituents. In this context, *Artemisia absinthium* seeds and seedlings were collected from three different sources (Ganderbal, Budgam and Bandipora) to characterize and evaluate for growth and yield parameters.

The main aim of evaluation of different sources is to determine the inherent differences among individuals and manipulate them in desired direction to obtain good result. Exposition of Tables 2-11 reveals significant variation among the three different sources of *Artemisia absinthium* on the basis of mean performance of all the growth and yield related characters. The mean performance of traits helps to identify the superior genotype among the existing populations. The mean performance also serves as a primary criterion for selection of desirable plants (Kumar *et al.*, 1979).

Perusal of data indicates significant variation in rooting percentage, number of roots per cutting and length of roots among different sources with maximum values recorded for Ganderbal source and minimum for Bandipora. The reason for maximum performance in Ganderbal source is that the experimental site falls in same area having similar micro-climatic conditions. Similar results for variation in root length have been reported by Kairon *et al.*, (2016) in *Sapindus mukorossi* progenies collected from different sources. Variations in root biomass among different sources have also been reported by Batkhuu *et al.*, (2006) in *Larix sibirica*. Likewise the current results are in parallel with the research findings of Mohit *et al.*, (2004) who reported significant variation in seedling traits among different seed sources, while studying *Albizia procera*. A significant variation in root length, root biomass, number of lateral roots has also been reported by Ghildiyal *et al.*, (2009) in *Pinus roxburghii* provenances of varied origins.

Critical examination of data also suggests a significant variation in germination percentage, survival percentage, shoot length, root length and biomass among different sources. Maximum values for all the growth and yield parameters studied were observed in experimental material procured from Ganderbal as compared to minimum recorded in Bandipora source which might be attributed to the fact that the experimental site was located in Ganderbal having similar soil and climatic conditions. *Artemisia absinthium* can grow well in the highlands with an altitude of 1000-1600 m asl, well drained soil with a pH of 5.5-8.5 (optimum pH 6-8), rainfall 700-1000 mm/year (Ban *et al.*, 1999). Ganderbal (1619m asl) had more suitable environment for *Artemisia absinthium* cultivation. Since the source and the site where *Artemisia absinthium* was raised were having the similar growing conditions, *Artemisia absinthium* raised from seedlings/ seeds procured from Ganderbal source show a superior performance in growth as well as yield traits. Variation in *Artemisia absinthium* with respect to the seedling traits among different

sources could be due to the fact that this species grow over a wide range of rainfall, temperature, altitude and soil types. Similar results were also observed by Mohit *et al.* (2004) in *Albizia procera*. The seed source and provenance variation in nursery and field is essentially genetic in origin (Snieszko and Stewart, 1989). Marchese *et al.* (2002) suggested that the genotypes from different geographical origins can vary in behaviour under the same photoperiod and temperature conditions. Nurhayati and Gusmaini (2013) also reported variations in fresh and dry weight of four *Artemisia* accessions. Significant variation in germination parameters of *Vateria indica* among different sources have been reported by Jagadish *et al.*, (2014). Batkhuu *et al.*, (2006) also reported significant differences as regards to germination, biomass and seedling emergence while evaluating seedlings of Larch grown from diverse seed sources of Mongolia. Variation among seed provenances with respect to seed and seedling traits (germination, survival, shoot length, root length etc.) have earlier been reported in many species including *Acacia karroo* (Abdelkhair *et al.*, 2003), *Pinus roxburghii* (Ghildiyal *et al.*, 2009), *Dalbergia melanoxylon* (Amri *et al.*, 2008), and *Celtis australis* (Singh *et al.*, 2006). In most plant species, seeds vary in their degree of germination between and within populations and between and within individuals (Mkonda *et al.*, 2003; Loha *et al.*, 2006). Causes of such variability- might be generally attributed either to (a) genetic characters of source population/plant (Shu *et al.*, 2012) or (b) impact of mother plant environment (Singh *et al.*, 2010). Gutterman (2000) stated that germination of seeds can be influenced by maternal factors, such as position of the seed in the fruit/tree, the age of the mother plant during seed maturation, as well as environmental factors such as day length, temperature, light quality, water availability and altitude. The results indicated that, the differences in the germination parameters could be due to wide variation in microclimate and local environmental conditions in the range of distribution of this species. Due to particular set of local environmental conditions, the genetic constitution of the species for the

particular traits must have changed resulting in geographically distinct clines (Kumar *et al.* 2004). As a result, racial variation among the populations of diverse origin did show association with locality factors such as latitude, longitude, altitude, precipitation, etc. (Shekar *et al.* 2002). Source variation tests are necessary to screen the naturally available genetic variation to select the best planting material for higher productivity (Bhat and Chauhan, 2002). Elucidation of data also revealed a non significant interaction of source of experimental material, IBA and potting media suggesting that all the aforementioned treatments are self sufficient in delineating the good agricultural practices for obtaining optimum yield in *Artemisia absinthium*.

5.2. Out-planting performance of *Artemisia absinthium* in field plantation

Growth and yield of herbal plants are closely connected with meteorological conditions and agrotechnical procedures (Azizi and Kahrizi, 2008., Acimovic, 2013). Although growth, yield and essential oil of medicinal plants are under control of genetic factors; however, these features are affected by the environmental factors too. Zaffaroni and Schneiter (1991) noted that three production variables that a producer can manipulate to influence the production of a given crop are plant population, row arrangement and hybrid selection. There are many factors that influence agronomic characteristics, biomass and essential oil yield of aromatic and medicinal plants. Among these plant spacing deserves special attention (Khazaie *et al.*, 2007; Ramamneh, 2009).

In light of the above mentioned facts, the present study on out planting performance of *Artemisia absinthium* in field plantation was undertaken to optimize the spacing required for achieving maximum leaf yield and essential oil. Perusal of data from tables 12-14 reveal a significant influence of spacing on leaf fresh weight, leaf dry weight and above ground biomass of *Artemisia absinthium* plants. Maximum values for all the yield parameters were recorded at a spacing of 40 cm x 60 cm

whereas minimum were recorded for 100 cm x 120 cm spacing. Similar results have been reported by Choudhari *et al.* (2013) who recorded highest dry leaf yield of 2.46 tonnes/ha at a spacing of 45cm x 60cm for *Artemisia annua*. Abirami *et al.* (2014) also reported that maximum values for yield parameters in *Artemisia annua* were observed at 45cm x 45cm spacing however the results were statistically at par with 45cm x 60 cm spacing. Our results are also in consonance with those of Nigussie *et al.* (2015) who reported higher yield at 40 cm x 60 cm as compared to higher spacing of 60 cm x 80 cm, 80 cm x 100cm and 100 cm x 120 cm in *Artemisia annua*. in our study *Artemisia absinthium* plants raised in the field at a spacing of 40 cm x 60 cm recorded the maximum yield owing to the fact that the plant density at such spacing was more (41666 plants per hectare) as compared to higher spacing of 60 cm x 80 cm (20833 plants per hectare), 80 cm x 100 cm (12500 plants per hectare) and 100 cm x 120 cm (8333 plants per hectare). Under conditions of sufficient soil moisture and nutrients, higher population is necessary to utilize all the growth factors efficiently. The present finding is in agreement with the result of Zewdinesh *et al.*, (2011) who found higher dry leaf yield at the highest plant density in *Artemisia annua*. Higher dry leaf yield per ha at higher planting density compared to the lower density on *A. annua* was also reported by Diemer and Griffee (2005). Singh (2002) explained that establishment of optimum population per unit area of the field is essential to get maximum yield. Optimum planting density is required to enable plants to take the best advantages of light, air, water and nutrients, and to prevent them from competition. An increase in biological yield with increasing plant population density was also reported by Nekonam and Razmjoo, 2007 and Najafi and Moghadam, 2002 on *Plantago ovata*.

Perusal of data also reveals a significant influence of planting material on growth and yield of *Artemisia absinthium*. Maximum values were recorded for plants raised from seedlings in contrast to those raised from cuttings. This might be

attributed to the fact that *Artemisia absinthium* profusely propagates from seed which is its natural mode of propagation and ensures the maximum survival and healthy growth of plants.

5.3. Extraction of oil from *Artemisia absinthium*

Results from the present study indicated a significant effect of different spacings on essential oil yield, as the maximum essential oil yield was obtained from leaves, stem and branches, seed as well as roots was recorded with a maximum population density at a spacing of 40cm x 60 cm (24.20 kg/ha) and minimum essential oil yield was recorded with minimum population density at higher spacing's of 60 cm x 80 cm,(4.03 kg/ha) 80 cm x 100 cm (1.44 kg/ha) and 100 cm x 120 cm (0.81 kg/ha). In agreement with this study, Abirami *et al.*, (2014) reported a maximum oil yield at 45cm x 45 cm in contrast to higher spacings of *Artemisia annua*. The present finding is also in agreement with the result of (Zewdinesh *et al.*, 2011) who found higher essential oil yield at the highest plant density in *A. annua*. Likewise, in corn mint, highest biomass and maximum essential oil yield were produced due to the narrow spacing's (Rao, 2002). The increasing in essential oil yield at higher densities i.e., at lower spacing may be due to the contribution of higher above ground biomass, fresh leaf yield and dry leaf yield at higher densities. Mert *et al.* (2002) also reported that the ecotype Samakaya of *Artemisia annua* gave highest oil yield at spacing of 15 plants/ m².

In this study data on essential oil yield also revealed that maximum oil yield was obtained from leaves followed by stem+branches and seeds. Roots yielded the minimum quantity of essential oil. Leaf being the economically most important part of *Artemisia absinthium* and the leaf mass is positively correlated with essential oil yield. Maximum oil yield of 24.20 kg/ha from leaves was recorded for *Artemisia absinthium* raised from seedlings of Ganderbal source at a spacing of 40 cm x 60 cm.

Similar yield of essential oil was recorded by Zigene *et al.* (2012) from six month old *Artemisia annua* plants. Likewise Nigussie *et al.* (2015) recorded a maximum oil yield of 23.39 kg/ha in *Artemisia annua* planted at a spacing of 40 cm x 60 cm. Reports also suggest a decrease in essential oil yield with the increase in spacing and a minimum of 5.59 kg/ha was observed at 100cm x 120 cm. Essential oil extracted from different parts of the plant was directly proportional to the essential oil yield. Maximum of 6.47 ml/kg were extracted from leaves and minimum of 0.52 ml/kg was obtained from roots of *Artemisia absinthium*. Tiwari *et al.*, (2011) reported maximum essential oil content (0.454 ml/ 100gm fresh leaf sample) in *Artemisia annua* at a planting density of 20,825 plants per hectare with plant to plant spacing of 50 cm × 80 cm.

5.3.1 Effect of source on growth and yield of *Artemisia absinthium*

The main aim of evaluation of different sources is to determine the inherent differences among individuals and manipulate them in desired direction to obtain good result. Exposition of results reveals significant variation among the three different sources of *Artemisia absinthium* on the basis of mean performance of all the growth and yield related characters. The mean performance of traits helps to identify the superior genotype among the existing populations. The mean performance also serves as a primary criterion for selection of desirable plants (Kumar *et al.*, 1979).

Perusal of data indicates significant variation in all growth and yield related parameters among different sources with maximum values recorded for Ganderbal source and minimum for Bandipora. The reason for maximum performance in Ganderbal source is that the experimental site falls in same area having similar micro-climatic conditions. *Artemisia absinthium* can grow well in the highlands with an altitude of 1000-1600 m asl, well drained soil with a pH of 5.5-8.5 (optimum pH 6-8), rainfall 700-1000 mm/year (Ban *et al.*, 1999). Ganderbal (1619m asl) had more

suitable environment for *Artemisia absinthium* cultivation. Since the source and the site where *Artemisia absinthium* was raised were the having the similar growing conditions, *Artemisia absinthium* raised from seedlings/ seeds procured from Ganderbal source show a superior performance in growth as well as yield traits. Variation in *Artemisia absinthium* with respect to the seedling traits among different sources could be due to the fact that this species grow over a wide range of rainfall, temperature, altitude, and soil types.. The seed source and provenance variation in nursery and field is essentially genetic in origin (Sniezko and Stewart, 1989). Marchese *et al.* (2002) suggested that the genotypes from different geographical origins can vary in behaviour under the same photoperiod and temperature conditions. Nurhayati and Gusmaini (2013) also reported variations in fresh and dry weight of four *Artemisia absinthium* accessions. Significant variation in germination parameters of *Vateria indica* among different sources have been reported by Jagadish *et al.*, (2014). Seeds vary in their degree of germination between and within populations and between and within individuals (Mkonda *et al.*, 2003; Loha *et al.*, 2006). Causes of such variability might be generally attributed either to (a) genetic characters of source population/plant (Shu *et al.*, 2012) or (b) impact of mother plant environment (Singh *et al.*, 2010). Gutterman (2000) stated that germination of seeds can be influenced by maternal factors, such as position of the seed in the fruit/tree, the age of the mother plant during seed maturation, as well as environmental factors such as day length, temperature, light quality, water availability and altitude. The current results indicate that, the differences in the germination parameters could be due to wide variation in microclimate and local environmental conditions in the range of distribution of this species. Due to particular set of local environmental conditions, the genetic constitution of the species for the particular traits must have changed resulting in geographically distinct clines (Kumar *et al.* 2004).

Results from the present study of *Artemisia absinthium* also indicate a significant effect of source on essential oil yield. Maximum essential oil yield from all parts of plants [Leaves (16.44 kg/ha, stem+branches (3.58 kg/ha), seed (1.27 kg/ha) and roots (0.72 kg/ha)] was recorded for Ganderbal source and minimum for Bandipora Source [Leaves (13.88 kg/ha, stem+branches (3.43 kg/ha), seed (1.02 kg/ha) and roots (0.57 kg/ha)]. Essential oil content was estimated to constitute about 0.76% of dry leaf weight and 0.26% of fresh leaf weight. Mahdavi *et al.*, (2014) reported similar range of essential oil content 0.91% to 0.96 % in *Artemisia absinthium* from three different altitudes of Iran. The content of essential oil in the *Artemisia absinthium* ranged from 0.40 to 0.88% (Geszprych *et al.*, 2011). The present findings are also in agreement with Wani *et al.*, (2014) who reported a essential oil yield of 0.156% on fresh weight basis of *Artemisia absinthium* collected from the Kulgam area of Kashmir, India. Racial variation among the populations of diverse origin did show association with locality factors such as latitude, longitude, altitude, precipitation, etc. (Shekar *et al.* 2002). Source variation tests are necessary to screen the naturally available genetic variation to select the best planting material for higher productivity (Bhat and Chauhan, 2002). Elucidation of data also revealed a non significant interaction of source of experimental material, IBA, potting media, spacing and planting material suggesting that all the aforementioned treatments are self sufficient in delineating the good agricultural practices for obtaining optimum yield in *Artemisia absinthium*.

Chapter-6

SUMMARY AND CONCLUSION

The present investigation entitled “**Standardization of cultivation, harvesting and processing techniques of *Artemisia absinthium***” was carried out at Centre for Medicinal plants, Faculty of Forestry, **Sher-e-Kashmir University of Agricultural Sciences and Technology**. The purpose of this study was to standardize the propagation techniques of *Artemisia absinthium*, a valuable medicinal plant widely used for the treatment of infectious and non-infectious health problems. Identification of superior lines among the existing populations and good propagation techniques are key parameters to increase the growth and yield of any plant species. Keeping this in mind, the present investigation was conducted to evaluate the different sources and to find out the role of IBA and potting media in influencing the growth and yield of *Artemisia absinthium*. Three experiments were conducted under proper statistical designs, the salient features of whose are summarized below.

The first experiment was conducted under Completely Randomized Design to ascertain the role of IBA in initiating and accelerating the root development of *Artemisia absinthium* cuttings. The conventional propagation of *Artemisia absinthium* by seeds is a constraint for its production due to its tiny seeds that require a symbiotic association within its own microflora for germination most of the seedlings raised from such seeds would have phenotypic variation making uniformity of growth and form difficult to attain. The experiment was conducted with five treatments viz., control, IBA@250 ppm, IBA@500ppm, IBA@750ppm and IBA@1000ppm to study the effect on rooting percentage, number of roots per cutting and root length of *Artemisia absinthium* cuttings of three sources (Ganderbal, Budgam and Bandipora). The results reveal that rooting percentage, number of roots per cutting and length of roots were significantly influenced when *Artemisia absinthium* cuttings were treated

with different IBA concentrations. Maximum values for rooting percentage (84.09% and 68.40%), number of roots per cuttings (5.49 and 4.72), root length (13.33 cm and 9.90 cm) were observed when *Artemisia absinthium* cuttings were treated with IBA@750ppm. Likewise maximum values for rooting percentage (68.40 %), number of roots per cutting (4.72) and root length (9.90 cm) were recorded for Ganderbal source. However non significant interaction effect of source and IBA on before mentioned growth parameters was observed.

Another part of the experiment to standardize optimum growing medium was carried under Completely Randomized Design and included three treatments *viz.* Potting media of Fine sand, Soil and Soil + Sand + FYM to evaluate their respective effects on germination percentage, survival percentage, Number of plants that survived out of germinated seeds, root length (cm), shoot length (cm), root shoot ratio and biomass (g/plant) of *Artemisia absinthium* seedlings procured from three sources (Ganderbal, Budgam and Bandipora). Potting media of soil+sand+FYM (1:1:1) was found superior than fine sand and soil alone to influence different growth and yield parameters of *Artemisia absinthium* seedlings. Higher values for germination percentage (46.55%), survival percentage (68.34%), number of plants that survived out of germinated seeds (36.35), shoot length (2.93 cm), root length (9.93 cm), root/shoot ratio (3.39), biomass (45.32 g/plant) were recorded in seedlings raised in polybags containing potting media of soil, sand and FYM in equal proportions. Similarly seedlings from Ganderbal source were found superior than Budgam and Bandipora. Maximum values for germination percentage (48.66%), survival percentage (67.16%), number of plants that survived out of germinated seeds (32.99), shoot length (2.59 cm), root length (8.25 cm), root/shoot ratio (3.19), biomass (43.19 g/plant) were observed in seedlings procured from Ganderbal source. Two way interactions between source and potting media had no significant effect on growth and yield parameters of *Artemisia absinthium* seedlings.

The second experiment on out planting performance of *Artemisia absinthium* seedlings was carried under Randomized Block Design included three factors viz. Planting material with two levels (Seedling and Cutting), Source with three levels (Ganderbal, Budgam and Bandipora), Spacing with four levels (40 cm x 60 cm, 60 cm x 80 cm, 80 cm x 100 cm and 100 cm x 120 cm). All the treatments significantly influenced the growth and yield parameters of *Artemisia absinthium* plants under field conditions. Maximum values for leaf fresh weight (9.25 tonnes/ha), Leaf dry weight (3.15 tonnes/ha), Above ground biomass (26.83 tonnes/ha) were observed when *Artemisia absinthium* seedlings procured from Ganderbal source were planted at a spacing of 40 cm x 60 cm. Although two way and three way interaction effects of all the factors on growth and yield parameters of *Artemisia absinthium* were found to be statistically non significant. The results revealed superiority of Ganderbal source, planting material of seedlings and spacing of 40 cm x 60 cm in influencing the growth and yield of *Artemisia absinthium* plants.

Experiment on estimation of oil from different parts of the *Artemisia absinthium* plants reveal that leaves are economically most important part of the plant as they yield maximum essential oil of 24.20 kg/ha when *Artemisia absinthium* plants procured from Ganderbal source raised from seedlings were planted at a spacing of 40 cm x 60 cm. Similarly stem + branches (4.03 kg/ha), seeds (1.44 kg/ha), roots (0.81 kg/ha) yielded the maximum essential oil in spacing of 40 cm x 60 cm in seedlings procured from Ganderbal source. Maximum essential oil (ml/kg) was also observed in leaves (6.47 ml/kg) followed by stem+branches (1.87 ml/kg), seeds (1.04 ml/kg) and roots (0.52 ml/kg) in *Artemisia absinthium* seedlings procured from Ganderbal source when planted at a spacing of 40 cm x 60 cm.

CONCLUSIONS

- ✓ Rooting percentage, No. of roots per cutting and Length of roots was recorded maximum in source Ganderbal among three different sources at an IBA concentration of 750 ppm.
- ✓ Germination percentage, survival percentage, No. of plants survived out of germinated seeds, shoot length, root length, root shoot ratio and biomass was observed maximum in plants procured from Ganderbal source in potting media (sand+soil+FYM) among three different sources.
- ✓ Maximum biomass was recorded at 40 cm x 60 cm spacing for planting of *Artemisia absinthium* seedlings.
- ✓ Maximum essential oil content was recorded in leaves in *Artemisia absinthium* seedlings procured from Ganderbal source when planted at a spacing of 40 cm x 60 cm.
- ✓

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CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner Dr. A. S. Shawl, Emeritus Scientist and Ex HOD CSIR-Indian Institute of Integrative Medicine (IIIM) during Viva-Voce examination held on January 4, 2018 have been incorporated in the manuscript entitled “**Standardization of cultivation, harvesting and processing techniques of *Artemisia absinthium***” submitted by **Mr.Sajad Ahmad (Regd. No. 2015-For-56-M)**.

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