EVALUATION OF GERMPLASM AND STANDARDIZATION OF PROPAGATION TECHNIQUES OF *Inula racemosa* Hook.f.

Thesis

by

HARPAL SINGH

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This is to certify that the thesis entitled, **"Evaluation of Germplasm and Standardization of Propagation Techniques of *Inula racemosa* Hook.f.**", submitted in partial fulfillment of the requirements for the award of degree of **Doctor of Philosophy in FORESTRY (Forest Products)** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.) is a record of bonafide research work carried out by **Mr. Harpal Singh (F-2009-05-D)** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of investigations has been fully acknowledged.

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This is to certify that the thesis entitled, **"Evaluation of Germplasm and Standardization of Propagation Techniques of *Inula racemosa* Hook. f.",** Submitted by **Mr. Harpal Singh (F-2009-05-D)** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.) in partial fulfillment of the requirements for the award of degree of **Doctor of Philosophy in FORESTRY (Forest Products)** has been approved by the Student's Advisory Committee after an oral examination of the same in collaboration with the external examiner

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Dated…….

Harpal Singh

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ABBREVIATIONS

%	:	Per cent
◦C	:	Degree Celsius
amsl	:	At mean sea level
cm	:	Centimetres
CRD	:	Completely Randomised Block Design
e.g.	:	For example
et al.	:	Co-workers
FYM	:	Farm Yard Manure
G	:	Germplasm site
GPS	:	Geographic Position System
g	:	Grams
GA_3	:	Gibberellic Acid
gm	:	Grams
H_2SO_4	:	Sulphuric acid
Ha	:	Hectare
HP	:	Himachal Pradesh
hr	:	Hours
i.e.	:	That is
IAA	:	Indol-3-Acetic Acid
IBA	:	Indole-3-Butryic Acid
J&K	:	Jammu & Kashmir
Kg	:	Kilogram
m	:	Meter
M	:	Molar
MeOH	:	Methanol
min:	:	Minutes
P	:	Pre-sowing treatment
ppm	:	Part(s) Per million
RBD	:	Randomised Block Design
RP-HPLC	:	Reversed Phase-High Performance Liquid Chromatography
S	:	Location Site
viz	:	Videlicet

Chapter-1

INTRODUCTION

Majority of the people all over the world are dependent on medicinal and aromatic plants as their principal health care resources. Many ancient cultures mention the healing properties of herbal medicines. The traditional systems of medicine, such as Ayurveda, Siddha and Unani are part of a time-tested culture and are still very well recognized by the people. A vast diversity of herbal ingredients, major proportion of which is derived from the wild, provide the resource base to the herbal industry (Kumar *et al*., 2011). Global demand for herbal medicines is accompanied by dwindling supply of medicinal plants due to over-harvesting, habitat loss and agricultural encroachment.

In the context of Indian agricultural scenario, medicinal and aromatic crops are firmly emerging on the scene from three different perspectives. Firstly, the traditional health care systems have become popular mainly due to the holistic treatment; lower cost of treatment and least side effects thereby resulting in the increased demand of these natural resources. Secondly, these herbal resources are collected from their natural habitats and under minimal supervised environment. As a result, the natural population of medicinal and aromatic plants in their natural habitats has started declining at an alarming rate. This over-exploitation has necessitated the cultivation of these plants under field conditions. Lastly, medicinal and aromatic crops have better economic opportunities as against the traditional field crops and can serve as a good option for the diversification of cropping systems.

In recent years herbal industry has been one of the major driving forces in the global economy. The global market for medicinal plants and herbal medicines is estimated to be worth US $ 80 billion a year. International export trade in medicinal plants from India is 32,600 tons a year (Jabeen *et al*., 2007; Shawl and Qazi, 2004). Presently most of the medicinal and aromatic plants are collected

from wild sources (forests) and very less quantity is sustainably produced and harvested through cultivation (Kumar *et al*., 2007).

The demand for medicinal plant-based raw materials is growing at the rate of 15 to 25% annually, and according to an estimate of WHO, the demand for medicinal plants is likely to increase more than US $5 trillion in 2050. In India, the medicinal plant-related trade is estimated to be approximately US $1 billion per year (Maheswari, 2011; Sharma, 2004; Joshi *et al*., 2004).

The Indian Himalayan Region (IHR) is a rich reservoir of biological diversity in the world. This region is a store house of high value medicinal & aromatic herbs and has a rich local tribal tradition of herbal medicine (Sharma *et al*., 2006). This region is represented by more than 1748 plant species, of which several are economically very important (Nautiyal *et al*., 2001).

Inula racemosa Hook. f. commonly known as pushkarmool or manu is an endangered perennial herb belonging to the family Asteraceae and is distributed in the North Western Himalayas between of 2000 to 3200 m (Anonymous, 1998). Pushkarmool has a narrow distributional range and is confined to Hindu-Kush Himalayan region across Afghanistan, Pakistan, India, China and Nepal. In India, it is mainly found in parts of Jammu & Kashmir, Himachal Pradesh and in Uttarakhand (Nayar and Sastry, 1988). The plant is about 1.5 m tall, stout herbaceous with radical, stalked, broad elliptical leaves. The stem is grooved, rough and very hairy bearing terminally borne yellow flower heads. Flowering in this plant takes place in July-August and seed ripens in August –October. Plant can be propagated through seeds and division of roots. Flowering is from July to August and the seeds ripen from August to October (Chauhan, 1999).

Out of twenty species of the genus *Inula* occurring wild in India, five are considered to be of economic importance. *Inula racemosa* has gained prominence as a medicinal and aromatic plant and is commercially cultivated in Lahul valley of Himachal Pradesh on small scale. The cultivation of Manu was at its peak in the 1960s (Kuniyal *et al*., 2004; Rawat *et al*., 2004). However, in the last few years, cultivation has drastically declined due to the introduction of other cash

crops like potato, pea etc. which provide greater economic returns. Small land holdings, lengthy cultivation cycle and fluctuating market prices are some of the reasons associated with decline in *Inula racemosa* cultivation in the region (Rawat and Everson, 2011; Sharma and Sharma, 2010)

Root is an official part of Pushkarmool. Fresh roots have a strong aromatic odour resembling orris and camphor; dried roots have a weak odour. In Kashmir roots are used as an adulterant of Kuth (*Saussurea lappa*). They contain inulin (10%) and an essential oil (1.3%) containing alantolactone. Alantolactone has antiseptic, expectorant and diuretic properties. The seeds of pushkarmool are bitter and aphrodisiac (Anonymous, 1959; Bhavaprakash, 1961). Roots have at least four sesquiterpene lactones, namely; alantolactone, isolantolactone, dihydroalantoctone and dihydroisolantolactone. Sesquiterpene lactones are the chief constituents which possess antiseptic, expectorant and diuretic properties *e.g*., beta-sitosterol, daucosterol and inunolide having healing properties. Sesquiterpene lactones have been found to be active against the human pathogenic fungi (Tan *et al*., 1998).

Inula racemosa is a commercially useful herb and paste of roots is effectively used in dressing the wounds and ulcers as it possess antiseptic properties. The paste of roots is especially recommended, to be applied on the chest in pleurisy and inflammatory conditions of pleura, to mitigate the pain (Kaul, 1997). This herb is used to mitigate *Vata-kappa Jawara* (a type of fever) as an indigenous medicine. The drug is considered more potent and less pungent in taste. It provides relief for *Vata*, nausea, swellings breathlessness, and chest pain (Charak, 1941). Internally, pushkarmool is used to boost appetite and digestion. Hence it is beneficial in anorexia and dyspepsia. Clinical reports of *Inula racemosa* confirm its use as hypoglycemic agent (Chaturvedi *et al*., 1995).This plant is also used in Tibetan medicine and it is said to have a sweet, bitter and acrid taste with a neutral potency. It is used in treatment of contagious fevers that have not fully ripened and pain in upper body, especially between the neck and the shoulders (Tsarong and Tsewang, 1992).

Due to fragile nature of its habitat and exploitation due to commercial medicinal properties, the species is facing the onslaught of indiscriminate unscientific exploitation, habitat destruction and competition. There is a speedy declination in its population density and diversity in the entire North West Himalayan range. Unabated as the plant extraction and habitat destruction continues to be, far are not days when these herbal gems will become extinct from globe. It is indeed a crucial situation for this species, calling for salvage of whatever is left and if not rescued now, irretrievable loss of this precious legacy from the globe will be the eventual and inevitable consequence (Wani *et al.*, 2006).

Germplasm is an important source for new plant types with desirable traits and increase in crop production (Yousaf *et al.*, 2005). Plant germplasm development and evaluation evolves through three successive stages: introduction, selection, and hybridization. Introduction involves screening and evaluation of plant introductions, foreign cultivars, and advanced breeding lines from other areas. The second stage is selection of superior-appearing plants from these introductions, multiplying them, and evaluating them in local conditions. The final stage is hybridization of selected parent lines, followed by selection for the best combination of traits from the two parents in each cross. One of the important factors *i.e.* ecological conditions plays major role in the cultivation of medicinal plants. Plants, which could withstand better under biotic and abiotic pressures, are thus the keys for sustainability in agriculture. Genes for such traits are often available in wild species and landraces and have thus to be exploited fully to achieve desired objectives.

Although existing potential of wild germplasm in India are tremendous, yet large areas are unexplored and untapped. A consortia approach would be desirable to augment, evaluate and sustainable use of the wild plant species for improvement of cultivated crops (Malik and Singh, 2006). Very limited scientific information is available on the potential medicinal herbs, which can be cultivated and utilized for different purposes.

Till now not much of the research work has been carried out on this commercial medicinal herb. Keeping in view the commercial importance of this plant present study **Evaluation of Germplasm and Standardization of Propagation Techniques of *Inula racemosa* Hook.f.** has been proposed to work out the ways and means for its evaluation and propagation for the development of appropriate cultivation techniques. The present work has been proposed with the following objectives:

OBJECTIVES:

i) Evaluation of *Inula racemosa* germplasm from different sites.

ii) To assess the propagation techniques of *Inula racemosa* under field and controlled conditions.

Chapter-2

REVIEW OF LITERATURE

The present work on "**Evaluation of germplasm and standardization of propagation techniques of *Inula racemosa* Hook.f.**" is based on studies conducted on germplasm collection from eight different sites of Himachal Pradesh and Jammu & Kashmir. The research trials were carried out in the experimental fields and in the laboratory. The available literature referred for planning and execution of this work for interpretation of results is reviewed as under:

2.1 SURVEY, COLLECTION AND EVALUATION OF GERMPLASM

Inula racemosa plant has not received any scientific study leading to improvement or selection; folk domestication has been aimed toward obtaining longer and more roots per plant. Though no standard forms are known as yet under cultivation, the domesticated type has sweeter odour and highest essential oil content than kuth roots (Arora *et al*., 1980; Anonymous, 1959). The surge in global demand for herbal medicines has been followed by a belated growth in international awareness about the dwindling density and diversity of herbal wealth (Shabir, 2011). Therefore, efforts should be made to identify the superior strains by screening available variability and locating genotypes with roots yielding higher percentages of essential oil. These steps should generate a large and stable market demand of potential medicinal plant as *Inula racemosa* and others.

General information on *Inula racemosa* Hook.f is available in the literature (Anonymous, 1959; Arora *et al*., 1980; Chauhan, 1999; Singh *et al*.,

2011; Amin *et al*., 2013). However, information regarding the morphological and quantitative variation of germplasm for selection of elite germplasm strains for identification of superior germplasm and strains for domestication yielding higher biomass and essential oil are lacking.

Germplasm is the sum total of all genes and their alleles present in a crop and its related species (Chawla, 2012). This is represented by a collection of various strains and related species of the concerned crop species. Evaluation of germplasm is important as geographic variation in plant morphology and is a function of phenotypic changes in response to local environmental conditions, genetic variation and evolution among populations, and the biogeography history of an individual species. Morphological and quantitative characteristics are constrained genetically, yet they also can be affected greatly by the local environment in which they develop (Thompson, 1991; Schlichting and Pigliucci, 1998). Morphological variation and geographical separation among populations are also prerequisite to the formation of subspecies and species (Losos and Glor, 2003).

Geographic variation in morphology reflects phenotypic responses to environmental gradients and evolutionary history of populations and species and may indicate local or regional changes in environmental conditions. The aim of germplasm evaluation and conservation is to ensure the availability of useful germplasm at any time.

Generally, medicinal plants are found more abundantly in the mountainous areas than in plains due to natural conducive habitat and suitable climatic conditions (Anwar and Masood, 1998). It is difficult to propagate high altitude medicinal and aromatic herbs due to their wide distribution, varied habitat and unsuitable environment for growth, ecological adaptations and different types of dormancy. Besides this conservation of germplasm and limited land resources are also the major constraints in their cultivation.

Inula racemosa Hook.f. is a perennial herb belonging to family Asteraceae and distributed in Hindu-Kush Himalayan region across Afghanistan,

Pakistan, India, Nepal, and extends up to Iran and Europe (Dawar, 1998). It is an oriental species in origin and distribution. In India, the species has been reported from Jammu and Kashmir, Himachal Pradesh and Uttar Pradesh (now in Uttarakhand) at an altitudinal range of 2800-3200m (Hooker, 1881; Kurup *et al.,* 1979; Nayar and Sastry, 1988). It is 1.5 to 2m tall with fragrant prominent roots and rootstock; stems are many in number, ascending from the base of rootstock. Leaves are leathery, rough above and densely hairy below, 25-50 cm long and 6-12 cm broad and shape is elliptic-lanceolate. Flower heads are yellowish in colour and they have bisexual florets and occur in terminal racemes. Flowering occurs from June-August and fruiting in October - November. Fruits are achenes cylindrical about 0.5 cm long (Agarwal, 1997; Kirtikar and Basu, 1984; Chauhan, 1999; Rawat and Everson, 2011).

Dawar (1998) collected *Inula racemosa* from Pakistan and described its morphology as: a stout perennial herb, 30-150 cm long; leaves elliptic to lanceolate, upper sessile, lower long winged petiolated, margin crenate- dentate; capitula 3.5-5.0cm in diameter, cymosely arranged; involucral bracts 5-6 seriate; marginal florets ligulate, 2-3 toothed, pistllate; central florets hermaphrodite, 5-toothed; cypselae 3-4 mm long, glabrous; pappus bristles uniseriate, 22-50 in number, 7-8mm long, reddish brown.

Shabir *et al.* (2013) studied among and within population variation in growth dynamics and floral sex ratios in *Inula racemosa;* a critically endangered medicinal herb of North West Himalayas. The altitude showed positive correlation with below-ground biomass and negatively correlated with the plant height, number of leaves, number of capitula and above ground biomass. Negative correlation of all morphological parameters to the increasing altitudes indicates better growth performance of this species at lower elevations.

Distribution and current conservation status of some important threatened medicinal plants of Ducksum Kokernag (Kashmir, Himalaya) was studied by Baig *et al*. (2012). *Inula racemosa* locally known as motocraz distributed from 3100-3800 m and official part used as roots/foliage. Phytosociological parameters revealed that at Ducksum (Kashmir Himalaya)

density was 0.38 (plants/m^2), relative density (RD) 0.05, frequency (22.22%), relative frequency (RF) 0.08 and important value index (IVI) 0.23%.

Wani *et al*. (2006) conducted work on phenological episodes and reproductive strategies of *Inula racemosa*. Flowering proceeded by pollen transfer to stigma and finally seed set was observed only in the natural populations while transplanted plants failed to show seed set. Self-incompatibility was confirmed during the studies. High pollen to ovule ratio suggested out breeding nature of the plant species. Pollination was observed to be effective by insects specially bees.

Distribution of *Inula racemosa* in Himalayan region

State	Place/District	Altitude (m)	Population Status	Reference (s)
Himachal Pradesh	Parwati valley (Kullu)	1600-4200	Wild	Chauhan,1999; Sharma and Sood, 2007
	Dhanshoh (Chamba)	3550	-	Gupta, 2011
	Megad Watershed (Lahaul&Spiti)	2200–5000	Domesticated	Rana *et al*., 2010
	Banks of Chandra, Bhaga and Chenab (Lahaul valley)	2400-3600	-	Kuniyal *et al*., 2004; Rawat *et al*., 2004; Rawat and Everson, 2011
	Khoksar (Lahaul&Spiti)	3200		
	Jahlma (Lahaul&Spiti)	3000	-	
	Hinsa (Lahaul&Spiti)	2700	-	
	Kuthar (Lahaul&Spiti)	2600	-	
	Pattan Valley (Lahul&Spiti)	3000	-	Sharma *et al*., 2006
	-do-	2950	-	Sharma and Sharma, 2010
	Kinnaur, Chamba, Kullu	upto2500	-	Aswal and Mehrotra,1994
Jammu & Kashmir	Gulmarg(Baramulla)	2300-3700	Wild	Wani *et al*, 2006; Baig *et al*., 2012
	Ducksumkokernag (Anantnag)	2700-3500	-	
	Pasi (Anantnag)	3000-3150	-	
	Brari-marg (Anantnag)	3240-3450	-	
	Thajwas (Sonmarg)	3050-3500	-	
	Lidarawat (Pulwama)	2500-3140	-	
	Gagarbal, Izmarg, Kanzalwan ,Gurez, Leh	2500-4500	Wild	Sharma, 2010; Kumar *et al*., 2011
	Herbal garden Kashmir University	1700	Domesticated	Wani *et al*., 2006

Morphological and quantitative characteristics of *Inula racemosa*

Research topic	Parameters	Authors
Microcharacteristics in *Inula racemosa*		Abid and Qaiser, 2004
Receptacular surface	Without scaly ridges	
Anther apices	Acute –obtuse	
Cypsela characters of *Inula racemosa*		Shekhar *et al.*, 2011
Shape	Oblongoid	
Size (mm)	3-4 × 0.5-0.75	
Colour	Dark brown	
Surface	Glabrous	
Number of ribs	16-24	
Pappus characters of *Inula racemosa*		
Series of Bristle	1	
Number	30-48	
Size (mm)	8-9	
Colour	Reddish brown	
Cypselae characters of *Inula racemosa*		Abid and Qaiser, 2002
Mean weight of 50 cypsela (gms)	0.275	
Shape	Oblong	
Surface (hairs)	Glabrous	
Number of ribs	16-24	
Colour	Dark brown	
Size (mm)	3.4×0.5-0.275	
Pappus characters		
Bristles (**Series**)	1	
Scales (**Series**)	0	
Number	30-48	
Size (mm)	7-8	
Colour	Reddish brown	
Carpopodium		
Shape	Slightly angular –narrow circular ring without any interruption	
Position	Basal-sub basal	
Diameter of carpopodium (µm)	463.62	
Diameter of foramen of carpopodium (µm)	275.75	
Endothecium pattern in *Inula racemosa*		Abid and Qaiser, 2004
EndothecialType	Transitional	
Capitula diameter, Arrangement	3.5-5.0 cm, cymosely	

Shabir (2011) studied reproductive biology of *Inula racemosa* Hook. f. and its morphological and functional traits. The floral morphology of *Inula racemosa* is especially adapted for insect attraction as the species possess 10-20 capitula per plant aggregated in racemes, each with the outer, colourful, "petal like" yellow ray florets that visually attract insects. Each disc floret possesses a fused corolla tube and the bell shape of the corolla serves to protect the

androecium and gynoecium before anthesis. The base of the corolla also serves as attachment points for the filaments of the stamens. The thin, membranous, scale-like pappus is the reduced form of the upper free limbs of the sepals, and apparently serves no functional purpose to disc florets of *Inula racemosa*.

Cytological investigations on Indian Compositae: IV Tribes Senecioneae, Eupatorieae, Vernonieae, and Inuleae done by Mehra and Remanandan (1975) reported 2n=10 number of chromosomes. However Koul and Gohil (1973); Shabir (2011) and Shabir *et al*., (2013) reported 2n=20 number of chromosomes.

Bisht *et al*. (2008) made an assessment of reproductive potential of different populations of *Angelica glauca* Edgew., a critically endangered Himalayan medicinal herb. The variability in reproductive characters of *A. glauca* were found having significant positive correlation with altitude.

2.1.1 Seed Characteristics

Pradhan and Badola (2011) made an assessment to study the seedling emergence and vigour for quality planting material in thirteen populations of *Swertia chirayita* which is reported to be a high value endangered medicinal herb, using substrate combinations. Populations differed significantly for seed characteristics, indicating considerable genetic diversity. Out of 13 populations tested, eight exhibited 100 per cent germination immediately after collection, which was negatively correlated with altitude of the sites where plants were growing. The results suggested an acceptable range of 16 to 42 per cent seed moisture content, before storage, as suitable criteria for *S. chirayita* populations in long term gene resource conservation. Seed from 10 plant populations showed a steady decline in germination over the storage period.

Pradhan and Badola (2012) studied the effects of microhabitat, light and temperature on seed germination and conservation implications of *Swertia chirayita.* It was summarised that microhabitats have a great influence on seed germination and seeds are light sensitive.

Ali (2008) made an assessment to study the propagation and germplasm evaluation on *Berberis aristata* DC. Status of *Berberis aristata* in its natural habitat, optimization of regeneration through seed, vegetative propagation. Further evaluation and comparison of different germplasms from different ecological zones was done.. The analysis of data depicted highly significant differences for germination per cent, germination energy, germination speed, peak value, mean daily germination, germination value, germination index, vigour index, plant height, collar diameter, fresh shoot weight, dry shoot weight, fresh root and dry root weight, which indicate considerable amount of variability for material under study.

2.2. SEED GERMINATION

2.2.1 Effect of pre-sowing treatments and growth regulators

Pre-sowing treatment has been used to enhance germination and increased seedling vigour in alpine species like *Heracleum candicans* (Joshi *et al*., 2004), *Aconitum heterophyllum* (Pandey *et al*., 2000) and *Podophyllum hexandrum* (Nadeem *et al*., 2000)

Water participates in many biochemical reactions and serves as a medium for the life processes. In seeds, water is an essential factor in the external environment for the stimulation of germination. Soaking the seeds in water at room temperature helps in softening the seed coats, removal of inhibitors and reduces the time required for germination and increases germination percentage (Hartmann and Kester, 1979). A number of treatments involve soaking of seeds in water or other liquid and combines the effects of softening hard seed coat and leaching out chemical inhibitors. Some seeds which have little resistance to germinate may respond well to soaking for 24 hours in water at ambient temperature (Kemp, 1975).

Sheikh (1980) studied effect of boiling water on germination of seeds of *Cassia fistula* and observed 67.5 per cent germination after 24 days. Bowen and Eusibio (1981) found that in *Acacia mangium* seeds in Sabah there was a close correlation between the initial temperature of water and the subsequent

germination. Germination increased progressively from 5 per cent after immersion in water at 30°C to 91 per cent immersion at 100°C.

Dharmalingam *et al.* (1971) reported that *Tephrosia purpurea* seeds pre-soaked with hot water at 50^0 C for five minutes improved germination. Similarly, Bhuyar *et al.* (2000) reported that treating the seeds of *Rauvolfia serpentina* with hot water (80°C for 5 min) and then cooling down to room temperature produced highest germination percentage and better seedling vigour index. Other study conducted by Gupta *et al*. (2001) showed that hot water treatment at 70°C for 10 minutes followed by scarification with concentrated H_2SO_4 for 5 minutes was most effective in breaking the seed coat dormancy in *Abutilon indicum*. This resulted in maximum germination (82%) as compared to control (8%). Gowda *et al*. (2003) reported that treating the seeds of *Embelia tsjeriam-cottam* with hot water for five minutes and then in cold water for 12h resulted in early and better germination (28%) compared to control (12%). Gupta (2003) obtained highest germination per cent of 88 and 82 in *Bryonopsis lanceolata* and *Bixa orellana,* respectively, when treated with hot water (65°C for 60 minutes) compared to control (40% and 36%, respectively).

Gibberellic acid is known to play an essential role in seed germination, stem elongation and flower development (Sozzi and Chiesa, 1995; Pupalla and Fowler, 2002; Fernandez *et al*., 2002). Effect of various dormancy breaking treatments on the germination of wild caper (*Capparis spinosa*) seed from the cold arid desert of trans-Himalayas was examined by Bhoyar *et al*., 2010. The role of dormancy breaking treatments, *viz* hot water treatment scarification, concentrated acids (H_2SO_4, HNO_3 and HCL), gibberellic acid, potassium nitrate, alcohol, acetone and gamma-rays irradiation on the germination of caper (*Capparis spinosa* L.) seeds. The results revealed that the seeds were epitomized by both physical and physiological type of dormancy that should be overcome to have maximum germination percentage. Highest germination of 62 per cent was obtained when seeds were pretreated with H_2SO_4 (40 min.) followed by 400 ppm of gibberellic acid soaking (2 hr). Similarly, seeds of *Gentiana scabra* on

treatment with 300 ppm GA_3 and *G. lutea* seeds with 500 ppm GA_3 gave 75 and 81 per cent germination, respectively (Kretschner and Franz, 1997).

Nautiyal *et al*. (2001) assessed germinability, productivity and cost benefit analysis of *Picrorhiza kurrooa* cultivated at lower altitudes. Germination was observed better inside polyhouse at 15-20^0 C temperature in sandy soil with litter treatment and high-moisture content. Vegetative propagation was done successfully through stolon segments by using hormonal as well as convenient and simple methods, *viz* water - dip treatment and use of high moisture trenches for rooting in cuttings, which can be easily used for cultivation purpose by local growers. Top segments were found to be more suitable for multiplication. It was endorced for mass cultivation that sandy loam soil covered with moss layer with higher moisture and 15-20^{0}c temperature are optimum conditions. Besides GA_3 and IAA treatments, water dip treatment for 48 hours was found suitable for surviving and rooting of stolon segments.

Liopa-Tsakalidi *et al.* (2012) studied effect of salicylic acid (SA) and gibberellic acid (GA_3) pre-soaking on seed germination of *Stevia rebaudiana* under salt stress and reported that growth regulators will help determining concentration of GA_3 suitable for the seed germination of Stevia. The maximum germination percentage with pre-soaking was recorded with 200 ppm GA_3, similar findings were reported by Darra and Saxena (1971), Jagadish *et al*. (1994), Aoyma *et al*. (1996), and Asrar (2011), who stated higher germination in pre-soaking with 200 ppm GA_3. Kumar *et al*. (2011) studied methods to break seed dormancy of *Andrographis paniculata* with hot water treatment and reported maximum germination percentage of 93 per cent. Analysis of variance indicated that both hormonal and hot water treatments had a significant effect on seed germination and final germination percentage.

Pradhan and Badola (2010) studied the role of chemical treatments to improve seedling emergence and vigour in *Swertia chirayita* and found that seedling emergence was promoted from 6 per cent (control) to 69 per cent (GA_3

250 µM). The seedling emergence rate was faster in GA_3 (250 µM) followed by $NaHClO_3$ (5 min) in all the seed sources.

According to Diaz and Martin (1971), gibberellins are known to stimulate germination of seeds where dormancy is imposed by a wide variety of mechanisms such as incomplete embryo development, mechanically resistant seed coats, presence of inhibitors etc. Shanmugavelu (1970), in his studies on the effect of GA_3 on the tree seeds, observed that seed germination was higher in some of the leguminous tree species with better shoot growth. He further confirmed that GA_3 treatment was superior to other growth regulators in jute seeds with respect to seed germination and shoot growth.

Treatment with gibberellic acid produced excellent germination in seeds of *Impatiens balsamina*, *Lavendula aungustifolia*, *Brassica rapa* and *Viola odorata* (Renard and Cleark, 1978).

Bhujbal (1979) reported highest germination (92.50%) with minimum period when dried stones of aonla were treated with 500 ppm GA_3. Further, studies conducted by Mukhopadyaya *et al*. (1990) found that in *Peltoforum ferrugenium,* in general 12 or14 hours of seed soaking in GA_3 200 ppm solution significantly increased the seed germination percentage and was superior to other treatments. Similar results with GA_3 were reported in several species like aonla (Dhankar and Singh, 1996; Wagh *et al*., 1998; Tendulkar, 1978; Hegde, 1991). Masoodi and Masoodi (2000) reported highest germination (71%) in *Ulmus wallichiana* (an endangered tree) seeds when treated with GA_3 100 ppm compared to control (48%). Gupta (2003) also while treating the seeds of *Embelia ribes* with GA_3 100 ppm reported maximum germination (92%) as compared to control (48%).

Biradar *et al*. (2005) observed better germination (70%) in Allahabad Safeda and Taiwan guava when treated with GA_3 at 100 ppm. The length, dry weight and vigour of seedling were also found maximum in this treatment.

Lalithkumar (2008) obtained better germination in Tulsi (*Ocimum sanctum*), Ashwagandha (*Withania somnifera*), Periwinkle (*Catharanthus roseus*)

and Kalmegh (*Andrographis paniculata*) seeds when treated with GA_3 at 250 ppm against the control.

Dhoran and Gudadhe (2012) investigated effect of plant growth regulators on seed germination, root, shoot and leaf length, weight and seedling vigour index *on Asparagus sprengeri* commonly known as *Asparagus* fern which is not a true fern and is propagated through seeds. They reported that GA_3 had a significant effect on germination rate as compared to control, IAA, IBA and NAA during light and dark period. The result indicated that GA_3 at 50 ppm gave best response but as the concentration increased above 60 ppm the germination decreased rapidly and vigour index also decreased during light and dark period.

Seeds of many species fail to germinate in spite of the presence of favorable environmental conditions. Jenick (1974) termed this state as seed dormancy. Donelly (1970) reported that besides environmental factors the genetic component of the species also causes dormancy. Schophymeyer (1974) reported that most of the shrub species have embryo dormancy in which germination is enhanced following a period of chilling at low temperature *i.e*. 2-5^{o}C.

Harrington (1970) and Stein *et al.* (1974) reported that fully ripened seeds retain the viability longer than seeds collected when immature. From the forester's point of view dormancy has some disadvantages. Delayed and irregular germination in the nursery is a serious impediment to efficient nursery management (Bonner *et al.,* 1974).

Butola and Badola (2004) reported poor germination in *Angelica glauca* seeds. Among 14 pre-sowing treatments, KNO_3 (150mM) and $NaHClO_3$ (30 minutes) significantly stimulated seed germination and reduced mean germination time under laboratory and nursery trials, as well as developed seedling vigour under nursery conditions. They further found that treatment of cuttings with IAA (0.25 & 1.25 mM) and IBA (0.25 mM) resulted in higher rooting/cutting, growth and biomass over control in *Angelica glauca.*

Studies conducted on seeds of *Gentiana scabra* on treatment with 300ppm GA_3 and *Gentiana lutea* seeds with 500 ppm GA_3 gave 75 to 81 percent germination (Kretschner and Franz, 1997).

Vashistha *et al.* (2009) reported that the *ex-situ* cultivation of *Angelica glauca* and *Angelica archangelica* is recommend for conservation and regular supply of raw material for pharmaceuticals and ethno-medicinal uses. Vegetative propagation of these species was carried out at Pothivas (2200 m amsl): a part of Western Himalaya, Uttarakhand, India. Three treatments, *viz*., IBA, IAA and GA_3 with different concentrations (100, 200 and 500 ppm) each were tried to stimulate sprouting and rooting. IBA 100 ppm showed better result in both the species.

Chauhan and Nautiyal, 2007 reported that seeds and rhizomes of *Nardostachys grandiflora* treated with 100 ppm and 200 ppm GA_3 (gibberellic acid) for 48 hours resulted in rapid germination and sprouting respectively. Loamy porous soil rich in organic matter like humus is considered the best for its growth, whereas seed sowing at depth of 0.5cm in soil: sand: FYM media @ 1:1:1 during October at lower altitude and during May at high altitude has been found suitable with application of GA_3 (100 ppm) and found best to enhance seed germination up to 90% (Anonymous, 2008).

Sanders (1926) described the propagation in *Inula racemosa* by seeds. Seeds showed good germination when sown in spring or autumn in a cold frame and germinated seedlings could be picked and shifted into individual pots when they are large enough to be handled. Thomas (1990) also reported propagation of *Inula racemosa*, using seeds and found that it took some years to become fully established.

Sharma and Sharma (2010) studied seed physiological aspects of pushkarmool *(Inula racemosa*), a threatened medicinal herb: response to storage, cold stratification, light and gibberlic acid. Cold – stratification and gibberlic acid treatments effectively alleviated seed dormancy. Data indicate a clear requirement of light for seed germination in this species, as there was no germination in the dark and under green light. Studies were also conducted by

Sharma *et al.* (2006) on germination behavior of some medicinal plants of Lahaul & Spiti cold desert (Himachal Pradesh). The efficacy of chilling, acid scarification, KNO_3 and GA_3 treatments were tried for germination.

Micropropagation and conservation of *Inula racemosa* was also studied by different workers and revealed that it can be successfully established under favourable controlled and optimum conditions (Kaloo and Shah 1990; Jabeen *et al.,* 2007; Kaur, 2010).

Kumar and Sharma (2012) conducted an experiment to study the effect of light and temperature on seed germination of important medicinal and aromatic plants *viz*., *Stevia rebaidiana, Salvia sclarea* and *Tagetes minuta*. The treatment comprises of two factors *viz*. light and temperature regimes under room temperature. Maximum seed germination was observed in the seed which were placed for 2 days open in room temperature and then placed at 20^0C in continuous light in all the three plant species in both media except *Salvia* seeds which recorded maximum germination (76%) when placed in room temperature under light condition in sand. Similarly Parmar *et al.* (2012) explored seeds germination and seedlings analysis of *Saussurea costus* in high and low altitudinal villages of district Uttarkashi (Uttarakhand). Successful seed germination, survival percentage and seedlings analysis of this species using both field and within the polyhouse techniques in low and high altitudinal villages was done. A significant increment in root length was recorded at high altitude. Highest percentage of seed germination and survival percentage was noticed at higher altitudes.

Kaushal and Rana (2004) conducted experiments to study the effect of growth regulators on germination, growth and yield of *Saussurea lappa*. Seed soaking treatments of GA_3 and IAA stimulated germination per cent, plant height, leaves per plant, root length, fresh and dry weight of roots. Maximum increase was obtained in all these traits at 200 ppm of GA_3 and 300 ppm of IAA. Comparing two growth regulators, GA_3 was found to be significantly superior to

improve root and shoot growth, while IAA was effective in promoting fresh and dry weight of roots in kuth which is of economic importance.

Amooaghaie (2009) studied the effect mechanism of moist-chilling and GA_3 on seed germination and subsequent seedling growth of *Ferula ovina.* The results showed that *Ferula ovina* seeds display an endogenous dormancy that can be released by moist-chilling treatment for a certain period. In this respect, the best treatment was moist-chilling for 6 weeks at 5 ± 1 °C or for 4 weeks of moist-chilling followed by soaking in 500 ppm GA_3 solution for 24 h. These treatments significantly increased germination percentage and decreased time to 50 per cent germination compared to control. Also, the characteristics of the obtained seedlings were much better than those of control. Moreover, the 6-week moist-chilled seeds contained the highest soluble protein concentration. The combination between GA_3 and moist-chilling treatments produced different effects on seed germination, soluble protein depending on the length of the moist-chilling period. GA_3 application on un-chilled seeds improves the germination process. It was concluded that treatment of moist-chilling for 6 weeks or 4 weeks followed by 500 ppm GA_3 is recommended for promoting the germination process of *Ferula ovina* seeds and improving growth characteristics of the subsequent seedlings.

Genova et al. (1997) studied on the germination of *Atropa belladonna* seeds. It was established that variable temperature (6 h at 30°C and 18 h at 15°C) significantly stimulated seed germination - 82.5%. A maximum germination was obtained by treatment with gibberellic acid (GA_3) 1mg/l H_2 O - 89.5%

Propagation of *Inula racemosa* by division of roots was studied by Arora *et al*. (1980). It is grown on small scale in Lahaul valley in North West Himalaya. Its cultivation is restricted to the borders of agricultural fields of wheat through root cuttings. The collar portion of the root gave better performance in sprouting and survival percentage. In general plant prefers porous soils for faster growth. Flower heads are borne in third year but the seed set remains poor possibly due to high sterility. The crop could be raised from seeds too but time period to require

maturity is much longer and hence cuttings are preferred. The root cuttings are planted, either in late autumn (October) or early spring season (May), in small deep pits, with application of farm yard manure or droppings of sheep and goats. Sprouting takes place in about six weeks. Availability of moisture due to melting of snow helps in growth initiation and the plant attains maximum growth in the second year. The plants grow even 1m in height in the third year and the roots are dug by September - October when the flower heads start drying. The fresh roots are later cleaned, cut into small pieces and sun-dried (Arora *et al*., 1980).

Shabir *et al*. (2010) developed vegetative and sexual multiplication protocol for commercialization of *Inula racemosa*. Split rhizome cuttings treated with varying concentrations of IAA, IBA, and GA_3 were used. Results indicate a maximum 88.89 % sprouting and rooting 77.78 % in 100 ppm of IAA. Also seeds show a broad range of pre-chilling requirements. Highest germination percentage (90%) was recorded when scarification and GA_3 (100ppm) were applied together. Study reveals that there was no germination in control.

Nautiyal *et al.* (2001), while studying the effect of 100 ppm and 200 ppm solutions of IAA, IBA, NAA and GA_3 for 48 hours on stolon cuttings of *Picrorhiza kurroa* observed that GA_3 and IAA treated top segments showed more than 90 per cent rooting. Similarly, Kaul and Kaul (1996) have attempted clonal propagation with hormonal treatment in *Picrorhiza kurroa* with varying success. In pot culture, IBA showed encouraging rooting response 60 per cent stolon splits rooted.

Thakur *et al*. (2010) conducted field trials to study the effect of different propagation and plant techniques on the performance of *Picrorhiza kurroa*. Maximum sprouting and survival was recorded from vegetatively propagated 6 cm stolon cuttings taken from top portion whereas, maximum rootstock yield was obtained with 10 cm stolon cuttings taken from middle portion of the plant. It was concluded that the stolon cuttings should be planted horizontally at 7.5 cm depth with the spacing of 30 cm×30 cm to get maximum rootstock yield/ ha.

Kumar *et al*. (2011) studied current status and potential prospects of medicinal plant sector in trans-Himalayan Ladakh. Studies revealed that seed germination was found to be 70-80 per cent with seed requirement of 200gm/ha, vegetative propagation by rootstocks (40,000 cuttings/ha), FYM @ 15 t/ha at the time of land preparation; light irrigation in every 3-4 weeks. Root was considered to be as an official part and harvesting period was October - November and total production was 8 t/ha (after 2nd year). Kuniyal *et al*. (2004) studied Kuth (*Saussurea lappa*) cultivation in the cold desert environment of the Lahaul valley, arising threats and need to revive socio-economic values. The findings reveal that this age-old practice now is in bottleneck. Main factors responsible for this setback to the species were the lengthy cultivation cycle, small land holdings, and even fluctuating and relatively low market prices.

Vashistha *et al*. (2006) studied conservation status, population and morphological variations between populations of *Angelica glauca* and *Angelica archangelica* in Garhwal Himalaya. Natural populations of two species of *A. glauca* and *A. archangelica* in sub alpine and alpine regions of Garhwal Himalaya were surveyed for the determination of threat status and evaluation of germplasm for domestication and cultivation. The study revealed that frequency of both species was more than 50 per cent in nature. However, density of individuals and area occupied were low as compared to other species of alpine and sub alpine region, indicating habitat loss and heavy exploitation. Based on species occurrence in selected areas, both species were identified as critically endangered to endangered in different areas. The results also revealed that natural distribution of these species was narrowing down due to habitat destruction. Efforts were also made to evaluate germplasm for domestication on the basis of morphological variations and yield of rhizomes under natural conditions as well as on the basis of phenotypic traits.

Ramdas *et al*. (2011) explored seed germination and seedling analysis of *Picrorhiza kurrooa* in Genwala and Bagori (Harsil) of district Uttarkashi from Uttarakhand under field and within the polyhouse techniques in low and high altitudinal villages. Vegetative propagation was achieved by rooting runner

cuttings. A significant increment in root length was recorded in high altitudinal polyhouse condition as compared to low altitude. In low altitudinal village Genwala, under open field condition seed germination percentage and survival percentage ranged from 9.00 per cent to 13.00 per cent and 9.00 per cent to 19.00 per cent while in low altitudinal village seed germination percentage and survival percentage within polyhouse were ranged from 17.00 per cent to 33.00 per cent and 20.50 per cent to 46.00 per cent respectively.

Mastana (2012) studied the propagation and harvesting of *Stevia rebaudiana* and effect of biofertilizers on seedling performance, vigour and growth regulators on rooting and growth of rooted cuttings. Findings revealed that IBA concentration 25×10^{-4} M formulated resulted in better induction of rooting by over 77.50 per cent as compared to control *i.e.* only 25.00 percent.

Dharmveer (2012) studied on cultivation practices of *Angelica glauca.* The effect of different soil media on seed germination, effect of spacing and fertilizers on growth and yield parameters. The results revealed that application of different soil media and field conditions had significant effects on seed germination. Spacing of 45×45 cm gave maximum dry root and essential oil content.

Farooqi *et al.* (1994) conducted an experiment to know the effect of IBA on *Rosa damascena* cuttings. They found the increasing trend of rooting percentage, number of roots per cutting, length of the longest root (cm), thickness of root (cm), fresh and dry weight of root with increasing concentration of IBA from 100 ppm to 300 ppm.

2.3 MEDICINAL USES AND CHEMICAL CONSTITUENTS

The roots of *Inula racemosa* are available as cut into pieces with diameter ranging from 0.4- 2.0 cm straight or slightly curved. Externally roots are grayish brown and internally yellowish brown in colour. The surface is rough due to longitudinally striations and cracks having an aromatic and camphoraceous odour (Jamna *et al*., 2012)

Study conducted by Kashman *et al.* (1967) and Purushothaman and Sarda. (1974) noted that the cultivated roots of *Inula racemosa* from Lahaul are superior to even the roots of the European species of *Inula helenium* or elecampane to find better market response.

Thymol derivatives from a root culture of *Inula helenium* were studied by Stojakowska *et al.* (2004) and reported that root culture of *I. helenium* was established from leaf explants of aseptic seedlings. An ethanol extract from the lyophilised roots was fractionated using different chromatogaraphic techniques. The main secondary metabolities found in the root culture were two thymol derivatives: 10-isobutyryloxy- 8, 9-epoxy-thymol isobutyrate and 10-isobutyryloxy-6-methoxy-8, 9-epoxy-thymol isobutyrate. The compounds were identified by spectral methods. Quantification of compound in plant material was done by analytical RP-HPLC.

Bokadia *et al.* (1986) assessed the chemical profile of roots of *Inula racemosa* and reported that the oil mainly contain sesquiterpenes (60%) wherein the heptadeca-1, 8, 11, 14-tetraene (aplotaxene-22%) was the most abundant constituent.

Kalsi *et al.* (1989) isolated the two new sesquiterpene lactones from the roots of *Inula racemosa* and identified them as alantodiene and isoalantodiene. Both lactones display biological activity as plant growth regulators. The sterostructures of these were determined by using spectral and chemical correlation.

Gholap and Kar (2005) reported to have hypoglycemic activity. Roots of *I. racemosa* are reported to contain sesquiterpene lactones, mainly alantolactones, isoalantolactone, alantolides, besides β- sitosterol, daucosterol. The essential oil from roots has a strong aroma and is reported to mainly contain sesquiterpenesaplotaxene.

A new epoxy alantolactone from *Inula racemosa* was extracted by Shah *et al.* (2009) in which sesquiterpine lactones are the chief constituents in the genus

Inula which possess antiseptic, expectorant, and diuretic properties. The other isolated compounds are Alkaloids, Diterpenoids, Seteroids and Terpenoids.

Cardioprotection evaluation by *Inula racemosa* in experimental model of myocardial ischemic injury was done by Ojha *et al*. (2010). Study clearly emphasizes the cardio protective effects of *Inula racemosa* and validates its traditional claims bracing the experimental and early reports which have demonstrated its usefulness in myocardial infarction. The study supports the notion that *Inula racemosa* consists of herbal origin β-blockers which may provide a future drug lead for cardiovascular diseases.

Zhang *et al.* (2010) extracted two new eudesmane-type sesquiterpene lactones isolated from the roots of *Inula racemosa* and their structures were elucidated as 3β-hydroxy-11α, 13-dihydroalantolactone (1) and 11α-hydroxy-eudesm-5-en-8β, 12-olide (2). Their cytotoxic activities against five human cancer cell lines had been tested and compound 2 exhibited weak cytotoxic activity against BEL-7402 and HCT-8 cell lines.

Burdi *et al*. (1990) reported fatty acids of *Inula grantoides*. The fatty acids were converted into methyl esters and identified by gas liquid chromatography- mass spectrometry as myristic, pentadecanoic, palmitic, margaric stearic, arachidiebehenic, tricosoic, lignoceric, hexadecanoic acid.

Chaturvedi *et al.* (1995) conducted comparative study of *Inula racemosa* and *Saussurea lappa* on the glucose level in albino rats. Results communicate that *Inula racemosa* reduces the blood sugar earlier as compared to *Saussurea lappa*. Maximum response in case of *Inula racemosa* was recorded between 2 to 4 hours after drug administration while for *Saussurea lappa*, it was 4 to 8 hours.

Jamna *et al*. (2012) evaluated comparative pharmacognostic study of *Inula racemosa* and its adulterant *Coffea tranvancorensis*. People in Kerala use the roots of *Pushkaramulla* instead *Pushkaramoola,* the former is an adulterant in order to ensure the use of only genuine and uniform material in preparation of herbal formulation, work on standardization was carried out. Macroscopial and

microscopial characteristics were worked out to identify the diagnostic features of the plant. Physico-chemical and ultra-violet analysis helped in the identification of genuine drug and also contributed towards establishing pharmacopeal standards.

Gnanasekaran (2012) carried out adaptogenic activity of siddha medicinal plant *Inula racemosa* roots. Forced swimming test (FST) is a screening model for antidepressants / adaptogens. Two swimming sessions were conducted: a 15 min pre-test followed 24 hr later by a 6 min test. The total duration of immobility behavior was recorded during the second 6 min test. Mouse was judged immobile, when it remained floating in water, in an upright position making only small movements to keep the head above water. The experimental animals were euthanized and their brains were removed immediately, and the prefrontal cortexes (PFC) were dissected out on ice for biochemical analysis. LD_{50} of the test drug was found to be greater than 2000mg/kg body weight. The animals treated with formulation of extract (100mg/kg) and (200mg/kg) showed significant decrease in the immobility period with simultaneous increase in anti-oxidant markers as well as adrenaline and serotonin levels which indicates positive adaptogenic activity of the extract *Inula racemosa (roots),* by forced swim test and resultant biochemical studies.

Pushkarmool commercially is a very important medicinal plant of the North western Himalayas. Traditionally, the plant is used in Ayurveda as an expectorant and resolvent in indurations. Considered a 'Rasayana' (rejuvenator, immunomodulator) by Ayurvedic physicians, the drug according to Bhavaprakasha is bitter pungent in taste. When administered it mitigates vatakaphajwara (fever caused by *vata pitta* imbalance), sotha (swelling), arachi (anorexia), swasa (breathlessness) and parswasoola (pain in the sides of the chest) (Bhavaprakasha, 1961).

Medicinally the powdered roots and dried foliage is used as anti-spasmodic, hypotensive and for treatment of cardiovascular and liver troubles. It is used for treatment of respiratory tract disorders, foul ulcers, and chronic

An overview of the methods used to assay chemical investigations of *Inula racemosa*

Methods to prepare essential oils	Assay methods	Isomers and their contents (%)	Reference (s)
Petroleum ether extract (with major constituents)	-	Alantolactone and Isolantolactone 5.7-6.2	Raghavan *et al*., 1969; Srivastava *et al*., 1971
Steam distillation	-	Steam volatile essential oil (1.3-2.6)	Singh *et al*., 1959; Anonymous, 1959
Soxhlet extraction (Column chromatography on Silica gel)	-	Alantolactone, isoalantolactone and terpenoids	Mehra *et al*., 1967
Steam distillation	GC-MS	Sesquiterpenes (60); heptadeca-1,8, 11,14-tetraene aplotaxene (22); Phenylacetonitrile (2) Lactones (83%)	Bokadia *et al*., 1986
EIMS: HS-ZAB mass spectrometer		Lignans and Sesquiterpene lactones	Tan *et al*., 1998
Simple Clevenger apparatus (Water distillation)	-	Essential oil (0.04)	Jabeen *et al*., 2007
Spectroscopic methods (Single-crystal X-ray diffraction analysis)	-	Nine sesquiterpeniods	Li-Wei-Xu & Yan-Ping Shi, 2011
Liquid chromatography (RP-HPLC) Resolution 2.2	-	Isoalantolactone and alantolactone	Sharma *et al*., 2011
Steam distillation combined with ether extraction	GC-MS	Elemene (26.77)	Yang *et al*., 2008 ; Wang *et al*., 2012
Steam distillation combined with ether extraction (IR,Spectrophotometer ; Column chromatography	GC-MS	Two new tri-nor-eudesmanolides: 8-oxo-tri-nor-eudesm-6-en—5α-ol and tri-nor-eudesm-5-en-7B,8β-diol	Zhang *et al*., 2013
Steam distillation	GC-MS	Aqueous root extract	Arumugam and Murugan , 2013
Soxhlet extraction (Column chromatography)	-	Alantolactone and Isoalantolactone	Kataria and Chahal, 2013

Chemical constituents of *Inula racemosa* and their medicinal properties

Chemical Constituents of essential oils (%)	Medicinal Properties	Reference(s)
Inulin (10) & roylene (3) (carbohydrates)	Decreases blood pressure & stimulates peristaltic movements of intestine	Wani *et al*., 2006; Anonymous 1959
Major constituents Sesquiterpene lactones (Alantolactone & Isoalantolactone); Sesquiterpenes (60), aplotaxene (22) & phenylacetonitrile (2)	Healing properties, cytotoxic activity against the K562 human leukemia cell line, Possesses strong antifungal, anthelmintic and hypolipidemic properties more potent and less toxic than *santonin*. Alantolactone kills *Ascaris* in 16 hr; antiseptic, expectorant, diuretic; a ringworm fungicide; and found to be beneficial in histamine induced bronchospasm.	Zhang *et al.,* 2010; Sharma *et al*., 2011
Minor-constituents Dihydroalantolactone, Inunolid, alkaloids, tannins and sugars.	Anthelmentic,andhypolipidemic properties; antiseptic, expectorant, diuretic & kills *Ascaris* in 16 hours, anti-inflammatory in animals to stimulate the immune system	Singh *et al*., 1980

Traditional use of *Inula racemoca* reported from India and other countries

Plant part	Uses	Reference(s)
Rhizomes and roots	Used as anthelmintic for children,antiasthmatic, antiseptic, anti-inflammatory & diuretic agents and digestive properties in India and Tibetin	Sharma *et al*., 2006
Pounded roots	Treatment of rheumatism, hypertension, cardiovascular and liver disease respiratory tract disorder, pulmonary infections, skin diseases, gastrointestinal disorders, fever and pain. Extract prepared from roots is frequently used for diarrhea in children and abdominal pain, dosage 0.5-1 ml once in day in alternate days till cure. For boils paste prepared from root powder is applied twice a day for seven days. Dried roots are chopped and boiled in water at low temperature till water turns brownish – red.One spoon of the decoction is taken daily as cure for boils.	Gholap and Kar, 2005; Rawat and Everson, 2011 Liu *et al*., 2001; Lal and Singh, 2008 ; Malik *et al*., 2011
Seeds	Aphrodisiac	
Veterinary medicine	Tonic and stomachic	
Flowers	Flowers used as offerings to various deities in religious ceremonies,	
Leaves and stems	Leaves and stem fodder and fuelwood	

Production and marketing profile of *Inula racemosa* Hook.f.

Source of supply	Cultivated and forests	Anonymous, 2002;Anonymous 2000
Demand 1999-2000	375.9 tonnes	
Demand 2004-2005	757.4 tonnes	
Average growth rate of demand	15.1% per annum	
Manufacturesrs purchase price	Rs 40 per kg	
Market demand 2006-07	3 tonnes per annum	
Production 1 hectare cropped area	80 qntls dry roots.	
	75-80 quintals fresh roots 25-30 quintal dry roots	Sepat *et al*., 2012
Estimated cost of cultivation /hectare	Rs 37 800	Anonymous, 2008
	Rs 36,000	Sepat *et al*., 2012
Root and fodder yield/ hectare	4260 kg and 1600 kg/hectare	Rawat and Everson, 2011
Market rate	Rs 175-200 per kg dried	Anonymous, 2013
Net profit per year	Rs 1,10,000 per year	Sepat *et al*., 2012

bronchitis and as an antiseptic. The roots collected for usage as an aromatic source. The dry are as an insect and pest repellants. Medicinally the powdered roots and dried foliage is used as an anti-spasmodic, hypertensive and for treatment of cardiovascular and liver troubles. It is used for treatment of respiratory tract disorders, foul ulcers, and chronic bronchitis and as an antiseptic (Anonymous, 1959).

The roots of *Inula racemosa* were widely used as an expectorant and as a home remedy for boils & skin infections. The fragrant roots which have a sharp pungent taste were priced as Rasayana by ancient Ayurvedic physicians

The root oil contains alantolactone which is used as anathematic and antiseptic. Roots contain inulin (10.1%) and roylene 3%. It decreases the blood pressure and stimulates peristaltic movements of intestine (Anonymous, 2001; Wani *et al*., 2006). The roots of *Inula racemosa* were widely used as an expectorant and as a home remedy for boils & skin infection.

Kumar *et al*. (2009) explore an ethnobotanical study of medicinal plants used by the locals in Kishtwar, Jammu and Kashmir, India. Study reveals that root oil of *Inula royleana* is mixed with kuth oil-root oil of *Saussurea lappa*. It produces fall in blood pressure and stimulates peristaltic movements of intestine.

2.3.1 Medicinal properties and Uses

The genus *Inula* (Asteraceae) is known for diverse biological activities viz., anticancer, antibacterial, hepatoprotactive, cytotoxic, and anti-inflammatory properties (Ali *et al*., 1992).

Inula racemosa find use in Indian System of Medicine for cardiac asthama cough, pulmonary infections and skin diseases and as adultrant for *Saussurea costus* (Sarin *et al.,* 1996)

The herb can be used both internally, as well as externally. The roots of *Inula* species are used for the medicinal purpose. Externally, the paste of its roots is used effectively in dressing the wounds and ulcers as the herb possesses antiseptic property. It also has therapeutic benefits in cardio respiratory and cardiovascular diseases (Patel *et al.,*1982). In Ayurvedic, Chinese and Mediterranean traditional system of medicine, *Inula* species are used in angina pain (Zhang *et al*., 2013). Ability of *Inula racemosa*, as β- blockers and antioxidant has generated interest to explore it as a cardio protective agent (Tripathi *et al.*, 1988)

Pushkaramool is one of the herbs mentioned in all Ayurvedic scriptures (Jamna, 2012. It possesses various synonyms like *kasari* an enemy of cough, *sulahara* pain killer, svasari an enemy of breathlessness. The great sage *Charaka* has categorized it as *hikkanigrahana*- stops hiccup and he has also cited it as the best medicament for pleurisy along with cough and asthama (Anonymous, 2004). Roots are bitter, acrid, thermogenic, cardiotonic, expectorant, alexipharmic, anodyne, anti-inflammatory, digestive, carminative, aphrodisiac, febrifuge and tonic (Ojha, 2010; Anonymous, 2004).

Inula racemosa is the highly praised panacea for cough, hiccup and bronchial asthama. It reconciles the pulmonary functions by abolishing the bronchospasm, relieving the mucous and hence, the obstruction in bronchial asthma. The herb restrains the itching sensation and oozing in the skin diseases and thus facilitates the wound healing. It is pacifying to the brain and helps in strengthening it in mental debility. The herb also accords a stimulant action to genital system in both the sexes. In males it works well as an aphrodisiac and in females, it augments the quantity of menstrual bleeding. Thus, it finds use both, in amenorrhea as well as dysmenorrhoea. It possesses a mild diuretic property hence, is used with benefit in dysuria (Zawar, 2011; Prajapati *et al.,* 2007).

Medicinally the powdered roots and dried foliage is used as anti-spasmodic, hypotensive and for treatment of cardiovascular and liver troubles. It is used for treatment of respiratory tract disorders, foul ulcers, and chronic bronchitis and as an antiseptic. The roots collected for usage as an aromatic source. The dry are as an insect and pest repellants. Also decreases the blood pressure and stimulates peristaltic movements of intestine (Anonymous, 2001; Wani *et al*., 2006).

Chapter-3

MATERIALS AND METHODS

The present investigations entitled "**Evaluation of germplasm and standardization of propagation techniques of *Inula racemosa* Hook. f."** were conducted at Research Centre Manali, Medicinal & Aromatic Plant Research Farm Shilly, Solan and Herbal garden UHF, Nauni, Department of Forest Products, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh during the year 2010-11 to 2011-12.

The details regarding Germplasm collection sites, methodology adopted for evaluation during the course of investigations are described as below:

3.1 Germplasm collection

3.2 Experimental sites

3.3 Experiment methodology

3.4 Statistical Analysis

3.1 GERMPLASM COLLECTION

The germplasm of *Inula racemosa* which included whole plant, seeds and roots were collected from different eight sites of domestic population from Himachal Pradesh and Jammu & Kashmir and geographical data was recorded with the help of GPS at individual site. Geographical features of selected sites for the collection of *Inula racemosa* are tabulated as below:

Geographical features of germplasms collected from different sites

Germplasm (site Code)	Collection material	Altitude (m-amsll)	Latitude	Longitude
G_1	**Keylong, HP**	3350	32°58′ 14.07" N	77°04′28.34" E
G_2	**Kardang , HP**	3550	32˚34' 17.18" N	77°04′28.34" E
G_3	**Dalang , HP**	3300	32˚40' 31.64" N	77° 00′22.99" E
G_4	**Sissu, HP**	3350	32°48 '28.70" N	77°11' 28.00" E
G_5	**Udaipur, HP**	3417	32° 59' 28.33" N	76˚39' 54.41" E
G_6	**Kukumseri HP**	3116	32˚42'19.55" N	76˚41' 23. 66" E
G_7	**Tangmerg , J&K**	2690	34˚02'39.21" N	74˚25' 29. 06" E
G_8	**Shopian , J&K**	2146	33˚42'31.96" N	74˚49' 29. 06" E

3.2 EXPERIMENTAL SITE

3.2.1 Location

The field experiments were conducted at two different experimental farms located Shilly (Solan) (1480 m amsl N 30°54'30" and 77°07'30"E) and Manali (Kullu) (1905 m above msl 32°15'30" N 77°10'35" E), Department of Forest Products and Regional Horticultural Research Station, Bajaura, Kullu (HP), respectively of Dr Y S Parmar University of Horticulture & Forestry,), and India.

3.2.2 Climate

The climate, in general is sub-temperate to temperate characterized by mild summer and relatively cool and dry winter. The average annual rainfall ranges between 800-1300 mm. The normal monsoon rain starts from the month of June and continues upto the month of September with pre-monsoon showers starting from mid-May. The meteorological data pertaining to the period of investigations has been are presented in Appendix-I.

3.3 EXPERIMENTAL METHODOLOGY

Survey of the conducted eight selected sites for germplasm collection was conducted and following observations were recorded as per Lawrence (1951) .and material was collected with proper identification and observations were taken. Various qualitative parameters pertaining to type of roots, stems and leaf were studied on the basis of the morphological features of plants as under:-

3.3.1 Observations recorded

a) Plant Height (cm)

Plant height of this species as measured from ground level to the tip of the fully grown ten representative plants at the ends of flowering stage and expressed in centimeters. Randomly ten plants were selected and the highest tip of the plant at the end of flowering stage was measured in terms of mean.

c) Number of stems

Number of stems per plant was counted and average value was taken by randomly selected ten plant samples.

Plate 1. Production and Cultivation; Medicinal and Aromatic plants Growers Society Keylong (Lahaul & Spiti)

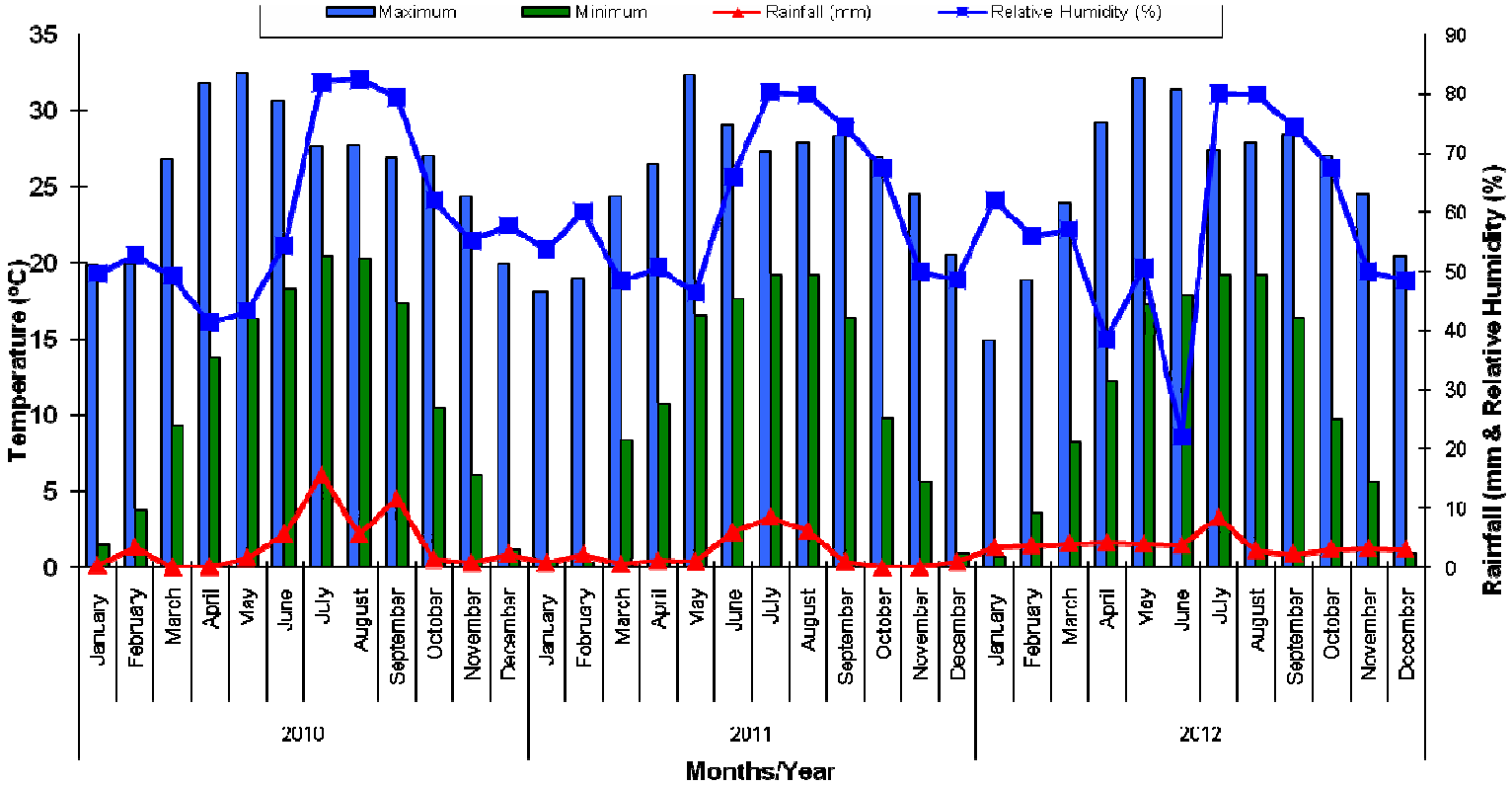


Fig. 1. Agro-meteorological data observed during 2010, 2011 and 2012 at Nauni-Solan (H.P.)

d) Leaf size (cm)

Length and breadth of leaves was taken in centimeters individualy per plant. Data regarding length (including petiole) and breadth (at widest point) of leaf was recorded in centimeters and has been reported as mean.

e) Number flower heads per plant

Number of flower heads per plant by ten randomly selected samples was counted.

f) **Root length (cm)**

Root length from point of emergence to the terminal point of the longest root was taken. The observations were recorded in centimeter of ten randomly selected samples and have been reported as mean.

g) Fresh root weight (g)

Fresh root was taken from randomly ten selected plants from each site and reported as mean in grams.

h) Essential oil (%)

250 grams of dry roots of *Inula racemosa* from each site was taken in 250 ml round bottom flask and 100 ml of water was added to it. The flask was fixed to Clevenger's Apparatus and was heated at 100° C until agitation commenced. Then temperature was lowered down to 70° C till the end of process. This process requires 5 hours for completion extraction of oil. The oil (%) was calculated as follows:

$$\text{Oil (\%)} = \frac{Y}{X} \times 100$$

Where, X = weight of sample used

Y = Number of unit of oil (ml)

Amount of oil = Y × 0.1ml

3.3.2 Seed characteristics of *Inula racemosa* seeds collected

About five hundred grams of ripened fruits were collected from *Inula racemosa* plants from eight different sites during August-September. Seeds were extracted from flowers and pressed in sealed polybags, washed in water, shade dried and seeds were stored by sealing in perforated polythene bag under refrigerated conditions (4±1°C). To study seed characteristics, four replications from each site were taken and each replication consisted of 50 seeds.

i) Test weight (g)

The weight of 100 seeds from each sample was taken and 1000-seed weight was calculated accordingly.

ii) Moisture percent

Seed moisture content was expressed on a wet basis and seed moisture is removed in oven till constant weight is achieved (Agrawal. 1987).

$$M = \frac{M_2 - M_3}{M_2 - M_1} \times 100 = \frac{\text{Loss in weight}}{\text{Initial weight of seed}} \times 100$$

Where

M Seed moisture content

M_1 Weight of the empty container with its cover

M_2 Weight of the container with its cover and seeds before drying

M_3 Weight of the container with its cover and seeds after drying

iii) Seed viability (%)

Seed viability was determined using Tetrazolium-test. A set of fifty seeds were placed on a moist filter paper for 24 hrs and then longitudinally sectioned to expose embryos. The sections were then incubated in dark in 1% aqueous solution of 2,3,5 triphenyl tetrazolium chloride for 24 hrs. Seeds showing strong stained embryos were considered viable.

Plate 2. Cultivated population of *Inula racemosa* from Pattan Valley of Lahaul-Spiti

Plate 3. Mature roots harvested from cultivated population *Inula racemosa*

Plate 4. Cultivated population of *Inula racemosa* from Kashmir Valley of Jammu and Kashmir

iv) Germination percent

Germination percent was calculated as the number of seeds sown and number of seeds germinated and expressed in percentage.

3.3.3 Pre sowing treatments on seeds germination in *Inula racemosa* in laboratory and nursery condition. The details of treatments are given in table as follows

Seeds collected from eight different sites were combined to form composite sample to study different parameters under laboratory and nursery conditions. The seeds were treated with Bavistine (0.1%) for half an hour as prophylactic treatment:

3.2 DETAILS OF TREATMENTS FOR PRE-SOWING TREATMENTS

i) Germination Studies

$$\text{Germination \%} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds kept for germination}} \times 100$$

P_1	Control
P_2	Hot Water (40°)
P_3	GA_3 50 ppm
P_4	GA_3 100 ppm
P_5	GA_3 150 ppm
P_6	IBA 50 ppm
P_7	IBA 100 ppm
P_8	IBA 150 ppm
Pre- sowing duration	
Hot Water (40°)	5 minutes
GA_3 (3 treatments)	24 hours
IBA (3 treatments)	24 hours
Replications	3
Total Pre-sowing treatments	8
Design	CRD

Preparation of GA_3, IBA solution

For making solution of 50ppm, 100ppm and 150ppm of GA3 and IBA, the stock solutions of 1000 ppm of GA_3 and IBA were first prepared separately by dissolving 1g of GA_3 and IBA thoroughly, in minimum volume of absolute

alcohol and then double distilled water was added to make 1 litre stock solution in each case. After this, 5ml, 10ml and 15ml of stock solution was diluted to 100 ml by adding double distilled water to make 50 ppm, 100ppm and 150 ppm of above solution respectively.

A) Laboratory Studies

Three replications having 50 seeds each from composite seed sample of *Inula racemosa* were given eight pre-sowing treatments as mentioned above. Seed germination studies were carried out in laboratory conditions in Petri plates with double layer filter papers as base at 25± 2 ˚C 85 percent relative humidity. Germinations parameters were recorded for a period of twenty eight days.

B) Nursery Studies

The pretreated *Inula racemosa* seeds as mentioned above were sown manually in RBD with three replications each containing 50 seeds at 10 cm × 5cm spacing in sunken nursery beds measuring 3m × 2m.

Beds were mulched with dry grass, irrigation was applied daily till germination was completed and thereafter, weekly irrigation was carried out till the commencement of rainy season. Weeding and hoeing were done as and when required.

3.3 OBSERVATIONS RECORDED

(a) Laboratory studies

The following germination observations were recorded under laboratory conditions.

i) Germination (%)

Germination percent was calculated as the number of seeds sown and number of seeds germinated, and expressed in percentage.

Plate 5. Germplasm of *Inula racemosa* from Kashmir Valley (J&K)

ii) Germination energy (%)

Germination energy (GE) was calculated on the basis of the percentage of the total number of seeds that had germinated when the germination reached its peak (generally taken upto the day when highest number of germination took place in 24 hours period).

$$GE\ (\%) = \frac{\text{Number of seeds germinated upto time of peak germination}}{\text{Total number of seeds sown}} \times 100$$

iii) Germination speed

Germination speed (GS) was determined by the method prescribed by Maguire (1962).

Germination speed = (n/t)

Where,

n = Number of seed newly germinating at time

t = Number of days from sowing

iv) Mean daily Germination

Mean daily germination or Daily germination speed (MDG or DGS)

Mean daily germination was calculated as the cumulative germination percentage of seeds at the end of the test divided by the number of days from sowing to the end of the test or the total per cent germination divided by total days in the test gives the final mean daily germination

V) Peak Value

Peak value was calculated as the maximum mean daily germination (MDG) reached at any time during the period of test* (Czabator, 1962; Shawl *et al*., 2007).

VI) Germination Value

Germination value (GV) is the index combining speed and completeness of seed germination. Daily germination counts were recorded and calculated as per (Czabator (1962; Shawl *et al*., 2007).

Germination value	=	PV x MDG
Where, PV	=	Peak value of germination
MDG	=	Mean daily germination

VII) Germination index

Germination index was calculated by dividing the total number of seeds germinated at the end of the experiment with the time taken for 50 percent germination.

VIII) Seedling height (cm)

Seedling height was recorded in centimeters from collar to the tip of stem.

IX) Collar diameter (cm)

Collar diameter of the seedling was measured using electronic vernier caliper and recorded in cms.

X) Root length (cm)

The length of tap root was recorded in centimeters using measuring scale by placing it horizontally on the ground.

XI) Root and shoot weight (g)

The seedlings were washed with water. Excess of water was wiped out by placing it between the folds of filter paper. Then the seedlings were cut at collar with a secateur and root and shoot weights were taken as fresh and dry after drying to constant weight in an oven at 70°C± and expressed in grams.

XIV) Seedling vigour index

It was calculated by multiplying germination per cent with length of each seedling separately as given by Abdul-Baki and Anderson, (1973).

SVI = Germination per cent **x** Seedling height

Plate 6. Roots of *Inula racemosa* collected from Lahul and Spiti Valley of Himachal Pradesh

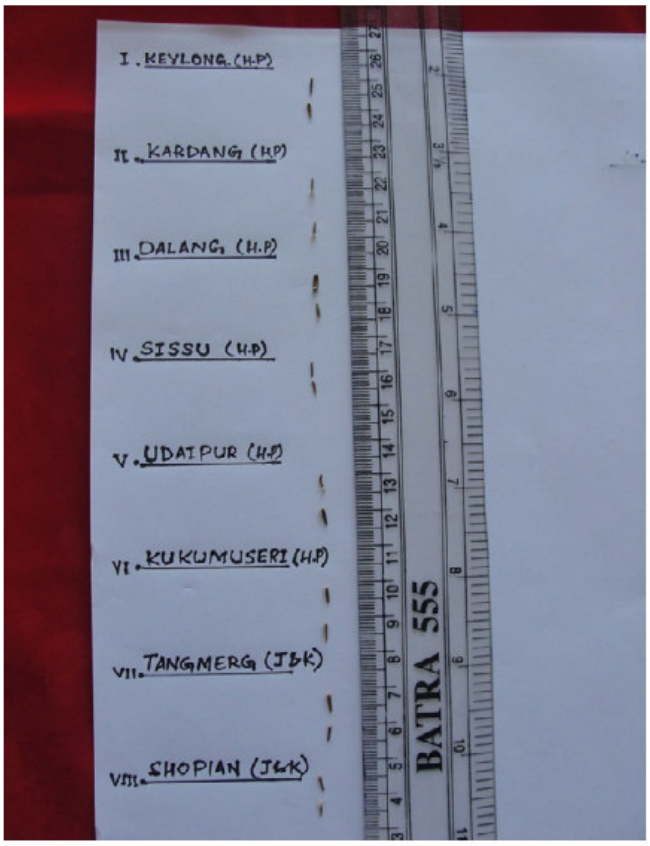


Plate 7. Seeds and collar bud used for propagation studies

Experiment 4: To study the effect of location site and germplasm collection sites on germination and growth of *Inula racemosa*

Number of germplasm sites	Cutting length	Spacing	Replication	Experimental Sites	Time period	Design
8	2.5 cm (collar bud)	60x65 cm	3	2	1 year	RBD factorial

Observations recorded:

a) Sprouting percent

Number of root cuttings that sprouted after planting were counted and percentage was calculated.

b) Number of shoots

Numbers of shoots were recorded at fully mature plant by visual scoring.

c) Number of leaves per plant

Number of leaves was recorded at flower initiation stage by visual scoring.

d) Number of flower heads per plant

Number of flower heads per plant was recorded at full bloom stage by visual scoring.

e) Number of seeds per flower head

Number of seeds per flower head was recorded at the time of harvesting and randomly selected plants per replication were recorded.

f) Length of main root (cm)

Length of main root was recorded on fully developed roots at the time of harvest randomly selected and taken as average per site collection.

g) Number of lateral roots

Number of lateral roots were recorded from fully developed roots and taken as average per site collection.

h) Root fresh weight (g)

Five mature and fully developed plants per replication were randomly selected for root fresh weight and data was recorded as mean.

i) Root dry weight (g)

The roots were dried at 80° C±1 in paper bags in an oven till constant weight was recorded.

STATISTICAL ANALYSIS

The entire data generated from the present investigations were subjected to statistical analysis as per methods described by Gomez and Gomez (1984). The least significant difference at 5 per cent level was used for testing the significant differences among treatments. The ASSEX software was used for statistical analysis. According, RBD, CRD and RBD factorial designs were employed for individual experiment as per technical programme during (2010-11 and 2011-12) the present investigations of study.

Chapter-4

EXPERIMENTAL RESULTS

The results emerged out of the present investigations entitled "**Evaluation of germplasm and standardization of propagation techniques of *Inula racemosa* Hook.f."** carried out at the experimental fields and in the laboratory of Department of Forest Products, Dr Y.S. Parmar University of Horticulture & Forestry, Nauni, Solan Himachal Pradesh during 2010-11 and 2011-12 are presented in this chapter under the following headings.

4.1 EXPERIMENT-I : Evaluation of *Inula racemosa* germplasm collected from different sites

4.1.1 Germplasm collection

The germplasm of *Inula racemosa* was collected from eight different cultivated places which include six from Himachal Pradesh and two from Jammu & Kashmir. In Himachal Pradesh three valleys were selected from Lahaul & Spiti district *i.e.* Ghar valley, Tinnan valley and Pattan valley. Out of these three selected valleys two germplasm collections each *i.e.* Keylong & Kardang (Ghar valley), Sissu & Dalang (Tinnan valley), Udaipur & Kukumseri (Pattan valley) were selected for germplasm evaluation. Similarly, two germplasm collections were selected from Jammu & Kashmir *i.e.* Tangmerg (Baramulla) and Shopian district.

4.1.2 Morphological and quantitative characteristics

The morphological characteristics of *Inula racemosa* collected from eight different germplasm collections during the year 2010-11 has been presented in Table (1)**.** The morphological characteristics studied included: plant height, number of stems per plant, leaf size, number of flower heads per plant, primary root length and root weight. Besides essential oil content (%) was also estimated.

4.1.2.1 Plant height (cm)

The studied conducted on this parameter revealed that the plant height was significantly affected by germplasm collections (Table 1). Maximum plant height

(204.90 cm) was observed in G_6 (Kukumseri, HP) which differed significantly from all other germplasm collections and minimum (106.30 cm) was recorded in G_4 (Sissu).

4.1.2.2 Number of stems per plant

The number of stems per plant differed significantly among different germplasm collections. The maximum number of stems per plant (4.74) was observed in site G_6 (Kukumseri, HP) and was found to be statistically different from all others. The lowest number of stems (2.25) per plant was found in G_3 (Dalang, HP) which was significantly found to be at par with G_2 (2.26).

4.1.2.3 Leaf length (cm)

The data registered for eight different germplasm collections revealed that the maximum leaf length (54.15 cm) was observed for collection G_5 (Udaipur, HP) which was statistically found to be at par with germplasm collection G_6 (53.76 cm). The minimum leaf length (24.24 cm) was observed for site G_3 (Dalang, HP), which differed significantly from all other collections.

4.1.2.4 Leaf Breadth (cm)

The significant difference was observed among different germplasm collections for leaf breadth (Table -1). The maximum leaf breadth (24.85 cm) was observed from germplasm collection G_6 (Kukumseri, HP), which differed significantly from all other collections. The minimum leaf breadth (10.82 cm) was observed from collection G_8 (Shopian, J&K) which was statistically found to be at par with germplasm collection G_7 (Tangmerg, J&K).

4.1.2.5 Number of flower heads per plant

The data pertaining to the number of flower heads per plant from different germplasm collections showed that maximum (22.15) number of flower heads per plant were observed for G_6 (Kukumseri, HP) which differed significantly from all others. The minimum (6.51) number of flower heads per plant were observed

in G_8 (Shopian, J&K) which was found to be statistically at par with G_7 (Tangmerg, J&K).

4.1.2.6 Primary root length (cm)

The data for primary root length of *Inula racemosa* revealed that the maximum primary root length (22.82 cm) was recorded from germplasm collection G_6 (Kukumseri, HP), which was statistically at par with G_2 (18.63 cm) and G_5 (17.52 cm). The minimum primary root length (11.72 cm) was recorded from G_8 (Shopian, J&K) which was statistically similar with germplasm collection sites G_1 (12.33 cm), G_3 (15.43 cm), G_4 (17.0 cm) and G_7 (16.29).

4.1.2.7 Fresh root weight (g)

The root fresh weight of different germplasm collections were found to be significant at 5% level of significance (Table-1). The maximum fresh root weight (659.30 g) was recorded from collection G_5 (Udaipur, HP) which was found to be statistically at par with germplasm collection G_6 (Kukumseri, HP) with a value of 636.50 g. The minimum fresh root weight (364.60 g) was observed from germplasm collection site G_7 (Tangmerg, J&K) which was found to be statistically at par with sites, G_1(432.50 g, HP), G_2(430.10 g), G_4 (418.50 g) and G_8 (370.20 g) *i.e.*(Keylong, HP), (Kardang, HP), (Sissu, HP) and (Shopian, J&K).

4.1.2.8 Essential oil content (%)

The essential oil content showed a significant difference among the germplasm collections sites. The maximum essential oil content of 1.96 per cent was observed from collection G_6 (Kukumseri, HP) followed by G_5 (Udaipur, HP) which differed significantly from each other. The minimum value of 1.82 per cent essential oil was obtained from G_8 (Shopian, J&K) and was found to be statistically at par with G_1 (Keylong, HP) and G_7 (Tangmerg, J&K) having values of 1.83 and 1.84 per cent respectively.

Table 1: Morphological and quantitative characteristics of *Inula racemosa* germplasm

Germplasm	Plant height (cm)	Number of Stems	Leaf length (cm)	Leaf Breadth (cm)	Flower heads per plant	Primary root length (cm)	Fresh root weight (g)	Essential oil content (%)
G_1 (Keylong, HP)	165.40	2.52	43.99	12.34	14.50	12.33	432.50	1.83
G_2 (Kardang , HP)	172.70	2.26	44.66	12.52	14.73	18.63	430.10	1.87
G_3 (Dalang , HP)	115.90	2.25	24.24	17.37	10.74	15.43	462.50	1.86
G_4 (Sissu, HP)	106.30	2.62	42.07	13.23	12.68	17.06	418.50	1.85
G_5 (Udaipur, HP)	195.50	4.23	54.15	18.64	14.44	17.52	659.30	1.93
G_6 (Kukumseri HP)	204.90	4.74	53.76	24.85	22.15	22.82	636.50	1.96
G_7 (Tangmerg , J&K)	144.70	2.40	47.23	11.29	7.26	16.29	364.60	1.84
G_8 (Shopian , J&K)	155.10	3.05	45.62	10.82	6.51	11.72	370.20	1.82
Mean	**157.56**	**2.73**	**44.46**	**15.13**	**12.88**	**16.48**	**471.78**	**1.87**
SEm±	**0.50**	**0.07**	**0.43**	**0.36**	**0.73**	**2.68**	**44.62**	**0.011**
$CD_{0.05}$	**1.04**	**0.14**	**0.91**	**0.75**	**1.52**	**5.59**	**92.79**	**0.023**

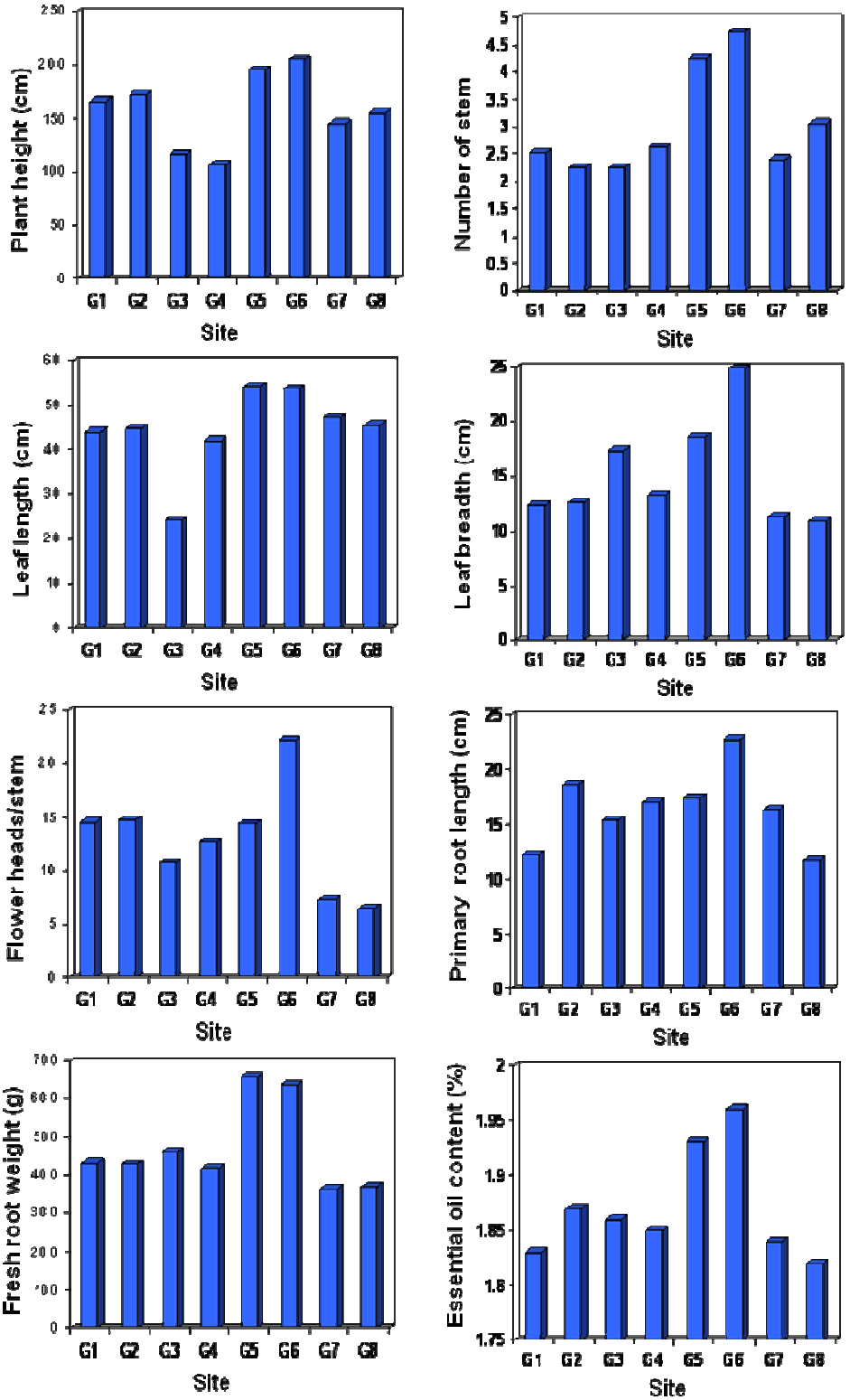


Fig. 2. Morphological and quantitative characteristics of different germplasm sites

4.2 EXPERIMENT-II : Seed characteristics and germination parameters of *Inula rcaemosa* germplasm

4.2.1 Seed characteristics

Data pertaining to seed characteristics and germination parameters of *Inula racemosa* collected from different germplasm collections during 2010-11 and 2011-12 is presented in Table 2.

i) **Fresh Seed Weight (g)** : Data for all the germplasm collections varied significantly for fresh seed weight for both the years of study (Table 2)**.** During 2010-11 maximum (1.49 g) fresh seed weight was observed from germplasm collection G_6 (Kukumseri, HP) and was found to be statistically different from all other collections. The minimum fresh seed weight (1.15 g) was recorded in G_7 (Tangmerg, J&K) and was statistically at par with G_8 (Shopian, J&K).

The maximum fresh seed weight (1.43 g) during 2011-12 was also recorded from germplasm collection G_6 (Kukumseri, HP) and was significantly found to be different from all other germplasm collections. The minimum fresh seed weight of (1.13 g) was observed from germplasm collection G_8 (Shopian, J&K) and was found to be statistically at par with germplasm collection sites G_1 (Keylong, HP) and G_2 (Kardang, HP).

ii) **Moisture Content (%):** The data pertaining to moisture content of *Inula racemosa* seed is presented in Table 2. During the year 2010-11 maximum value of 24.93 per cent was recorded in G_6 (Kukumseri, HP). The minimum moisture content (17.72 %) was observed from germplasm collection site G_8 (Shopian, J&K) which was found to be statistically at par with germplasm collection G_7 (Tangmerg, J&K) with value of 17.95 per cent seed moisture content.

Table 2: Fresh Seed weight, Moisture content, Seed viability and Germination percent of *Inula racemosa* germplasm

Germplasm	Fresh Seed Weight (g)		Moisture Content (%)		Seed Viability (%)		Germination (%)	
	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12
G_1 (Keylong, HP)	1.18	1.14	18.76	18.25	77.30 (61.55)	77.48 (61.67)	66.03 (54.35)	66.02 (54.35)
G_1 (Kardang , HP)	1.17	1.14	18.47	18.22	75.75 (60.50)	76.78 (61.20)	64.93 (53.69)	76.95 (59.65)
G_3 (Dalang , HP)	1.23	1.22	18.23	18.17	77.23 (61.50)	76.04 (60.69)	66.23 (54.47)	72.62 (55.76)
G_4 (Sissu, HP)	1.22	1.27	21.67	22.42	76.01 (60.69)	74.51 (59.68)	64.97 (53.71)	87.51 (67.79)
G_5 (Udaipur, HP)	1.37	1.35	24.16	21.63	84.52 (66.83)	84.04 (66.46)	74.26 (59.52)	87.93 (69.50)
G_6 (Kukumseri HP)	1.49	1.43	24.93	24.85	87.20 (69.04)	86.16 (68.16)	77.51 (61.70)	77.50 (61.69)
G_7 (Tangmerg , J&K)	1.15	1.16	17.95	17.73	67.54 (55.27)	67.52 (55.26)	67.53 (55.27)	65.41 (54.16)
G_8 (Shopian , J&K)	1.16	1.13	17.72	17.62	68.32 (55.75)	68.24 (55.70)	63.66 (52.93)	62.24 (53.30)
Mean	**1.25**	**1.23**	**20.24**	**19.86**	**76.73(61.39)**	**76.35 (61.10)**	**68.14 (55.71)**	**74.52 (59.25)**
SEm±	**0.006**	**0.0043**	**0.12**	**0.099**	**0.29**	**0.099**	**0.007**	**0.013**
$CD_{0.05}$	**0.012**	**0.0089**	**0.25**	**0.26**	**0.60**	**0.208**	**0.015**	**0.027**

* Figures in parentheses are arc sine transformed values

Plate 8. Effect of different pre-sowing treatments on seed germination

A) IBA 150 ppm B) IBA 50 ppm

C) Hot Water D) Control

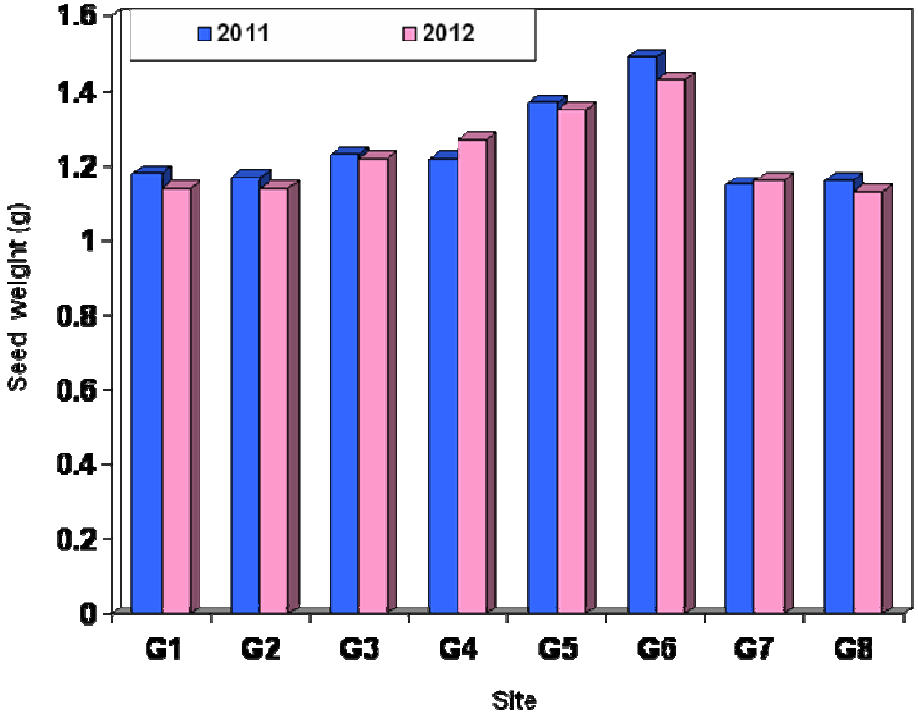


Fig. 3. Fresh Seed weight of *Inula racemosa*.

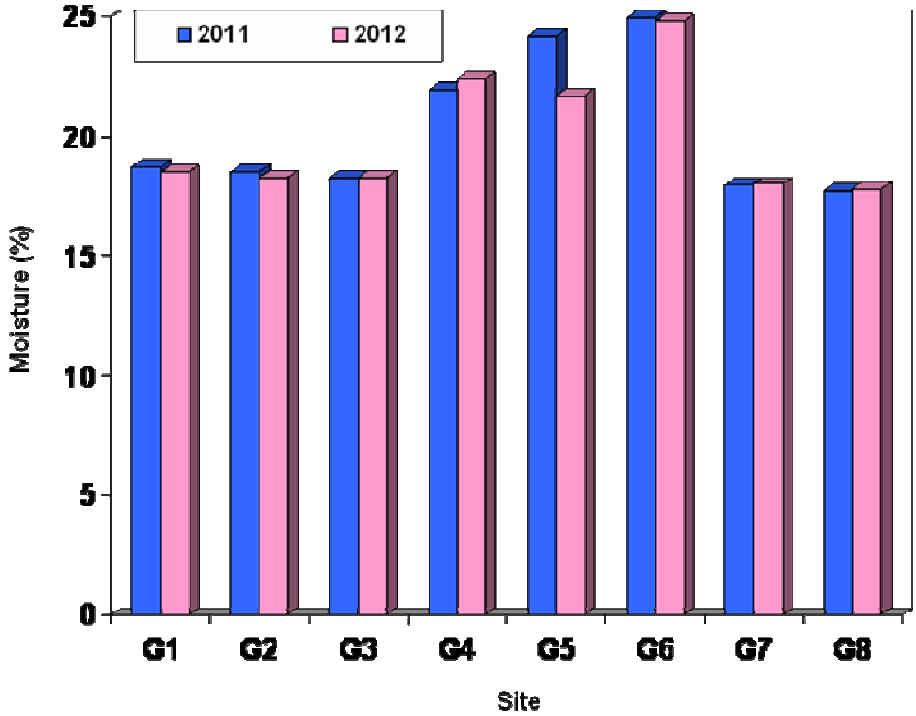


Fig. 4. Fresh Moisture (%) of *Inula racemosa*.

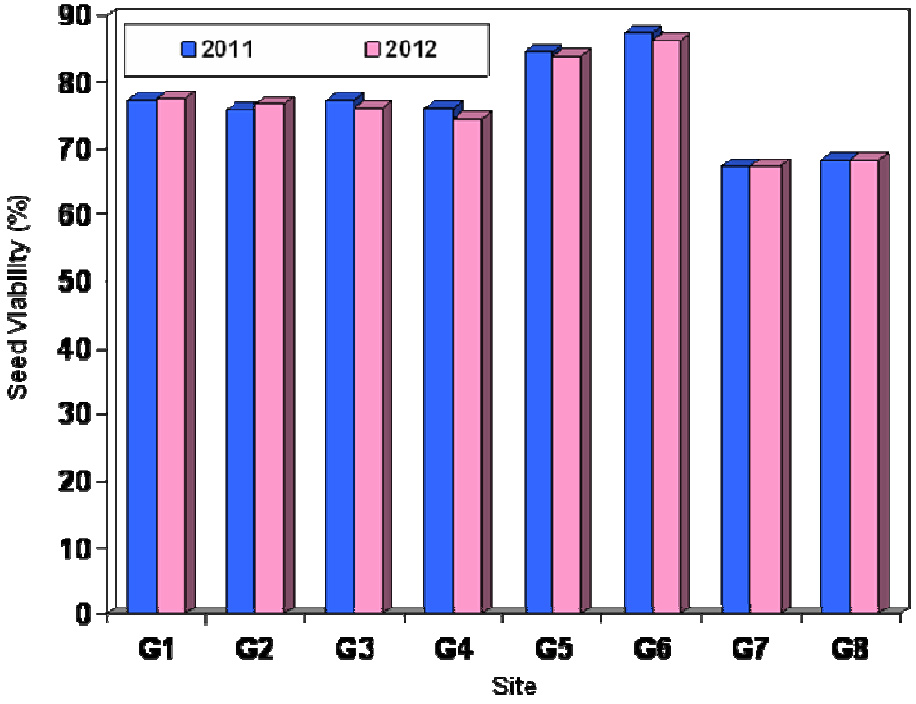


Fig. 5. Fresh Seed viability (%) of *inula racemosa*

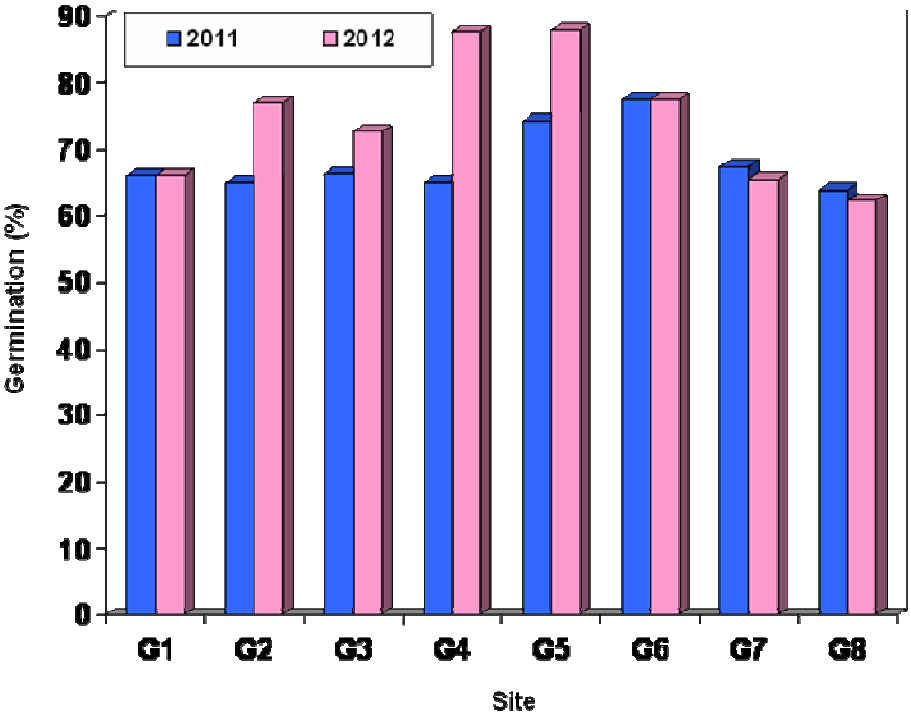


Fig. 6. Fresh Germination (%) of *inula racemosa*

The maximum moisture content (24.85 %) during 2011-12 was also observed from germplasm collection G_6 (Kukumseri, HP) and was found to be statistically different from all others. The minimum moisture content (17.62 %) was recorded from germplasm collection G_8 (Shopian J&K) and was found to be statistically at par with G_7 (Tangmerg, J&K).

iii) Seed Viability per cent (%): It was evident from the data in given in Table 2 that seed collected from different germplasm collections depicted significant affect on seed viability of *Inula racemosa*. The significantly highest seed viability of 87.20 (69.04) per cent during 2010-11 was recorded in G_6 (Kukumseri, HP). The minimum seed viability 67.54 (55.27) per cent was observed in G_7 (Tangmerg, J&K) which was statistically at par with G_8 with value of 68.32 (55.75) per cent.

The data pertaining to seed viability of *Inula racemosa* during 2011-12 presented in Table 2 revealed that maximum value of 86.16 (68.16) per cent seed viability was also obtained from collection G_6 (Kukumseri, HP). Similarly, the minimum seed viability of 67.52 (55.26) per cent was obtained from site G_7 (Tangmerg, J&K).

iv) Germination per cent: The germination per cent was significantly affected by seeds collected from different germplasm (Table 2). In 2010-11 significantly highest germination of value 87.74 (69.50) per cent was recorded in germplasm collection G_5 (Udaipur, HP) which was found to be significantly different from all other collections. The least value of 52.76 (46.58) per cent was recorded in S_6 (Kukumseri, HP) and was found to be significantly different from all other germplasm.

During the year 2011-12 highest germination of 87.93 (69.57) per cent was recorded from G_5 (Udaipur, HP) and was found to be statistically different from all other germplasm collections. The lowest germination of 51.17 (45.67) per cent was obtained from germplasm collection G_8 (Keylong, HP) and was found to be statistically different from all other sites.

4.3 EXPERIMENT-III: Effect of pre-sowing treatments on germination parameters of *Inula racemosa*

4.3.1 Effect of pre-sowing treatments on germination parameters under laboratory conditions

The perusal of data in Table 3, 4 and 5 revealed the effect of eight pre-sowing treatments on various germination parameters of *Inula racemosa* composite seed.

i) Germination per cent: It was apparent from the data in Table 3 that germination per cent was significantly affected by different pre-sowing treatments. During 2010-11 highest germination percentage of value 87.74 (69.50) per cent was observed when seeds were treated with 150 ppm GA_3 which was significantly different from all other pre-sowing treatments. The least germination of 52.76 (46.58) per cent was recorded for treatment P_6 (IBA 50 ppm) and was found to be statistically at par with treatment P_1 [53.33 % (46.91)] *i.e.,* control.

During 2011-12 highest germination of 87.93 (69.67) per cent was also recorded from pre-sowing treatment P_5 (GA_3 150 ppm) and was found to be significantly different from all other treatments. The least germination of 51.17 (45.67) per cent was obtained from P_1 (control) and was found to be significantly different from all other pre-sowing treatments.

ii) Germination Energy per cent: The data presented in Table 3 revealed significant effect of pre-sowing treatments on seed germination energy of *Inula racemosa*. During 2010-11 the maximum 19.20 (4.38) per cent germination energy was recorded in treatment P_5 (150 ppm GA_3) which was found to be significantly different from all other values. The lowest value of 9.18 (3.02) per cent was recorded when seeds were treated with IBA 150 ppm (P_8) and was found to be statistically different from all other pre-sowing treatments.

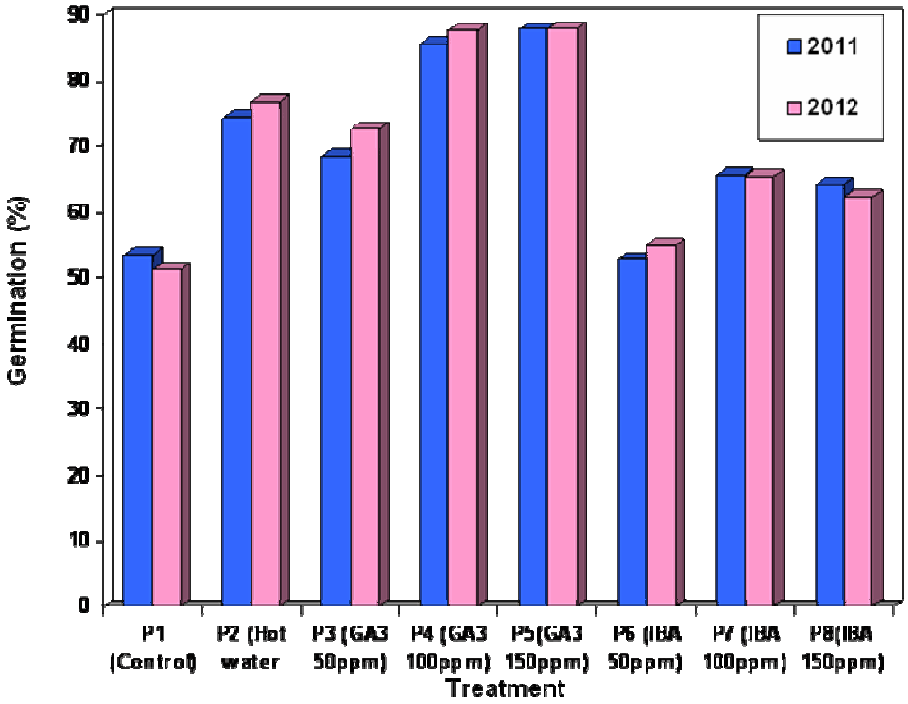


Fig. 7. Effect of pre-sowing treatments on Germination percent of *Inula racemosa* seeds under laboratory conditions

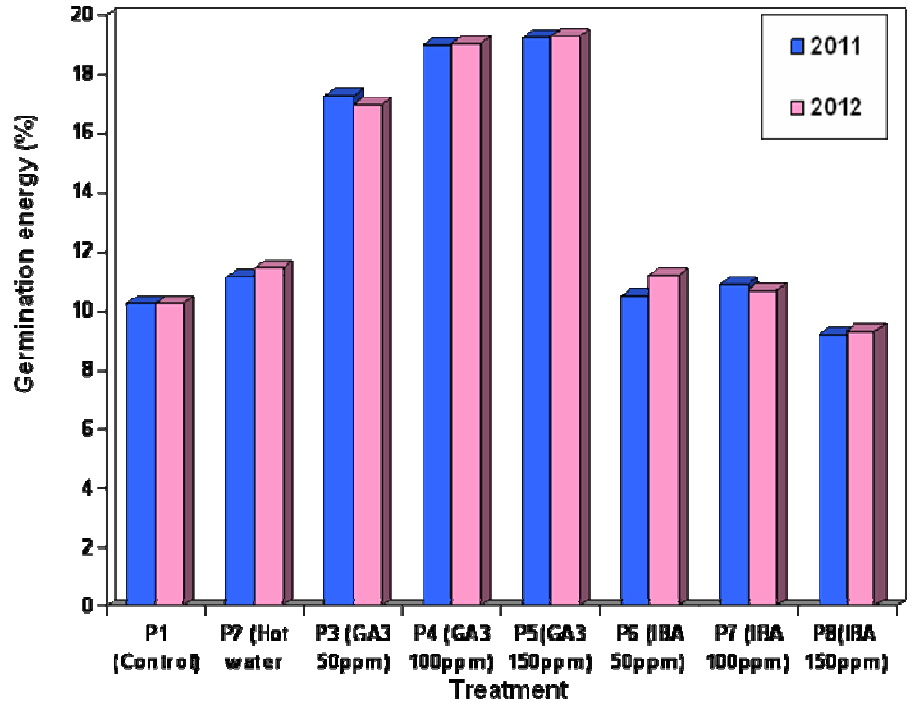


Fig. 8. Effect of pre-sowing treatments on Germination energy (%) of *Inula racemosa* seeds under laboratory conditions

Table 3: Effect of pre-sowing treatments on Germination Per cent, Germination Energy Per cent of *Inula racemosa* seeds under laboratory conditions

Treatments	Germination Per cent		Germination Energy Per cent	
	2010-11	2011-12	2010-11	2011-12
P_1 (Control)	53.33 (46.91)	51.17 (45.67)	10.24 (3.20)	10.26 (3.20)
P_2 (Hot water))	74.47 (59.65)	76.95 (61.31)	11.15 (3.33)	11.47 (3.38)
P_3 (GA_3 50ppm)	68.33 (55.76)	72.62 (58.45)	17.27 (4.15)	16.95 (4.11)
P_4 (GA_3 100ppm)	85.59 (67.69)	87.51 (69.31)	18.95 (4.35)	19.26 (4.39)
P_5(GA_3 150ppm)	87.74 (69.50)	87.93 (69.67)	19.20 (4.38)	19.00(4.36)
P_6 (IBA 50ppm)	52.76 (46.58)	54.87 (47.80)	10.48 (3.24)	11.22 (3.35)
P_7 (IBA 100ppm)	65.69 (54.16)	65.41 (53.98)	10.85 (3.29)	10.68 (3.26)
P_8 (IBA 150ppm)	64.28 (53.30)	62.24 (52.09)	9.18 (3.02)	9.34 (3.06)
Mean	**68.77(56.69)**	**69.84(57.29)**	**13.42(3.62)**	**13.52(3.64)**
SEm+	**0.50**	**0.013**	**0.0016**	**0.0013**
$CD_{0.05}$	**1.06**	**0.027**	**0.0033**	**0.0028**

*Figures in parentheses are arc sine transformed values
**Figures in parentheses are square root transformed values

During 2011-12 the maximum germination energy of 19.26 (4.39) per cent was recorded in treatment P_4 (100 ppm GA_3) which was found to be significantly different from all other values, followed by P_5 (GA_3 150 ppm) with value 19.00 (4.36) per cent. The lowest value of 9.34 (3.06) per cent was recorded when seeds were treated with IBA 150 ppm (P_8) and was found to be statistically different from all other pre-sowing treatments.

iii) Germination Speed: The pre-sowing treatments exerted significant effect on germination speed of *Inula racemosa* seeds (Table 4). During 2010-11 significantly highest germination speed of 0.67 resulted when seeds were treated with 50 ppm and 100 ppm IBA (P_6 and P_7) GA_3 (P_5), which was statistically at par with 150 ppm GA_3 (P_4). The minimum value of 0.38 was recorded in P_1 (control) which was statistically at par with P_2 (0.42) treatment.

During 2011-12 maximum germination speed (0.68) was obtained from treatment (P_6) IBA 150 ppm and was found to be statistically at par with treatment P_5 (GA_3 150 ppm). Similarly, the minimum value of 0.40 germination speed was recorded from pre-sowing treatment P_1 (control) and was found to be statistically different from all other pre-sowing treatments.

Table 4: Effect of pre-sowing treatments on Germination Speed and Peak Value of *Inula racemosa* seeds under laboratory conditions

Treatments	Germination Speed		Peak Value	
	2010-11	2011-12	2010-11	2011-12
P_1 (Control)	0.38	0.40	1.35	1.53
P_2 (Hot water)	0.42	0.42	3.21	3.14
P_3 (GA_3 50ppm)	0.52	0.52	4.45	4.21
P_4 (GA_3 100ppm)	0.62	0.64	5.15	5.36
P_5(GA_3 150ppm)	0.65	0.66	5.38	5.37
P_6 (IBA 50ppm)	0.67	0.68	2.28	2.54
P_7 (IBA 100ppm)	0.67	0.65	2.16	2.18
P_8(IBA 150ppm)	0.46	0.47	2.58	2.57
Mean	**0.70**	**0.56**	**3.32**	**3.36**
SEm±	**0.008**	**0.007**	**0.015**	**0.007**
$CD_{0.05}$	**0.017**	**0.014**	**0.031**	**0.016**

iv) Peak Value: The data pertaining to peak value is presented in Table 4. During the year 2010-11 maximum peak value of 5.38 was recorded in treatment P_5 (GA_3 150 ppm) and was found to be significantly different from all other pre-sowing treatments. The minimum peak value of 1.35 was recorded in treatment P_1 (control) and was found to be statistically different from all other pre-sowing treatments.

During 2011-12 maximum peak value of 5.37 was also obtained from pre-sowing treatment P_5 (GA_3 150 ppm) and was found to be statistically at par with P_4. Similarly minimum peak value of 1.53 was recorded from pre-sowing

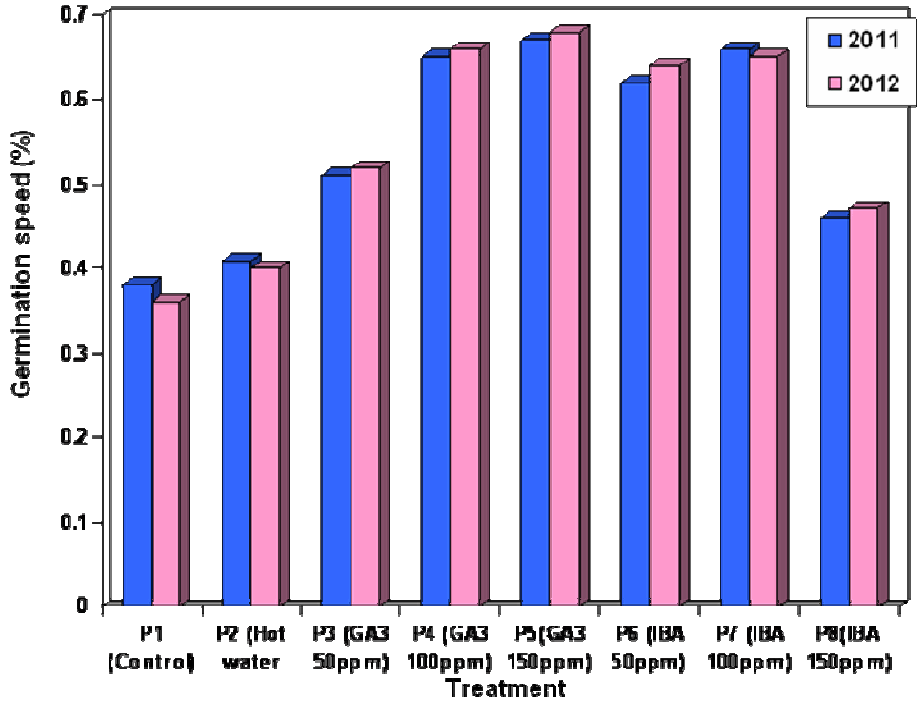


Fig. 9. Effect of pre-sowing treatments on Germination speed of *Inula racemosa* seeds under laboratory conditions

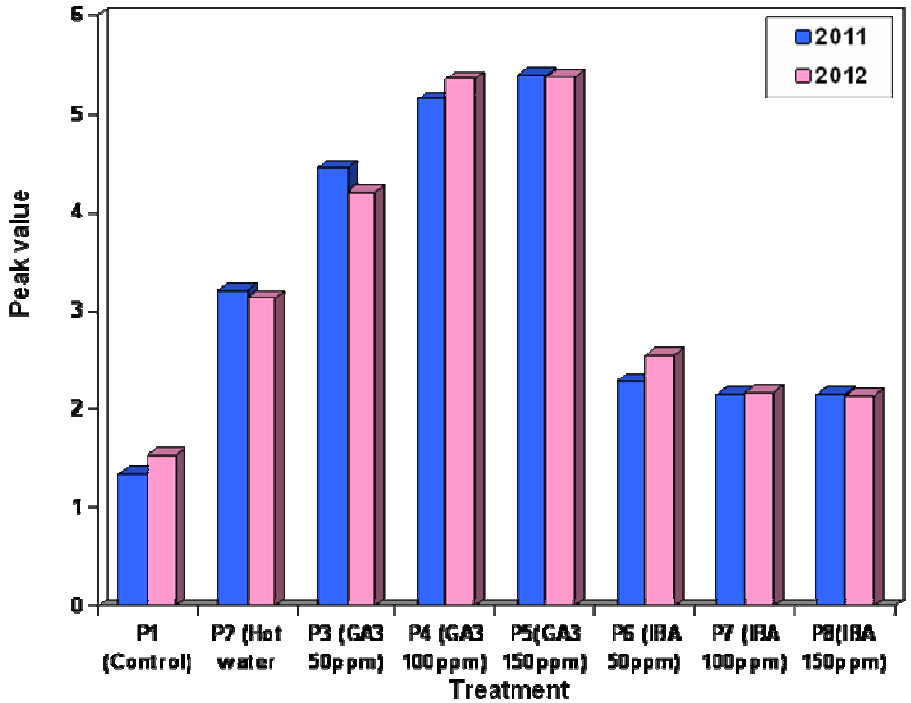


Fig. 10. Effect of pre-sowing treatments on peak value of *Inula racemosa* seeds under laboratory conditions

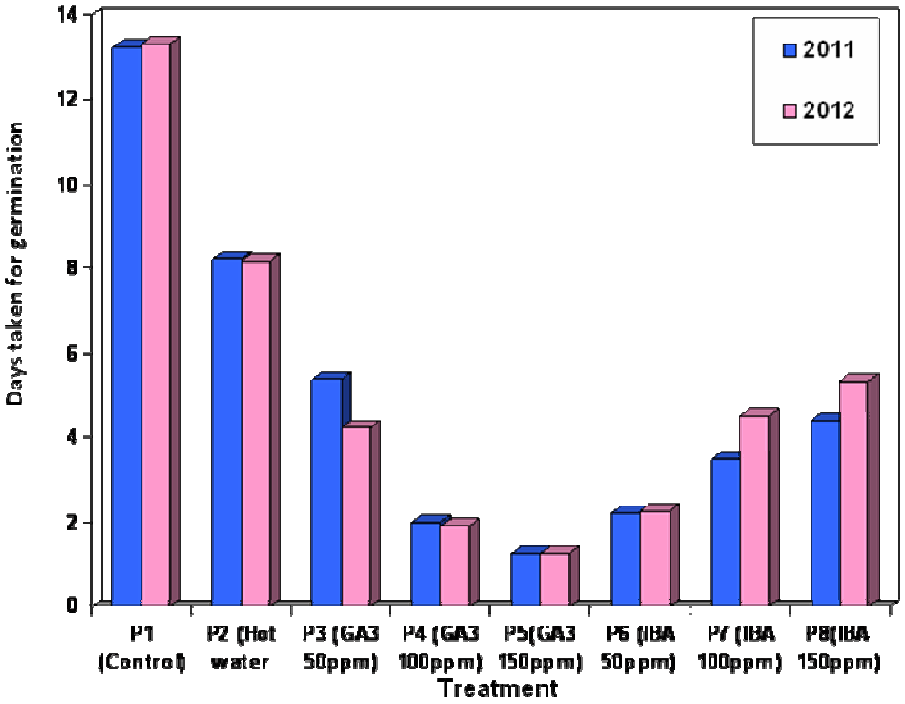


Fig. 11. Effect of pre-sowing treatments on days taken for germination of *Inula racemosa* seeds under laboratory conditions

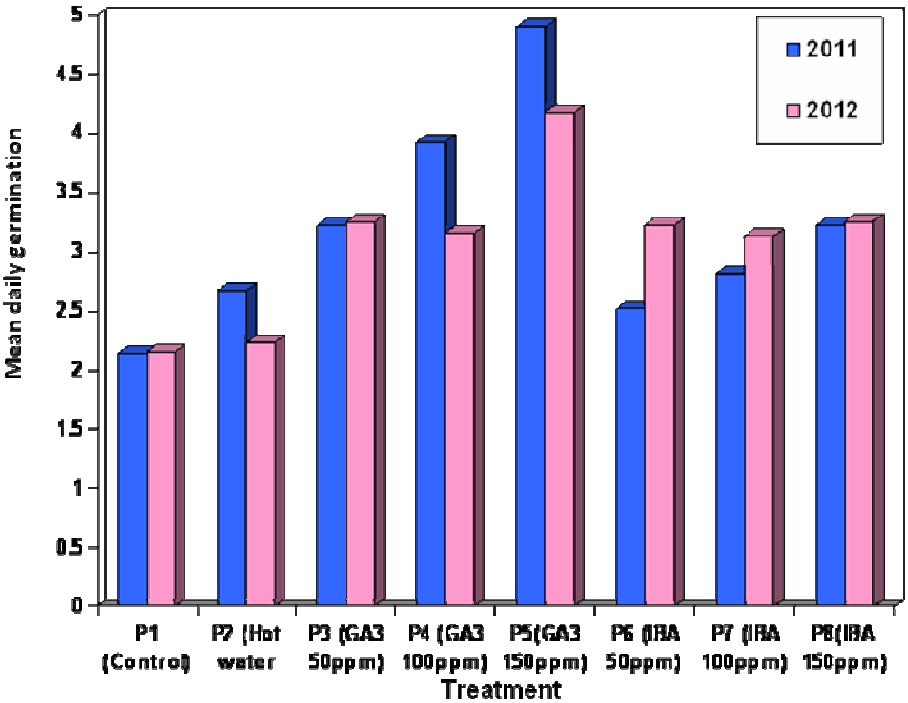


Fig. 12. Effect of pre-sowing treatments on mean daily germination of *Inula racemosa* seeds under laboratory conditions

treatment P_1 (control) and was found to be significantly different from all other pre-sowing treatments.

Table 5: Effect of pre-sowing treatments on Days taken for Germination and Mean Daily Germination of *Inula racemosa* seeds under laboratory conditions

Treatments	Days taken for Germination		Mean Daily Germination	
	2010-11	2011-12	2010-11	2011-12
P_1 (Control)	13.25	13.33	2.14	2.15
P_2 (Hot water))	8.24	8.17	2.68	2.23
P_3 (GA_3 50ppm)	5.38	4.22	3.22	3.25
P_4 (GA_3 100ppm)	1.98	1.90	3.93	3.16
P_5(GA_3 150ppm)	1.25	1.23	4.90	4.17
P_6 (IBA 50ppm)	2.23	2.24	2.52	3.22
P_7 (IBA 100ppm)	3.48	4.48	2.81	3.13
P_8(IBA 150ppm)	4.42	5.33	2.22	3.16
Mean	**5.03**	**5.11**	**3.05**	**3.06**
SEm±	**0.058**	**0.080**	**0.082**	**0.007**
$CD_{0.05}$	**0.122**	**0.170**	**0.173**	**0.156**

v) Days taken for Germination: The data presented in Table 5 indicated that pre-sowing treatments have shown significant effect on number of days taken for germination. It was evident from the tabulated data that during 2010-11 minimum number of days taken for first germination were 1.25 when seeds were treated with 150 ppm GA_3 (P_5) and was found to be significantly different from all other pre-sowing treatments. The maximum number of days (13.25) taken for germination was recorded from treatment P_1 (control) and were found to be significantly different from all other pre-sowing treatments.

It was also observed during 2011-12 that minimum days (1.23) taken for germination of seeds of *Inula racemosa* composite sample was recorded from treatment P_5 (150 ppm GA_3) and was found statistically different from all other pre-sowing treatments. Similarly, maximum days (13.33) taken for onset of seed

germination was recorded from pre-sowing treatment P_1 (control) and was found to be significantly different from all other pre-sowing treatments.

vi) Mean Daily Germination: The data in Table 5 indicated that pre-sowing treatments had shown significant effect on mean daily germination of *Inula racemosa* seeds. During the year 2010-11 the maximum mean daily germination of 4.90 was recorded when seeds were treated with 150 ppm GA_3 (P_5). The minimum value of 2.14 was recorded in treatment P_1 (control) and was found to be significantly different from all other pre-sowing treatments.

During 2011-12 also maximum (4.17) mean daily germination was recorded in treatment P_5 (150 ppm GA_3) and was found to be significantly different from all other pre-sowing treatments. Similarly, minimum mean daily germination (2.15) was recorded in treatment P_1 (control) and was found to be significantly at par with treatment P_2 (2.23).

Table 6: Effect of pre-sowing treatments on Germination Value and Germination Index of *Inula racemosa* seeds in laboratory conditions

Treatments	Germination Value		Germination Index	
	2010-11	2011-12	2010-11	2011-12
P_1 (Control)	2.86	2.84	0.52	0.57
P_2 (Hot water)	8.64	8.64	0.83	0.84
P_3 (GA_3 50ppm)	14.44	14.46	1.13	1.15
P_4 (GA_3 100ppm)	20.14	20.18	1.33	1.32
P_5(GA_3 150ppm)	26.45	26.64	1.35	1.34
P_6 (IBA 50ppm)	5.78	5.78	1.04	1.13
P_7 (IBA 100ppm)	5.86	5.87	1.03	1.10
P_8(IBA 150ppm)	5.76	5.45	0.67	0.76
Mean	**11.24**	**11.23**	**0.99**	**0.86**
SEm±	**0.02**	**0.05**	**0.010**	**0.010**
$CD_{0.05}$	**0.03**	**0.09**	**0.021**	**0.021**

vii) Germination Value: Data pertaining to germination value is given in Table 6. It was evident from the table that during 2010-11 the maximum

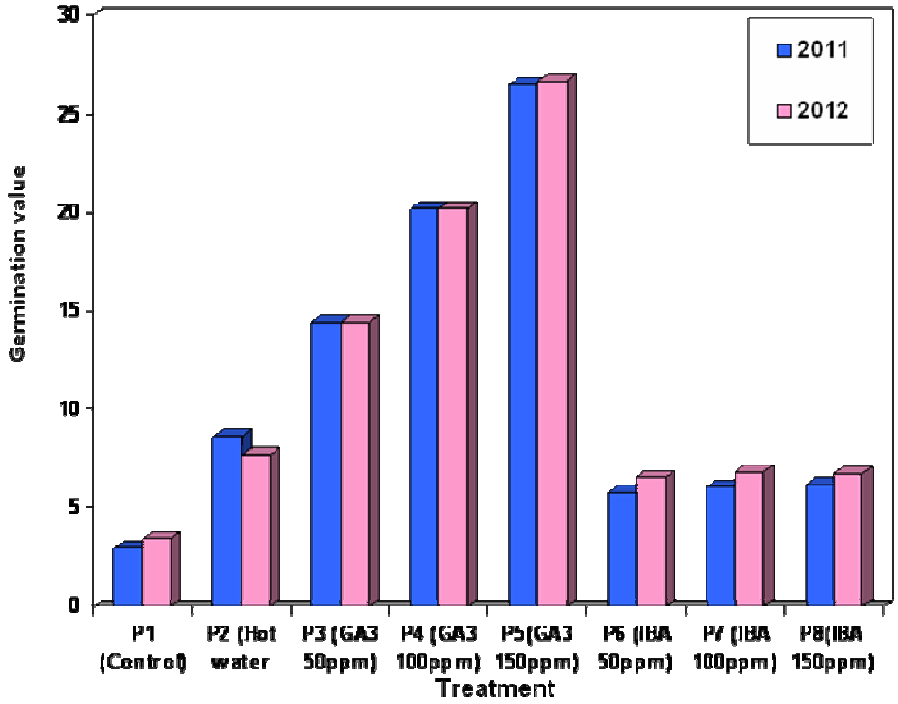


Fig. 13. Effect of pre-sowing treatments on germination value of *Inula racemosa* seeds under laboratory conditions

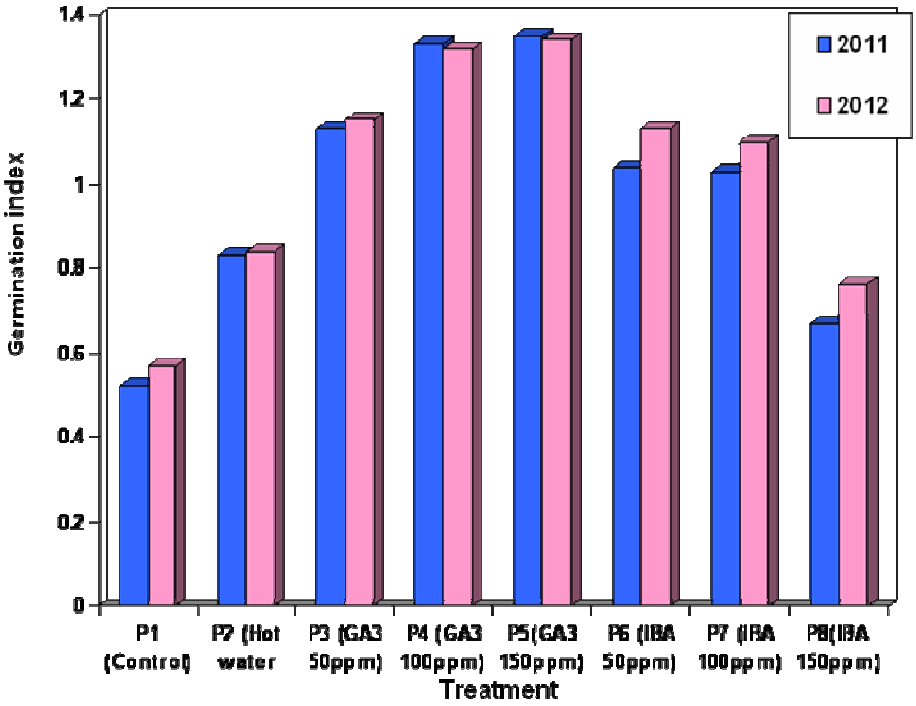


Fig. 14. Effect of pre-sowing treatments on germination index of *Inula racemosa* seeds under laboratory conditions

germination value (26.45) resulted when seeds were treated with 150 ppm GA_3 (P_5). The significantly minimum value of 2.87 was recorded in treatment P_1 (control) and was found to be significantly different from all other pre-sowing treatments.

During 2011-12 maximum germination value (26.64) was also recorded in pre-sowing treatment P_5 (GA_3 150 ppm) and was found to be significantly different from all other treatments. Similarly, minimum germination value (2.84) was recorded in treatment P_1 (control) and was found to be significantly different from all other treatments.

viii) Germination index: The Pre-sowing treatments exerted significant effect on germination index of *Inula racemosa* seeds as presented in Table 6. During 2010-11 the significantly highest value of 1.35 was recorded when seeds were treated with 150 ppm GA_3 (P_5) which was statistically found to be at par with P_4 treatment 100 ppm GA_3 (1.33). The significantly lowest value of 0.52 was obtained in treatment P_1 (control) and was significantly different from all other treatments.

During the year 2011-12 also the maximum germination index of 1.34 was recorded in treatment P_5 (GA_3 150 ppm) and was statistically at par with treatment P_4 (GA_3 100 ppm). Similarly, minimum germination index of 0.57 was recorded in treatment P_1 (control) and was statistically different from all other pre-sowing treatments.

4.3.2 Effect of pre-sowing treatments on germination parameters under field conditions

The perusal of data in Table 7-11 revealed the effect of eight pre-sowing treatments on various germination and survival parameters on composite seeds of *Inula racemosa* in field conditions.

i) Germination per cent: The data presented in Table -7 revealed that pre-sowing treatments showed significant effect on germination per cent of *Inula racemosa* seeds under nursery conditions. During 2010-11 the maximum

germination of 84.74 per cent (67.09) resulted when seeds were treated with 150 ppm GA_3 (P_5) which was statistically different from all other treatments. The significantly least value of 55.00 per cent (47.87) was observed in P_1 (control) and was found to be statistically different from all other treatments.

Table 7. Effect of pre-sowing treatments on Germination per cent and Seedling height of *Inula racemosa* under field conditions

Treatments	Germination per cent		Seedling height (cm)	
	2010-11	2011-12	2010-11	2011-12
P_1 (Control)	55.00 (47.87)	52.78 (46.61)	12.14	15.32
P_2 (Hot water)	61.17 (51.45)	62.11 (52.0)	16.28	17.98
P_3 (GA_3 50ppm)	66.57 (54.69)	65.94 (54.31)	32.28	36.27
P_4 (GA_3 100ppm)	84.53 (66.86)	86.62 (68.55)	37.05	38.67
P_5(GA_3 150ppm)	84.74 (67.09)	87.07 (68.99)	47.25	48.65
P_6 (IBA 50ppm)	72.66 (58.48)	72.55 (58.41)	36.24	37.21
P_7 (IBA 100ppm)	72.86 (58.61)	72.11 (58.13)	32.31	32.28
P_8(IBA 150ppm)	71.87 (57.97)	70.45 (57.07)	32.06	34.86
Mean	**71.18 (57.88)**	**71.20 (58.00)**	**30.70**	**32.65**
SEm±	**0.009**	**0.162**	**0.016**	**0.0126**
$CD_{0.05}$	**0.018**	**0.343**	**0.034**	**0.0269**

*Figures in parentheses are arc sine transformed values

During the year 2011-12 the maximum germination of 87.07 per cent (68.99) was recorded from treatment (P_5) 150 ppm GA_3 and was found to be significantly different from all other treatments. Similarly, least germination 52.78 per cent (46.61) was observed from treatment P_1 (control) and was found to be significantly different from all other treatments.

ii) Seedling height (cm): Data pertaining to seedling height differed significantly among the different pre-sowing eight treatments (Table 7). During 2010-11 the maximum seedling height of 47.25 cm was observed from treatment P_5 (GA_3 150 ppm). The significantly minimum value of 12.14 cm was observed with treatment P_1 (control) and was found to be significantly different from all other treatments.

Plate 9. Radical leaves of *Inula racemosa*

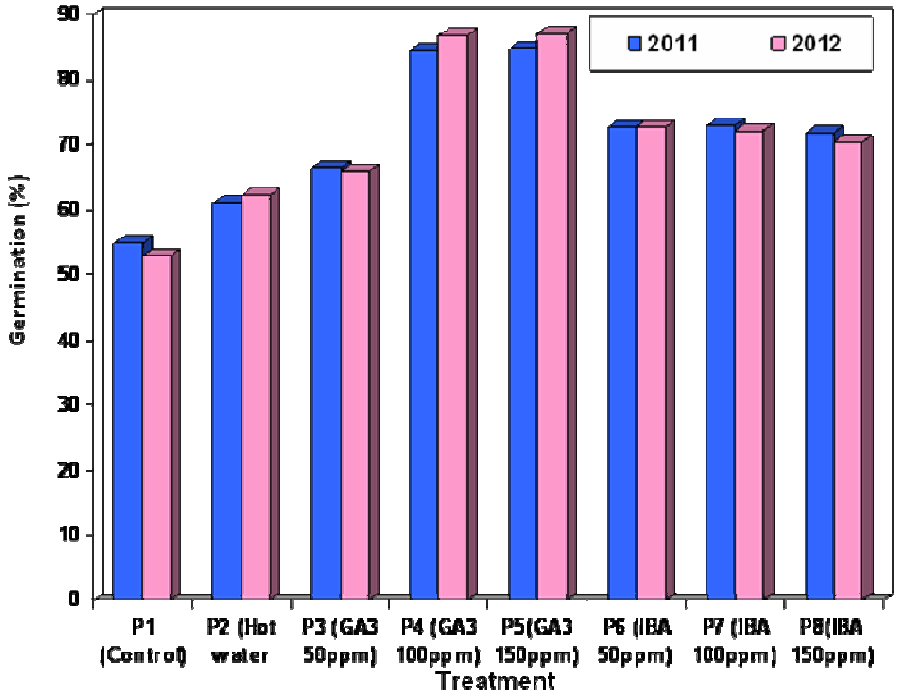


Fig. 15. Effect of pre-sowing treatments on germination (%) of *Inula racemosa* seeds under field conditions

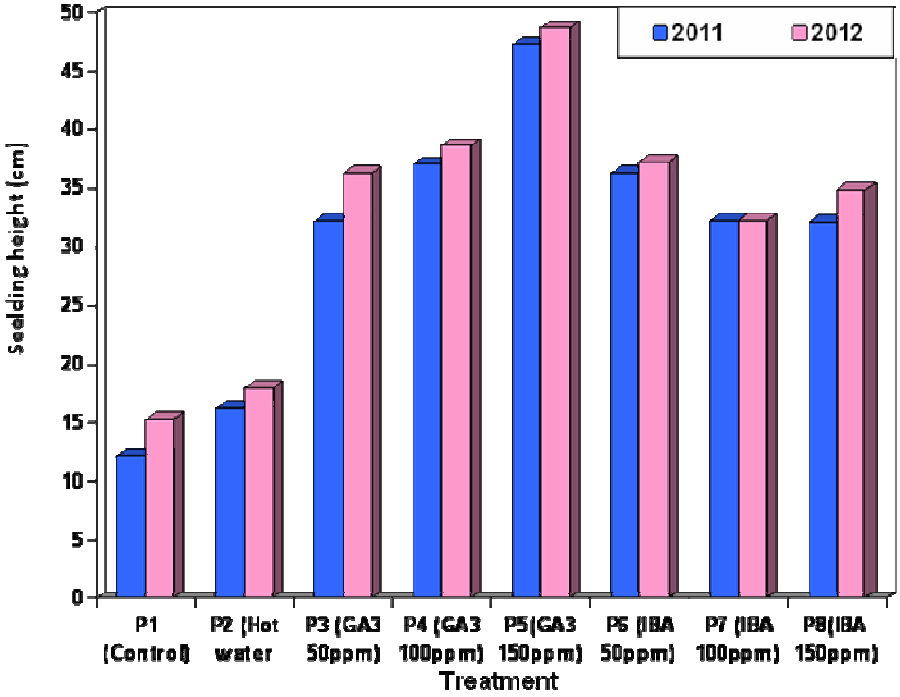


Fig. 16. Effect of pre-sowing treatments on seedling height of *Inula racemosa* seeds under field conditions

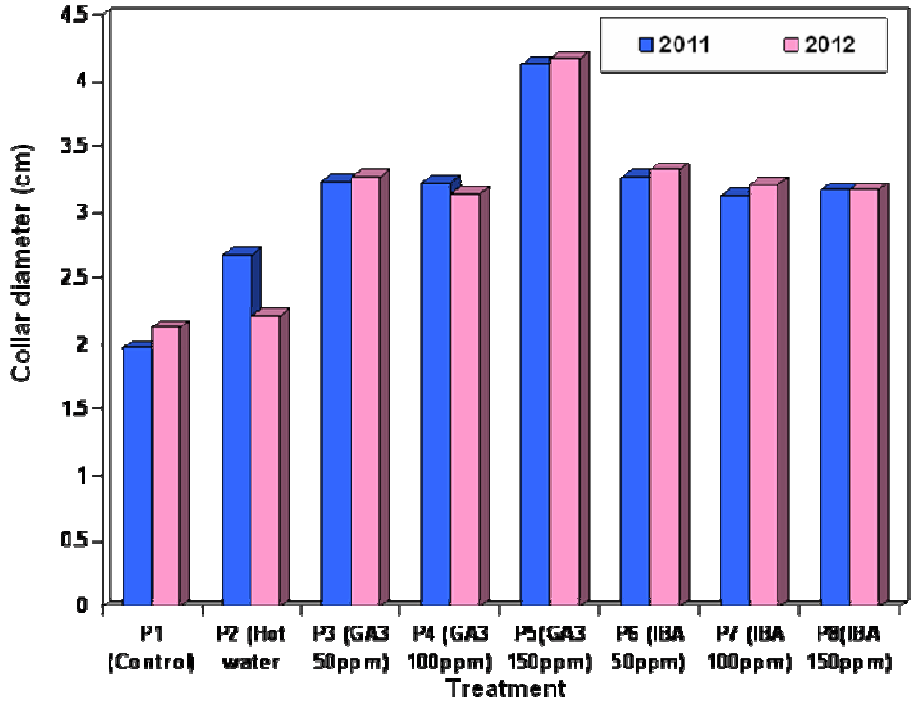


Fig. 17. Effect of pre-sowing treatments on collar diameter (mm) of *Inula racemosa* seeds under nursery conditions

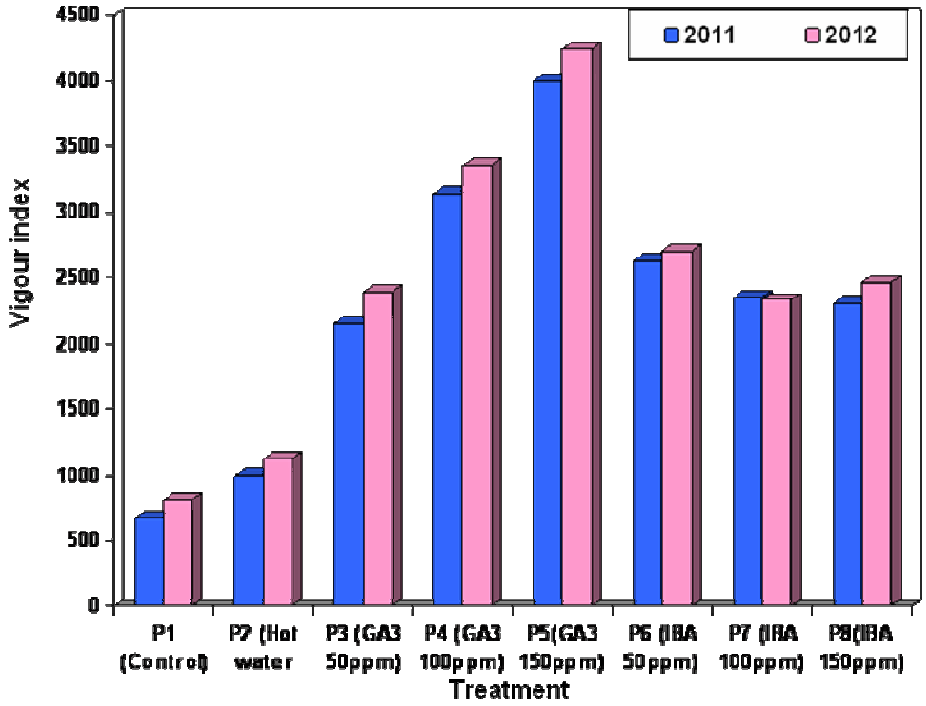


Fig. 18. Effect of pre-sowing treatments on vigour index of *Inula racemosa* seeds under nursery conditions

During 2011-12 the maximum seedling height (48.65) was also observed from treatment P_5 (GA_3 150 ppm) and was significantly different from all other pre-sowing treatments. Similarly, minimum seedling height of 15.32 cm was observed from P_1 (control) which was statistically different from all other treatments.

Table 8. Effect of pre-sowing treatments on Collar Diameter & Seedling Vigour Index of *Inula racemosa* under field conditions

Treatments	Collar Diameter (cm)		Seedling Vigour Index	
	2010-11	2011-12	2010-11	2011-12
P_1 (Control)	1.97	2.13	667.70	808.60
P_2 (Hot water)	2.67	2.21	995.80	1117.00
P_3 (GA_3 50ppm)	3.24	3.26	2149.00	2392.00
P_4 (GA_3 100ppm)	3.23	3.14	3132.00	3350.00
P_5(GA_3 150ppm)	4.12	4.17	4004.00	4236.00
P_6 (IBA 50ppm)	3.27	3.32	2633.00	2700.00
P_7 (IBA 100ppm)	3.12	3.21	2347.00	2328.00
P_8(IBA 150ppm)	3.16	3.16	2304.00	2789.00
Mean	**3.10**	**3.08**	**2279.06**	**2130.95**
SEm±	**0.014**	**0.009**	**325.05**	**325.52**
$CD_{0.05}$	**0.29**	**0.019**	**689.08**	**690.06**

iii) Collar diameter (cm): It was observed from data presented in Table 8 that pre-sowing treatments had significant effect on collar diameter. During 2010-11 the significantly maximum collar diameter was recorded when seedlings were raised from seeds treated with GA_3 150 ppm (P_5) with a value of 4.12 cm and was found to be significantly different from all other treatments. The significantly minimum value of 1.97 cm was observed in P_1 (control) and was found to be significantly different from all other pre-sowing treatments.

During 2011-12 maximum (4.17cm) collar diameter was also recorded in pre-sowing treatment P_5 (GA_3 150 ppm) and was found to be significantly different from all other pre-sowing treatments. Similarly, minimum collar diameter (2.13 cm) was recorded in P_1 (control).

iv) Seedling Vigour Index: A cursory glance on year wise data presented on seedling vigour index (Table 8) revealed significant effect of pre-sowing treatments on this parameter. During 2010-11 the highest seedling vigour index (4004.0) was observed when seeds were raised from treatment P_5 (GA_3 150 ppm) which was significantly different from all other treatments. The minimum seedling vigour index (667.70) was observed in P_1 (control) and was significantly similar with P_2 (995.80) treatment.

During 2011-12, the highest seedling vigour index (4236.0) was also observed when seeds were raised from treatment P_5 (GA_3 150 ppm) and showed a significantly different effect from all other treatments. The minimum seedling vigour index (808.60) was observed in P_1 (control) and was statistically at par with P_2 (1117.00).

Table 9. Effect of pre-sowing treatments on Fresh Shoot Weight and Dry Shoot Weight of *Inula racemosa* in field conditions

Treatments	Fresh Shoot Weight		Dry Shoot Weight	
	2010-11	2011-12	2010-11	2011-12
P_1 (Control)	10.82	8.34	5.43	4.12
P_2 (Hot water)	16.82	18.52	8.43	9.21
P_3 (GA_3 50ppm)	28.15	28.45	14.14	14.21
P_4 (GA_3 100ppm)	26.73	26.18	13.34	13.07
P_5(GA_3 150ppm)	54.10	52.77	27.77	26.35
P_6 (IBA 50ppm)	40.20	42.76	20.05	21.35
P_7 (IBA 100ppm)	29.47	28.53	14.73	12.62
P_8(IBA 150ppm)	23.47	25.93	12.37	12.95
Mean	**28.72**	**28.94**	**14.53**	**12.99**
SEm±	**0.0136**	**0.0131**	**0.182**	**0.018**
$CD_{0.05}$	**0.0289**	**0.0278**	**0.387**	**0.039**

v) Fresh Shoot Weight (g): The perusal of year wise data in Table 9 revealed significantly different effect of pre-sowing treatments on fresh shoot

Plate 10. Ten months old plants of *Inula racemosa* after A) GA_3 150 ppm treatment and B) Control

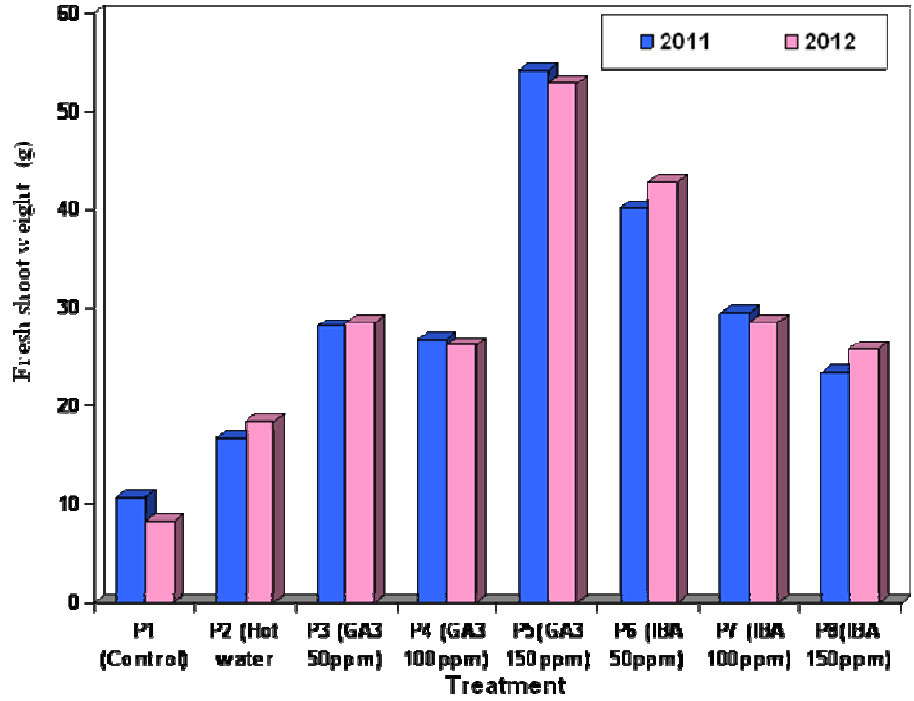


Fig. 19. Effect of pre-sowing treatments on fresh shoot weight (g) of *Inula racemosa* seeds under field conditions

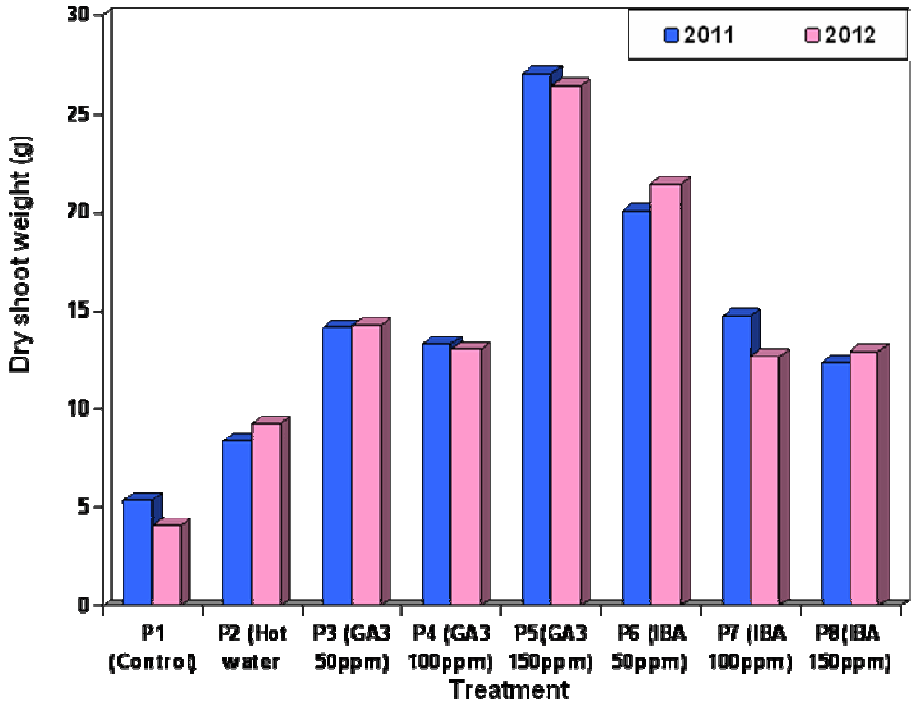


Fig. 20. Effect of pre-sowing treatments on dry shoot weight of *Inula racemosa* seeds under field conditions

weight and dry shoot of *Inula racemosa*. During 2010-11 maximum fresh shoot weight of 54.10 g was recorded when seedlings were raised from seeds treated with P_5 (150 ppm GA_3) and was found to be statistically different from all other treatments. The minimum value of 10.82 g was recorded when seeds were raised from control (P_1) and showed a significant difference among all the treatments.

During 2011-12 also, highest fresh shoot weight (52.77 g) was observed from treatment P_5 (150 ppm GA_3) and was found to be statistically different from all other treatments. Similarly, lowest fresh shoot (8.34 g) weight was recorded in P_1 (control) and was found to be significantly different from all other treatments.

vi) Dry Shoot Weight (g): It appeared from data (Table 9) that significantly highest dry shoot weight of 26.77 g was observed for seedlings raised from seeds treated with 150 ppm GA_3 (P_5) during 2010-11. The significantly lowest value of 5.43 g was recorded when seeds were raised from untreated seeds (control).

During 2011-12 maximum dry shoot weight of 26.35 g was also observed from pre-sowing treatment P_5 (150 ppm GA_3) and was found to be statistically different from all other treatments. Similarly, least dry shoot weight (4.12 g) was recorded in (P_1) control and was found to be significantly different from all other treatments.

vii) Fresh Root Weight (g): Year wise data on fresh root weight and dry root weight is tabulated in Table 10. The maximum fresh root weight of 9.22 g was obtained in treatment P_5 (GA_3 150 ppm) and was significantly different from all other treatments. Significantly minimum value for fresh root weight (3.17 g) was noticed with untreated raised seedlings (P_1) and was found to be significantly different from all other treatments.

During 2011-12 also, maximum fresh root weight of 9.33 g was observed in treatment P_5 ($GA_3$150 ppm) and was found to be statistically different from all other treatments. Similarly, minimum fresh root weight (3.86 g) was observed with pre-sowing treatment P_1 *i.e.,* from control.

Table 10. Effect of pre-sowing treatments on Fresh Root Weight and Dry Root Weight of *Inula racemosa* in field conditions

Treatments	Fresh Root Weight (g)		Dry Root Weight (g)	
	2010-11	2011-12	2010-11	2011-12
P_1 (Control)	3.17	3.86	1.59	1.93
P_2 (Hot water))	6.54	5.87	3.27	2.92
P_3 (GA_3 50ppm)	4.54	5.33	2.28	2.68
P_4 (GA_3 100ppm)	7.14	7.44	3.57	3.72
P_5(GA_3 150ppm)	9.22	9.32	4.61	4.64
P_6 (IBA 50ppm)	6.20	6.52	3.08	4.25
P_7 (IBA 100ppm)	8.41	8.63	4.21	4.31
P_8(IBA 150ppm)	7.15	6.33	3.54	3.14
Mean	**5.75**	**6.67**	**3.27**	**3.45**
SEm±	**0.015**	**0.015**	**0.015**	**0.017**
$CD_{0.05}$	**0.033**	**0.032**	**0.031**	**0.037**

viii) Dry Root Weight (g): It appeared from the data in Table 10 that significantly highest dry root weight of 4.61 g was observed for seedlings raised from seeds treated with 150 ppm GA_3 (P_5) during 2010-11. The significantly lowest value of 1.59 g was recorded from untreated seeds *i.e*., from control.

During 2011-12 also, maximum dry root weight (4.64 g) was also observed in the treatment P_5 (150 ppm GA_3) which showed a significant difference among all other treatments. Similarly, minimum dry root weight (1.93 g) was recorded when there was given no treatment *i.e.,* control (P_1) and showed a significant difference among all the treatments.

ix) Root length (cm): Year wise data on root length of *Inula racemosa* from different pre-sowing treatments is presented in Table 11. The data revealed that during 2010-11 maximum root length of 10.86 cm was obtained from treatment P_5 (GA_3 150 ppm) was statistically different from all other pre-sowing treatments. The minimum root length (5.24cm) was recorded when there was given no pre-sowing treatment *i.e.,* control (P_1).

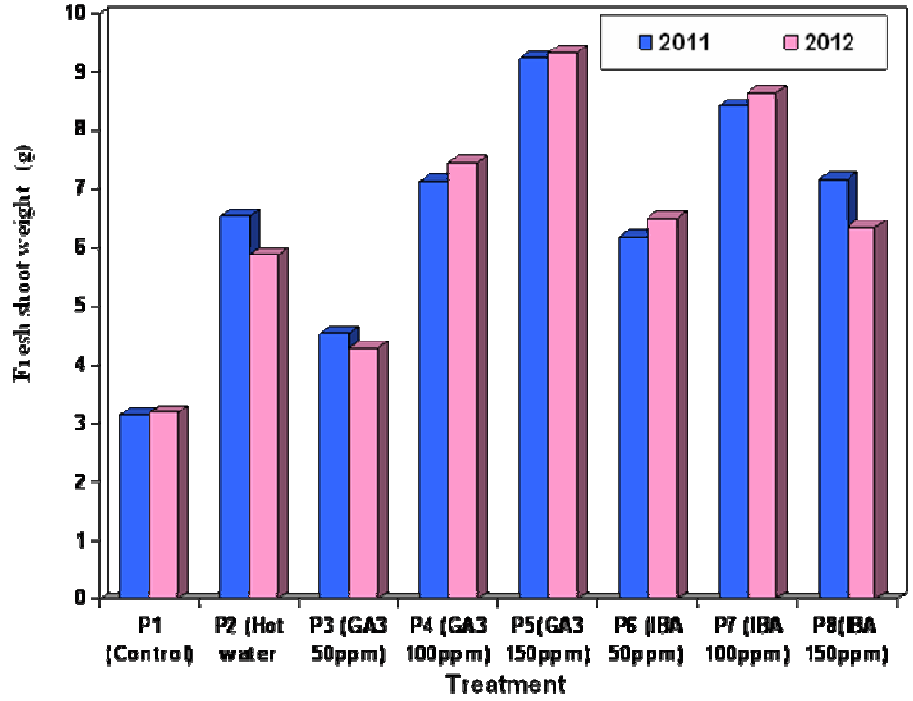


Fig. 21. Effect of pre-sowing treatments on fresh root weight (g) of *Inula racemosa* seeds under field conditions

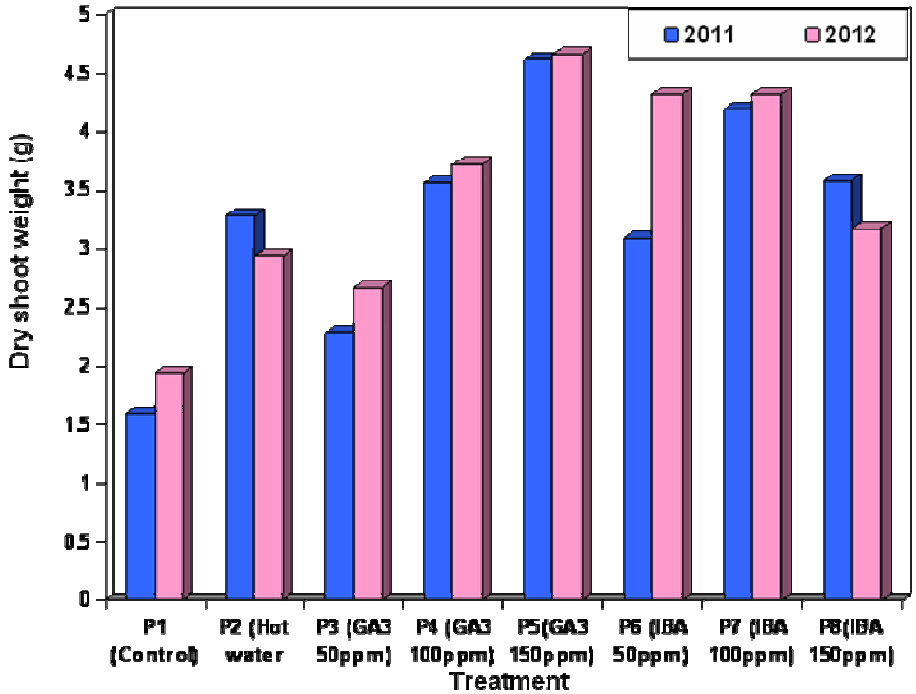


Fig. 22. Effect of pre-sowing treatments on dry root weight of *Inula racemosa* seeds under field conditions

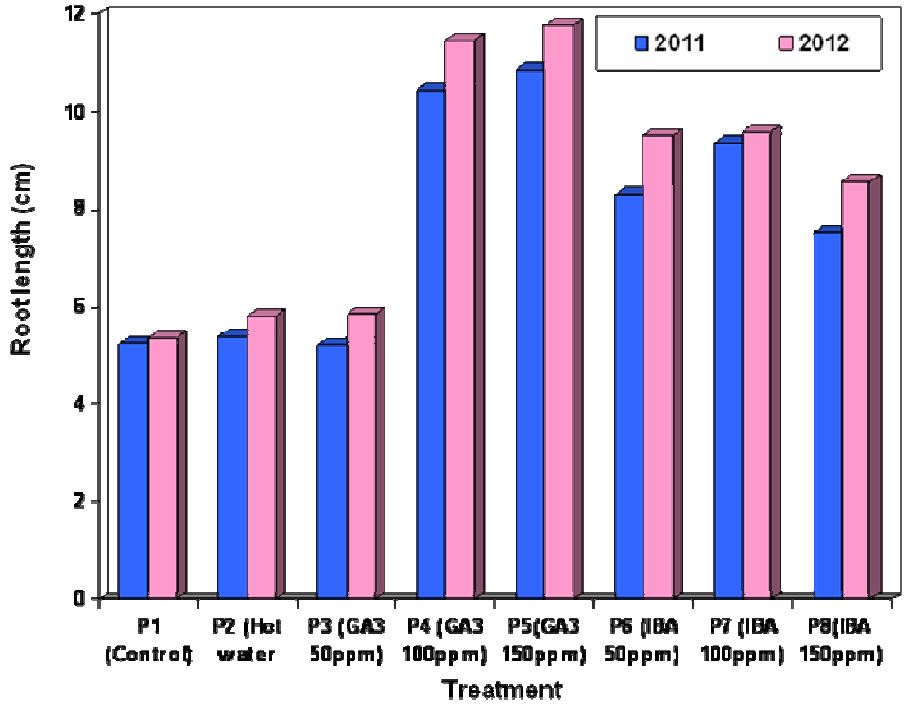


Fig. 23. Effect of pre-sowing treatments on root length (cm) of *Inula racemosa* seeds under field conditions

Table 11. Effect of pre-sowing treatments on Root length of *Inula racemosa* in field conditions

Treatments	Root Length (cm)	
	2010-11	2011-12
P_1 (Control)	5.24	5.36
P_2 (Hot water))	5.41	5.81
P_3 (GA_3 50ppm)	5.21	5.84
P_4 (GA_3 100ppm)	10.44	11.47
P_5 (GA_3 150ppm)	10.86	11.77
P_6 (IBA 50ppm)	8.27	9.49
P_7 (IBA 100ppm)	9.33	9.58
P_8 (IBA 150ppm)	7.47	8.54
Mean	**7.78**	**8.48**
SEm±	**0.013**	**0.013**
$CD_{0.05}$	**0.027**	**0.026**

During 2011-12 also, maximum root length of 11.77 cm was recorded from pre-sowing treatment P_5 (GA_3 150 ppm) and was found to be statistically different from all other treatments. Similarly, minimum root length 5.36 cm was observed when there was given no pre-sowing treatment *i.e.,* from control (P_1).

4.4 EXPERIMENT-IV: Effect of Location and germplasm collection sites on the germination and growth of *Inula racemosa*

The location wise data based on different germplasm collections on sprouting per cent, plant height, number of shoots, number of leaves, number of heads per plant, number of seeds per head, length of main root, number of lateral roots, root fresh weight and root dry weight was recorded and the results are presented as below (Table 12).

i) Sprouting per cent: The effect of site and germplasm collection on the sprouting per cent of *Inula racemosa* (Table-12) showed significant difference among germplasm at 5% level of significance. The maximum sprouting 95.35 per cent was observed from germplasm collection site G_5 (Udaipur, HP). The minimum sprouting of 80.08 per cent was obtained from germplasm collection site G_8 (Tangmerg, J&K).

Under the two different locations maximum sprouting of 87.04 per cent was recorded from S_2 (Manali) as compared to S_1 (Shilly) (86.13 %) and was found to be statistically non significant.

Table 12. Effect of Locations sites and Germplasm collection on sprouting percent of *Inula racemosa* in (2011-2012)

Locations / Germplasm	Sprouting (%)		
	S_1 (Shilly)	**S_2 (Manali)**	**Mean**
G_1 (Keylong, HP)	88.72	88.28	**88.50**
G_2 (Kardang , HP)	86.56	84.93	**85.74**
G_3 (Dalang , HP)	88.16	86.62	**87.39**
G_4 (Sissu, HP)	86.78	83.49	**85.14**
G_5 (Udaipur, HP)	95.43	95.27	**95.35**
G_6 (Kukumseri HP)	85.48	95.02	**90.25**
G_7 (Tangmerg , J&K)	78.26	82.24	**80.25**
G_8 (Shopian , J&K)	79.67	80.50	**80.08**
Mean	**86.13**	**87.04**	

Treatments	**SEd±**	**$CD_{0.05}$**
G (Germplasm)	0.92	1.87
S (Location)	0.46	NS
GxS	1.30	2.65

The interaction among germplasm and location were found to be significant at 5 % level of significance. The maximum sprouting per cent was observed from $G5_{\times}S_1$ (95.43 %) which was found to be statistically at par with $G_{5\times}S_2$ (95.27 %) and $G6_{\times}S_2$ (95.02 %). Minimum value of 78.26 per cent was recorded in $G_{7\times}S_1$ which was statistically at par with $G_{8\times}S_1$ (79.67 %) and $G_{8\times}S_2$ (80.50 %).

ii) Number of Shoots per plant: Appraisal of data in Table 13 revealed that maximum number of shoots were recorded in germplasm collection site G_6 (4.0) which was statistically at par with G_5 (3.75) germplasm collection sites. The minimum number of shoots was recorded from G_7 with a value of 1.98 shoots per plant and found to be statistically different.

Plate 11. Field trial of *Inula racemosa* conducted at Manali -Kullu (H.P.)

Plate 12. Field trial of *Inula racemosa* conducted at Shilly-Solan (H.P.)

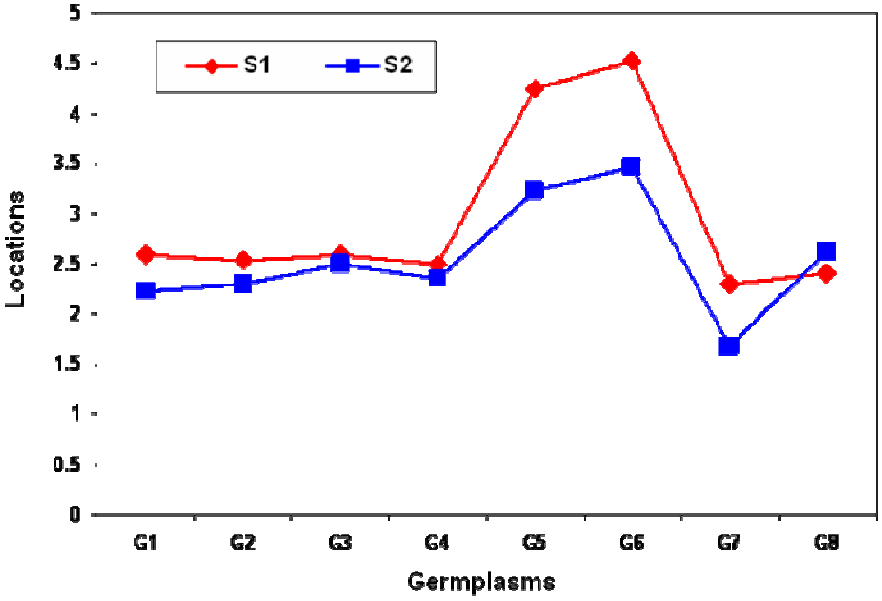


Fig. 24. Effect of sites and germplasm collection on number of shoots/plant of *Inula racemosa* in 2011-2012

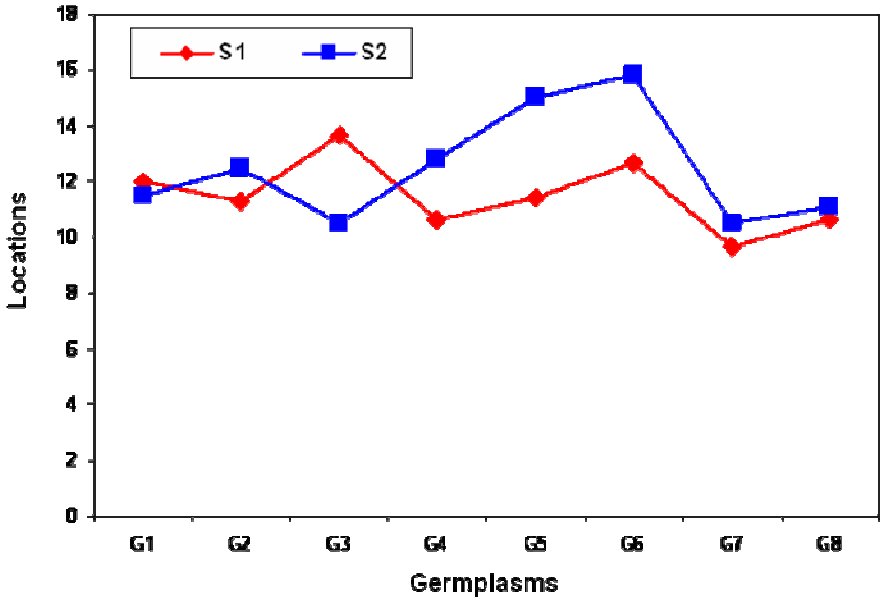


Fig. 25. Effect of sites and germplasm collection on number of leaves of *Inula racemosa* in 2011-2012

Under the two different locations significantly maximum (2.98) number of shoots was recorded from location S_1 (Shilly) and minimum (2.56) from S_2 (Manali).

The interactions among germplasm and location were found to be significant at 5 % level of significance. The maximum number (4.53) of shoots per plant was observed from $G_{6\times}S_1$ which was found to be statistically at par with $G_{5\times}S_1$ (4.26) and minimum number of shoots (1.67) was observed in $G_{7\times}S_2$ which was found to be statistically at par with $G_{7\times}S_1$.

Table 13. Effect Location sites and Germplasm collection on number of shoots per plant of *Inula racemosa* (2011-2012)

Locations / Germplasm	Number of shoots		
	S_1 (Shilly)	**S_2 (Manali)**	**Mean**
G_1 (Keylong, HP)	2.60	2.23	**2.42**
G_2 (Kardang , HP)	2.53	2.30	**2.41**
G_3 (Dalang , HP)	2.60	2.50	**2.67**
G_4 (Sissu, HP)	2.50	2.36	**2.43**
G_5 (Udaipur, HP)	4.26	3.23	**3.75**
G_6 (Kukumseri HP)	4.53	3.46	**4.0**
G_7 (Tangmerg , J&K)	2.30	1.67	**1.98**
G_8 (Shopian , J&K)	2.40	2.63	**2.52**
Mean	**2.98**	**2.56**	

Treatments	**SEd±**	**$CD_{0.05}$**
G (Site)	0.12	0.25
S (Location)	0.062	0.13
GxS	0.17	0.35

iii) Number of Leaves: A perusal of data presented in Table 14 revealed that maximum value for number of leaves (13.73) were observed from germplasm collection site G_6 (Udaipur, HP) which was statistically at par with G_5 (13.37) and

G_4 (12.25). The minimum (10.08) number of leaves were recorded from G_8 (Tangmerg, J&K) site collection.

Under locations, S_2 showed maximum (12.47) number of leaves as compared to S_1 (11.79) and there was no significant difference among the locations.

The interactions between germplasm collection sites and locations were found to be statistically significant. The maximum number of leaves (15.80) were recorded in $G_{6\times}S_2$ which was statistically similar with $G_{3\times}S_1$ (13.67), $G_{5\times}S_2$(15.07) and minimum number of leaves 10.50 were recorded in $G_{7\times}S_2$ which was found to be statistically at par with $G_{1\times}S_1$ (12.0), $G_{2\times}S_2$ (11.33), $G_{4\times}S_1$,(11.67), $G_{5\times}S_1$ (11.67), $G_{7\times}S_1$ (11.67), $G_{8\times}S_1$(10.67), , $G_{1\times}S_2$ (11.47) $G_{2\times}S_1$(12.50), $G_{3\times}S_2$ (10.53), $G_{7\times}S_2$,(10.50) and $G_{8\times}S_2$ (11.10).

Table 14. Effect of Location sites and Germplasm collection on number of Leaves of *Inula racemosa* (2011-2012)

Locations / Germplasm	Number of Leaves		
	S_1 (Shilly)	**S_2 (Manali)**	**Mean**
G_1 (Keylong, HP)	12.00	11.47	**11.73**
G_2 (Kardang , HP)	11.33	12.50	**11.92**
G_3 (Dalang , HP)	13.67	10.53	**12.10**
G_4 (Sissu, HP)	11.67	12.83	**12.25**
G_5 (Udaipur, HP)	11.67	15.07	**13.37**
G_6 (Kukumseri HP)	11.67	15.80	**13.73**
G_7 (Tangmerg , J&K)	11.67	10.50	**10.08**
G_8 (Shopian , J&K)	10.67	11.10	**10.88**
Mean	**11.79**	**12.47**	

Treatments	**SEd±**	**$CD_{0.05}$**
G (Germplasm)	0.78	1.65
S (Location)	0.39	NS
GxS	1.106	2.26

iv) Number of Flower heads per Plant: A critical scrutiny of data presented in Table 15 revealed that maximum number of flower heads were obtained from

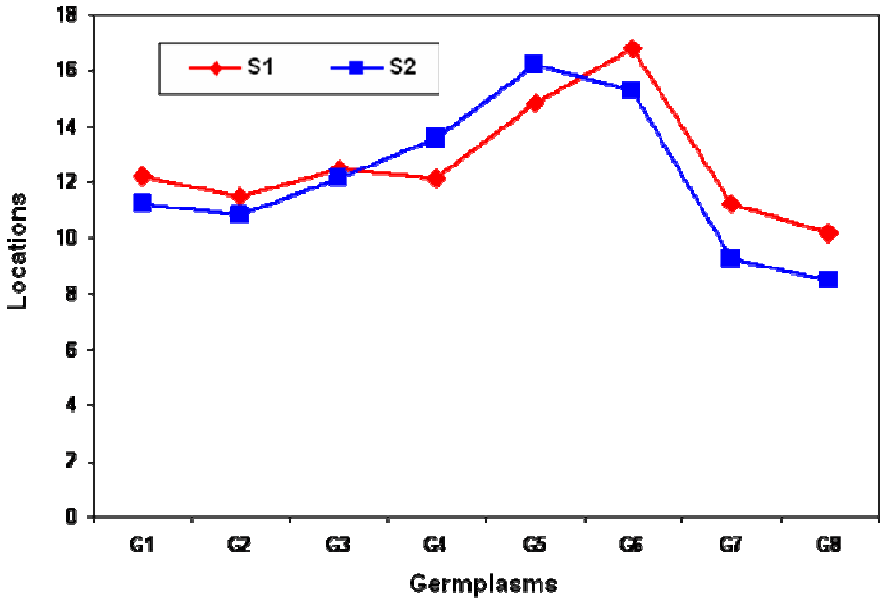


Fig. 26. Effect of sites and germplasm collection number of heads of *Inula racemosa* in 2011-2012

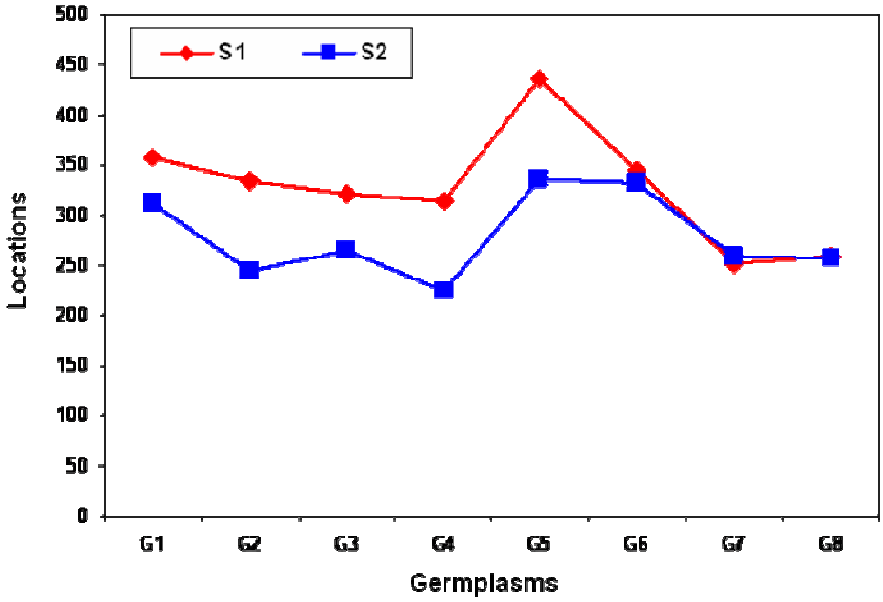


Fig. 27. Effect of sites and germplasm collection on number of seeds/head of *Inula racemosa* in 2011-2012

germplasm collection site G_6 (Kukumseri, HP) with 16.08 flower heads per plant which was significantly highest for all other values. The minimum (9.33) flower heads per plant were recorded from germplasm collection site G_8 (Shopian, J&K).

Under location, S_1 showed maximum (12.68) number of flower heads per plant as compared to S_2 with 12.14 numbers of flower heads per plant.

Among the interactions effect between germplasm collection and locations maximum (16.84) number of flower heads per plant was obtained from $G_{6\times}S_1$ which was significantly at par with $G_{5\times}S_2$(16.22). The minimum (8.50) number of flower heads per plant was obtained from $G_{8\times}S_2$ which was found to be statistically at par with $G_{7\times}S_2$ (9.29)

Table 15. Effect of Location sites and Germplasm collection on number of Flower heads per plant of *Inula racemosa* (2011-2012)

Locations / Germplasm	Number of Flower heads per plant		
	S_1 (Shilly)	**S_2 (Manali)**	**Mean**
G_1 (Keylong, HP)	12.23	11.23	**11.73**
G_2 (Kardang , HP)	11.49	10.81	**11.15**
G_3 (Dalang , HP)	12.47	12.18	**12.32**
G_4 (Sissu, HP)	12.17	13.59	**12.88**
G_5 (Udaipur, HP)	14.83	16.22	**15.52**
G_6 (Kukumseri HP)	16.84	15.32	**16.08**
G_7 (Tangmerg , J&K)	11.23	9.29	**10.26**
G_8 (Shopian , J&K)	10.15	8.50	**9.33**
Mean	**12.68**	**12.14**	

Treatments	**SEd±**	**$CD_{0.05}$**
G (Germplasm)	0.38	0.76
S (Location)	0.18	0.38
GxS	0.526	1.07

v) Number of seeds per head: Data presented in Table 16 revealed that maximum (385.90) number of seeds was recorded from germplasm site G_5 (Udaipur, HP) which was statistically higher from all the values. The significantly minimum number (247.60) of seeds per flower head was obtained from G_7 (Shopian, HP) which was statistically at par with site G_8 (258.40)) 258.40 per head.

Under the locations, S_1 showed maximum (327.20) number of seeds per head as compared to S_2 (276.60).

Among the interaction effect between germplasm collection site and locations, maximum number of seeds per head were observed from $G_{5\times}S_1$ (435.80) which was statistically different from all other values. The minimum (223.70) number of seeds per head was recorded from $G_{4\times}S_2$ which was found to be statistically similar with $G_{2\times}S_2$ (244.30)

Table 16. Effect of sites and germplasm collection on number of seeds per head of *Inula racemosa* (2011-2012)

Locations / Germplasm	Number of Seeds per head		
	S_1 (Shilly)	**S_2 (Manali)**	**Mean**
G_1 (Keylong, HP)	357.60	311.20	**334.40**
G_2 (Kardang , HP)	333.90	244.30	**289.10**
G_3 (Dalang , HP)	320.50	265.00	**292.70**
G_4 (Sissu, HP)	313.50	223.70	**268.60**
G_5 (Udaipur, HP)	435.80	335.90	**385.90**
G_6 (Kukumseri HP)	345.50	331.40	**338.50**
G_7 (Tangmerg , J&K)	252.30	258.60	**247.60**
G_8 (Shopian , J&K)	258.60	258.20	**258.40**
Mean	**327.20**	**276.60**	

Treatments	**SEd±**	**$CD_{0.05}$**
G(Germplasm)	7.96	16.25
S (Location)	3.97	8.12
GxS	11.25	22.97

vi) Primary root length: Appraisal of data in Table 17 revealed that maximum length of primary root was recorded in G_6 (25.74 cm) which was statistically higher than all other values. The minimum length of primary root was recorded in G_2 (18.69 cm).

Under locations, maximum (23.40 cm) value for primary root length was recorded for location S_2, followed by S_1 (17.94 cm).

Among the interactions effect between germplasm collection site and locations maximum primary root length of 31.79 cm was recorded from $G_{6\times}S_2$

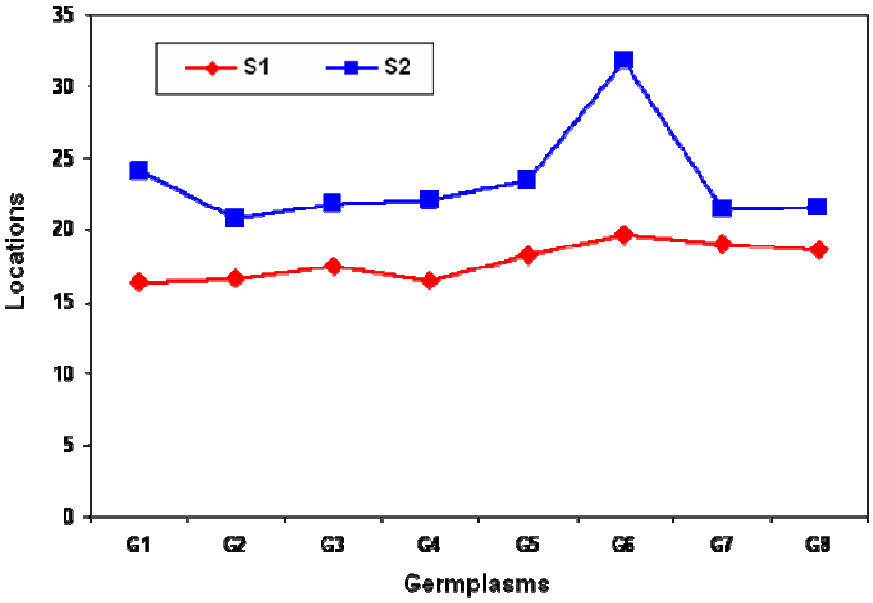


Fig. 28. Effect of sites and germplasm collection on length of primary roots *Inula racemosa* in 2011-2012

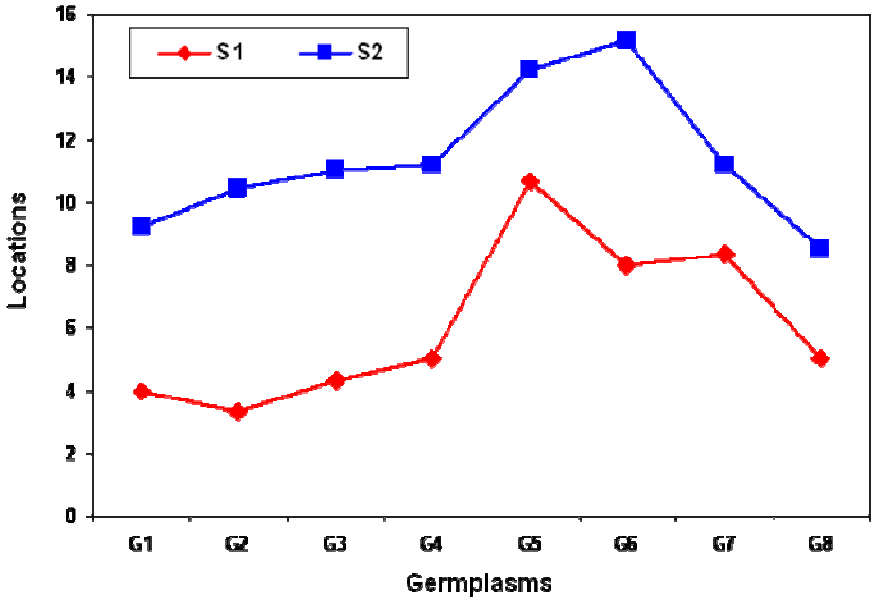


Fig. 29. Effect of sites and germplasm collection on number of lateral roots of*Inula racemosa* in 2011-2012

31.79 cm, where as minimum primary root length was observed from interaction $G_1 \times S_1$ with 16.30 cm which was found to be statistically at par with $G_{2\times}S_1$(16.56 cm) and $G_{4\times}S_1$ (16.46 cm).

Table 17. Effect of Location sites and Germplasm collection on primary root length of *Inula racemosa* (2011-2012)

Locations / Germplasm	Primary root length (cm)		
	S_1 (Shilly)	**S_2 (Manali)**	**Mean**
G_1 (Keylong, HP)	16.30	24.13	**20.21**
G_2 (Kardang , HP)	16.56	20.83	**18.69**
G_3 (Dalang , HP)	17.56	21.85	**19.70**
G_4 (Sissu, HP)	16.46	22.13	**19.29**
G_5 (Udaipur, HP)	18.25	23.47	**20.86**
G_6 (Kukumseri HP)	19.70	31.79	**25.74**
G_7 (Tangmerg , J&K)	19.06	21.46	**20.26**
G_8 (Shopian , J&K)	19.67	21.53	**20.60**
Mean	**17.94**	**23.40**	**20.21**

Treatments	**SEd±**	**$CD_{0.05}$**
G (Germplasm)	0.32	0.65
S (Location)	0.16	0.33
GxS	0.45	0.91

vii) Number of Lateral Roots: Data pertaining to number of lateral roots presented in Table 18 revealed that maximum (12.45) number of roots were recorded from germplasm collection site G_5 (Udaipur, HP) which was significantly at par with site G_6 (Kukumseri, HP) with 11.58 number of lateral roots. Minimum number of lateral roots (6.61) were recorded from germplasm collection site G_1 (Keylong, HP) and was statistically found to be at par with G_2 (6.89), G_3 (7.69) and G_8 (6.77).

Under locations maximum number of lateral roots (11.38) was found from S_2 and minimum (6.08) from S_1

The interaction among the germplasm collection sites and the location was found to be significant at 5% level of significance. The maximum number of lateral roots was found in $G_1 \times S_2$ (15.17) which was observed to be statistically at

par with $G_5 \times S_2$ (14.24), whereas the minimum number (3.33) of lateral roots were recorded in $G_2 \times S_1$ which was found to be significantly at par with $G_1 \times S_1$ (4.00) and $G_3 \times S_1$ (4.33).

Table 18. Effect of Location sites and Germplasm collection on number of lateral roots of *Inula racemosa* (2011-2012)

Locations / Germplasm	Number of lateral roots		
	S_1 (Shilly)	**S_2 (Manali)**	**Mean**
G_1 (Keylong, HP)	4.00	9.23	**6.61**
G_2 (Kardang , HP)	3.33	10.46	**6.89**
G_3 (Dalang , HP)	4.33	11.06	**7.69**
G_4 (Sissu, HP)	5.00	11.19	**8.09**
G_5 (Udaipur, HP)	10.67	14.24	**12.45**
G_6 (Kukumseri HP)	8.00	15.17	**11.58**
G_7 (Tangmerg , J&K)	8.33	11.19	**9.76**
G_8 (Shopian , J&K)	5.00	8.53	**6.77**
Mean	**6.08**	**11.38**	

Treatments	**SEd±**	**$CD_{0.05}$**
G (Germplasm)	0.61	1.24
S (Location)	0.31	0.62
GxS	0.86	1.76

viii) Fresh Root Weight (g): Perusal of data presented in Table 19 revealed that germplasm collection sites and location had significant effect on the fresh root of *Inula racemosa*. Maximum fresh root weight of 313.0 g was recorded from germplasm collection site G_5 (Udaipur, HP) and was statistically higher than all other recorded values. The minimum fresh root weight of value 197.60 g was recorded from germplasm collection site G_8 (Shopian, J&K).

The critical scrutiny of the data revealed significant difference among two locations for fresh root weight. The fresh root weight was recorded to be significantly higher (244.80 g) in S1 (Shilly) as compared to S_2 (239.80 g)

The interaction among germplasm collection sites and location was found to be significant at 5% of significance level. The data given in Table -19 shows that the highest fresh root weight of 313.40 g was noticed in $G_5 \times S_1$ which was

Plate 13. Root, Flower heads and seeds yield from *Inula racemosa*

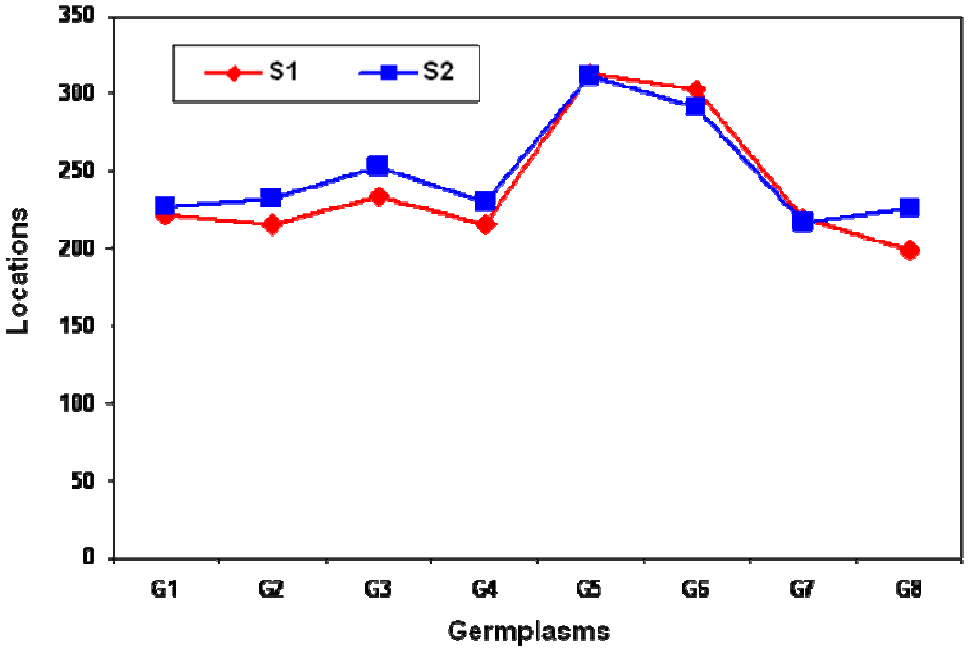


Fig. 30. Effect of sites and germplasm collection on root fresh weight of *Inula racemosa* in 2011-2012

statistically similar $G_5 \times S_2$ (312.70 g) and lowest fresh root weight of 197.60 g was recorded in $G_8 \times S_1$ which was recorded to be significantly at par with $G_8 \times S_1$(198.0 g).

Table 19. Effect of Location sites and Germplasm collection on Fresh Root Weight of *Inula racemosa* (2011-2012)

Locations / Germplasm	Fresh Root Weight		
	S_1 (Shilly)	**S_2 (Manali)**	**Mean**
G_1 (Keylong, HP)	221.0	225.40	**223.20**
G_2 (Kardang , HP)	215.0	231.80	**223.40**
G_3 (Dalang , HP)	234.0	252.90	**243.40**
G_4 (Sissu, HP)	215.70	228.60	**222.20**
G_5 (Udaipur, HP)	313.40	312.70	**313.0**
G_6 (Kukumseri HP)	302.70	291.70	**297.30**
G_7 (Tangmerg , J&K)	218.60	217.30	**218.0**
G_8 (Shopian , J&K)	197.60	198.0	**197.80**
Mean	**244.80**	**239.80**	

Treatments	**SEd±**	**$CD_{0.05}$**
G(Germplasm)	0.61	1.24
S (Location)	0.30	0.62
GxS	0.86	1.75

ix) Dry Root Weight: An inquisition of data given in Table 20 revealed that maximum dry root weight 148.80 g was obtained from germplasm collection site G_5 (Udaipur, HP) and was statistically higher from all other recorded values for this parameter. The minimum value for dry root weight (97.67 g) was obtained for the site G_8 (Shopian, J&K).

Under locations studied significantly maximum value (122.20 g) for dry root weight was recorded from location S_1 and minimum (114.0 g) was recorded from location S_2.

The interaction between germplasm collection sites and location sites was found to be statistically significant. The maximum dry root weight (155.30 g) was found in $G_5 \times S_2$, whereas minimum dry root weight (97.43 g) was observed in $G8 \times S_1$ which was found to be statistically at par with $G_8 \times S_1$ (97.92 g) .

Table 20. Effect of Location sites and Germplasm collection on Dry Root Weight of *Inula racemosa* (2011-2012)

Locations / Germplasm	Root dry weight		
	S_1 (Shilly)	**S_2 (Manali)**	**Mean**
G_1 (Keylong, HP)	105.50	113.10	109.30
G_2 (Kardang , HP)	103.30	116.50	109.90
G_3 (Dalang , HP)	116.60	127.40	122.00
G_4 (Sissu, HP)	105.90	115.30	110.60
G_5 (Udaipur, HP)	142.20	155.30	148.80
G_6 (Kukumseri, HP)	132.60	145.10	138.80
G_7 (Tangmerg, J&K)	108.20	107.00	107.60
G_8 (Shopian, J&K)	97.43	97.92	97.67
Mean	**122.20**	**114.00**	

Treatments	**SEd±**	**$CD_{0.05}$**
G(Germplasm)	0.65	1.34
S (Location)	0.33	0.67
GxS	0.93	1.89

Chapter-5

DISCUSSION

The results obtained during the present investigations on "**Evaluation of germplasm and standardization of propagation techniques of *Inula racemosa* Hook.f."** have been discussed in this chapter as under:

5.1 EVALUATION OF *Inula racemosa* GERMPLASM

5.1.1 Germplasm collection

In the present study, germplasm of *Inula racemosa* collected from different eight sites cultivated sites has been evaluated on the basis of morphological and quantitative characters including the percentage of essential oil in roots.

Critical analysis of the results of present work have revealed that the germplasm of *Inula racemosa* collected from different sites have shown a significant variation in morphological and quantitative parameters. Maximum plant height (204.90 cm), number of stems (4.74), leaf length (54.15 cm), leaf breadth (24.85), flower heads per plant (22.15), primary root length (22.82 cm), and essential oil content 1.96 per cent have been observed in germplasm collection site G_6 (Kukumseri, HP). However, maximum fresh root weight of 659.30 g has been recorded for G_5 (Udaipur, HP) and found to be statistically at par with the site G_6 (Kukumseri, HP). The performance on sandy loam and alluvial soils has been reported to be better but that growth and yield being highest on blackish sandy loam soil (Rawat and Everson, 2011). The variability among populations is indicative of differential selection pressures across environments and can affect phenotypic and genotypic characteristics in different ways (Petit and Thompson, 1998). Studies suggest that in various mountainous areas altitude and latitude plays a significant role in determining vegetative and reproductive characters (Airi *et al*., 1997). The relationship between altitude and reproductive / vegetative characters has also been reported in earlier studies (Mac

Arthur and Wilsom 1967; Bhatt, 2004). In extreme climate and ecological conditions, plants of alpine zone possess attractive appearance, interesting mode of perennation and special morphological, physiological and adaptation features. Indian Himalyan region harbors several medicinal plants and more importantly, the plants of higher zone synthesize secondary metabolites of medicinal importance and therefore, offer great possibilities of having novel bimolecular and even larger quantity of active components (Nautiyal *et al*., 2002). Plants grow in a modular manner and are indeterminate in their growth which allows them to be highly plastic in both morphology and reproduction. Moreover, because buds act as the germ, any mutations acquired will be passed along through subsequent cell divisions. It is this mode of growth that allows the large degree of morphological plasticity, characteristic of plant growth, resulting in wide intraspecific differences in many features of plant architecture (Silvertown and Lovett, 1993).

Shabir *et al*., (2013) studied among and within population variation in growth dynamics and floral sex ratios in *Inula racemosa* from North West Himalayas and reported distinct directional trends in growth morphology, architecture and fitness-related traits across different populations of *Inula racemosa* growing along a steep altitudinal gradient.

Cultivated roots of *Inula racemosa* from Lahaul and Spiti have been reported superior to even the roots of the European species of *Inula helenium* or elecampane (Kashman *et al*., 1967; Purushothaman and Sarda 1974). Root is the official part of the *Inula racemosa* and still as an aromatic plant domestic forms of this incipient cultigen have been selected by the natives from the wild types which occur amongst stony, alpine scrub vegetation in cold arid habitat between 2700-3500 m. As essential oil obtained from the roots is of great importance for medicinal and perfumery industry (Singh *et al*., 2011). Our results have revealed more essential oil percentage than these reported earlier (Agnihotri *et al*., 2004; Dhramaveer, 2012) which suggests the potential of cultivation of best germplasm for getting higher economic returns.

Plate 14. Quadrangular seeds with bristly pappus

Interestingly, the high altitude populations of medicinal plants such as those from Lahaul & Spiti are known to yield markedly superior active principles compared to their lower altitude counterparts (Sharma *et al*., 2006)

5.2 SEED CHARACTERISTICS AND GERMINATION BEHAVIOUR

5.2.1 Seed characteristics and germination parameters

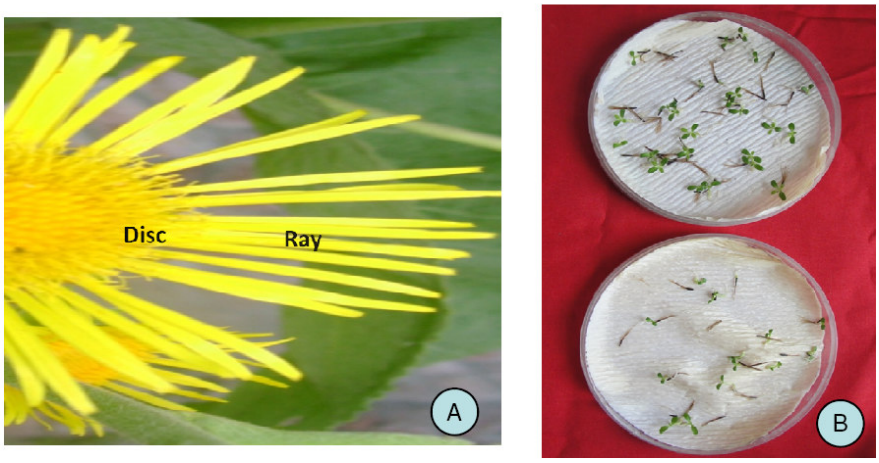
A seed source is an important factor in determining the seed quality (Savenko and Pudzarova, 1970; Doikov, 1973). Success in establishment and productivity of plants is, generally, determined by the species used and the different seed sources of the species. Many studies on provenance and seed source have been made or are currently underway (Wright and Baldwin, 1957; Wells and Wakeley, 1970 ; Lacaze, 1977; Vashistha *et al*., 2013) in determining the best species and seed sources within species. The source information, leading to the reliability and availability of the desired source of seed, needs to be determined. Species that exist in highly specific habitats often produce seeds with highly specialized adaptations (Navarro and Guitian, 2003). A congenial microhabitat may provide a higher chance of establishing a large gregarious population, even for a rare and endangered species (Pradhan and Badola, 2008).

The results of the present study have revealed significant differences for seed characteristics *viz*. seed weight, moisture per cent, seed viability per cent and germination per cent for the two consecutive years (2010-11 & 2011-12), thereby indicating a large amount of variation among the seed sources (Table 2). The maximum seed weight of 1.49 g and 1.43 g have been recorded for germplasm collection site G_6 (Kukumseri, HP). For seed moisture content the maximum value of 24.93 per cent and 24.85 per cent have been recorded in G_6 (Kukumseri, HP) followed by G_5 (Udaipur, HP). These findings are in accordance with Gabriel (1978); Pradhan and Badola (2008) who reported that provenances from higher altitudes and cooler temperate zones produce heavier seeds. In addition, several studies have indicated that environmental conditions of the female parent in a stand can have a strong influence on weight and size of seed (Sorensen and Campbell, 1985; Sorenson and Franklin, 1977).

The response of different plant populations on seed germination provides helpful clues on the genetic make-up of the species and its existence in the natural settings (Baskin and Baskin, 1998) which is essential to select elite seeds (Jayshanker et *al*., 1999) for ex-situ conservation of gene resources. Seed storage, and frequent testing to monitor losses in germination rate, which might adversely affect nursery recovery rates, is considered as one of the most efficient methods (Gonzalez-Benito *et al*., 1998; Williams *et al*., 2007) to select elite populations. However, poor seed germination has been shown to be one of the limiting factors (Butola and Badola, 2004; 2006). Due to inappropriate storage, germination capacity of a species may decline during the first few months after collection whereas; proper storage may be effective over a considerable storage period (Butola and Badola, 2004).

Germination is the sequential series of physiological and morphogenetic events that result in the transformation of an embryo into a seedling (Berlyn, 1992). It is considered as the most important quality test in evaluating the planting value of a seed. The ability of seed to produce normal seedlings and plants is measured in terms of germination test. The ultimate objective of seed germination testing is to obtain information with respect to the planting value of the seed and to provide results, which could be used to compare the value of different seed sources. A number of environmental factors together with the make-up of a seed affect germination phenomenon (Jagetiya and Pankajpurohit, 2006)

The analysis of data depicted has shown highly significant differences for germination per cent, seed viability, and moisture content (Table 2) suggesting huge variation and thus there exists a scope for improvement of these traits. Perusal of data on highest germination and seed viability per cent in site G_4 (Sissu, HP), G_5 (Udaipur, HP) and G_6 (Kukumseri, HP) have shown a direct relation with moisture content. Germination values are the function of seed size and weight (Czabator, 1962; Dunlapp and Barnett, 1983). Significant variation in seed viability besides germination per cent between the sites are in conformity



Disc floret

Plate 15. A) Capitulum, B) Germination of *Inula racemosa seeds;* C) Disc floret showing stigmas

with, those found in fir and spruce (Singh and Singh, 1981), *Acacia* species (Mathur *et al*., 1984) and *Dalbergia sissoo* (Vakshasya *et al*., 1992).

Seed size (length and width), along with seed weight have strong influence on seed germination (Baldwin, 1942; Dunlapp and Barnett, 1983). Generally, larger seeds germinate fast and more completely than the smaller ones due to more endosperm nutrient pool (Kandya, 1978). Hence the germination per cent and related traits may be ascribed to have significant difference due to seed size and seed weight. Khalil (1973) has concluded that heavier seeds produce more viable and faster growing seedlings in *Picea maliana*. Fins and Libby (1982) in *Sequoia dendren*, having variable seed germination without any clear geographic trends. The above findings are in line with the works of Chaisurisri *et al.* (1994) in Sitka spruce and Clair and Adams (1991) in Douglas fir. Seeds of endangered species *Podophyllum hexandrum* from alpine regions have been reported to exhibit greater germination than those from the subalpine regions.

In dry seeds, enzymatic reactions may play small role in seed ageing because dry seeds lack active enzymatic metabolism. However, certain non-enzymatic reactions such as Amadori and Maillard reactions are known to occur even at very low moisture content (Ali, 2008).

5.2.2 Seed dormancy

Seed dormancy and poor germination are considered to be a barrier to the regeneration and *ex situ* cultivation of commercially important plant species. The freshly collected seeds of *Inula racemosa*, have found to be completely dormant; there has been no germination despite 100 per cent seed viability and which loss gradually after two year storage (Sharma and Sharma, 2010). Failure of seed germination should not be taken as an indication of dormancy. Many a time seeds may not germinate because of certain environmental conditions like sufficient moisture or temperature. Thus our results are improving the results Shabir et *al.,* (2010) obtained for this species from wild population.

5.3 EFFECT OF PRE-SOWING TREATMENTS ON GERMINATION PARAMETERS

Pre-sowing treatments are the various physical, chemical and mechanical methods advocated to break dormancy in seeds, which aim at softening the hard endocarp, reducing the effect of mesocarp, leaching out of inhibitor locations on the seed or seed coat and shortening dormancy period, thus, achieving fast, uniform and desired seed germination percentage. Germination response of seeds to growth regulators is highly viable, which generally depends on both internal and external factors. The growth regulators may easily penetrate the seeds at their optimum concentration and also be available at the site of action. So these are vitally important and their application to seeds intensifies the metabolism, improve the germination and plant productivity (Kulkarni and Ganapathi, 2003). The analysis of the data (Table 3) has depicted highly significant differences for Germination per cent, Germination energy, Germination speed, Peak value, Mean daily germination, Germination value, Germination index, and minimum Number of days taken for germination.

Appraisal of data in Table-3 on the experiment conducted under laboratory conditions reveals that maximum (87.74 and 87.93) per cent germination per cent, germination energy (19.20 and 19.00) per cent, Maximum germination speed (0.68) is noticed in treatment P_6 (IBA 50 ppm) which is statistically at par with pre-sowing treatment P_5 (GA_3 150 ppm) with value of 0.66 during both the years of study *i.e.* 2010-11 and 2011-12 whereas, minimum in control. Similar findings were observed by Kaushal and Rana, 2004. This may be due to the fact that the action of GA_3 applied could have sustained itself till the plants reached vegetative stage. Further, this could be related to an elaboration of an endo-membrane system and regulated synthesis of proteins required for germination, which in turn could have contributed additionally to the amino acid rescue and protein turnover during active metabolism, later in plant life (Shah, 2007). Appreciable reduction in mean germination time with concentration of GA_3 was also reported by Pradhan and Badola, 2008. Previous studies have shown that GA_3 enhances the germination of seeds exhibiting physiological, morphological or morpho-physiological dormancy (Ganai and

Nawchoo, 2002; Shivakumar *et al.,* 2006). The efficacy of GA_3 treatment in breaking dormancy depends on the concentration and length of incubation.

The effectiveness of GA_3 and chilling treatment in causing dormancy removal of freshly harvested seeds of *Inula racemosa* has also been reported earlier (Sharma *et al*., 2006). The effects of gibberellins (GA_3) could be ascribed to stimulate activities of various hydrolytic enzymes and consequently increased availability of nutrients for embryo growth. GAs also regulates seed germination by losing the mechanical restraints of the testa and endosperm to permit easy protrusion of the radical. Generally, the micropylar endosperm acts as a barrier to radical expansion and therefore contributes to seed dormancy. In several species, endosperm weakening has been found to be associated with induction of cell-wall remodeling enzymes, including endo *β*-mannanase, *β*-1,3-glucanases, pectin methylesterases, polygalacturonase and others (Linkies *et al*., 2010; Holdsworth *et al*., 2008). GAs also promote seed germination of dormant seeds by overcoming germination constraints existing in seeds by requiring after-ripening (Grappin *et al*., 2000), light and cold (Casal and Sanchez, 1998). Low - temperature treatment is known to activate GA synthesis and /or increase the sensitivity of the embryo towards GA. Due to these changes the embryo is able to penetrate the covering structure (Halinska and Lwak, 1987; Sharma and Sharma, 2010; Shabir, 2011).

5.3.1 Effect of pre-sowing treatments on seedling parameters

Highly significant differences for germination per cent, seedling height, collar diameter, seedling vigour index, fresh shoot weight, dry shoot weight, fresh root weight, dry root weight and root length under field conditions have been obtained (Table-7) which indicate considerable amount of variability in the germplasm of composite seed sample material under study.

Appraisal of data for both the consecutive years (2010-11 and 2011-12) under field conditions maximum (84.74 and 87.07 %) germination per cent has resulted when seeds have been treated with 150 ppm GA_3 (P_5) and minimum value of 55.00 per cent has been obtained in P_1 (control). The same treatment *i.e*.

P_5 (GA_3 150 ppm) has also shown maximum values for seedling height (47.25 and 48.65 cm), collar diameter (4.12 and 4.17 cm), and seedling vigour index (4004.00 and 4236.00) as compared to treatment P_1 (control). Maximum fresh shoot weight (54.10 and 52.77 g) and dry shoot weight (27.77 and 26.35 g) has been seen under treatment P_5 (GA_3 150 ppm) against treatment P_1 in control. The maximum fresh root weight (9.22 and 9.33g) and maximum dry root weight (4.61 and 4.31g) is also noticed in treatment P_5 (GA_3 150 ppm) as compared to control. It is clear from the earlier findings of Dhoran and Gudadhe, (2012) while working on effect of plant growth regulators on seed germination and seedling vigour in *Asparagus sprengeri* the treatment of GA_3 was effective in breaking seed dormancy and reaching the germination rate and high vigour index.

Comparatively of all the pre-sowing treatments, both GA_3 and IBA growth regulators are superior to improve root and shoot growth in *Inula racemosa* which is of economic importance and these findings are in accordance with earlier reports on Kuth, the same family plant and used as adulterant (Kaushal and Rana, 2004). The collar diameter increased in treatment P_5 and decreased in IBA, with increase in IBA concentration (Ali, 2008). The increase in collar diameter might be due to more number of leaves and vigorous root system, which might have resulted in more carbohydrate production and assimilation.

5.3.2 EFFECT OF LOCATION SITE AND GERMPLASM COLLECTION ON GERMINATION AND GROWTH OF *Inula racemosa*

Testing vegetative cultivation such as propagation through tubers or root segments may be a useful approach for reducing the long cultivation cycle (Kuniyal *et al*., 2004). However, these should be tested in the field before recommending them to farmers. Moreover, mass scale propagation and selection of elites is also possible through this method (Kuniyal *et al*. 2010).

In the current scenario competition of demographic issues along with lack of resources, cultivable land is the main issue for global concern. It is not only difficult but also impossible to maintain the gene-bank or germplasm of a species every off season.

Mainly three constraints are found in the cultivation of rare, endangered and threatened medicinal plants like, *Aconitum heterophyllum*, *Angelica glauca*, *Pdophyllum hexandrum*, *Inula rcaemosa and Picrorhiza kurroo.* These are lengthy cultivation cycle, small land holdings, low and fluctuating market prices (Nautiyal *et al.,* 2001; Sharma & Sharma, 2010 and Rawat and Everson, 2011).

The negligence of people due to labour cost, fragile ecosystem and physiographic factors due to cold desert valley are the important factors to cultivate the manu or pushkarmool on large scale. However, efforts has been made for long time to maintain the germplasm of important medicinal plants for sustainable conservation.

The roots of *Inula racemosa* are used in traditional medicine, but are of great economic importance due to large demand by the pharmaceutical industry. Therefore it is not possible to maintain the germplasm of *Inula racemosa* for mass cultivation and domestication through vegetative propagation every time due to the constraints in sexual propagation as seeds are not viable due to high sterility, self incompatibility and very little germination period (Arora *et al*., 1980; Chauhan, 1999; Shabir, 2011). Hence in the present study it has been found that the collar portion or collar bud of the plant with one eye bud segment and by giving uniform treatment (IBA 150 ppm) of growth regulator have been used for the successful establishment of the potential medicinal plant for large scale propagation and introduction with suitable way.

The present investigation revealed a significant influence of IBA 150 ppm on sprouting per cent, number of shoots, number of leaves, and number of flower heads, seed production, primary root length (cm), lateral roots, fresh weight and dry weight (g) of roots for the germplasm collected from different sites. The basis for this may be the potentiality of IBA in root induction and the enhancement of hydrolysis of nutrient reserves by auxin treatments. Similar trend was also observed by (Shabir *et al*., 2010; Shabir, 2011) in *Inula racemosa.* The probable reason for increase in biomass may be the better utilization of carbohydrates,

nitrogen and other nutrients which has been aided by growth regulators (Chandramouli, 2001).

Similar results were shown by Butola and Badola, (2007) for *Angelica glauca* and *Heracleum candicans* percentage of rooting after IAA and IBA as compared to control shows the higher potential induction in adventitious root development as reported earlier in *Oreganum vulgare* (Kuris *et al.,* 1980). Moreover, the chances of survival and growth performance of vegetatively propagated individuals are better implying that a reasonable number of plantlets could be raised and their survival could be ensured by using different concentrations of IAA and IBA.

Present investigations on effect of location sites and germplasm collection sites for *Inula racemosa* conducted at two different land forms for yield and better survival reveals that microclimate, light and temperature on germination and growth of *Inula racemosa* is showing significant effects. In plants, the emergence of seedlings and their survival varies greatly from one habitat to another. Highly specific habitats often produce highly specialized adaptations even for rare and endangered species (Navarro and Guitian, 2003). Both internal and external environmental factors strongly influence germination and establishment (Baskin and Baskin 1998, Uysal *et al*., 2006). The importance of the ecological conditions prevailing in a given habitat through the observed variability in germination among germplasm collected from different microhabitats or sources is often restriced to locations that meet specific environmental conditions. These are often referred to as 'safe-sites' or regeneration niches in natural environment (Pradhan and Badola, 2012). Therefore, preservation of such safe-sites along with the restoration of habitat is crucial for conservation of the species.

According to the present investigation on germplasm evaluation and propagation studies of *Inula racemosa* it has been found a sun loving plant and need and open sunny location for establishment with no drainage or water logging condition as root is the official part. Comparing to both the location sites under study with germplasm collected from different sites, it is evident that there

is a significant difference for location site S_1 (Shilly) and S_2 (Manali) and germplasm site with respect to interaction under parameter sprouting percent, number of shoots, number of leaves, flower heads per plant number of seeds produced per head number of lateral roots, fresh root weight and dry root weight. It might be due to the acclimatization of plant for both the location sites due to easily adaptability and local climatic factors like microhabitat, and elite germplasm. Similar findings are reported by Nautiyal *et al*. (2001) and Thakur *et al*. (2010) while working on performance of *Picrorhiza kurroa.*

Primary objective of germplasm evaluation is to produce more rooting percentage while using rhizome segment *i.e.* eye bud from collar portion will lead to a successful beginning to domestication of this little known aromatic plant of Lahaul valley (Arora *et al*., 1980). In fact it has been introduced from some other places for domestication but accordingly ecological conditions has been adopted by it in the fragile system of cold desert *i.e.* Lahaul Valley. Among the eight sites studied for germplasm collection and evaluation and it is also noticed that Udaipur and Kukumseri (Pattan valley) are maintaining seed banks of Manu or Pushkarmool in small areas of the Lahaul valley.

To encourage the cultivation of this endangered medicinal plant, establishment of value-addition centres and farmers federations; stabilize and strenghthen of the existing market are the main issues (Rawat and Everson, 2011)

Plants of alpine regions have various morphological means of adaptations against adverse climatic conditions. Plant phenology in alpine region is strongly influenced by variation in microenvironments related to microtopography (Bliss, 1966). Therefore variations of the plants are the product of interaction, between genotype and environment.

Chapter-6

SUMMARY AND CONCLUSION

The present work entitled **"Evaluation of germplasm and standardisation of propagation techniques of *Inula racemosa* Hook. f.**" was conducted during 2010-11 and 2011-12 with the the following objectives:

i) **Evaluation of *Inula racemosa* germplasms from different sites;**

ii) **To assess the propagation techniques of *Inula racemosa* under field and controlled conditions**.

For achieving these objectives field trials were conducted in the experimental fields as well as laboratory of the Department of Forest Products. The plant is highly priced for its roots which are a source of raw material for pharmaceutical and perfumery industry. The main compounds of essential oil are alantolactone & isoalantolactone.

Knowledge regarding present findings from the study will lead to the successful introduction of medicinal herbs on large scale so as to uplift of the livelihood of the people especially in the upper regions of Himalayan zone and to find out the possible track for various industries as raw material to check out the adulteration in present scenario.

The salient results of the "**Evaluation of germplasm and standardisation of propagation techniques of *Inula racemosa* Hook. f.**" are summarised as under**:**

6.1 EVALUATION OF *Inula racemosa* GERMPLASM COLLECTED FROM DIFFERENT SITES

- The maximum plant height, leaf size, number of stems per plant and number of flower heads per plant, primary root length, fresh root weight and essential oil content were recorded in germplasm site collection G_5

and G_6 *i.e.*, Udaipur and Kukumseri (Pattan valley) Lahaul & Spiti from Himachal Pradesh

- Maximum fresh seed weight, moisture content, seed viability and germination percent during 2010-11 and 2011-12 were recorded in germplasm collection sites G_4, G_5 and G_6 *i.e.*, Udaipur and Kukumseri and Sissu of Lahaul & Spiti from Himachal Pradesh.

6.2.1 Effect of pre-sowing treatments on germination parameters under laboratory conditions

- The maximum germination per cent was recorded in treatment (P_5) GA_3 150 ppm and the minimum in control during both the years 2010-11 and 2011-12. Maximum germination energy per cent was also recorded in treatment (P_5) 150 ppm GA_3.
- The maximum germination speed was recorded in treatment (P_6 IBA 50 ppm) and (P_7 IBA 100 ppm) and minimum in control during both the study years.
- The maximum peak value was recorded in treatment P_5 (GA_3 150 ppm) with value of 5.38 and 5.37 during both the years and minimum in control.
- The minimum days taken for first germination was recorded in treatment (P_5) GA_3 150 ppm and maximum days taken for germination was recorded in control during both the years 2010-11 and 2011-12.
- The maximum mean daily germination was recorded in treatment (P_5) GA_3 150 ppm and minimum in control during both the years the years 2010-11 and 2011-12.
- Maximum germination value and germination index was recorded in treatment P_5 (GA_3 150 ppm) and minimum in control for both the years during 2010-11 and 2011-12.

6.2.3 Effect of pre-sowing treatments on germination parameters under field conditions

- The maximum germination per cent and seedling height was recorded in seeds having pre-sowing treatment P_5 (GA_3 150 ppm) and minimum was recorded in control during both the years 2010-11 and 2011-12.

- The maximum collar diameter (4.12 cm and 4.17 cm) was recorded in pre-sowing treatment P_5 (150 ppm GA_3) during 2010-11 and 2011-12. The maximum seedling vigour iIndex was also recorded in treatment P_5 (150 ppm GA_3) during both the years 2010-11 and 2011-12.
- The maximum fresh shoot weight (54.10 g and 52.77 g) and dry shoot weight (27.77 g and 26.35 g) was recorded in treatment P_5 (GA_3 150 ppm) and minimum under control for both the years during 2010-11 and 2011-12 respectively.
- The maximum Fresh Root Weight (9.22 g and 9.32 g) and Dry Root Weight (4.61 g and 4.64 g) was recorded in seeds given treatment in P_5 (GA_3 150 ppm) and minimum under control for both the years during 2010-11 and 2011-12 respectively.
- The maximum root length (10.86 cm and 11.77 g) was recorded in treatment P_5 (GA_3 150 ppm) and minimum under control for both the years during 2010-11 and 2011-12 respectively.

6.2.4 Effect of Location sites and germplasm collection sites on the germination and growth of *Inula racemosa*

- The maximum sprouting 95.35 per cent was observed from germplasm collection site G_5 (Udaipur, HP) and minimum sprouting of 80.08 per cent was obtained from germplasm collection site G_8 (Tangmerg, J&K). Under the two different locations maximum sprouting of 87.04 per cent was recorded from S_2 (Manali) as compared to S_1 (Shilly) with value of 86.13 per cent and was found to be non significant. Among the interactions maximum sprouting per cent was observed from $G_5 \times S_1$ (95.43 %) and minimum value of 78.26 per cent was recorded in $G_7 \times S_1$.
- The maximum number of shoots were recorded in germplasm collection site G_6 (4.0) and minimum number of shoots was recorded from G_7 with a value of 1.98 shoots per plant. Under the two different locations significantly maximum (2.98) number of shoots was recorded from location S_1 (Shilly) and minimum (2.56) from location site S_2 (Manali). Among the interactions germplasm and location maximum (4.53) number

of shoots per plant was observed from $G_{6\times}S_1$ and minimum number of shoots was observed in $G_{7\times}S_2$ with 1.67 number of roots.

- The maximum value for number of leaves (13.73) were observed from germplasm collection site G_6 (Udaipur, HP) and minimum (10.08) number of leaves were recorded from G_8 (Tangmerg, J&K) site collection. Under locations, S_2 (Manali) showed maximum (12.47) number of leaves as compared to S_1 (Shilly) 11.79 and there was no significant difference among the locations sites. Among the interactions between germplasm collection sites and locations maximum number of leaves (15.80) were recorded in $G_{6\times}S_2$ which and minimum number of leaves 10.50 were recorded in $G_{7\times}S_2$.
- The maximum number of flower heads were obtained from germplasm collection site G_6 (Kukumseri, HP) with 16.08 flower heads per plant which was significantly highest for all other values and the minimum (9.33) flower heads per plant were recorded from germplasm collection site G_8 (Shopian, J&K). Under locations, S_1 (Shilly) showed maximum (12.68) number of flower heads per plant as compared to S_2 (Manali) with 12.14 numbers of flower heads per plant. Among the interactions effect between germplasm collection and locations maximum (16.84) number of flower heads per plant was obtained from $G_{6\times}S_1$ and minimum (8.50) number of flower heads per plant was obtained from $G_{8\times}S_2$.
- The maximum primary root length was recorded in G_6 (25.74 cm) which was statistically higher than all other values and the minimum length of primary root was recorded in G_2 (18.69 cm).Under locations, maximum (23.40 cm) value for primary root length was recorded for location S_2, followed by 17.94 cm for S_1(Shilly). Among the interactions effect between germplasm collection site and locations maximum primary root length of 31.79 cm was recorded from $G_{6\times}S_2$ (31.79 cm), where as minimum primary root length was observed from interaction $G_1\times S_1$ with 16.30 cm.
- The maximum number of lateral roots (12.45) were recorded from germplasm collection site G_5 (Udaipur, HP) and minimum number of lateral roots (6.61) were recorded from germplasm collection site G_1

(Keylong, HP) . Under locations maximum number of lateral roots (11.38) was found from S_2 (Manali) and minimum (6.08) from S_1 (Shilly). Among the interaction among the germplasm collection sites and the location sites, maximum number of lateral roots was found in $G_1 \times S_2$ (15.17) whereas, the minimum number (3.33) of lateral roots were recorded in $G_2 \times S_1$.

- The maximum fresh root weight of 313.0 g was recorded from germplasm collection site G_5 (Udaipur, HP) and the minimum fresh root weight of value 197.60 g was recorded from germplasm collection site G_8 (Shopian, J&K). Under the locations fresh root weight was recorded to be significantly maximum (244.80 g) in S_1 (Shilly) and minimum (239.80 g) at location site Manali S_2 (239.80 g). Among the interaction among the germplasm collection sites, maximum fresh root weight of 313.40 g was noticed in $G_5 \times S_1$ and lowest fresh root weight of 197.60 g was recorded in $G_8 \times S_1$.
- The maximum dry root weight 148.80 g was obtained from germplasm collection site G_5 (Udaipur, HP) and minimum value for dry root weight (97.67 g) was obtained for the site G_8 (Shopian, J&K) .Under locations studied significantly maximum value (122.20 g) for dry root weight was recorded from location S_1 and minimum (114.0 g) was recorded from location S_2. The interaction between germplasm collection sites and location sites was found to be statistically significant. The maximum dry root weight (155.30 g) was found in $G_5 \times S_2$, whereas minimum dry root weight (97.43 g) was observed in $G_8 \times S_1$.

CONCLUSION

On the basis of results obtained in the present investigation, it can be concluded that:

i) Germplasm collection sites G_5 (Udaipur, HP) and G_6 (Kukumseri, HP) and Himachal Pradesh have registered maximum value for morphological and quantitative parameters recorded under present study of investigation

for higher yield in terms of biomass and essential oil content comparatively than other sites.

ii) Seeds collected from germplasm collection sites Udaipur and Kukumseri, Pattan valley of Himachal Pradesh have higher seed weight, germination per cent and seed viability.

ii) Under pre-sowing treatments, plant growth hormone GA_3 (150 ppm) has shown maximum germination per cent, vigour index and higher biomass yield as compared to control.

iii) Maximum germination speed of 0.67 and 0.68 has been obtained from pre-sowing treatment (P_6) IBA 50 ppm during 2010-11 and 2011-12.

iv) Maximum peak value is recorded in treatment P_5 (GA_3 150 ppm) during both the year of study.

v) Maximum value of 13.25 days taken for first germination has been obtained in treatment P_1 i.e. control and minimum value of 1.25 and 1.23 is noticed in treatment P_5 (GA_3 150 ppm) during both the years.

vi) Multlocation trials conducted at two different locations, it has been found that maximum sprouting per cent and fresh root and shoot weight were noticed from germplasm sites G_5 (Udaipur) and G_6 (Kukumseri, HP) Lahaul and Spiti *i.e.* Pattan valley of Himachal Pradesh.

vii) Under location sites S_1 (Shilly) and (S_2) Manali maximum sprouting per cent was recorded from S_2 (Manali) and primary root length was also recorded from S_2 (Manali).

viii) Among the interactions maximum sprouting per cent is registered G_5 x S_1 and maximum root and shoot biomass is noticed for $G_5 \times S_2$ and G_6 x S_2.

Chapter-7

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Title of Thesis	:	**"Evaluation of Germplasm and Standardization of Propagation Techniques of *Inula racemosa* Hook.f."**
Name of the Student	:	**Harpal Singh**
Admission Number	:	F-2009-05-D
Major Advisor	:	**Dr. Bhupender Dutt**
Major Field	:	Forest Products
Minor Field(s)	:	i) Botany ii) Tree Improvent
Degree Awarded	:	Ph.D. Forestry (Forest Products)
Year of Award of Degree	:	2014
No. of Pages in Thesis	:	105+II
No. of words in Abstract	:	464

ABSTRACT

The present investigations entitled "**Evaluation of Germplasm and Standardization of Propagation Techniques of *Inula racemosa* Hook.f.**" were conducted in the laboratory and experimental fields of Department of Forest Products, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, (H.P) at Herbal Garden Nauni, Shilly (Solan) and Manali (Kullu) during 2010-11 and 2011-12. Observations on morphological and quantitative characteristics of *Inula racemosa* were recorded from eight different sites *i.e*. six from Himachal Pradesh (Lahaul & Spiti) *i.e.* Keylong, Kardang , Dalang, Sissu, Udaipur and Kukumseri and two from Jammu & Kashmir (Kashmir valley) *i.e.* Tangmerg and Shopian. The results of morphological and quantitative parameters of *Inula racemosa* showed maximum plant height (204.90 cm), maximum leaf length (54.15 cm), maximum leaf breadth (24.85 cm), number of stems (4.74), fresh root weight (659.30 g) and essential oil content (1.96 %) from germplasm collection site G_5 (Udaipur) and G_6 (Kukumseri) *i.e*. from Pattan valley (Lahaul & Spiti) Himachal Pradesh. For seed characteristics maximum seed weight, moisture content, seed viability and germination per cent was also registered for germplasm site collection G_5 (Udaipur) and G_6 (Kukumseri). Among the pre-sowing treatments under laboratory and field conditions maximum germination per cent was recorded in treatment P_4 (GA_3 150 ppm) and minimum in control. Maximum germination energy (19.20) per cent was recorded in treatment P_5 (GA_3 150 ppm) and minimum in P_8 (IBA 150 ppm). The maximum germination speed was recorded in treatment P_6 (IBA 50 ppm) and minimum in control. The maximum mean daily germination was recorded in treatment P_5 (GA_3 150 ppm). The maximum germination per cent and seedling height was recorded in seeds treated with P_5 (GA_3 150 ppm). The maximum collar diameter (4.17 cm) and seedling vigour index (4236.0) was recorded in pre-sowing treatment P_5 (150 ppm GA_3). The maximum fresh shoot weight (54.10 g) and dry shoot weight (27.77 g) was recorded in treatment P_5 (GA_3 150 ppm). The maximum fresh root weight (9.32 g) and dry root weight (4.64 g) was recorded in seeds treated with P_5 (GA_3 150 ppm). The maximum root length (11.77 g) was recorded in treatment P_5 (GA_3 150 ppm). The effect of location site on the germination and growth of *Inula racemosa* at two different location sites was found to be significant. The maximum sprouting per cent, (95.35) per cent, number of shoots (4.00), number of leaves (13.73), number of flower heads (16.08) maximum primary root length (25.74 cm), fresh root weight (313.00 g) and dry root weight (148.80) was observed from germplasm collection site G_5 (Udaipur, HP) and G_6 (Kukumseri, HP) minimum sprouting of 80.08 per cent was obtained from germplasm collection site G_8 (Tangmerg, J&K). Among the interactions maximum sprouting per cent was observed from $G_5 \times S_1$ (95.43 %) minimum value of 78.26 per cent was recorded in $G_7 \times S_1$.

Signature of Major Advisor **Signature of Student**

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APPENDIX-I

EXPERIMENT-I

Anova for Morphological and quantitative characteristics of *Inula racemosa* germplasm (2010-11)

Source of variance	df	Mean Sum of Square			
		Plant Height	Number of stems	Leaf length	Leaf breadth
Treatment (A)	**7**	4862.9	3.8546	345.27	92.133
Replication (B)	**3**	5.6402E-01	1.3045E-02	6.4080E-01	4.3086E-02
A×B	**21**	5.0279E-01	9.0472E--03	3.8405E-01	2.5963E-01
Total	**31**				

Anova for Morphological and quantitative characteristics of *Inula racemosa* germplasm (2010-11)

Source of variance	df	Mean Sum of Square			
		Flower heads/plant	Primary root length	Fresh root weight	Essential oil content
Treatment (A)	**7**	97.804	49.899	6.5871E+04	6.2246E-03
Replication (B)	**3**	1.1263	11.170	3993.3	1.0365E-03
A×B	**21**	1.0675	14.449	3982.5	2.4598E-04
Total	**31**				

EXPERIMENT-II

ANOVA for Effect of pre-sowing treatments on I*nula racemosa* seeds under laboratory conditions (2010-11 AND 2011-12)

Source of variance	df	Mean Sum of Square							
		Fresh seed weight		Moisture (%)		Seed viability		Germination (%)	
		2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12
Treatment (A)	**7**	5.7542E-02	4.9214E-02	4.0882E-01	1.3591	90.767	82.472	99.589	39.833
Replication (B)	**3**	6.9792E-05	4.1667E-05	3.4826E-04	7.2199E-01	1.7433E-01	1.5314E-02	1.9946E-04	7.5525E-05
A×B	**21**	7.4554E-05	3.6905E-05	3.5158E-04	9.0558E-04	1.6972E-01	1.9946E-02	2.8041E-04	1.0608E-04
Total	**31**								

APPENDIX-II

Agro-meteorological data observed during 2010, 2011 and 2012

Years / Months	Temperature (^{0}C)		Rainfall (mm)	Relative Humidity (%)
	Maximum	Minimum		
2010				
January	19.82	1.52	0.37	49.52
February	19.92	3.73	3.46	52.66
March	26.82	9.28	0.03	49.21
April	31.79	13.79	0.09	41.43
May	32.52	16.35	1.55	43.19
June	30.63	18.25	5.63	54.40
July	27.68	20.47	15.63	81.97
August	27.71	20.23	5.53	82.42
September	26.85	17.32	11.55	79.33
October	27.07	10.47	1.35	61.98
November	24.38	6.13	0.73	55.20
December	19.89	1.20	2.26	57.82
2011				
January	18.11	0.43	0.75	53.66
February	19.02	0.28	2.20	59.98
March	24.38	8.35	0.59	48.39
April	26.48	10.68	1.12	50.62
May	32.31	16.55	1.02	46.35
June	29.09	17.68	5.94	65.92
July	27.35	19.23	8.50	80.24
August	27.92	19.21	6.12	79.87
September	28.35	16.39	1.00	74.38
October	26.97	9.83	0.00	67.31
November	24.53	5.63	0.00	49.88
December	20.54	0.94	0.94	48.52
2012				
January	14.9	0.7	3.4	62.0
February	18.9	3.6	3.7	56.0
March	23.9	8.2	4.1	57
April	29.2	12.2	4.2	38.5
May	32.1	17.3	4.0	50.5
June	31.4	17.9	3.8	21.9
July	27.40	19.20	8.4	80.00
August	27.9	19.2	2.9	79.9
September	28.4	16.4	2.3	74.4
October	27.0	9.8	3.1	67.3
November	24.5	5.6	3.2	49.9
December	20.5	0.9	3.1	48.5

Source: Meteorological observatory, Department of Environmental Sciences, Dr. Y S Parmar University of Horticulture and Forestry, Nauni, Solan (HP) - 173 230.

EXPERIMENT-III

ANOVA for effect of pre-sowing treatments on I*nula racemosa* seeds under laboratory conditions (2010-11 AND 2011-12)

Source of variance	df	Mean Sum of Square							
		Germination (%)		Germination Energy (%)		Germination speed		Peak value	
		2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12
Treatment (A)	7	218.81	577.68	4.4923	4.7501	2.4970E-01	2.2570E-01	2.3851E+07	5.0287E+06
Replication (B)									
A×B	16	3.7565-01	4.8333E-04	2.2812E-02	9.8750E-02	1.5417E-04	1.5000E-04	1.8816E+07	1.2072E+04
Total	23								

Anova for effect of pre-sowing treatments on *Inula racemosa* seeds under laboratory conditions (2010-11 and 2011-12)

Source of variance	df	Mean Sum of Square							
		Days taken for germination		Mean daily germination		Germination value		Germination index	
		2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12
Treatment (A)	7	48.070	47.932	2.6722	1.02057	2.2570E-01	2.2560E-01	2.851E+07	5.0287E+06
Replication (B)									
A×B	16	5.0000E-05	9.5833E-05	1.0000E-04	8.0875E-03	1.5000E-04	1.5000E-03	1.8816E+07	1.2072+04
Total	23								

Anova for effect of pre-sowing treatments on *Inula racemosa* seeds under field conditions

Source of variance	df	Mean Sum of Square							
		Germination (%)		Seedling Height		Collar Diametr		Seedling Vigour Index	
		2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12
Treatment (A)	7	1406.2	1447.0	387.53	362.87	1.1088	1.2647	3.4685E+06	3.6624E+06
Replication (B)									
A×B	16	1.7129	1.4119	3.9167E-04	2.4167E-04	2.7500E-04	1.2500E-04	1.5849E+05	1.5894E+05
Total	23								

Anova for Table 5-6 Effect of pre-sowing treatments on *Inula racemosa* seeds under field conditions

Source of variance	df	Mean Sum of Square							
		Fresh Shoot Weight		Dry Shoot Weigt		Fresh Root Weigt		Dry Root Weight	
		2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12
Treatment (A)	7	39.854	46.847	8.183E-01	5.8689	11.539	9.4086	2.448	2.374
Replication (B)									
A×B	16	3.9958E-02	1.0727E-02	3.2740E-04	4.2765E-03	3.5833E-04	3.3333E-04	4.1508E-02	2.3750E-04
Total	23								

Anova for Effect of pre-sowing treatments on *Inula racemosa* seeds under field conditions

Source of variance	df	Mean Sum of Square	
		Root Length	
		2010-11	2011-12
Treatment (A)	7	18.638	17.822
Replication (B)			
A×B	16	2.3750E-04	2.7917E-04
Total	23		

EXPERIMENT –IV

Anova for Effect of Locations sites and Germplasm collection on sprouting percent of *Inula racemosa* in (2011-2012)

Source of variance	df	Mean Sum of Square			
		Sprouting (%)	Number of shoots	Number of flower heads/plant	Number of seeds/head
Treatment (A)	7	129.04	3.0257	5.9724	33.888
Interval (B)	1	9.0808	2.1252	5.6033	3.3974
Replication (C)	2	2.1097	5.1458E-02	1.8958E-02	3.0853
A×B	7	21.748	3.1473E-01	8.4176	2.5772
A×B×C	30	1.8530	4.6792E-02	1.8352	4.1530E-01
Total	47				

Source of variance	df	Mean Sum of Square			
		Length of primary root	Number of lateral roots	Root fresh weight	Root dry weight
Treatment (A)	7	1.3405E+04	49.312	31.043	8927.3
Interval (B)	1	3.0737E+04	2463.0	337.19	825.35
Replication (C)	2	79.874	1.5988	5.5994	96.618
A×B	7	2377.6	34.944	4.6531	262.55
A×B×C	30	189.90	4.7413	1.0982	33.215
Total	47				

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(**Harpal Singh**)