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Research Article

Germplasm Evaluation and Interactions Studies of *Inula racemosa* Hook.f. Little Known Medicinal and Aromatic Plant of Himalayan Region

Harpal Singh*, Bhupender Dutt and K. R. Sharma
*Department of Agriculture, Baba Farid College, Bathinda, Punjab
Department of Forest Products, Dr Yashwant Singh Parmar University of Horticulture and Forestry Nauni Solan H.P.
*Corresponding Author E-mail: hari85@ymail.com
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ABSTRACT

Investigations 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. For germplasm evaluation germplasm collected from different sites the 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). 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 %) and minimum value of 78.26 per cent was recorded in $G_7 \times S_1$ For large scale domestication, effect of location site on germplasm collection by conducting multilocation trials for successfull introduction of Inula racemosa was also conducted in two different places of Himachal Pradesh.

Key words: *Inula racemosa, Germplasm, Conservation, Evaluation, Sesquiterpenes lactones, endangered*

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INTRODUCTION

Germplasm is an important source for new plant types with desirable traits and increase in crop production[27]. 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 thus stand 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.

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[24]. This region is represented by more than 1748 plant species, of which several are economically very important[17].

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[29]. As a result, the natural population of medicinal and aromatic plants in their natural habitats has started declining at an alarming rate. This overexploitation 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[8,25]. 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[13].

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[16,23,9].

Inula is a genus of herbs rarely shrubs, distributed in Europe, Africa and Asia, mostly in temperate regions. About twenty species occur in India. Bioactive compounds and medicinal properties are available[2,26]. *Inula racemosa* Hook. f. commonly known as pushkarmool or manu is a rare, critically endangered threatened perennial herb belonging to the family Asteraceae and is distributed in the North Western Himalayas between of 2000 to 3200 amsl[3,18]. 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[18]. 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[6]. 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[14,20]. However, in the last few years, cultivation has drastically declined due to the introduction of other cash crops.

MATERIAL AND METHODS

Collection and Post harvest approaches

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. Survey of the conducted eight selected sites for germplasm collection was conducted and observations were recorded as per Lawrence[15]. 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,) India. 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.

For essential oil estimation 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

Table 1: 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

The entire data generated from the present investigations were subjected to statistical analysis as per methods described by Gomez and Gomez[12]. 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 the present investigations of study.

RESULTS AND DISCUSSIONS

Morphological and quantitative parameters of *Inula racemosa*

Inula racemosa is a highly valued medicinal plant found in temperate and cold climatic zones. Efforts made for its domestication in sub-temperate to temperate needs well organized and deep research on its aspects. However, some efforts have been made to suggest successful introduction of this rare, endangered and potential little known medicinal and aromatic plant. The studied conducted revealed that the plant height was significantly affected by germplasm collections. 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) (Table 1).The number of stems per plant differed significantly among different germplasm collections. 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 data registered for eight different germplasm collections revealed that the maximum leaf length (54.15 cm) was observed for collection G_5 (Udaipur, HP). The maximum leaf breadth (24.85 cm) was observed from germplasm collection G_6 (Kukumseri, HP).

Morphological and phenological studies on single plant based[22] are available on wild species but no literature is available on scientific and systematic domestication[21]. 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 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). The altitude and eco-edaphic conditions seem to play an important role in the phenological behaviour of the species. It is evident by the fact that at higher altitudes with low temperature and late melting of snow cover, the plants enter into the vegetative and reproductive phases of life cycle relatively later than the plants grown at lower altitudes.

Table 2: 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**

Root is considered to be an official part of this herb and fresh roots have a strong aromatic odour resembling orris and camphor and contain inulin (10%) - a flexible oligosaccharide and an essential oil (1.3%)[2,28]. The root fresh weight of different germplasm collections were found to be significant at 5% level of significance and maximum fresh root weight (659.30 g) was recorded from collection G_5 (Udaipur, HP). Harvesting of

roots is done after 2-3 years as it attains maturity and 75-80 quintals of fresh root and 25-30 quintal dried roots have been reported from one hectare of *Inula racemosa* crop[21].

Being medicinal and aromatic plant roots have to be found steam volatile essential oil (1.3-2.6%) and petroleum ether extract (5.7-6.2%)[5,4]. 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.

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[10,19]. As essential oil obtained from the roots is of great importance for medicinal and perfumery industry[26]. Our results have revealed more essential oil percentage than these reported earlier[1,7] which suggests the potential of cultivation of best germplasm for getting higher economic returns.

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[24,26].

Seed characteristics and germination parameters of germplasm collections

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.

A seed source is an important factor in determining the seed quality. 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 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. A congenial microhabitat may provide a higher chance of establishing a large gregarious population, even for a rare and endangered species.

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; Pradhan and Badola 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.

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 which is essential to select elite seeds 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 to select elite populations. However, poor seed germination has been shown to be one of the limiting factors. 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.

Germination is the sequential series of physiological and morphogenetic events that result in the transformation of an embryo into a seedling. 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

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. Significant variation in seed viability besides germination per cent between the sites are in conformity

Table 3: Fresh Seed weight, Moisture content, Seed viability and Germination percent of *Inula racemosa* germplasm

Germplasm	Fresh Seed Weight (g)	Moisture Content (%)	Seed Viability (%)	Germination (%)
G_1 (Keylong, HP)	1.18	18.76	77.30 (61.55)	66.03 (54.35)
G_1 (Kardang , HP)	1.17	18.47	75.75 (60.50)	64.93 (53.69)
G_3 (Dalang , HP)	1.23	18.23	77.23 (61.50)	66.23 (54.47)
G_4 (Sissu, HP)	1.22	21.67	76.01 (60.69)	64.97 (53.71)
G_5 (Udaipur, HP)	1.37	24.16	84.52 (66.83)	74.26 (59.52)
G_6 (Kukumseri HP)	1.49	24.93	87.20 (69.04)	77.51 (61.70)
G_7 (Tangmerg , J&K)	1.15	17.95	67.54 (55.27)	67.53 (55.27)
G_8 (Shopian , J&K)	1.16	17.72	68.32 (55.75)	63.66 (52.93)
Mean	1.25	20.24	76.73(61.39)	68.14 (55.71)
SEm±	0.006	0.12	0.29	0.007
$CD_{0.05}$	0.012	0.25	0.60	0.015

* Figures in parentheses are arc sine transformed values

Effect of Location sites and germplasm collection sites on the 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[14]. 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[14].

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[17,24,20].

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 [4,6,22].

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.

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. Both internal and external environmental factors strongly influence germination and establishment. 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. 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*.[17], and Thakur *et al*., 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[4]. 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[20].

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. Therefore variations of the plants are the product of interaction, between genotype and environment.

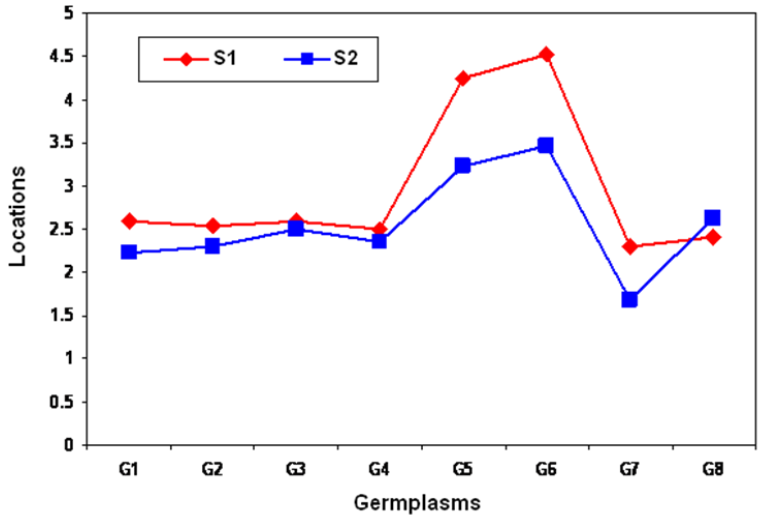

Fig. 1: Effect of sites and germplasm collection on number of shoots/ plant of *Inula racemosa*

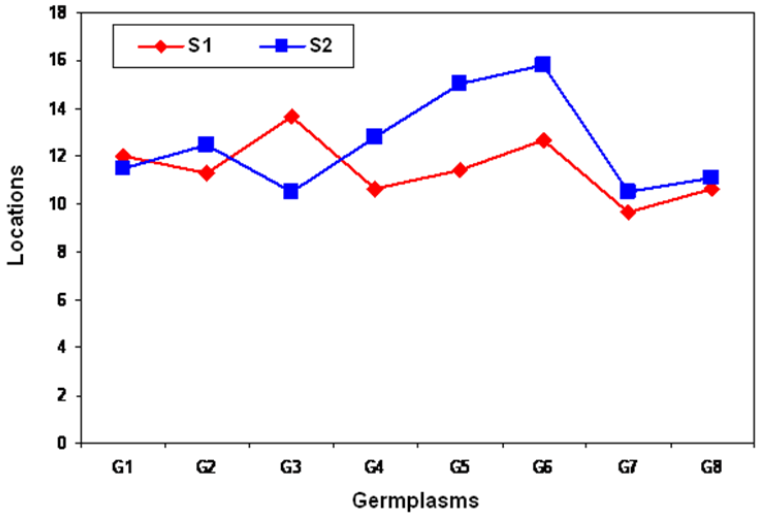

Fig. 2: Effect of sites and germplasm collection on number of leaves of *Inula racemosa*

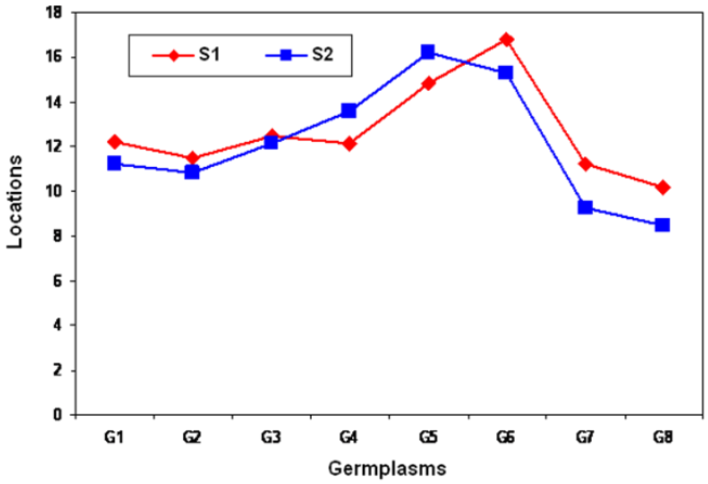

Fig. 3: Effect of sites and germplasm collection on number of flower heads of *Inula racemosa*

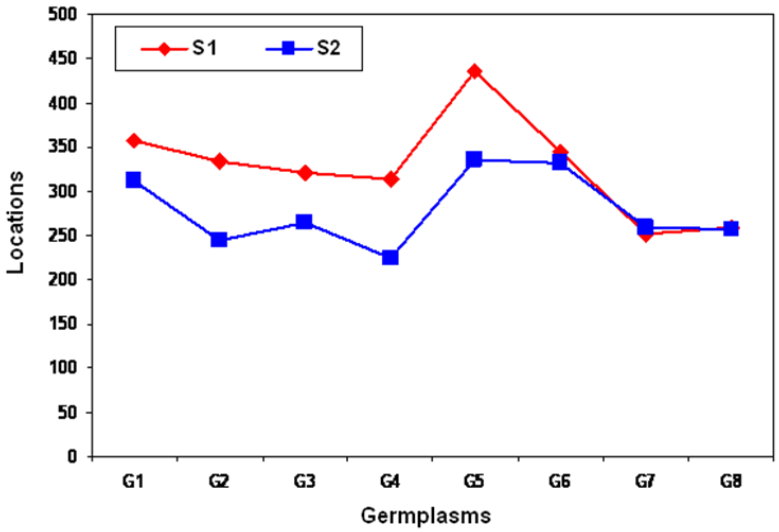

Fig. 4: Effect of sites and germplasm collection on number of seeds/heads of *Inula racemosa*

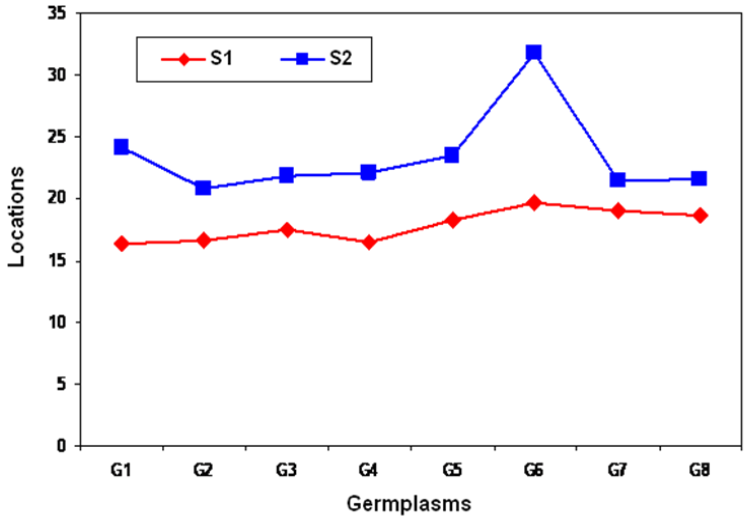

Fig. 5: Effect of sites and germplasm collection on primary root length of *Inula racemosa*

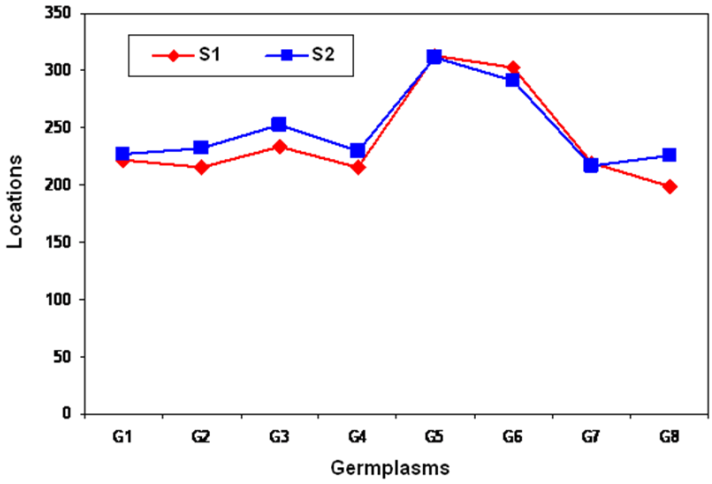

Fig. 6: Effect of sites and germplasm collection on fresh root weight of *Inula racemosa*

CONCLUSION

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. 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 qualitative 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.

iii) 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

iv) 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)

vi) 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.

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