CHAPTER 3

Review of Literature

3.1 Introduction

Human dependence on the traditional medicine to cure various diseases is an age old practice. Even now, majority of the people especially from developing countries (80%) largely depends on the traditional medicine for their primary health care needs (IUCN 1993; WHO 2002; Badola and Aitken, 2003) for several reasons such as low cost, easy availability, high efficacy, non-occurrence of side effects, environmental friendliness, lasting curative properties, etc. Being highly effective, the pharmaceutical industries have diverted their attention towards deriving basic compositions from traditional medicines to prepare modern drugs, the source for which is the medicinal plants. Dhar *et al.*, (2000) reviewed that *ca.* 175 species (most of them are threatened) of medicinal plants from the Indian Himalayan Region (IHR) are used for the preparation of over 316 drug formulations. With increasing human population, several new diseases have emerged leading to the discovery of new medicinal drugs thereby increasing the demand for the raw materials. This resulted in unprecedented harveting pressure on many of the high value medicinal plants in their natural habitats, as 90% of the raw material demands of the pharmaceuticals are met from the wild, leading to over exploitation of the existing resources. Un-scientific and unsustainable over-harvesting in the wild has caused severe damages to the quality and quantity of natural populations of medicinal plants, thereby accelerating the threat of their extinction and/or loss or genetic diversity (Badola and Aitken, 2010). The over extraction have resulted in wiping out of the entire populations of many of the important species from the nature in many parts, due to which, it has become very difficult for the pharmaceutical companies to obtain the required amount of raw materials as the demands for medicinal plant species have increased by 50%, whereas their availability in nature has declined by 26% (Anonymous, 2001). To overcome these problems, the pharmaceutical sector has substantially increased the prices for the raw materials, thus aggravating the extraction pressure, leading to non-recoverable damage to the biodiversity, as many species have already shown sign of extinction and many are assessed as threatened. This has forced the biodiversity scientists all over the world to come together and raise concern over the declining populations of the rare medicinal plants and finding ways to conserve endangered medicinal plant species (Badola and Pal, 2002). Therefore, the conservation of these high value threatened medicinal plants has become the priority of the environmental policies in many countries (Zhang *et al.*, 2010; Badola, 2009). Thus the need of the hour is to assess the availability and distribution pattern and identification of the suitable habitats of the threatened medicinal plants in the wild, and equally important to develop suitable propagation and mass multiplication protocols, strengthening both *in-situ* and *ex-situ* conservation of prioritized Himalaya taxa, such as *Swertia chirayita* (Badola and Pal, 2002).

This review of literature tries to look into the findings of the studies made on the medicinal plants from Indian Himalayan region and other parts of the world on the aspects falling within the objectives of the present study.

3.2 Population assessment and habitat characteristics of medicinal plants

The population assessment has been identified as an important tool for the prediction of extinction risk and the future population size (Guedje *et al.*, 2003) of the important plant species. In addition to assessing resource availability, the micro-habitat assessment is very important which helps in determining performance of the species under different growing conditions and provides vital information on the specialized ecological requirements of the species (Badola and Pradhan, 2010a,b; Uniyal *et al.*, 2002b), which is crucial for developing conservation management strategies and suitable agro-techniques for cultivation of the important plant species (Shrestha and Jha, 2008). Assessment of the site specific populations with those covering micro-habitats will strengthen conservation approaches for threatened plant species, especially at *in-situ* level, which further may provides clues for taking not only elite populations but the type of potential micro-habitats within the targeted sites for their management. Successful conservation and management planning of the threatened plant species also depends on the insight of the effect of the natural or man-made change on habitat (Hegland *et al.*, 2001), their ecological requirements, status of the existing populations (Kalliovirta *et al.*, 2006) and identification of the superior germplasm, etc. Moreover, the identification of potentially viable populations provides an opportunity to conserve the important medicinal plants through various propagation methods (Bisht *et al.*, 2006; Butola and Badola, 2006a).

In recent years, several studies covering population structure and habitat assessment of threatened medicinal plants have appeared from different localized parts focusing either on single taxa or a group of taxa from IHR, Western Ghat (India) and other regions. Bhadula *et al.*, (1996) through their study in 6 populations revealed restricted distribution and small population size of *Podophyllum hexandrum* in Garhwal Himalaya. Furthermore, their long term observation revealed decrease in population size as well as disappearance of the species from some of the populations. Airi *et al.*, (1997, 2000) assessed the status of *P. hexandrum* and *Nardostachys jatamansi* in western Himalaya applying vertical belt transect method using stratified random quadrats. They recorded very low availability as well as patchy or scattered distribution of *P. hexandrum* in all the populations and high availability of *N. Jatamansi* on the west facing on dripping moss-laden rocks and moist boulders. They concluded *N. jatamansi* to be restricted to some specialized habitat and are subject to destructive harvesting, in addition. Nautiyal *et al.*, (2003a) evaluated the threat status of *Nardostachys jatamansi* in the Garhwal Himalaya and found that the species had restricted distribution in small pockets and are vulnerable. They also pointed out that the species performing well on moist, rocky or boulder habitat with rich organic content.

The population structure of *Angelica galuca* in western Himalaya and part of Trans-Himalaya applying different quadrat approach (Bisht *et al.*, 2006), disclosed the low density of the species in all the 8 populations studied, with random distribution in 63% of the total sites. Vashistha *et al.*, (2006) reported very low availability of *A. glauca* and *A. archangelica* in Garhwal Himalaya and categorized them as critically endangered to endangered and stressed on immediate conservation initiative. Nautiyal *et al.*, (2004), Bhatt *et al.*, (2005a) and Giri *et al.*, (2008) divulged increasing habitat disturbance with time leading to decline in population of *Dactylorhiza hatagirea* in Garhwal Himalaya and western Himalaya regions. For Himachal Pradesh, a study assessed and reviewed the conservation status of 17 high value threatened medicinal plant species by taking habitat, quantitative availability in nature and known conservation status (Butola and Badola, 2008a).

Bhatt *et al.*, (2007) revealed the low availability of *Swertia angustifolia* in Kumaon Himalaya; they recorded random distribution of the species; however had high frequency of occurrence and good performance of the species on the southeast facing dry slope under the canopy. Small population size and patchy or scattered distribution was attributed to the habitat degradation in *Gaultheria fragrantissima* in Darjeeling region (Bantawa and Mondal, 2008). Very low density and frequency percent was assessed for *Malaxis muscifera,* a medicinal orchid in Garhwal Himalaya, attributing to several reasons, viz., over collection, poor regeneration, low seed germination and seedling establishment, habitat loss, grazing, forest fire, competition, etc, and suggesting the species as rare and adapted to specific microhabitat condition, i.e., moss-laden moist slope (Chauhan *et al.*, 2008).

Duchok *et al.*, (2005) studied the impact of disturbance in the form of fruit/seed harvesting, firewood collection, etc., on population structure and regeneration of *Illicium griffithii,* a medicinal tree from the eastern Himalaya. Their study disclosed low regeneration of the species as well as decreasing number of adult individuals. Similarly, Laloo *et al.*, (2006) studied the status of four important medicinal tree species viz., *Camellia caduca, Cinnamomum pauciflorum, Erithroxylum kunthianum* and *Picrasama javanica* in eastern Himalaya. They recorded high regeneration of all the four species in disturbed sites; however, its conversion into sapling was highly affected.

The availability and habitat preferences of different medicinal plants in the Kumaon Himalaya and the western Himalaya indicated their species specific habitat requirement (Uniyal *et al.*, 1998, 2002). Kala (2000) studied the distribution pattern, population structure and conservation status of rare and endangered medicinal plant species in Spiti sub-division of Himachal Pradesh in the Indian trans-Himalaya. He recorded the localized and patchy distribution of 23 rare and endangered medicinal plants distributed over 10 major habitat types. He also observed the large differences in the number of rare and endangered medicinal plant species within six different zones and the species number was high in areas close to the Great Himalayan range. In determining the population status of *Aconitum balfourii, A. heterophyllum* and *A. violaceum* in Garhwal Himalaya, Nautiyal *et al.* (2002) recorded fragmented distribution in *A. balfourii* and *A. heterophyllum* due to specific habitat requirement and uninterrupted harvesting, while good availability in majority of populations in *A. violaceum*. Very low density of the targeted medicinal plant species in Kedarnath Wildlife Sanctuary was assessed and attributed to the species specific habitat requirements, restricted distribution and high pressure, legal and illegal exploitation etc. (Semwal *et al.*, 2007); the study further highlighted the positive responses of the species to the conservation effort.

Remarkable impacts of habitat disturbance as well as unsustainable harvesting technique on the distribution, availability and regeneration of the important medicinal plants in Gyasumdo valley, Nepal were reported (Ghimire *et al.*, 1999). In *Aconitum naviculare* and *Neopicrorhiza scrophulariiflora* habitat assessment study revealed that the populations of either species were fragmented and small with complete absence of *A. naviculare* from the open habitat, confining to alpine scrub due to collection and livestocks trampling; whereas, for *N. scrophulariiflora*, receding of the glacier was the emerging threat to its habitat (Shrestha and Jha, 2008). Assessing the high density of *A. naviculare* in Manang district (Central Nepal), a study reported the disturbance intensity dependent variability in plant availability amongst the populations, as the populations were either fragmented or confined to single patch and more common in open areas than within the patches of shrubs (Shrestha and Jha, 2010). Duraisamy and Paulsamy (2010) observed the variations in population density of six medicinal plant species from Nilgiri hills; very low density was related to the severity in climatic condition and illegal exploitation.

The review of literature discloses that there is hardly any publication on the systematic evaluation of *Swertia chirayita* in its natural habitats, except Bhatt *et al.*, (2006) who assessed availability of *S. chirayita* in 5 populations from central Himalaya. They accounted very low availability of the species in the area which were randomly distributed in 3 populations and showed regular distribution in 2 populations. Semwal *et al.* (2007) found the localized distribution of *S. chirayita* restricted to single habitat in entire study area in Kedarnath Wildlife Sanctuary, central Himalaya. A complete lack of substantial population data poses a question on a perceived estimated assessment in categorization of *S. chirayita* under endangered or vulnerable category. Nevertheless, maximum studies on the medicinal plants are conducted either in the central or the western Himalaya region of the IHR; however, meager efforts have been made on the assessment on the availability and habitat characteristics of the important medicinal plant from the eastern Himalaya region of the IHR, including *S. chirayita.* In this research, an attempt has been made to assess the availability of *S. chirayita* covering 22 populations covering all the four districts in Sikkim, India. Further, its suitable microhabitat have been identified which will be useful in species recovery programme for the conservation of *S. chirayita.*

3.3 Morphological variations amongst populations

Habitat degradation results in fragmentary and isolated distribution of the plant species which may develop different morphological and physiological characters in a plant (Ouinsavi and Sokpan, 2010). In such fragmented and isolated natural plant communities, a reduced gene flow level consequently leads to the loss of genetic diversity thereby increase the extinction risk of important plant species (Ouinsavi and Sokpan, 2010). In addition to altitude and seasonal variation (Stech and Tesitel, 2005), adaptation to varying climatic and habitat conditions may also be reflected upon the variability in morphological characters viz., plant height, numbers of branches, scent and colour of flower, number, shape and size, pigmentation of leaves, etc., the study of such variation has become significant in conservation biology. Assessing variability in plant morphology is the traditional way of determining diversity within and amongst plant populations (Bayorbor *et al.*, 2010). Different populations of a plant species may exhibit noticeable inter and intra variations

(Bhadula *et al*, 1996) and identifying such morphological variation, specific to each targeted taxa, may have high importance in identifying superior germplasm in conservation programme (Badola, 2002) and can easily be identified in the field.

Considerable amount of work is available on the assessment of morphological variatiosn amongst plant population (Davis and Gilmartin, 1985; Van, 1992; Cordell *et al.*, 1998; Ralph *et al.*, 1998; Allen *et al.*, 1996; Casas *et al.*, 1999; Krishnan *et al.*, 2000; Lynn and Waldren, 2001; Ojeda *et al.*, 2001; Sapir *et al.*, 2002; Bruschi, 2003; Ellison *et al.*, 2004; Puijalon and Bornette, 2004; Karam *et al.*, 2006; Del *et al.*, 2007; Tesitel and Stech, 2007; Pavlova, 2009; Serebryanaya and Shipunov 2009; Thiebaut and Nino, 2009; Bayorbor *et al.*, 2010; Ouinsavi and Sokpan, 2010). Yet, few scattered literatures are available with regard to assessment of morphological variation (plant architecture) in medicinal plants.

Inter and intra variation in plant morphology, viz., plant height, leaf characteristics, fruit weight, seed weight, seed colour, etc., in different populations of *P. hexandrum* from Garhwal Himalaya is reported and related to growing conditions (Bhadula *et a*l., 1996). Similar variations in morphological characters such as, number of leaves/plant, leaf shape, etc., in *P. hexandrum* was observed by Sharma *et al.* (2000). Vriations in plant morphology viz., plant height, stem diameter, rhizome length, rhizome diameter, leaf area, etc., in the same species from Kumaon Himalaya has been reported (Airi *et al.* 1997). The inter-population variation in plant morphology has also been reported in *Aconitum atrox* (Kuniyal *et al.*, 2002). To identify the suitable germplasm for domestication, Nautiyal *et al.* (2003a) evaluated the morphological characters of *Nardostachys jatamansi* from 6 populations from Garhwal Himalaya. They found the availability of superior germplasm on moist rocky or bouldery habitats with rich carbon and nitrogen contents.

Morphological variation in *Astilbe rivularis* and *Plantago major* amongst population in Darjeeling Himalaya indicated the decrease in leaf length and width, stem length and diameter, and root length and diameter with increase in altitude in *P. major* and noticed variation in recorded morphological parts in *A. rivularis* (Chettri *et al.*, 2005).

Vashistha *et al.* (2006) observed significant difference in morphological characters in both *Angelica glauca* and *A. archanglica* from Garhwal Himalaya and recorded superior germplasm of both the species on moist habitats with rich humus content. By examining the morphological variations in populations of *Picrorhiza kurrooa* from Garhwal Himalaya, Purohit H. *et al.* (2008) presumed that in addition to microhabitat conditions, soil nutrient plays a significant role in determining variability in plant morphology.

On the basis of morphological assessment of *Gaultheria fragrantissima*, Bantawa and Mondal (2008) concluded the population of Maneybhanjyang, Darjeeling to be superior germplasm compared to other assessed populations and can be commercially exploited. Significant variations among the genotype in *Andrographis paniculata* were observed, indicating maximum variation in leaf colour Sharma S.N. *et al.*, (2009). Morphological characters of *Acacia caesia* var. *caesia* from different populations from Western Ghat revealed that the arid condition suppresses the vegetative growth but enhances reproductive ability in plant species (Satishkumar *et al.*, 2010). However, for the morphological variability amongst 28 populations in *Prunella vulgaris* in China, geographical isolation was not the only contributing factor (Liao *et al.*, 2010).

The above information reveals that not much work has been done in this regard in case of medicinal plants from north-east Indian states including Sikkim, and for *S. chirayita*, no literature on the assessment of morphological variation amongst natural populations is available, except Raskoti and Shakya (2004) from Nepal. At international level also, such studies on medicinal plants are missing except few from China. The current effort targets to assess the existing variation in morphological characteristics amongst different populations of *S. chirayita* in Sikkim (India).

3.4 Propagation of medicinal plants under laboratory condition

3.4.1 Effect of microhabitat, light and temperature on seed germination

Seed germination in the field conditions is strongly influenced by internal as well as external environmental factors (Baskin and Baskin, 2001) including seed traits and microhabitat conditions (Kitajima, 2007) Seed responses greatly varies depending on the type of microhabitat it is exposed too (Navarro and Guitian, 2003), because some microhabitat may offer better condition than others, while some provide appropriate condition at different times (Bisigato and Bertiller, 1999; Guariguata, 2000; Nilsson *et al.*, 2000; Oleskog and Sahlen, 2000; Isselstein *et al.*, 2002; Castro *et al.*, 2005). In addition, the temperature, light and soil humidity greatly impacts the seed germination and seedling establishment (Baskin and Baskin, 1988). The optimal growth response and development in plants depends on the amount of light they receive (Maloof *et al.*, 2000).

In natural habitats, several monitoring of seedling emergence as affected by the microhabitats (Eckrt *et al.*, 1986; Smith and Capelle, 1992; Caccia and Ballare, 1998; Ibanez and Schupp, 2002; Castro, 2006; Gul *et al.*, 2007; Sharma *et al.*, 2009; Yu *et al.*, 2009) and the effect of light and temperature on the seed germination/seedling emergence (Jensen, 1973; Thompson *et al.*, 1977; Ellis, 1980; Probert *et al.*, 1985; Washitani and Saeki, 1986; Tooren and Pon, 1988; Meyer *et al.*, 1989; Dickie *et al.*, 1990; Bai and Romo, 1995; Baskin *et al.*, 1995; Navarro and Guitian, 2003; Pinheiro and Borghetti, 2003; Zaddy *et al.*, 2003; Godoi and Takaki, 2004; Sugahara and Takaki, 2004; Ren *et al.*, 2005; Tilki and Cicek, 2005; Albrecht 2006; Cicek and Tilki, 2006; Serrano-Bernardo *et al.*, 2007; Yilmaz and Aksoy, 2007; Kandari *et al.*, 2008; Simao and Takaki, 2008; Socolowski *et al.*, 2008; Silveira *et al.*, 2010; Vieria *et al.*, 2010) are reported from other regions. In India, especially on Himalayan medicinal plants, insufficient attention has been given on those aspects. Amongst few, Sultan *et al.*, (2006) studied the effect of light on the seed germination of *Podophyllum hexandrum*; Kandari *et al.*, (2008) observed the effect of temperature and light on the seed germination of *Arnebia benthamii*; and Sharma *et al.*, (2009) studied the effect of microhabitats on seedling emergence of *Cinnamomum tamala* in natural habitat.

Extensive review of literature revealed that not much work on this aspect of microhabitat, light, temperature effect on the seed germination in medicinal plants, especially on *S. chirayita* have been carried out so far.

3.4.2 Effect of storage period, storage condition and storage temperature on seed germination

For the sustenance and conservation of threatened medicinal plant resources in nature, it is imperative to have knowledge on seed biology (Vazquez Yanes and Orozco-Segovia, 1993). Studies have suggested that the seed germination varies within and amongst the populations, and understanding on such variability offers useful clues on the genetic make-up of the species and its existence in the natural settings (Baskin and Baskin, 2001). Seeds do not emerge immediately after ripening and seed sowing immediately after harvesting is very rare (Oneykwelu and Fayose, 2007), the process follows drying and storage at least for some period of time. Many plant species do not develop seeds every year, hence, the seed storage serve as important back-up for the threatened and high value plant species and is a necessary initiative to support the long term conservation of genetic resources. Seed deterioration during storage is the natural phenomenon (Nasreen *et al.*, 2000); however, inappropriate storage aggravates the process (Romanas, 1991) whereas, suitable storage minimizes the rate of seed deterioration. Therefore, to attain long term viability in seeds, storage conditions play a significant role (Bonner, 1990; Takos, 1999; McCormack, 2004).

From India, a significant numbers of publications are available on the effect of long term storage, storage condition and storage temperature on the seed germination (Mukherjie, 1966; Sastry *et al.*, 2006, 2007; Rao *et al.*, 2009; Suthur *et al.*, 2009) and other regions (Muenscher, 1936; Jones, 1962; Harrison, 1966; Janssen, 1973; Roberts, 1973; Ellis and Roberts, 1980; Scoweroft, 1988; Barnett and Momonov, 1989; Dickie *et al.*, 1990; Ellis *et al.*, 1991ac; Wang *et al.*, 1993; Zarnstorff *et al.*, 1994; Ramiro *et* *al.*, 1995; Garcia-Fayose and Verdu, 1998; Sivritepe and Dourado, 1998; Walters and Engels, 1998; Acharya *et al.*, 1999; Gonzalez and Mendoza, 1999; Dahiya *et al.*, 2000; Kitchen and Monsen, 2001; Takos and Merou, 2001; Chen and Chian, 2002; Cupic *et al.*, 2005; Espinar *et al.*, 2005; Liu *et al.*, 2005; Albrecht, 2006; Tilki and Cicek, 2005; Cicek and Tilki, 2006; Maara *et al.*, 2006; Martinkova *et al.*, 2006; Ma and Liang, 2007; Beardmore *et al.*, 2008; Huang *et al.*, 2008; Ekpong, 2009; Getys and Dumroese, 2009; Sakcali and Serin, 2009; Salimi, 2010; Silveira *et al.*, 2010; Liu *et al.*, 2011). Nevertheless, in respect to medicinal plants, very few works have been done to assess the effect of storage period, temperature and condition on the germination potential of the medicinal plants from India as well as other regions.

Significant difference in the germination between different storage periods in seeds of *Strychnos nux-vomica,* and further 92% germination in the seeds, with 10% initial moisture content, after storage for 30 weeks at ambient room temperature was observed (Sivakumar *et al.*, 2006). Chauhan and Nautiyal (2007) stored the seeds of *Nardostachys jatamansi* collected from 6 different populations in two different conditions, refrigerator (0 to 4^0C) and room temperature (10 to 35^0C) and tested its viability after every two months for 2 years. They observed significant fall in the moisture content, seed viability and germination percentage with increase in storage period and noticed the loss of seed viability at faster rate in room temperature than the refrigerator. Further, it was revealed that, in either condition, the seeds stored in polythene bag retained their viability for longer period, relatively. They discovered that the seeds stored at room temperature lost viability in majority of the populations after one year; however seeds stored in refrigerator showed over 60% germination by the end of 2^{nd} year.

Budvytyte (2001) tested the seed viability of 6 medicinal plants species and 4 vegetable species prior and after storage for 1 year subjecting to ultra-desiccation and freezing. For medicinal plants, he observed fall in germination percent in ultra-desiccated seeds stored in freezer compared to initial test in 4 species, while for two species, increase in germination occurred. Similarly, for vegetable species, seed germination improved in majority of the species, and related such improvement snd breaking of seed dormancy to ultra-desiccation and freeze storage. Steady decline in seed viability within the period of one year storage from 95% to15% was recorded in *Newbouldia laevis* (Ehiagbonare and Onyebi, 2009).

The review shows that the studies on the long term effect of storage and storage conditions are missing for *S. chirayita*. In addition, studies examining the differences in germination potential amongst populations for single species are very rare (Thompson, 1981).

3.4.3 Chemical stimulation of seeds germination

Poor seed germination have been one of the several causes endangering the existence of the plant species including many of the important medicinal plants. Hence, the control and timing of the germination process is crucial for the survival of the plant populations whose principle mode of reproduction is from the seeds (Harper, 1977). The seed germination requirements are species-specific (Harper *et al.*, 1970; Stebbins, 1971; Fenner, 1985; Meyer and Monsen, 1991; Schutz and Milberg, 1997); seed remains inactive until it receives appropriate environment triggering the embryo growth. Seeds often respond to specific blend of light, temperature, and soil moisture that are most favorable for their establishment (Baskin and Baskin, 2001). Some seed germinates instantly once exposed to appropriate environment, while many attain dormancy which may prolonged to several years (Hartman *et al.* 1997; Pons, 2000). Over the years several methods have employed to improve germination and in breaking dormancy in seeds which includes scarification to chemical or hormonal treatments etc., depending on the size and texture of the seeds.

For Indian Himalaya, several publications have appeared reporting stimulatory effect of chemicals and growth regulators on the seed germination of medicinal plants (Bhadwar and Sharma, 1963; Choudhary *et al.*, 1996; Kant and Vashist, 1998; Pandey *et al.*, 2000; Beigh *et al.*, 2002; Joshi and Dhar, 2003; Manjkhola *et al.*, 2003; Butola and Badola, 2004ab; Bhatt *et al.*, 2005b; Bhuwan *et al.*, 2006; Sharma *et al.*, 2006; Sivkumar *et al.*, 2006; Sultan *et al.*, 2006; Chauhan and Nautiyal, 2007; Kandari *et al.*, 2007, 2008; Sreenivasulu *et al.*, 2008; Vashistha *et al.*, 2009; Joshi and Pant,

2010) and other regions (Genova *et al.*, 1997; Prasad BN., 1999; Coelho *et al.*, 2000; Abdel-Hady *et al.*, 2008; Ali *et al.*, 2010; Amri, 2010).

Chaudhary *et al.* (1996) observed no effect of GA_3 (5 and 10 mM) on breaking seed dormancy in *Podophyllum hexandrum*. In *Nardostachys jatamansi* from 4 populations in Garhwal Himalaya, Kant and Vashist (1998) used 4 different concentrations of GA_3 (100, 500, 1000 and 1500 ppm) to test its efficacy on seed germination and found GA_3 (100.ppm) most stimulatory, which varied amongst populations. Pandey *et al.*, (2000) disclosed significant improvement in seed germination in *Aconitum heterophyllum* with GA_3 (250 μM) but was inhibitory to *A. balfourii*. They further reported that seed germination significantly improved in *A. balforii* on pretreatment with KNO_3 (50 and 150 μM), whereas, the treatment showed marginal effect in *A. heterophyllum*.

The efficacy of KNO_3 and GA_3 (100, 500 and 1000 ppm) on seed germination in *Heracleum candicans* from 4 populations from Himachal Pradesh was reported; all the concentrations of KNO3 was stimulatory over control, however, highest seed germination obtained for GA_3 100 ppm (Joshi and Dhar, 2003).

In *H. candicans*, Butola and Badola (2004a) also studied the seed germination enhancing properties of GA_3 (25, 100 and 250 μM), KNO_3 (50 and 100 mM) and $NaHClO_3$ (15, 30 and 45 minutes), and recorded effectivness of all treatment over control except for GA_3 (100 μM), with best germination and lowest mean germination time for GA_3 (250 μM), KNO_3 (100 mM) and $NaHClO_3$ (30 minutes). Another study showed that GA_3 (25 μM), KNO_3 (150 mM) and $NaHClO_3$ (30 minutes) treatments were highly stimulatory on the seeds of *Angelica glauca* for germination and reducing mean germination time using over control (Butola and Badola, 2004b). In *Arnebia benthamii* seeds collected from alpine and sub-alpine population from Western Himalaya, Manjkhola *et al.*, (2003)assessed the effect of GA_3 (100, 200 and 400 μM) and KNO_3 (50 and 100 mM) on seed germination and revealed the inhibitory effect of GA_3 in alpine population, while stimulatory effect in sub-alpine population.

In *Swertia angustifolia* effectiveness of GA_3 (100, 200 and 400 μM) and KNO_3 (100, 200 and 400 mM) with variability in seed germination with particular concentration of GA_3 as well as KNO_3 amongst the population were observed (Bhatt *et al.*, 2005b); the study further disclosed GA_3 (100 μM) and KNO_3 (200 mM) to be the best treatment.

In *Picrorhiza kurrooa,* 11-fold increase in seed germination using GA_3 (250 μM) was reported (Bhuwan *et al.*, 2006). Bisht *et al.*, (2006) observed the variability in stimulatory effect of KNO_3 (100, 500 and 1000 ppm) and GA_3 (100, 500 and 1000 ppm) on seed germination on *Angelica glauca* amongst populations. However, they obtained highest seed germination with KNO_3 (500 ppm) and GA_3 (100 ppm) for the majority of the populations. However, the efficacy of KNO_3 (0.2%) and GA_3 (10^{-3} and 10^{-4} M) on seed germination in seven medicinal plants from Lahaul and Spiti (Himachal Pradesh) was tested (Sharma *et al.*, 2006), which observed GA_3 (10^{-3} M) to be more effectivefor all the medicinal plants; however, KNO_3 (0.2%) was effective only for *Inula racemosa* and *Saussurea costus*, and proved detrimental to the remaining species.

Maximum seed germination (90%) and lowest mean germination time using GA_3 (100 ppm) was observed in *Nardostachys jatamansi* (Chauhan and Nautiyal, 2007). In *Angelica glauca* and *Pleorospermum angelicoides*, Kandari *et al.*, (2007) examined the effect of GA_3 (100, 200 and 300 ppm) under light and dark condition and different temperature regimes (15^0, 20^0, and 25^0C). They detected higher concentration of GA_3 to be relatively effective at low temperature in dark condition in both the species; the GA_3 (100 ppm) incubated at 25^0C under light condition was highly effective in improving seed germination in both the species. In *Arnebia benthamii* higher concentration of GA_3 was found to be highly effective at low temperature, and lower concentration to be effective at high temperature both in dark and light condition; however, increase in mean germination time with increasing concentration of GA_3 in both dark and light conditions was observed (Kandari *et al.*, 2008). Improvement in seed germination and reduction in mean germination time with KNO_3 and $NaHClO_3$ over control is reported in 4 populations of *Angelica archangelica* from Garhwal Himalaya (Vashistha *et al.*, 2009).

Of the 4 concentrations of GA_3 (0.35, 0.50, 1.00 and 1.50 mg/l), Genova *et al.*, (1997) recorded 1.00 mg/l concentration at an exposition of 24h producing the optimal effect on germination process in *Atropa belladonna.* Improvement in seed germination with all concentrations of KNO_3 (0.01, 0.02 and 0.03%) in *Descurainia sophia over* control, with highest value with KNO_3 (0.02%) was observed (Ali *et al.*, 2010).

Similar scattered reports are available on stimulatory effect of chemicals, growth regulators and chilling effect on seed germination in *Swertia chirayita* (Raina *et al.*, 1994; Prasad, 1999; Bhatt *et al.*, 2007; Mukherjee *et al.*, 2008; Maji *et al.*, 2009). Prasad (1999) revealed that in *S. chirayita*, seeds treated with GA_3 (50–400 ppm) can achieve high seed germination but higher concentration can have inhibitory effect. Bhatt *et al.*, (2007) observed significant improvement in seed germination and reduction in mean germination time with GA_3 (100 ppm) in *S. chirayita* and found variability amongst the populations.

The review of literature does not show any detailed work focused on seed germination in plants growing in *ex-situ* plots within natural habitats and compared to those obtained from nursery conditions, using stimulatory chemical treatments for seed germination improvement.

3.5 Propagation of medicinal plants under nursery condition

3.5.1 Influence of chemical treatments on seedling emergence and vigour

The risk of extinction of threatened species in nature can be reduced by reinforcing the *ex-situ* raised individuals (Bowes, 1999). To raise them, expensive techniques are not necessary, but identifying suitable technique, based on species requirements such as pre-sowing chemical treatments, would minimize the wasting of the resources (Butola and Badola, 2004b, 2007; Fay, 1994; Benson *et al.*, 2000) as well as there would be an option for the production of uniform and vigorous seedlings. There have been insufficent studies covering this aspect in medicinal plants. The productions of uniform and vigorous seedlings have been achieved in some of the Himalayan medicinal herbs (Kattimani *et al.*, 1999; Nadeem *et al.*, 2000; Butola and Badola, 2004b; 2006a, 2007).

Nadeem *et al.*, (2000) in their study treated the seeds of *Podophyllum hexandrum* with GA_3 (10 and 100 μM) and $NaHClO_3$ (30 minutes) before sowing. They recorded the improvement in seed germination with $NaHClO_3$ over control; whereas, GA_3 was ineffective. In another set of experiment, they observed seed treatment with GA_3 (250 μM) resulting in high seed germination compared to GA_3 (25 μM) and control. Butola and Badola (2004b) treated the seeds of *Angelica glauca* with GA_3 (25 μM) and $NaHClO_3$ (30 min) prior to sowing in nursery condition (net house, farm yard manure and sandy loam soil, equal proportion), and found both the treatments to be beneficial in enhancing seedling emergence and seedling vigour under nursery condition over control. However, they noticed $NaHClO_3$ (30 minutes) treatment to be more effective than GA_3 (25 μM) in improving seedling emergence and seedling vigour in *A. glauca*.

In *Heracleum candicans*, using seeds from two populations, Butola and Badola (2006a) observed the effects of pre-sowing treatment of GA_3 (250 μM) and $NaHClO_3$ (30 minutes) and recorded both the treatments to be effective over control in augmenting seedling emergence and vigour in net house; nevertheless, likewise their previous experiment (Butola and Badola, 2004a), they obtained higher seedling emergence and vigour with $NaHClO_3$ (30 minutes) than GA_3 (25 μM).

Effect of $NaHClO_3$ (15, 30 and 45 minutes) on seedling emergence, vigour and survival in *Angelica glauca* and *Aconitum heterophyllum* in nursery condition under poly house was assessed, discovering $NaHClO_3$ to be effective in breaking seed dormancy, increasing seedling vigour and maintaining higher seedling survival in both the species in general (Butola and Badola, 2007). However, experiment acquired high seedling emergence, seedling vigour and seedling survival with $NaHClO_3$ (30 minutes) in *A. glauca* and $NaHClO_3$ (15 minutes) in *A. heterophyllum* over control.

3.6 Growth and plant phenology

Phenology involves the recording of recurring seasonal events. Such recording in plants provides information about different growth phases and their periodicity, length of growing seasons and of the dormancy (Badola, 1994; Jeffrey *et al.*, 2009). Such studies impart knowledge on the morphological and functional traits offering clues about adaptability mechanisms of the plants under varied and periodically altering climatic conditions in which they grow, and thus help in developing management and conservation plans for valuable plant species (Badola, 1994; Martinez-Calvo *et al.*, 1999; Baumgartner and Hartmann, 2000; Bhat and Murali, 2001; Sanz-Cortes *et al.*, 2002; Hamann, 2004; Tebar *et al.*, 2004; Badola, 2010). In addition, the phenology is also pertinent to other sectors like agriculture, socio-economy and public health within the context of global change (Penuelas and Filella, 2001). Studies suggest that variation in altitude (Butola and Badola, 2006c) and hike in temperature is responsible for such changes in plant phenology (Badola, 1994; Menzel *et al.*, 2006; Parmesan and Yohe, 2003; Hamann, 2004; Walther, 2004).

Over the years, several studies on plant phenological observations have appeared from differnt regions (Holway and Ward, 1965; Wang, 1993; Kubinova and Krahulec, 2009; Martinez-Calvo *et al.*, 1999; Sanz-Cortes *et al.*, 2002; Ramirez, 2002; Tebar *et al.*, 2004; Schaber and Badeck, 2005; Tredici, 2007; Fatimah and Ahmed, 2009; Hudson *et al.*, 2009; Pirzad *et al.*, 2010) including India (Sundriyal *et al.*, 1987; Lodhiyal *et al.*, 1998; Bhat and Murali, 2000; Badola, 2010).

For one of critically endangered Himalayan herb, *Dactylorhiza hatagirea*, Butola and Badola (2006c) performed the comparative analysis of phenophases between domesticated and wild plants. They noticed earlier initiation of phenophases in domesticated plants than wild plants of *D. hatagirea*. Duraisamy and Paulsamy (2010) made phenological observation in six medicinal plant species from Nilgiri Hill, and recorded no variation in initiation and completion of phenophases in all the studied medicinal plants except seed dispersal which was very late in *Anaphalis elliptica*. Vashistha *et al.*, (2010) examined the phenological attributes of *Angelica* *glauca* and *A. archangelica* under cultivation at two different altitudes (2200 m asl and 3600 m asl). They recorded variation in phenophases between the two altitudes and stressed on the need of developing location specific strategies for cultivation and management of the species.

The review of literature indicates that such phenological studies are limited in medicinal plants. There is no literature available on the phenological and periodical growth recordings in *S. chirayita*.

3.7 Development of agro-techniques, harvesting techniques and economic viability of cultivation of medicinal plants

To restrain the over exploitation of medicinal plants for meeting the demand of the pharmaceuticals industries, *ex-situ* cultivation is the only answer which is considered as appropriate tool to *in-situ* conservation of the threatened medicinal plants (Badola and Pal, 2002; Badola, 2009). *Ex-situ* cultivation of medicinal plants further provides better economic returns compared to wild collection, it reduces the chances of adulteration, incorrect identification, etc. It has several advantages over the cultivation of traditional crops, (1) the cost of cultivation of medicinal plants are very low; (2) can be cultivated in degraded and marginal lands, and also as inter crops; (3) can be sold both in national and international market in higher prices; (4) has less chances of pest attacks, diseases and being grazed; and (5) can be stored with minute precautionary measures for long time. However, for cultivation and high productivity, development of quality planting material and appropriate growing media or substrate combinations is imperative (Badola and Pokhriyal, 2001; Badola and Butola, 2003; Butola and Badola, 2005, 2006c). In recent years, suitable agro-techniques for the successful domestication using different growing media has been reported from Indian Himalaya and other regions for many of the important medicinal plants. However, different researcher used varied parts of the medicinal plants such as seeds, rhizome cuttings etc., for their trials.

A comparative study on growth and estimated the productivity of *Aconitum balfourii* and *A. heterophyllum* propagated through tubers under poly house and open field at 3600 m was conducted, recording high growth in plants under poly house than the open fields in either species with estimated multifold profit from poly house compared to open fields. (Nautiyal and Purohit, 2000)

Effect of inorganic fertilizers on growth and yield of *Valeriana wallichii* crop indicated an increase in the total plant biomass and weight of roots (on dry weight basis) with application of NPK fertilizers at 150:75:75 kg/ha compared to lower levels or untreated control (Singh *et al.*, 2000). Study recorded increase in yield of dry roots, root/shoot ratio, and proportion of roots in the whole plant biomass with advancement in age after transplanting. In addition, better crop growth under nylon net-shade compared to natural shade and open condition was noticed.

The germinability and productivity in *Picrorhiza kurrooa* by using both seeds and stolon segments as propagule at 1800m and 2200m asl was assessed, recording high germination under poly house as well as in open beds at both the altitudes (Nautiyal *et al.*, 2001). Study further recorded that year-wise productivity significantly increased during third year of cultivation with high dose (60 kg) of litter treatment in crop raised from both seeds and stolon at either altitude; however, the production was higher in crop raised through vegetative segment than the seeds at both the altitudes.

Nautiyal *et. al.* (2003b) applied the similar approach for carrying out cultivation of *Rheum emodi* at 1800 m asl and 2200 m asl under poly house using nursery soil treated with farm yard manure and forest humus in various concentration. They also sowed the seeds in open beds; however they used only single concentration of the treatments and observed maximum seedling emergence during October in both poly house (nursery soil treated with forest litter, 1:2) and open beds (treated with forest litter), and during June in both poly house (nursery soil treated with farm yard manure, 1:2) and open beds (treated with farm yard manure). They also recorded survival and production under different treatment after transplantation and found highest seedling survival under forest litter treatment in 40 and 60kg concentration at both the altitude. Of the three different treatments used, yearly productivity was recorded higher with forest litter treatment (60kg per 2m x 2m) by them comparatively at both the altitudes.

The seedling emergence, growth and productivity in *Heracleum candicans* using three different treatments (farm yard manure, compost and forest humus) recorded highest seedling emergence in forest humus treated soil; while seedling growth, survival rate and productivity was recorded higher in compost treated soil (Badola and Butola, 2003); the study concluded that the compost treatment to be most beneficial in enhancing crop productivity and increasing economic benefits in *H. candicans*.

The effect of ploughing depths (30, 45 and 60 cm) on growth and productivity was examined in *Heracleum candicans*, using nursery beds with black sandy loam soil amended with farm yard manure and forest humus; the study obtained highest survival percent in bed with ploughing depth of 60 cm and 30 cm in the first year and second year, respectively (Badola and Butola, 2005). The positive impact of increasing ploughing depth on seedling growth and overall productivity in *H. candicans* was revealed; study recommended deep ploughing for better growth, high productivity and high economic return from cultivation of *H. candicans* (Badola and Butola, 2005).

Chauhan and Nautiyal (2005) carried out the cultivation of *Nardostachys jatamansi* at three different altitudes viz., 1800 m, 2200 m and 3600 m using vegetative propagules and seedling transplantation methods under different treatments. They observed increasing economic yield with the addition of farm yard manure in all the conditions (horizontal bed, vertical bed and poly house bed) and altitudes compared to control; nevertheless, they found significantly high yield in horizontal ridge condition at 3600 m; As per them, cultivation was commercially viable at 2200 m and 3600m in most of the treatments. They disclosed highest profit at 3600m under poly house condition and comparable profit at 2200 m from both vegetatively grown as well as seedling transplanted crop.

In their study, Butola and Badola (2006b) assessed the seedling emergence, growth and vigour in *Angelica glauca* and *Heracleum candicans* using 5 different growing media under poly house and net house. The study revealed that all the substrate combinations to be effective in improving seedling emergence, survival, growth and biomass over control. They identified sandy loam soil + forest humus combination and sandy loam soil + vermin-compost combinations to be highly suitable media for *H. candicans* and *A. glauca*, respectively. They further reported the poly house to be suitable condition for getting higher seedling emergence, better seedling growth and survival in *A. glauca*; they stressed that, though for *H. .candicans*, polyhouse appeared suitable condition for higher seedling emergence, the seedlings should be transferred to the net-house for better growth and survival.

The domestication trial for *Dactylorhiza hatagirea* using both seeds and young tubers in soil amended beds mixed with farm yard manure and forest humus disclosed the failure of seeds to germinate; however, study recorded 90% sprouting from tubers with 70% survival frequency, and on comparing, domesticated plants were found better performing with respect to growth and productivity than the plants collected from the wild (Butola and Badola, 2006c).

Collected from 3000 m and 3600 m, the seeds of *Aconitum heterophyllum*, *Angelica glauca* and *Heracleum candicans*, sown in a mixture containing sandy loam soil and farm yard manure under poly house, net house and open condition recorded the maximum seedling emergence as well as lowest mean emergence time under polyhouse condition in all three species; however, growth parameters were high under net house and open condition for *A. heterophyllum* and *H. candicans*, but in the case of *A. glauca*, better growth was obtained under poly house (Butola and Badola, 2008b). The study concluded that each species differ in their growth requirements.

Experiment conducted on *Andographis paniculata* to study the impact of spacing (30 x 30 cm, 30 x 45 cm, 45 x 45 cm and 45 x 60 cm) and harvesting methods (destructive: uproot method and non-destructive: cut method) on quality and productivity of *A. paniculata* revealed that spacing, harvesting time and technique plays a major role on productivity and quality of herb, and found a significant difference in the yield of the herb in relation to harvesting techniques (Pandey and Mandal, 2008).

Sher *et al.*, (2005) made cultivation trials of six important medicinal plants at different altitudes (1200 m, 1400 m, 1600 m and 1900 m) through vegetative propagation method using rhizome. They recorded highest survival in *Viola serpense* and *Valeriana jatamansi*, as economically feasible species. Sher *et al.*, (2010a) conducted the domestication and cultivation study of six important high altitude medicinal plants using their earlier approach (Sher *et al.*, 2005) at different altitude (1400 m, 1600 m, 1800 m and 2200 m), and recorded highest survival in *Linnum ustatissimum* followed by *Carum carvi*, and concluded their cultivation economically viable for having high market price. Sher *at al.*, (2010d) using the same approach evaluated the growth performance six medicinal plant species at 1200 m, 1400 m, 1600 m and 1900 m altitudes and observed highest survival leading to high productivity in *Hypericum perforatum* and *Bunium persicum* and revealed their economic feasibility of cultivation..

Swertia chirayita has been largely neglected with regard to such studies. The present study attempts (i) to develop suitable agro-technique for developing uniform and vigorous seedlings, (2) to evaluate the cultivation prospect and viability of *S. chirayita* which would be very helpful in species conservation.