Reproductive Biology of *Valeriana jatamansi* Jones

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By

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Dated.....

CERTIFICATE

Certified that the Dissertation entitled "**Reproductive Biology of *Valeriana jatamansi* Jones**" submitted to the University of Kashmir, Hazratbal, Srinagar for award of degree of Master of Philosophy in Botany, embodies original research work carried out by **Aabid Mohi-ud-Din Rather** under our joint supervision. This work has not been submitted in part or in full for this or any other degree before.

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Contents

Introduction

Biodiversity is one of the vital treasures of any nation. A healthy biological diversity can contribute immensely to the economy of a nation. The candle of life is burning on this planet with flames of diverse colors. This variety is endorsed/measured by different estimates and the values varying with different reports and authorities (Heywood, 1995). The diversity of living organisms or biodiversity is of paramount importance for keeping the various processes and phenomena operative in the biosphere. In other words it means that survival of a species assures the survival of many other species. The importance of biodiversity can be smelled from the fact that stability and sustenance of the biosphere is furnished by the golden shining and valuable word biodiversity. So the system (biosphere) being delicate, balanced, co-ordinate, interdependent in terms of biotic and abiotic interactions cannot afford to lose even a single species. India is recognized one of the mega biodiversity countries of the world and nurtures enormous plant diversity. However, this plant wealth is eroding at a fast pace due to habitat loss, fragmentation, over-exploitation, invasion of exotics, pollution and climate change. The biosphere has lost some valuable species and many more are threatened with the throat cutter "Extinction". According to estimates tropical forests alone are losing one species per day. The erosion of species richness is going to erode the valuable genomes, genes, ecosystem balance, ecosystem stability and a host of other characteristics which are hard to retrieve back. The anthropogenic interferences have deflected the natural directions, posing threat and thirst to the charming ecosystems. The population explosion and economic development, the world over has been the basic and fundamental reason for the depletion of natural resources. Growing awareness of the importance of plant diversity and rapid decline of these valuable plants, have given an unprecedented impetus for their monitoring and conservation (Victor, 2010). Any conservation approach has to be based on in-depth study of plant

reproductive biology, as failure of reproductive processes to cope with the environmental changes is one of the fundamental reason for species loss and various reproductive characteristics such as seed dispersal, germination capacity, survival rate of seedlings and adults, age at flowering, reproductive life span and number of flowers and seeds refer to set of responses that allow a species to adapt to a particular environment (Moza and Bhatnagar, 2007). Reproductive biology is one of the fundamental fields for development of productive conservation protocol for elite and threatened medicinal plants and there is always a buzz all over the globe to unravel the basic and detailed information about the reproductive biology of important species preferably for their genetic improvement through hybridization as well as for development of conservation protocol. To develop a suitable prescription and panacea, understanding of reproductive biology, seed biology and breeding behaviour is regarded to be of nuclear importance as well as the central element (Wafai and Nawchoo, 2001). A central element of effective conservation of plants is knowledge of the reproductive system in all its manifestations-compatibility, breeding systems, pollination, dispersal etc. Conservation or restoration programs will not be effective without an understanding of breeding systems, pollination and dispersal.

Sexual reproduction is the only natural process that incorporates variability and ensures survival of species under adverse conditions, but successful reproduction depends upon the successful pollination which is dependent upon many biotic and abiotic factors. Plants have coevolved with their pollinators and large ecological changes can decouple their coinciding flowering and breeding cycles (Wilcock and Neiland, 2002). Pollinator behaviour and plant mating systems are influenced by a variety of plant traits, including flower morphology and phenology, self-incompatibility, and inflorescence architecture (Richards, 1986, Harder and Barret, 1996). In turn plant mating system is the primary determinant of plant population genetic structure which is of the fact

that inbreeding species are expected to have less genetic diversity and heterozygosity within populations and more genetic differentiation among populations than out-crossing species (Allard *et al*., 1968). Other important aspects such as seed dispersal and germination are the phases of reproductive cycle that are typically of great significance for the species fitness. Plants are immobile, and therefore rely on abiotic and biotic vectors to transport pollen (gametes) for sexual reproduction. An inadequate quantity and quality of pollen can reduce plant reproductive success i.e. seed quality and quantity, (Ashman *et al*., 2004). Variations in seed dispersal efficacy or seed viability are often interpreted as a reflection adaptation to specific ecological conditions (Nishitani and Masuzawa, 1996). Many studies have also demonstrated that seed size and/or weight may be good predictor of various performances including germination capacity (Naylor,1993), resistance to intra and inter-specific completion (Houssard and Escarre, 1991), dormancy period (Stamp,1990), distance dispersed with respect to mother plant (Augspurger and Franson, 1986) and seedling survival (Marshall,1987). Any deviation from the normal mode of sexual reproduction together with continued adverse conditions, herbivory, habitat degradation, extraction and stiff inter and intra specific competition threatens the survival and existence of species. Of these questions, detailed information on the different aspects of reproductive cycle of rare and threatened medicinal species may contribute to improve understanding of the phenomena of rarity and at the same time assist conservation management decisions for the species. In order to address issues related to conservation or to resolve questions of viability, extinction, conservation and management of endangered and vulnerable species, the analysis of their reproductive biology is a must.

The Himalaya harbors many beautiful cities in its lap. This hospitable approach is not restricted only to human settlements, but it also provides home to large number of plant and animal species. Himalaya is credited all over the

world as a treasure of medicinal and aromatic plants, which in turn prove as treasures of bioactive agents. Among these a good fraction of species are critically endangered. These medicinal plants taking refuge in the sub alpine and alpine zones are facing the brunt of varied threats.

One of these Himalayan critically endangered list of gems is *Valeriana jatamansi* belonging to family Valerianaceae, which is distributed in all the temperate ranges except Australia (Bennet, 1987), growing at an altitude of 1500- 3000m asl. It is an important medicinal herb of N.W. Himalayas being used in the treatment of epilepsy, leprosy, hysteria and asthma. The active principle of this plant besides having antibacterial and antiprozal activity can be taken as a remedy for snake bite as well as scorpion sting (Chopra *et al*.,1956) It can also be used as potential anti-tumor agents (Bounthanh *et al.,* 1981).

Valeriana jatamansi Jones Syn. *Valeriana wallichi* (Bennet, 1987), popularly known as Indian valerian (English), Mushkibala (Kashmiri), Sughanthdawal or Tagar (Sanskrit), (Raina and Srivastava, 1992), is being labeled as critically endangered due to over-exploitation of rhizomes for its medicinal value, habitat degradation and other biotic interferences in its distribution ranges. The species witnessed a tremendous decline in its population size. It tells the tale of biotic interferences, which have brought it to the brink of extinction. If left as such and exploited at the same rate, in near future, the species will disappear forever. Thus, convention on international trade on endangered species notified *Valeriana jatamansi* in its schedule for conservation.

Keeping in view the immense medicinal importance and critically endangered status of *Valeriana jatamansi* it becomes necessary to study various reproductive constraints if any which in turn will prove to be helpful in planning the conservation strategies. Nonetheless, the mounting demand of this plant species from various sources necessitates its domestication and propagation in a big way outside its natural habitat. A thorough understanding

of their reproductive and growth biology as well as identification of biological and ecological constraints leading to their reducing fitness, restricted distribution, or even extinction is, therefore essential so that one is able to predict their behavior under *ex situ* cultivation and develop strategies for their successful conservation. This devised programme of the study on *Valeriana jatamansi* can help in developing certain protocols to combat the problems that impede regeneration. Further, the study of reproductive biology can provide important paradigm for conservation, reclamation and restoration of *Valeriana jatamansi*.

Review of Literature

Valeriana jatamansi or Indian Valerian of the family Valerianaceae is an important medicinal plant used in several indigenous systems of medicine practiced in India (Anonymous, 1976). This species has been listed as critically endangered species in Western Himalayas (Kaul & Handa, 2000) and is therefore of considerable interest for various studies which centre around the following aspects

2.1. Taxonomy and geographical distribution

Taxonomy and conservation go hand in hand. We cannot conserve organisms that we cannot identify and our understanding the consequences of environmental change and degradation are compromised fatally, if we cannot recognize and describe the interacting components of natural ecosystems (Mace, 2004).

The familial status of Valerianaceae is debatable in light of recent studies (Bell and Donoghue, 2005). Judd *et al.* (1994) on the basis of morphological and anatomical similarities merged it in Caprifoliaceae. However, morphological studies depicted the close relationship between both the groups, pinpointing the evolution of Valerianaceae from caprilioid ancestor. The preposition of it as a separate family in turn depicts it to comprise of either 9 genera (Gunn *et al*., 1992) or 13 genera (Cronquist, 1981).

Valerianaceae comprises of @ 350 species distributed throughout the World (except Australia and New Zealand), mostly at high elevations and with many species in alpine zones (Backlund and Moritz, 1998). Bell (2004) labeled Valerianaceae as a natural group of 350 species of cosmopolitan distribution comprising of 13 genera with 200 species chiefly confined to temperate regions. *Valeriana jatamansi* Jones (Syn. *Valeriana wallichi)* popularly known as Indian Valerian (English), Mushkibala (Hindi), Suganthdhawal or Tagara (Sanskrit), is distributed in all the temperate regions except Australia, (Jain,

1968; Bennet, 1987). Several species of *Valeriana* have also been reported from Andean Chile, Brazil, South Africa and Sub-tropical Asia. About 12 species of genus *Valeriana* have been reported from India (Anonymous, 1976). Rao *et al.* (1977) have reported 10 species of *Valeriana* from India. Out of these *Valeriana jatamansi* has been reported to be widely distributed in temperate Himalayas at an altitude ranging from 1500m in Khasi Hills to 3000m in Jammu and Kashmir, Himachal Pradesh and Bhutan (Kritikar and Basu, 1975).

Mukerjee, (1953) while assessing distribution of *Valeriana officinalis* reported it to be growing only in restricted sites of North Kashmir at an altitude of 2400 to 2700m. However, *Valeriana jatamansi* according to Chauhan and Khosla (1988) is sporadically found in whole N.W. Himalayas.

Polunin and Stainton, (1987) reported the distribution of *Valeriana jatamansi* from Afghanistan to South west China and Burma. The herb was seen to grow at an altitude of 1500 to 2600m where in the surroundings were mainly comprising of Bedula (*Fiscus* species), Laliguron (*Rhododendron arboreum*).

The herbarium records of KASH, Department of Botany, University of Kashmir represents 5 species of the genus *Valeriana* reported from different localities of Kashmir valley which include Shajnar, Dara, Harwan, Gulmarg, Yusmarg, Dacksun, Ferozpur, Sonamarg (Naqashi and Dar, 1982-1986- KASH Herbarium Collection).

2.2. Species morphology

Valeriana jatamansi is a perennial herb with pubescent stem, radical leaves, several long petiolated cordate-ovate, cauline few or much smaller entire or pinnate, fruits hairy or nearly glabrous. Root stocks thick, horizontal with thick descending fibers; stem 6-18cm, radical leaves often 1-3cm in diameter, deeply cordate, usually acute toothed. Cymes corymbosely panicled; bracts small oblong or linear persistent, calyx limb in flower obscure, unrolling in fruit into

5-15 plumose bristles united at base equal or sub-gibbous, flowers white, stamens 3, ovary 3celled, 1 ovuled, stigma shortly 2-3 fid or sub-entire, fruits oblong lanceolate, compressed crowned by persistent pappus calyx (Hooker, 1881).

Kokwar, (1968) described Valerianaceae member as annuals (occasionally biennial) or perennial herbs, rarely subshrubs; often with strongly scented rhizomes. Leaves opposite sometimes forming basal rosettes, exstipulate, often pinnately much divided but sometimes entire, cauline leaves sometimes few, small or none; basal leaves pinnatifid, base often sheathing. Inflorescence a many flowered compound dichasial cyme, thyrse or monochasium, sometimes condensed and capitate, bracteate and usually bracteolate. Flowers hermaphrodite or unisexual by abortion (plants then dioecious as in some *Valeriana* spp.), irregular or almost regular usually 5-merous. Calyx often small or absolute at the time of flowering, sometimes enlarging as the fruit matures, and then variously lobed; lobes often forming a pappus. Corolla funnel shaped or tubular, often attunated at the base. Stamens 1-4, epipetalous, alternating with the corolla lobes, anthers versatile, 2 or 4-lobed, 2 or 4-thecous, pollen grains tricolpate, echinate ovary inferior, tricarpellate, 3 locular but only one locule fertile; ovule solitary and pendulous, anatropous, style single and slender; stigma 2-3 lobed, fruit a 1- seeded achene.

Polunin and Stainton, (1987) reported corolla of *Valeriana* as funnel shaped, limb 5 lobed, spreading, stamens 3, style slender, undivided, fruit is indehiscent bearing a single hairy achene.

2.3. Phenology

Robertson, (1924) proposed that a natural group of flowering plant has a definite position. It begins at a given time period, attains peak at a given point and retards until all of its members are out of bloom, showing complete senescence and complete seasonal growth. Their seasons do not coincide but they often overlap.

Flowering phenology is an important life history trait because the timing of reproduction and the schedule of reproductive expenditures across time can strongly influence individual fitness (Primack, 1985; and Rathcke and Lacey, 1985; Fenner, 1998) and hence flowering phenology is of fundamental interest for understanding of species interaction and community functions. Thus phenology in general and reproductive phenology in particular is a critical and important trait of a plant because it determines the growth, developmental pattern and number of reproductive isolations or speciation over time (Bronstein *et al.*, 1990). The timing of flowering within and among individuals is of fundamental biological importance because of its influence on total seed production and ultimately on fitness (Stefan and Durka, 2007). The competition for pollinator services among plant populations and communities is determined by floral forms or by phenological variations (Campbell, 1985; Fishman and Wyatt, 1999; Caruso, 2000; Brown *et al.*, 2002). Further plant species within different communities usually depict overlapping flowering periods and often share generalist pollinators, thereby suggesting that whenever these diverse plant communities persist, plant reproduction is not limited by pollinator availability (Ramirez *et al.*, 1998; Gross *et al.*, 2000).

Kanon, (1978) while studying the floral phenology of *Valeriana officinalis* reported that majority of the flowers open before midday where in the most suitable time for the pollination was between 8:00 to 10:00 h while the stigma were most receptive one day after anthesis.

Sobral, (2000) investigating the flowering phenology of *Valeriana eupatoria* and *Valeriana tujuvensis* reported asynchronization from flower to flower in an inflorescence and plant to plant in a population in the former while in the latter flowering and fruiting was reported to proceed simultaneously during July to December.

Faivre and Windus, (2002) reported that in *Valeriana ciliata* peak flowering takes place during April in Southern population in Ohio while it occurs during

June in Northern population of the same locality, thus depicting habitat dependent variability in phenological episodes of the species.

Chauhan *et al.* (2008) investigated the phenology of *Nardostachys jatamansi*, and reported that flowering takes place during July-August wherein anthers disperse pollen grains after 24 hours of anthesis.

2.4. Pollen production and pollen ovule ratio

Pollen-ovule ratio reflects pollination efficiency, i.e., the likelihood of a pollen grain reaching a stigma (Cruden, 1977). P/O ratio is also used to examine how breeding system, sexual system, pollen vectors and dispersal units influence pollen grain number (Cruden, 2000). He also reported that there is substantial decrease in P/O ratio from xenogamy to facultative xenogamy to autogamy suggesting that there is a cost associated with changes in sexual system. The P/O ratio of the wind pollinated plants are substantially higher than those of animal pollinated plants. The P/O ratio of the plants whose pollen is dispersed in tetrads, polyads or pollinia are substantially lower than those of species whose pollen is dispersed as monads (Cruden, 2000). The P/O' s of plants that provide only pollen as reward are higher than those that provide nectar as a reward. In general more efficient the transfer of pollen, the lower the P/O ratio should be. Thus it logically depicts that cleistogamous flowers will have lower P/O's than xenogamous flowers i.e., P/O's are correlated with the breeding systems (Cruden, 2000).

Layton and Ganders (1984) analyzed the pollen ovule ratio in two species of family Valerianaceae and reported that wind pollinated species (*Plectritis brachystemon*) produce more pollen grains than insect pollinated (*Plectritis congesta*). The latter had fewer ovules per flower than the former. He concluded that differences in pollen-ovule ratio may possibly reflect the differences in pollination efficiency.

Clarke (1978) reported that in *Valeriana officinalis* the flower produce a highly variable number of male and female gametes. Each flower on an average bears 2-3 anthers, each with 947-960 pollen grains and whole flowers on an average bearing 1994-2880 pollen grains. He concluded that pollen-ovule ratio is somewhat nearer to the ratio typical of some autogamous taxa.

Webb, (1984) investigated the variation in pollen grain number in many homoecious species and reported differences in number of pollen grains per anther and/or number of anthers per flower. He also reported that in species with other sexual systems the percentage of male flowers per plant may also vary. Likewise ovule number may vary as a function of the number of ovules per carpel or ovary, carpels per flower, female flowers per plant.

Variations in shape and size of pollen grains between different families and even different species are also found in nature. Backlund and Nilsson, (1997) have reported spheroidal, tectate to tricolpate pollen grains in the families Caprifoliaceae, Dipsacaceae and Valerianaceae.

Parveen and Qaiser, (2007) on the basis of exine ornamentation recognized three distinct pollen types viz.; Valeriana jatamansi-type, Valeriana hardwikii-type and Valeriana-dentata type. They reported that Valerianaceae pollen grains are usually radially symmetrical, isopolar, mostly prolate-spheroidal to sub-prolate, often oblate-spheroidal rarely prolate with tricolpate sexine thicker or thinner than nexine.

2.5. Breeding system

Plants cannot choose their mates; nevertheless they have diverse methods by which genetic structure of their populations and the patterns of their evolution is influenced by their mating patterns. Plant breeding systems are under genetic control and can themselves be selected for. They are fluid and respond to selection pressures in an infinite variety of subtle and interrelated ways because breeding systems are genetically controlled and affect genotype structure

(Richards, 1986). Thus breeding system is the purposeful manipulation of plant species in order to create desired genotypes and phenotypes for specific purposes. Mating strategies in flowering plants are governed by several classes of floral adaptations. Floral design and display primarily influence the quantity and quality of pollen dispersed during pollination, whereas physiological mechanisms operative in pistil screen pollen receipt by rejecting certain male gametophytes, especially self pollen, (Barret, 1998), thus selective mechanisms that influence the evolution of plant mating strategies include inbreeding depression, pollen discounting and optimal allocation of resources to female and male function.

Layton and Ganders (1984) investigated the genetic consequences of contrasting breeding systems in *Plectris* (Valerianaceae). The study revealed the consequences of contrasting breeding systems in two closely related taxa; *Plectris congesta* (Lindl) D.C. and *Plectris brachystemon* F & M (Valerinaceae). The studies revealed that due to large flowers and a nectiferous spur in the former, insect (pollinator) visitation was active. Thus, even though self-compatible, it was strongly protandrous also. The numerous flowers on the inflorescence may thus open simultaneously which will facilitate geitenogamous pollination. In contrast, *Plectris brachystemon* bears small flowers with spurred corolla though in some population the spur is reduced to a mere swelling, the flower were not protandrous and hence pollinators were not dominantly visiting the flowers. The differences in floral character strongly suggest contrasting breeding strategies which was further investigated by Gander *et al.* (1977a,b).

Wyatt, (1982), Richards (1986) and Harder and Barret, (1996) while assessing reproductive biology of different plant species reported that plant mating systems and pollinator behavior are influenced by many morpho-reproductive characters, like phenology, self incompatibility and inflorescence architecture.

They concluded that mating system is a primary determinant of a genetic structure of a plant population.

Faivre and Windus, (2002) during their study on Prairie Valerian (*Valeriana ciliata*) in Mid Western Fens reported that genetic variation within populations of *Valeriana ciliata* may be influenced by its breeding system. The authors argued that the gynodioecious species had greatest genetic diversity followed by the species. They conclude that breeding system has a profound effect on genetic diversity.

2.5.1. Phenomenon of gynodioecism

Gynodioecism is a sexual system characterized by the presence of female plants together with hermaphrodite plants in a population of a species (Couvet *et al.*, 1990). Young, (1972) labeled gynodioecy as a out breeding mechanism since female flowered plants must be pollinated by hermaphrodites in order to produce the seeds. This breeding system according to Valdeyron *et al.* (1973) serves to promote out crossing particularly in those species which show strong tendency towards inbreeding.

Charlesworth, (1981); Delannay *et al.* (1981) and Ross and Gregorius, (1985) have reported that in a gynodioecious species female plants have an advantage over hermaphrodite because of their avoidance of inbreeding. This is put forth by authors as, transmission of genes by hermaphrodites occurs through both ovules and pollens, while the females do so only through ovules. The second hypothesis is based on resources allocation argument which states that since females do not produce male reproductive structures, therefore, they allocate more resources towards female function which results in better quality of seeds and progeny. Apart from these two factors in the maintenance of females in otherwise hermaphroditic populations pleiotropic effect of sterility genes can also be playing a role in such activity (Van Damme, 1984; Poot, 1997).

Although gynodioecy is typically associated with self compatibility as reported in *Plantago ovata* (Ross, 1970), *Hemophila menzeisii* (Ganders, 1978) and *Silence acaulis* (Shykoff, 1988). It has also been reported in self incompatible species like *Plantago lanceolata* (Ross, 1970) and in *Spachea membranacea* (Steiner, 1985).

2.6. Pollination system

One of the most exquisite features of many flowering plants is their interaction with different species of pollinating insects (Olesen and Warncke, 1989). Out crossing plants mate only with the assistance of pollen vectors so that the abundance and efficiency of vectors determine mating success (Burd, 1994; Larson and Barret, 2000). In recent years pollination failure in small, isolated population has been identified as a potential threat to the long term persistence of declining plant species (Rathcke and Jules, 1993). The size and density of a plant population may affect interactions with pollinators and pollen transfer in several ways. First, increase in size and density of a population increases pollinator visits to plants, as well as the amount of pollen received per flower, (Powell and Powell, 1987). Second, pollinator foraging behavior may change the size and density of the population (Zimmerman, 1981; Goulson, 2000), thereby affecting the composition of pollen deposited. If the overall visitation rate decreases with decreasing population size or density, then this may increase standing crop of nectar and pollen in individual flowers. This in turn may induce pollinators to visit more flowers per plant (Pyke, 1978; Harder, 1990). This depicts that relative abundance of different pollinator species may vary with plant population size (Sowing, 1989) which can influence both the quality and composition of pollen deposited.

Leppik, (1953) on the basis of innate symmetry preference hypothesis reported that the beetles, honey bees, moths and the butterflies dominantly visited primarily zygomorphic forms. Kanon, (1978) on the basis of pollination system

analysis reported *Valeriana officinalis* as an entemophilous plant with geitenogamy and autogamy as the two modes of pollination.

Titz and Titz, (1981) while assessing the pollination system of *Valeriana officinalis* reported two distinct types the diploid exaltate type adopted to mesophilous and tetraploid collina type adopted to xenophilous to mesophilous conditions. The studies of Proctor *et al.*, (1996) revealed that *Valeriana officinalis* is better adapted to butterfly pollination where in lepidoteran is the dominant visitor. Further they reported that *Valeriana dioca* was visited by dipterans and culcidae (mosquito family). The closer observation of *Valeriana ulginosa* in New England have revealed lepidoterans, dipterans and other insect groups as main pollinators. Faivre and Windus, (2002) while assessing reproductive strategies of *Valeriana ciliata* reported that small bees, flies and ants were the dominant visitors to its inflorescence and some or one of these may be the effective pollinator of the species.

Neal *et al.* (1998) on the basis of a syndrome concept proposed that the pollination mechanism of a given species is generally dependent upon pollinator type. They reported that non symmetrical floral symmetry, particularly medial zygomorphy, has been associated with melitophily (pollination by bees) and ornithophilly (pollination by birds).

Pollination mechanism of *Nardostachys jatamansi* was investigated by Chauhan *et al.* (2008). They reported that the fruit set was 40% in passive autogamy, 70% in active autogamy, 53.33% in Xenogamy and 86.67% in geitenogamy as well as in open pollination experiments. The species even though dominantly self pollinated is also dependent on pollinators for optimum pollination thereby depicting that the cross pollination may have adaptive value for species as it compensates the failure of autogamy and also maintains genetic variability in the population. Main pollinators of Nardostachys have been identified as small insects like flies (Ericksen, 1989).

Verma *et al*. (2011) reported that pollen grains of *Valeriana wallichi* are sticky and pollination is effected by insects belonging to orders Hymenoptera and Diptera which visit flowers in large numbers for pollen and nectar, and disperse the pollen from anthers before stigma becomes available for selfing.

2.7. Seed Biology

In most angiosperms, flowering date varies among plants within a population, and such variation may result in differences in reproductive output among plants like fruit: flower ratio (Dieringer, 1991; Kelly and Levin, 2000), seed: ovule ratio (Widden, 1991), number of seeds produced by a plant (Schmitt, 1983; Kelly and Levin, 2000) and number of seeds per fruit produced by a plant (Widden, 1991). The reproductive strategies among plants may evolve from fitness tradeoff between seed dispersal and seedling establishment. Large seeds of small crops generally are not dispersed as compared to small seeds of large crops, but large seeds contain more reserves for seedling establishment (Preciak, 2002). However, variation in seed size vs. number can reflect a compromise between seed dispersal (i.e., movement of seeds away from parent plants) and seedling establishment including seed germination and seedling emergence, growth and survival (Sollabanks, 1992). Seeds play an important role in the development of civilization by supplying food, feed and natural products and traditional medicines, thus acquiring knowledge of seed biology has been a priority for most cultures (Jaimie *et al.*, 2005).

Weins, (1984) on the basis of studies on seed productivity reported that seed ovule ratio of out crossing perennials tends to be lower than selfing species. However, Argen and Willson, (1991) have reported that in gynodioecious species, seed production in hermaphrodite plants was lower than in female plants. It was reported that in gynodioecious *Gernium maculatum* female plants produce 1.6 times more seeds than hermaphrodites while in *Geranium sylvaticum*, females usually produce fewer and smaller seeds than hermaphrodites

Lubbers and Christensen, (1986) analyzed the intra seasonal variation in seed production among flowers and plants of *Thalictrum thalictroids*. The studies revealed that the mean seed number and percent seed set were lower in the flowers that open late in the season than in those that open earlier. Further the low seed set was primarily reported in flowers that are positioned laterally and open later than the centrally located flowers. The studies also revealed that the plants flowering earlier in the season produce more flowers, ovules and seeds than those flower latter. However, the percent seed set per plant did not change indicating that the temporal differences in total seed output can be traced largely to variation in total ovule number. The authors concluded that seed output may also be influenced by limitations which in turn will have greatest effect on total seed production.

According to Aswanthaiah *et al.* (1993) germination test is useful in evaluating the planting value of a seed lot. Gorbunov, (1979) while studying the biomorphic characteristics of 5 *Valeriana* species reported better percentage of seed germination at 3-4^{o}C, 7-9^{o}C alternatively with 18-20^{o}C than the germination at constant temperature of 18-20^{o}C. The polyploidy species viz. *Valeriana cardamines* and *Valeriana eriophylla*, according to the author are more suitable for cultivation than the diploid *Valeriana alpestris* and *Valeriana allarifolia*.

Vashist and Kant, (1998) conducted studies on seed viability, seed germination and seed storage of *Nardostachys jatamansi*. They report that GA_3 Treatment at 100 ppm resulted in 85% germination. They further reported that freshly harvested seeds showed 65% germination after 20 days. After one month storage % age decreased to 60% for seeds in cold storage and 45% for those stored at room temperature and corresponding values of germination after six months storage are 25% and 10% respectively.

Mattana *et al.* (2010) on the basis of studies on *Centranthus* (Valerianaceae) reported that the effects of a range of constant temperature 5-25^{o}C and two

alternating regimes 25/10^{o}C and 30/15^{o}C on seed germination. They argued that seed and seedling mass of *Centranthus ruber* were higher than for *Centranthus amazonum* and the lack of a persistent soil seed bank detected for *Centranthus amazonum* increases vulnerability to extinction for this species.

2.8. Resource allocation

Every organism allocates its resources to various functions and storage organs which in turn govern the maintenance, growth and reproduction (Wilson, 1983). The maintenance includes survival activities such as regulation of water movement, avoidance of predation and disease, baseline metabolism and resistance to competitors. The growth is multidimensional and includes increases in biomass wherein some organisms depict better growth as juveniles, while others achieve growth during every span of life cycle. Reproduction includes acquisition of the mates, production of the gametes and the parental care. Reproductive effort has been labeled as the total resource budget of an organism devoted to reproduction (Wilson, 1983).

Bernath *et al*. (1973) reported that *Valeriana officinalis* shows greater response to change in nitrogen supply than other nutrients. All the macro-nutrients (NPK) had equal influence on root morphology and tissue structure. Further the increasing levels reduced the root surface area but raised the proportion of thicker roots. Bink (1980) while assessing the effect of fertilization on the root yield and valtrate content in *Cantranthus ruber* reported best yield with NPK and NK registering 10.37Kg/hac and 9.94 Kg/ha of root and 1.22 percent and 1.37 per cent valtrate (valepotriates) contents respectively. Further he reported that NP and PK did not increase the root yield as compared to control but slightly did increase valtrate concentration.

Pal *et al*. (1984) on the basis of their studies reported that application of higher dose of nitrogen in *Valeriana officinalis* increased the height, number of branches and spread of plant and flower yield. Addition of phosphorus fertilizers did not show any effect on plant growth or yield of flowers. Smaller

plant populations increased the height, number of branches and spread of plant while as the bigger populations per unit area increased the number of flowers.

Vats *et al.* (2002) have reported that plants grown in open sunny conditions have thicker leaves, higher wax content and high net photosynthetic rate than those grown under shade, thereby predicting that *Valeriana jatamansi* is more adopted to open habitats to meet the ever increasing industrial demand. Chauhan *et al.* (2005) analyzed the impact of various agronomic factors on the growth of *Nardostaychs jatamansi* grown in rows with varied spacing. The studies depicted that the row spacing of 40-50 cm and 20-30cms plant to plant spacing along with an addition of 30-40 FYM (farmyard manure) yielded 70-75 tones/ha fresh root mass and 20-25 tones/ha dried root/mass as compared to control.

2.9. Cytology

The assessment of available cytological data depicts 47 species of *Valeriana*, whose ploidy has been fully assessed (Federov, 1974). Among these 19 species have base member X=8 while 13 species have X=7. The chromosome number in these 47 species range from 2n=2x=14 to 2n=4x=56.Among these 7 species depict 2n=4x=32 (x=8) and nine species depict 2n=4x=28 (x=7). Perusal of literature reveals that the family Valerianaceae exhibits a dysploid series of five basic chromosomes numbers viz.; X=15 in American *Valerianella*, X=13 in *Nardostachys*, X=11in *Patrinia* and *Valeriana. Cieltica*, X=8 in *Cantranthus, Fedia, Valeriana* and *Valerianella* and X=7 in *Valeriana* species and *Valerianella* species (Engel, 1976). Polyploidization events are common with a few genera being exclusively polyploid viz. *Centranthus* (tetraploid) and *Fedia* (tetraploid). While a few species of *Patrinia* and *Valeriana* exhibit various ploidy levels ranging from diploidy to octoploidy. Hence both polyploidy and dysploidy seem to have played a significant role in the differentiation and evolution of these plants.

2.10. *In vivo*/*In vitro* studies

Propagation is the practice of rapidly multiplying stock plant material to produce large number of progeny plants. It is used to multiply novel plants such as those that have been genetically modified or bred through conventional plant breeding methods. It is also used to provide a sufficient number of plantlets which do not produce seeds or do not respond well to vegetative reproduction (Horn, 1992).

Kaur *et al.* (1999) through *in vitro* propagations established productive method for rapid and mass multiplication of *Valeriana jatamansi* through induction of shoot proliferation from shoot buds. The optimum results were obtained by using solid media supplemented with benzyl adenine alone or in combination with the indole acetic acid or naphthalene acetic acid. Further, culturing on media supplemented with BA and IAA or NAA facilitated the shoot formation which produced roots on the same .medium within 3-4 weeks. The survival of progenials was hundred percent.

Luissa *et al.* (2002) developed a practical method for the multiplication of *Valeriana glechomifolia*. They also assessed the valtrate synthesis in the plantlet. Doing their study the auxiliary buds and shoot tips were cultured in 0.3µ Murrashige and Skoog basal medium supplemented either with 0.4mg BAP or without plant growth regulators. The cultured segment grew on both media producing roots after 3-4 weeks.

Singh *et al.* (2005) assessed the impact of transplantation time on the growth and yield of *Valeriana jatamansi*. The maximum plant yield in terms of height, aerial biomass, underground biomass, rhizome yield, and root yield was obtained after 9 months of transplantation. The assessment of impact of habitat conditions depicted highest values for plant height, biomass, underground biomass, rhizome yield and root when plantation was carried out on ridges.

2.11. Medicinal properties

Chopra *et al.* (1956) reported that rhizome and roots of *Valeriana jatamansi* contain an essential oil-valepotriate, which has antibacterial and antiprozoal activity. The isolation of valepotriates and determination of its medicinal properties resulted in wide spread use of this compound as a sedative in west Germany under the trade name "valmane" (Thies, 1966). The valmane comprises of standarized mixture of valepotriates containing valtrate (15%), didrovaltrate (80%) and acevaltrate (5%). Although some clinical testing of these alkaloids were carried out earlier also, but the first report on their medicinal properties was published by Von Eickstedt and Rehman, (1969).

Uniyal *et al.* (1967) on the basis of collection of ethenic information reported that the tribes of Tehri-Garwal regard *Valeriana* as a sacred plant. It is used in the preparation of ubtan (a cosmetic) in certain ceremonies and also used as an insect repellent.

Kritikar and Basu, (1975) have reported that the roots and rhizomes of *Valeriana* are useful in the treatment of epilepsy, hysteria and asthma. They also reported that roots of *Valeriana jatamansi*, in combination with other drugs as a remedy for snake bite and scorpion sting.

Bounthanh *et al.* (1981) conducted tests on the cytotoxity of some valepotriates for their use as potential anti-tumor agents. *In vitro* experiments, using cultural rat hepatoma cells, showed that the valtrate and didrovaltrate suspension were highly cytotoxic but Baldrinal did not have such activity. Didrovaltrate was found to reduce tumor size after 24 hours of its application.

Pande *et al.* (1994) reported roots of *Valeriana jatamansi* are acrid and bitter with a flavor. These are used as carminative, laxative, antiperiodic, hypnotic and aphrodisiac. The roots are also used for curing diseases of blood, burning sensation, leprosy, cholera, skin disease throat troubles and ulcers. Further the roots increase the lusture of eyes, promote growth and blackness of hair and are

also useful in the treatment of cough, chest pain and kidney troubles. Prakash and Mahrotra (1994) reported that *Valeriana jatamansi* rhizome and roots have been used in the treatment of hysteria, epilepsy, asthma. However, Gupta *et al.*, (1996) assessed that the drug Valerian extracted from roots of *Valeriana* is being used immemorial for curing different diseases. Valerian is also used as a traditional sedation, antispasmodic and tranquilizer (Diapher and Hindwarch, 2004).

2.12. Conservation status

The ever increasing exploitation of medicinal plants and habitat destruction are the main causes of plant extinction. The growing public awareness of resources exploitation and its impact on surrounding biodiversity has necessitated creation of suitable protective strategies so that natural wealth is maintained.

Wyatt (1981) while assessing the status of *Valeriana* reported that *Valeriana jatamansi* and *Valeriana officinalis* are over exploited due to their use in wide range of diseases nationally and internationally. The ever increasing demand has enforced indiscriminate collection of rhizomes by various agencies, researchers and locals causing drastic threat to the wild population. The population assessment of *Valeriana* has revealed that on an average there is a decrease of about 30-40 plants per 100sq mts. which is increasing with every passing year. A study by Wyatt (1981) reveals that they are totally wiped out from some previously recorded localities, comparatively at lower altitude while their distribution in the inaccessible terrain has dissected and shrunken. The authors suggested both *in-situ* and *ex-situ* conservation strategies to protect the genus from extinction. Verma *et al.* (2011) reported that *Valeriana wallichi* is exploited for its rhizome, which is the source of active principle-valeportriates, for which plant is sought after and has been depleting from its natural habitats at a fast pace and hence is of immediate concern to observe conservation strategy.

Material and Methods

During the present study various methods and scientific approaches were carried out in the field, laboratory and in natural habitats to understand the reproductive biology of *Valeriana jatamansi*. Being categorized as critically endangered, it catches our immediate attention to study its reproductive biology so as to develop any conservational protocol for its survival and widespread growth. The data on various aspects of reproductive biology were recorded during the year 2009-2010 along the following lines.

3.1. Field surveys and Exploration

An extensive exploration of different Kashmir Himalayan habitats was carried out to identify specific areas across different geological conditions covering a wide range of habitats. Among various populations three sites were selected for further studies on the basis of following criteria:

- Accessibility of the site
- Habitat structure.
- Plant density

The salient features of the selected sites are depicted in Table 1.

Table 1: Salient features of some selected sites for studies on *Valeriana jatamansi*

Habitat character	Population		
	Gulmarg	**Ferozpora**	**KUBG***
Soil type	Humus rich	Humus rich	Sandy loam
Altitude (asl)	2650m	2150 m	1595m
Slope	Moderate to steep	Moderate to steep	Moderate
Forest range	Jehlum valley forest division	Jehlum valley forest division	-
Latitude/longitude	34º 04´/N 74º 20´/E	34º 04´/N 74º18´/E	34º 30´/N 75º 30´/E
Direction with reference to Srinagar	North west	North west	-
Habitat	Shady moist slopes	Shady rocky slopes	Open, plain with partial shade
Threat factor	Grazing, extraction, Habitat degradation and fragmentation	Grazing, extraction, Habitat degradation and fragmentation	-

KUBG- Kashmir University Botanical Garden (Transplant site)

3.2. Collection, Preservation and Storage

Giving due cognizance to the threat status of the species plant collections were carried out judiciously throughout the course of the present study.

The young seedling of the *Valeriana jatamansi*, underground rhizomes, floral parts and seeds were collected in different seasons. Part of the material was used for laboratory analysis and part of it was sown in the Kashmir University Botanical Garden (KUBG).The voucher specimens were identified & deposited in Kashmir University Herbarium (KASH)

3.3. Species morphology and phenotypic variability

Various populations were analyzed for plant structure, number of shoots per plant, rhizome shape and dimensions, plant height, leaf number and dimensions, flower structure and dimensions, structure of sex organs and seed size and number. Both qualitative and quantitative parameters were studied on the basis of morphological features given by Lawrance (1951), Kaufman *et al.* (1989), Weberling (1989) and Nath (1996). 20 plants were selected for the current study at each study site in order to study phenotypic variability and species morphology by following proper procedure. The studies were carried out in both *ex situ* as well as in natural habitats and photographs were taken using pentex K1000 camera and Olympus zoom stereo microscope. ANOVA using SPSS (11.5) software was used to calculate and compare the final means within and across different populations at three study sites.

3.4. Phenology

Studies on initiation and duration of various phenological events viz. vegetative phase, (Sprouting of the underground part, initiation and duration of vegetative phase), sexual phase (initiation and duration of bud formation, anthesis, sigma receptivity, pollen shedding, seed development) and senescence of aerial shoot were carried out in natural population as well as on *ex situ* population (in

KUBG). Randomly selected plants (15 plants at each study site) were tagged in different populations and monitored during the growing season of years, 2009 and 2010.

3.5. Reproductive Biology

3.5.1. Pollen viability and pollen ovule ratio

To test viability, pollen grains from ready to dehisce anthers of 10 randomly selected flowers were stained in 1% acetocarmine and 1% aniline blue – lactophenol, (Swanson and Sohmer, 1976). The stained, healthy and plump pollens were recorded as viable.

Also 15 mature flowers just to anthesize were collected at random for estimating P/O ratios. Pollen quantity was estimated by squashing one anther (several times) in 10 drops of distilled water in a cavity block and shaken with a glass rod. The following equation was followed to calculate the number of pollen per flower and per plant.

$$p \times q = r$$

$$r \times s = t$$

p* =No. of pollen per drop of water

q = No. of drops of water drops taken initially in which anther was squashed

r = No. of pollen per anther

s = No. of anthers per flower

t = total pollen count per flower

P* represents the mean pollen count per drop of water

Average number of ovules per pistil was counted using dissection microscope.

Pollen- ovule ratio was calculated following Cruden's (1977) method as follows:

$$P/O = \frac{\text{Pollen count per anther x No.of anthers per flower}}{\text{Number of ovules per flower}}$$ (Cruden, 1977)

3.5.2. Stigma receptivity

Stigmas at different developmental stages were fixed in 1:3 acetic alcohol (Carnoy's fixative, 1886). Subsequently they were stained in 1% cotton blue-lactophenol. The stigmas were allowed to remain in the stain for about an hour (Hauser and Morrison, 1964). These were observed under microscope (with 15x and 40x combination) and stigmas carrying the germinating pollen grains were recorded as receptive and hence taken as index of degree of receptivity. Stigmas with germinating pollen grains were photographed at different developmental stages.

3.5.3. *In vitro* Pollen germination

The *in vitro* pollen germination was analysed in six different media containing boric acid 100mg/l, calcium nitrate 300mg/l and 15% sucrose (Shivanna and Rangaswamy, 1992) in various permutations and combinations (Table 2). After preparation of this medium, ten drops of the medium were added into cavity block and mixed thoroughly with glass rod and covered with the lid. Proper care was taken to prevent contamination of one medium with other media. The cultures were incubated in the laboratory conditions at 22 ± 2^{o}C for 1-6 h. The pollen tube lengths were estimated using ocular and stage micrometer. Photographs of pollen grains with long pollen tubes have been taken using zoom stereo microscope.

Table 2: Media composition for *in vitro* pollen germination of *Valeriana jatamansi*

S. No	Medium*	Ratio of Nutrients
1	S	-
2	S+B+C	1:1:1
3	S+B+C	1:1:2
4	S+B+C	1:2:1
5	S+B+C	1:2:2
6	S+B+C	2:1:1
7	S+B+C	2:2:1

*S-sucrose, B- boric acid, C- calcium nitrate

The percentage pollen germination was calculated as;

$$\% \text{ age pollen grain germinated} = \frac{\text{No of Pollen grains germinated}}{\text{Total No of Pollen grains observed}} \times 100$$

(Cruden, 1977)

3.5.4. Nature of breeding system

Nature of breeding system operative in the species was unraveled by controlled pollination and bagging experiments. For this 10 plants were individually selected randomly in each case at each study site and were assessed for both experiments. The following experiments were conducted;

- Whole inflorescence bearing unemasculated flowers were selected, tagged and allowed to open pollinate

- Whole inflorescences bearing unemasculated flowers bagged to prevent open pollination and enforce selfing
- Physically isolating individual plants of female and bisexual flowering types from other plants of the species so as to prevent any chance of immigration of pollen to the experimental plants
- In order to check for apomictic seed development in female individuals and to avoid pollination, female individuals were bagged by butter paper bags

3.5.5. Pollination Mechanism

Pollination mechanisms were studied by observing the foraging behavior of various insects visiting the flowers for reward. The representative specimen of visitor were collected and identified in the Department of Zoology, University of Kashmir. The insects with maximum efficiency of visitation were labeled as major pollinators.

The efficiency of insect visitation was observed during different periods of a day and different weather conditions. The insect visiting efficiency (IVE) was calculated as following Bingham and Orthner, (1998)

$$IVE = \frac{\text{Number of flowers visited by the insect in one time}}{\text{Total number of flowers available}}$$

(Bingham and Orthner, 1998)

For obtaining a reliable estimate of IVE this study was repeated many times during the flowering period.

3.5.6. Flower Characteristics

Qualitative and quantitative characters of flowers which include number, length, breadth, color, structure and arrangement of sepals, petals, androecium and gynoecium were studied at full bloom stage. Structure and arrangement of these various floral parts were studied following methodology of Lawrance, (1951), Kaufman *et al.* (1989) and Nath, (1996). Randomly selected 20 flowers

from each study site were selected to carry out the flower study in terms of both qualitative and quantitative traits. Comparison of female and hermaphrodite flowers were carried out in different populations using ANOVA following SPSS (11.5) software.

3.6 Seed biology

3.6.1. Seed set

Seed set was calculated following Lubbers and Christensen's (1986) formula:

$$\% \text{ Seed set} = \frac{\text{Total number of seeds produced}}{\text{Total number of ovules borne by a plant}} \text{ x } 100$$

Further, before the onset of experimentation, number of flowers/inflorescence axis/ plant was counted in the selected plants. After the experiment was over number of seeds/fruit set per inflorescence axis/plant was counted and percentage fruit/seed set calculated. Since the flower in this species is with only one ovule/ovary, there was obviously no difference between the number of seeds and fruit set/plant.

3.6.2. *In vitro* seed germination

For *in vitro* seed germination studies were carried out on randomly collected seeds from natural as well as *ex situ* populations. Each replicate composed of 20 seeds were used and were washed with 0.1% mercuric chloride for 5-7 minutes followed by washing 4-5 times with distilled water. These were subjected to different physical and chemical treatments (Table 3) at an average temperature of 15-20^0 C in petriplates on moist what man filter paper.

For each treatment, six replicates, each with a set of control to compare the percentage germination and mean germination time (MGT) were used. MGT was calculated following Joshi and Dhar (2003) equation:

$$\text{MGT} = \Sigma \frac{\text{nxd}}{\text{N}}$$

Where n = Number of seeds germinated after each incubation period in days d N = Total number of seeds germinated at the end of experiment.

In vivo seed germination was assessed using 20 seeds sown in soil pots (containing sandy loam soil) as well as in the field with 1×1 m bed size and 35 ×45 cm spacing to test the germination.

Table 3: Physical and chemical treatments to test seed germination of *Valeriana jatamansi*.

S. No	Treatment	Time period	Concentration(mM)
1	Chilling		
a	Wet chilling	20 days	-
		40 days	-
		75 days	-
		90 days	-
b	Dry chilling	20 days	-
		40 days	-
		75 days	-
		90 days	-
2	Conc. H_2So_4 dip.	-	-
3.	GA_3	-	0.25
		-	0.5
		-	1.0
		-	1.5
		-	2.0
4	Thiourea	-	0.25
		-	0.5

		-	1.0
		-	1.5
		-	2.0
5	Kinetin	-	0.25
		-	0.5
		-	1.0
		-	1.5
		-	2.0
6	Scarification	-	-
7	Control	-	-

3.6.3. Seed size

Seed size was calculated by weighing randomly selected 100 seeds from each population, following Agarwal and Dadlani (1988) formula

$$\text{Average seed size (weight)} = \frac{\text{Weight of 'N' Seeds}}{\text{N}}$$

(Agarwal and Dadlani,1988)

t-test was done to elucidate the comparative size and weight of hermaphrodite and female seeds.

3.6.4. Seed viability test

Seeds are soaked overnight at room temperature and are cut longitudinally to expose the embryos. After preparing the desired number of seeds (mean 20), they were soaked in 1% tetrazolium solution (TZ) of ph 6 to 7 and are kept in dark at 30^0 C for 3-4 hours. After developing color, the TZ solution is drained

by rinsing seeds 2-3 times with water. The intensity of the red colour in the stained seed depends upon the amount of formazon formed, which in turn is regulated by dehydrogenase activity. In a healthy seed the intensity of colour is expected to be more. (Moor, 1973) and hence percentage viability is determined.

3.7. Modes of propagation

To study the methods of propagation operative in species 15-20 plants were tagged at the onset of the growing season 2009 and monitored thorough out the growing season 2009 and 2010.

Occurrence of seedling in the natural populations served as an index of successful recruitment of several seedlings originating from seeds (Sexual propagation).

3.7.1. *In Vivo* Propagation

The fresh underground parts (rhizomes) were collected from natural population in the 1st week of April and split longitudinally containing a portion of shoot apex.

The split cuttings were treated with 25mM, 50mM, 100mM IAA, IBA and GA_3 for 48 hours and were sown in sandy loam soils at Herbal Garden, University of Kashmir, Srinagar to observe the leafy shoot generation, survival and comparison with control (untreated cutting).

3.8. Resource partitioning and reproductive effort

Healthy 10-15 mature flowering individuals from each study site were harvested for the study of resource partitioning in different organs of a plant. The plants were fragmented into individual parts such as roots, rhizome, Leaves, stem and inflorescence whose fresh weights (weighing as fresh) and dry weights (after oven-drying for 48 hours at 80^{o}C, Kawano and Masuda 1980), were worked out using electric balance. The reproductive effort (RE) was calculated by Abrahamson and Gadgil (1973) method.

$$RE = \frac{\text{Dry Weight of inflorescence}}{\text{Total dry weight of the above ground parts}}$$

3.9. Pollen mother cell meiosis

For investigation of pollen mother cell meiosis, buds of suitable size were collected from all the three study sites in the morning hours between 7 to 9 am and were fixed in Carnoy's fixative (absolute alcohol, glacial acetic acid and chloroform with composition 6:3:1) for a period of 24 hours. After proper fixation time the plant material was washed with 70% alcohol to remove all traces of the fixative. Then the fixed material was stored in freshly prepared 70% absolute alcohol at 4^{o}C under refrigerated conditions. Then anthers were squashed in 2% propionocarmine to observe phases with countable chromosomes numbers (metaphase and anaphase) in order to calculate "n" and "2n". Propiono-carmine was prepared by taking 45ml of 45% propionic acid with 55 ml of distilled water to which 2gm of carmine powder was added to prepare 100ml of 2% propiono-carmine.

3.10. Development of agrotechniques and impact of habitat

To make the cultivation of this species possible in an effective way at lower altitudes, the response was observed in the following soil textural classes recommended by Nautiyal *et al.* (2001) and fertilizer applications were given to each (Table 4).

a. Natural soil (from natural habitat)
b. Loam: organic manure (2:1)
c. Loam soil (garden soil)
d. Sandy loam (2:1)
e. Sandy loam (1:2)
f. Sand: silt: clay (1:2:2)

Young seedlings (of the same age) were collected from natural habitats in April – May and sown in the mentioned soil types. Each set of pots were irrigated at

different intervals and after young plants had stabilized and adopted, various inorganic fertilizers (Table 4) in different concentration were applied each with a set of control. The plants were kept in shade, partial shade (less light intensity) and open sun (more light intensity) to observe the morphological variations with respect to requisite conditions and harvested (at the full bloom) to determine dry biomass (after oven dried at 80^{o}C for 72 h; Singh and Purohit, 2003) and compared with the control.

Table 4: Different fertilizers applied to the young plants of *Valeriana jatamansi*

Nitrogen (Urea)	Potassium (Potassium phosphate)	Phosphorus (DAP)	NPK
139.86mg (300mg)	67.34mg (300mg)	117.42 mg (500 mg)	321.62

Fertilizers were given to per plant.

3.10. Scanning electron microscopy (SEM) Studies

Double sided conductive tap was fixed to the stub and the pollen from mature ready to dehisce anthers were dusted over it. The dusted material sputter coated with gold was observed under SEM (S-3000 H). Mature and dry seeds were loaded on the stub and sputters coated and were observed under SEM at the University Scientific Instrumentation Centre (USIC), University of Kashmir.

Results

Valeriana jatamansi Jones (Valerianaceae) is perennial, critically endangered medicinal herb distributed in sub-temperate to temperate regions-ranging in an altitude from 1200-3000m asl. It is commonly known as Mushkibala (Kashmiri), Tagar (Sanskrit), Indian Valerian (English) and Suganthdhawal (Tamil). Owing to its immense medicinal properties, over-exploitation of rhizome and roots for medicinal use and consequent degradation of natural habitats are major threats which the herb at present is facing. Since the species is categorized as critically endangered, it necessitates that *in situ* as well as *ex situ* conservation strategies are devised to prevent its extinction and this is where study of reproductive biology comes into play. It is only after assessing the reproductive strategy the conservational policies can be planned and programmed for this or any other related species.

4.1. DISTRIBUTION

4.1.1 Kashmir Himalayas

During the present investigation the species was found sporadically distributed in the mountain ranges of Kashmir Himalayas confined to sub-temperate and temperate regions, thriving best in moist shady slopes, rocky slopes, land slide areas ranging in an altitude of 1200-3000m asl. The species was mainly found in Gulmarg, Ferozpora, Yusmarg, Duksum, Sonamarg, Phalgam, Naranag and Dara. Out of these; two natural sites viz.; Ferozpora and Gulmarg and one transplant population at KUBG (Kashmir University Botanical Garden) were selected for the present investigation. The characteristics of the selected sites are summarized in section 3, Table 1.

4.1.2. Species Morphology

This species is a tufted, hairy herbaceous perennial, gynodiecious herb with hermaphrodite plants ranging from 13.0-37.70 cm in height and female plants found usually dwarf than hermaphrodite with heights from 10.90-29.50cm.

The plant is characterized by thick horizontal rhizome with diameter ranging from 2.0-4.40cm with 10-37 roots per stock. Basal radical leaves are long stalked, deeply cordate -ovate, usually toothed or sinuate up to 3.20-8.30cm long and 2.40-7.50cm broad. Cauline leaves are only a few, much smaller, entire or sometimes pinnate of 1.90-2.70cm in length and 1.60-2.30cm in breadth. Flowers are white or tinged with pink in terminal corymbs with 8-13 female flowers per inflorescence and 8-14 hermaphrodite flowers per inflorescence which are larger and broader than female flowers and are ranging from 0.30-0.40cm across. Calyx is represented by inwardly curved ring which opens into plumose pappus at fruit setting stage. However, corolla is five lobed with rotate, white or pinkish depending on the availability of light. Gynoecium is tricarpellary syncarpous represented by trifid stigmas in females and unifid in hermaphrodites, ovary seemingly unilocular with single ovule, ovary inferior and seed is one seeded achene. Hermaphrodite and female plants do not differ much in vegetative characters but can only be segregated during flowering phase, as flowering axis is seasonal which dries up during senescence. These female and hermaphrodite plants can be segregated by quantitative flower morphological features as depicted in Table 1.

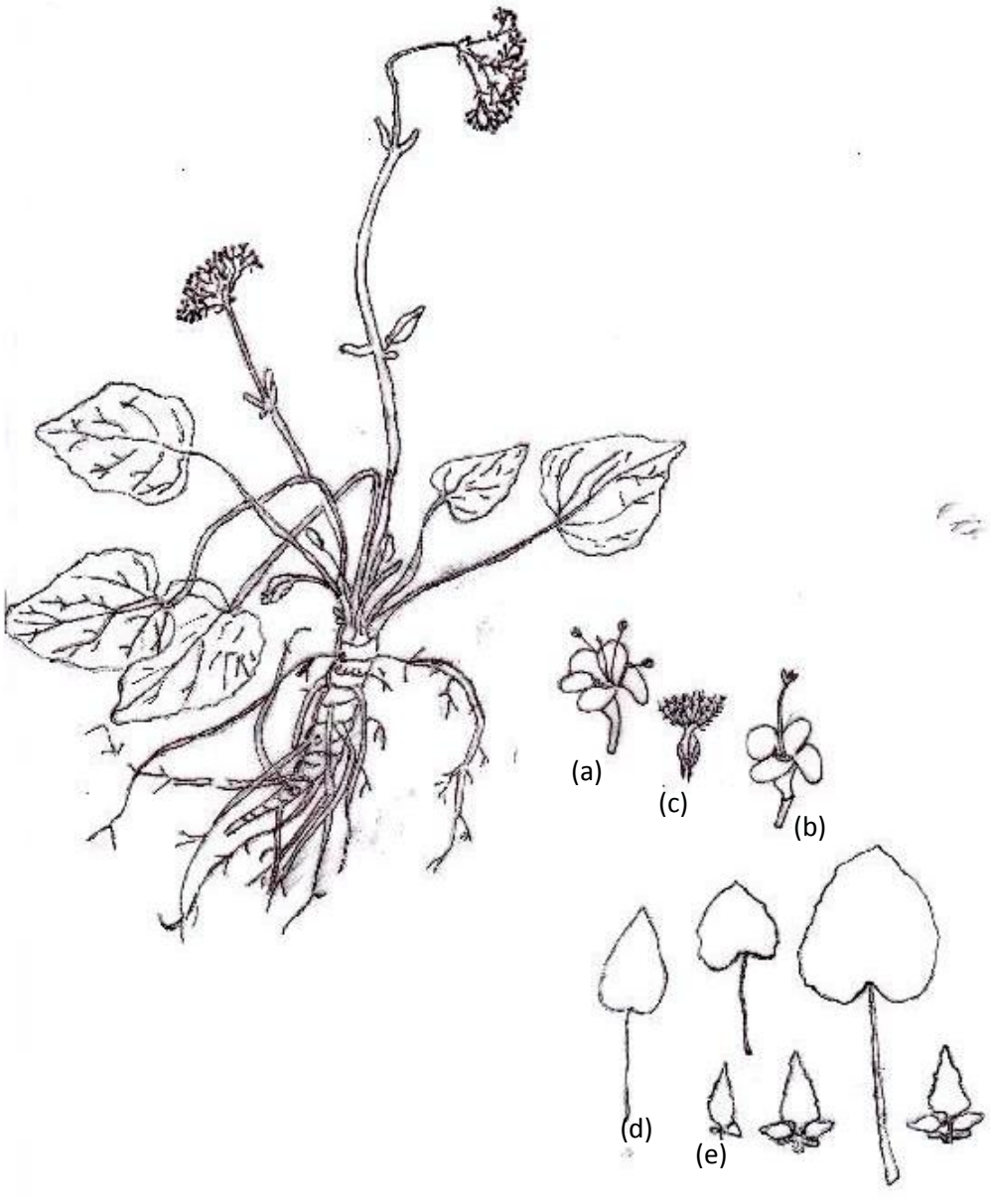


Plate 1: Morphological characters of *Valeriana jatamansi*

(a) Hermaphrodite flower
(b) Female flower
(c) Parachute type of seed
(d) Radical leaves (basal)
(e) Cauline leaves (middle)

(a)

(b)

Plate 2

a) Unifid stigma of hermaphrodite flower
b) Trifid stigma of female flower

Table 1: Differential quantitative flower characters of hermaphrodite and female plant

S. No	Characters	Plant type	
		Female	**Hermaphrodite**
1.	**Androecium**	Absent	Stamens 3, epipetalous, opposite to corolla lobes.
2.	**Flower**	Pistillate white (large) or tinged with pink (small).	Bisexual white, long prominently larger than pistillate flower.
3.	**Stigma**	Trifid	Unifid
4.	**Number of petals**	4	5

4.2. PHENOTYPIC VARIABILITY

The species is variable with respect to its quantitative traits. Phenotypic variability was observed in two natural populations namely, Gulmarg and Ferozpora and the same was then compared with the transplant population at Botanical Garden Kashmir University (KUBG).

Plants were randomly selected in all the populations and the traits analyzed include-plant height, number of leaves per plant, internode length, leaf dimensions, number of flowers per inflorescence, flower dimensions, rhizome dimensions and number of roots per stock.

The phenotypic data gathered in different populations is described in detail;

4.2.1. Plant Height

The hermaphrodite plants from Gulmarg population were observed to be 14.81±2.44cm tall. While female plants were 14.04±1.79cm tall which is

statistically at par with Ferozpora population with hermaphrodite 15.63±2.27cm and female plants 14.16±2.00cm tall. The transplants were relatively observed to be more vigorous compared to natural populations, registering plant height 21.58±2.14cm in hermaphrodite and 19.13±2.98cm in females. It was also observed that plants inhabiting shady, moist and fertile or humus rich soils attain vigorous growth, while plants growing under open sunny conditions and on rocky slopes were observed to be on the other extreme. (Table 2, 3)

4.2.2. Leaf dimensions

Valeriana jatamansi produce two types of leaves- basal (radical) leaves which arise from rhizomatous portion and cauline (middle and apical) leaves which arise from the stem. Basal leaves were 5.74±1.33cm in length and 4.60±0.89cm in breadth in hermaphrodite plants while in female plants these are 5.41±1.50cm in length and 4.54±1.25cm in breadth in transplant population. Basal leaf length and breadth of Gulmarg population was 4.94±1.06cm, 4.11±1.04cm in hermaphrodite and 4.52±0.95cm, 4.09±1.13cm in females respectively. Among Ferozpora populations the basal leaf length was 4.31±0.82cm and breadth 3.67±0.95cm in hermaphrodite plants, while in female plants 4.22±0.74cm length and 3.62±0.63cm breadth was observed. (Table 2, 3)

4.2.3. Root stock

Root Stock, consisting of rhizome and roots, is characterized by underground thick horizontal rhizome with descending adventitious fibrous roots. Rhizome surface has nodes and internodes and terminates in a tuft consisting of leaf and flowering shoot bases. Average length of rhizome of transplant population was 7.00±2.41cm with a diameter of 3.23±0.72cm in hermaphrodite plants while length of 6.96±1.87cm and a diameter of 3.20±0.61cm were recorded in female plants.

Among natural populations, plants inhabiting Ferozpora were observed to have shortest rhizome length of 4.51±1.29 cm with the diameter of 2.32±0.77cm (in hermaphrodite) and 4.43±0.68 cm rhizome length with diameter of 2.50±0.45cm (in females).The plants inhabiting Gulmarg were intermediate between the two populations (5.32±1.51cm length and diameter of 3.01±0.46cm in hermaphrodite, 3.01±0.46cm length and diameter of 3.01±0.46cm in females).

Maximum number of roots per stock was observed in KUBG population with mean of 24.57±2.85 roots per stock (in hermaphrodite) and 24.00±2.59 (female plants). The plants of Gulmarg population depicted 21.90±2.06 (hermaphrodite plants) and 18.52±2.53 (female plants) roots per stock. However, lowest number of roots per stock was observed in plants inhabiting Ferozpora depicting only 15.87±2.17 (hermaphrodite plants) and 13.95±2.17 (female plants) roots per stock. Thus it is evident from the data that plants growing under dense canopy and humus rich moist soils as well as loose and fertile soil of KUBG showed better rhizome development. (Table 2, 3)

4.2.4. Inflorescence

In both hermaphrodite and female plants, the flowers are arranged in terminal corymbs. The average inflorescence length in hermaphrodite plants is 3.23±0.67cm and diameter (across) of 2.55±0.35 cm, while average inflorescence length in female plants is 3.16±0.56cm and diameter (across) of 2.42±0.22cm.

Female flowers are mostly pinkish and markedly smaller than the hermaphrodite flowers which are white colored. Average hermaphrodite and female flower number per plant varies from *ex situ* to natural sites because number of ramets per genet and number of inflorescence axis per genet varies considerably from *ex situ* to natural sites which ranges from 6-12 ramets per genet in transplanted population and 4-7 (Gulmarg), 3-6 (Ferozpora) ramets per genet. (Table 2,3)

Table 2: Phenotypic variability of hermaphrodite individual (*Valeriana jatamansi)* across three different sites

Morphological Feature		Hermaphrodite Plant			ANOVA	
		Populations				
S.No.	**Trait**	**Gulmarg**	**Ferozpora**	**KUBG**	**F**	**P**
1	**Plant height(cm)**	14.81±2.44*	15.63±2.27	21.58±2.14	13.84	.00
2.	**Petiole length(cm)**	5.57±1.37	5.09±1.06	6.41±1.51	5.33	.01
3	**Leaf dimensions**					
3.1	**Basal leaves (radical leaves)**					
(a)	**Lamina length(cm)**	4.94±1.06	4.31±0.82	5.74±1.33	9.15	.00
(b)	**Lamina breadth(cm)**	4.11±1.04	3.67±0.95	4.60±0.89	8.15	.00
3.2	**Middle leaves (cauline leaves)**					
(a)	**Lamina length(cm)**	2.16±0.38	2.04±0.43	2.21±0.33	1.12	.33
(b)	**Lamina breadth(cm)**	1.97±0.21	1.96±0.23	1.99±0.19	0.10	.90
3.3	**No.of leaves per genet**	13.71±2.73	13.28±1.89	26.43±2.65	18.96	.00
4.	**Internode length(cm)**	4.02±0.80	4.34±0.70	5.52±1.38	12.83	.00
5.	**Infloresence**					
(a)	**Length(cm)**	2.76±0.38	2.63±0.47	3.23±0.67	7.73	.00
(b)	**Diameter(cm)**	2.51±0.30	2.49±0.24	2.55±0.35	0.18	.83
5.1.	**Number of flowers per inflorescence**	11.00±1.70	9.81±1.54	12.09±1.67	10.23	.00
5.2.	**Number of infloresences axis per ramet**	6.86±1.06	6.67±1.11	7.24±0.77	1.81	.17
5.3.	**Number of flowers per ramet**	74.67±12.84	64.57±11.19	87.24±13.81	16.89	.00
5.4	**No.of flowers per genet**	480.00±95.77	402.71±88.05	695.57±98.35	112.64	.00
6.	**Root stock**					
6.1	**Number of roots per genet**	21.90±2.06	15.86±2.17	24.57±2.85	10.84	.00

6.2.	**Root length(cm)**	6.67±1.53	4.49±1.22	6.12±2.09	9.79	.00
6.3	**Rhizome length(cm)**	5.32±1.51	4.51±1.29	7.00±2.41	10.32	.00
6.4.	**Rhizome diameter(cm)**	3.01±0.46	2.32±0.77	3.23±0.72	10.54	.00
7.	**Ramets per genet**	5.43±0.92	4.09±0.94	8.09±1.41	69.91	.00

*Mean± Standard Deviation, df =2.

Table 3: Phenotypic variability of female individual (*Valeriana jatamansi)* across three different sites

Morphological Feature		**Female Plant Populations**			**ANOVA**	
					F	**P**
S.No.	**Traits**	**Gulmarg**	**Ferozpora**	**KUBG**		
1	**Plant height(cm)**	14.04±1.79*	14.16±2.00	19.13±2.98	16.62	.00
2	**Petiole length(cm)**	5.02±1.18	4.96±0.63	6.31±1.96	6.58	.00
3.	**Basal leaves (radical leaves)**					
(a)	**Lamina length(cm)**	4.52±0.95	4.22±0.74	5.41±1.50	6.56	.00
(b)	**Lamina breadth(cm)**	4.09±1.13	3.62±0.63	4.54±1.25	4.07	.02
3.2	**Middle leaves (cauline leaves)**					
(a)	**Lamina length(cm)**	2.13±0.27	2.08±0.14	2.20±0.19	1.76	.18
(b)	**Lamina breadth(cm)**	1.95±0.16	1.97±0.19	1.98±0.21	0.12	.88
3.3	**No. of leaves per genet**	13.71±3.73	13.28±2.98	25.42±3.13	17.47	.00
4.	**Internode length(cm)**	3.77±0.60	3.87±0.69	5.33±1.59	14.17	.00
5.	**Infloresence**					
(a)	**Length(cm)**	2.72±0.39	2.58±0.40	3.17±0.56	9.25	.00

(b)	**Diameter(cm)**	2.42±0.22	2.40±0.19	2.42±0.22	.03	.97
5.1	**Number of flowers per inflorescence**	10.52±1.60	9.43±1.75	10.90±1.76	4.25	.01
5.2	**Number of infloresences axis per Ramet**	6.05±0.67	5.19±1.03	6.09±1.13	5.83	.00
5.3	**Number of flowers per ramet**	62.09±16.12	49.33±11.89	66.38±15.83	7.60	.00
5.4	**No. of flowers per genet**	339.23±110.03	218.85±80.18	555.90±122.66	54.72	.00
6	**Root stock**					
6.1	**Number of roots per genet**	18.52±2.53	13.95±2.17	24.00±2.59	19.18	.00
6.2	**Root length(cm)**	5.68±1.47	4.42±0.79	6.07±1.73	8.13	.00
6.3	**Rhizome length(cm)**	5.56±0.88	4.43±0.68	6.96±1.88	21.37	.00
6.4	**Rhizome diameter(cm)**	3.01±0.46	2.50±0.45	3.20±0.61	10.61	.00
7.	**Ramets per genet**	5.48±1.12	4.38±0.80	8.52±1.50	69.60	.00

*Mean ± Standard Deviation, df=2

The data revealed that plant characters are statistically significant ($p \leq 0.05$) depicting that most traits of KUBG are significant with respect to other two natural populations (Ferozpora and Gulmarg) which seem to be statistically at par with each other.

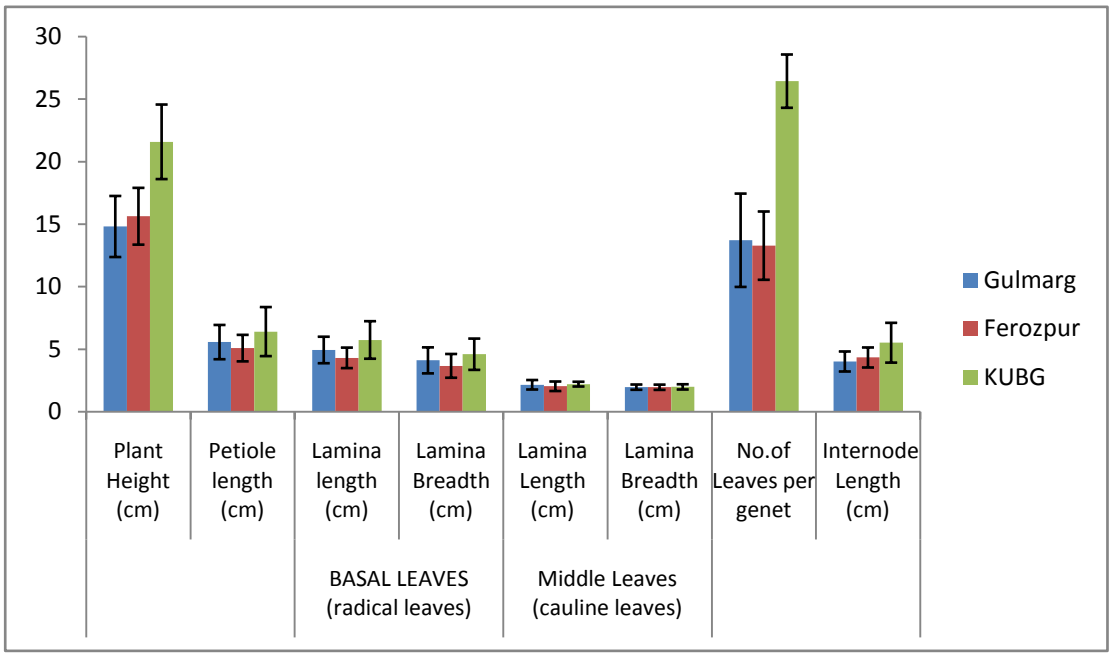


Fig. 1: Depicting phenotypic variability of hermaphrodite individual (*Valeriana jatamansi)* across three different sites

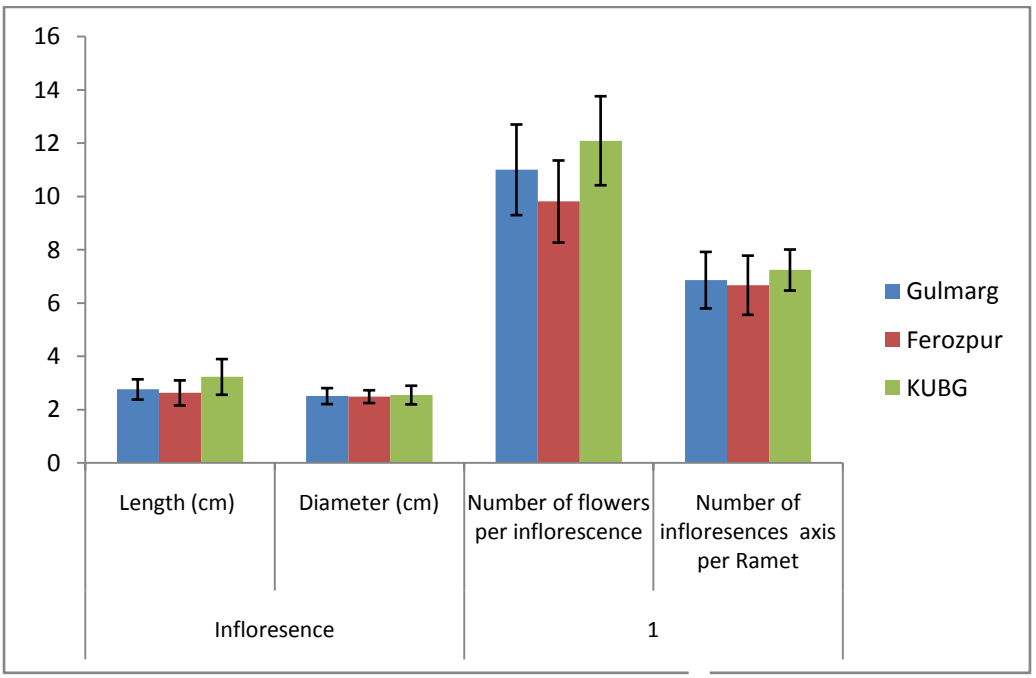


Fig.1.1: Showing number and dimensions of inflorescence of hermaphrodite individual across three different sites

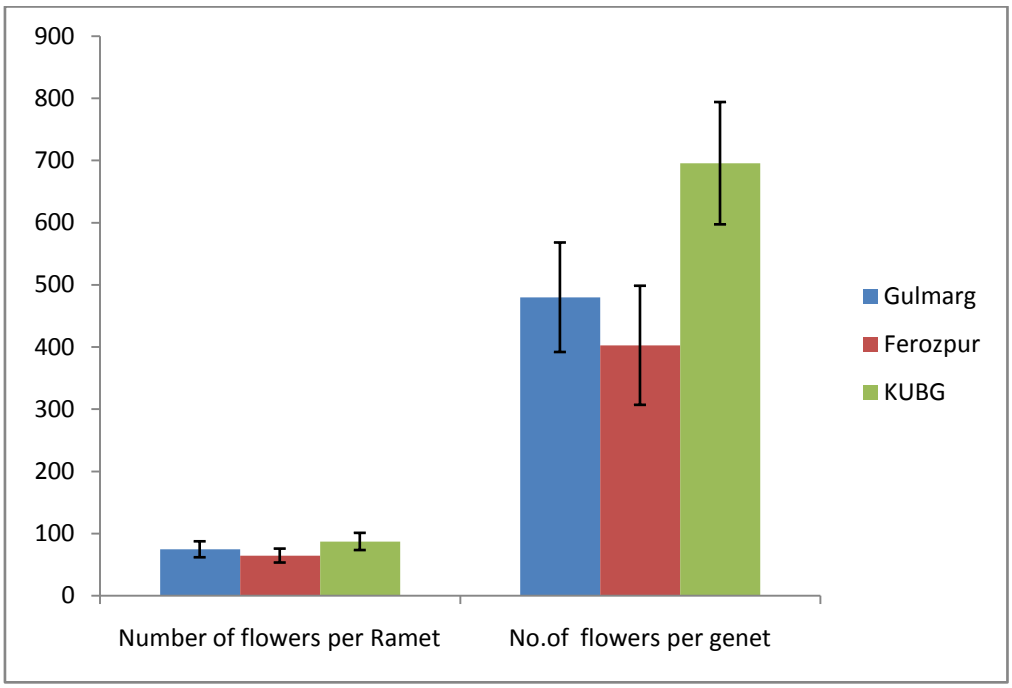


Fig.1.2: Showing number of flowers of hermaphrodite individual across three different sites

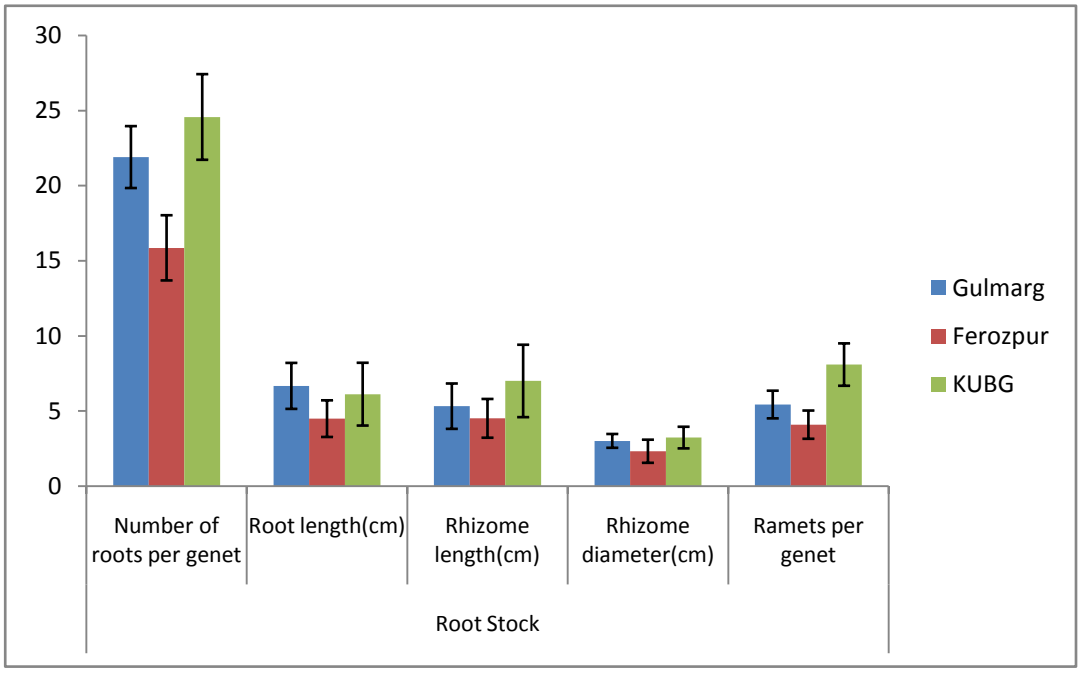


Fig.1.3: Showing root stock dimensions of hermaphrodite individual across three different sites

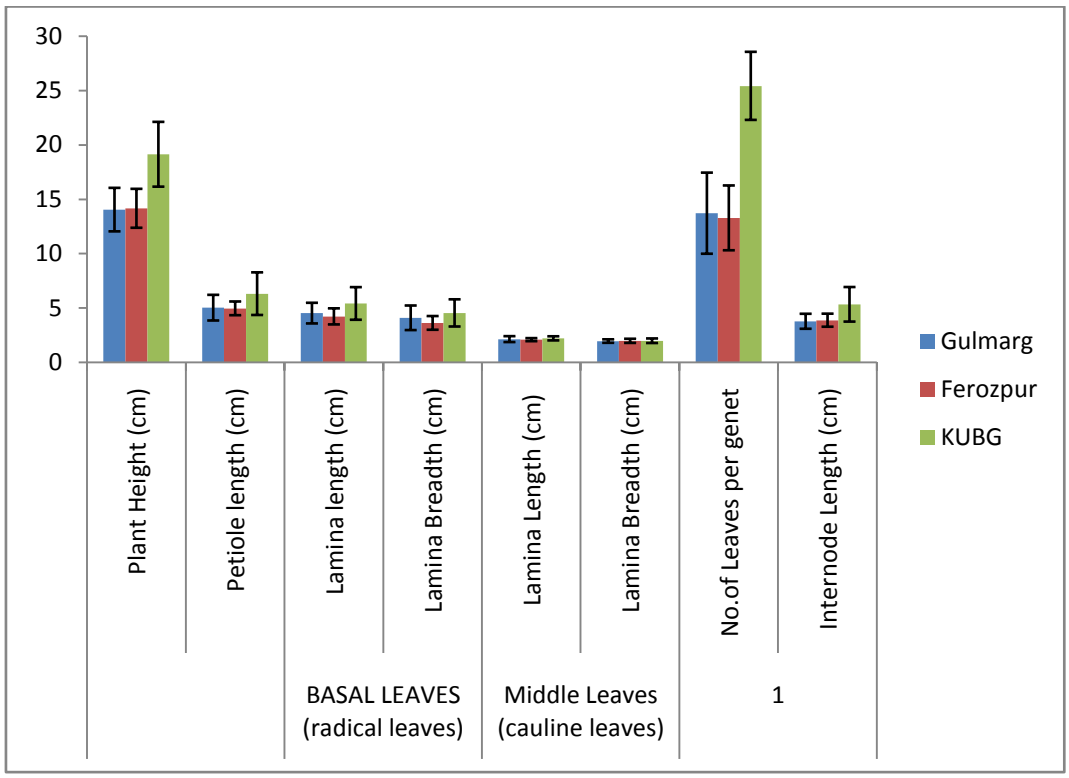


Fig. 2: Depicting phenotypic variability of female individual across three different sites

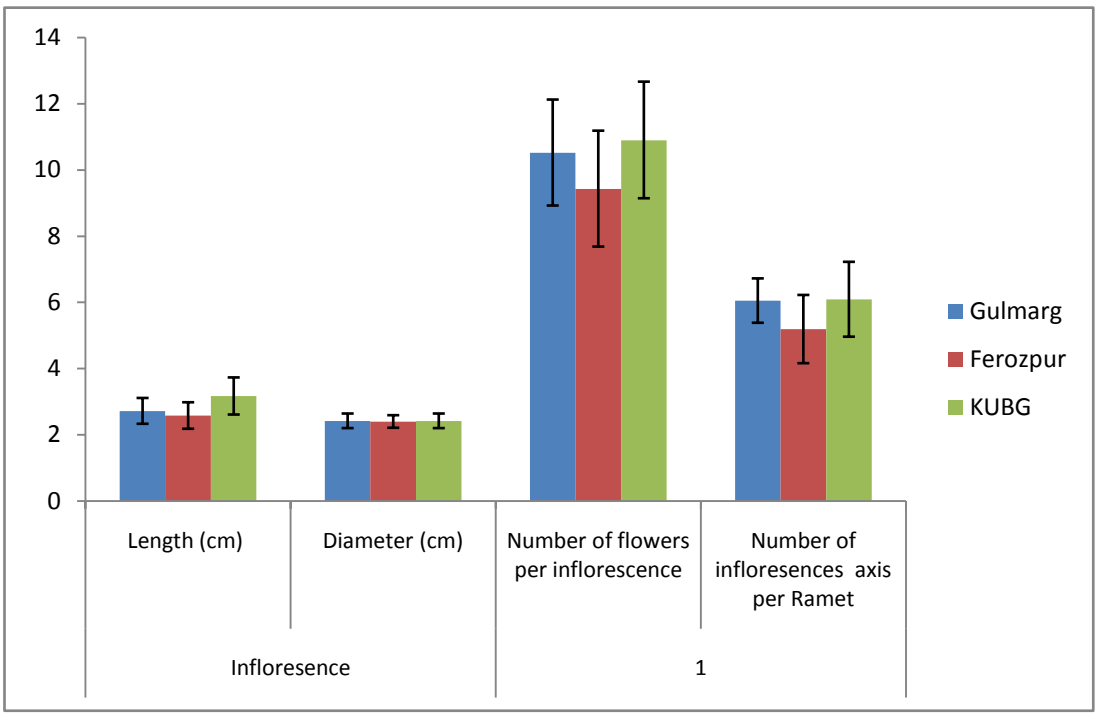


Fig. 2.1: Depicting inflorescence dimensions of female individual across three different sites

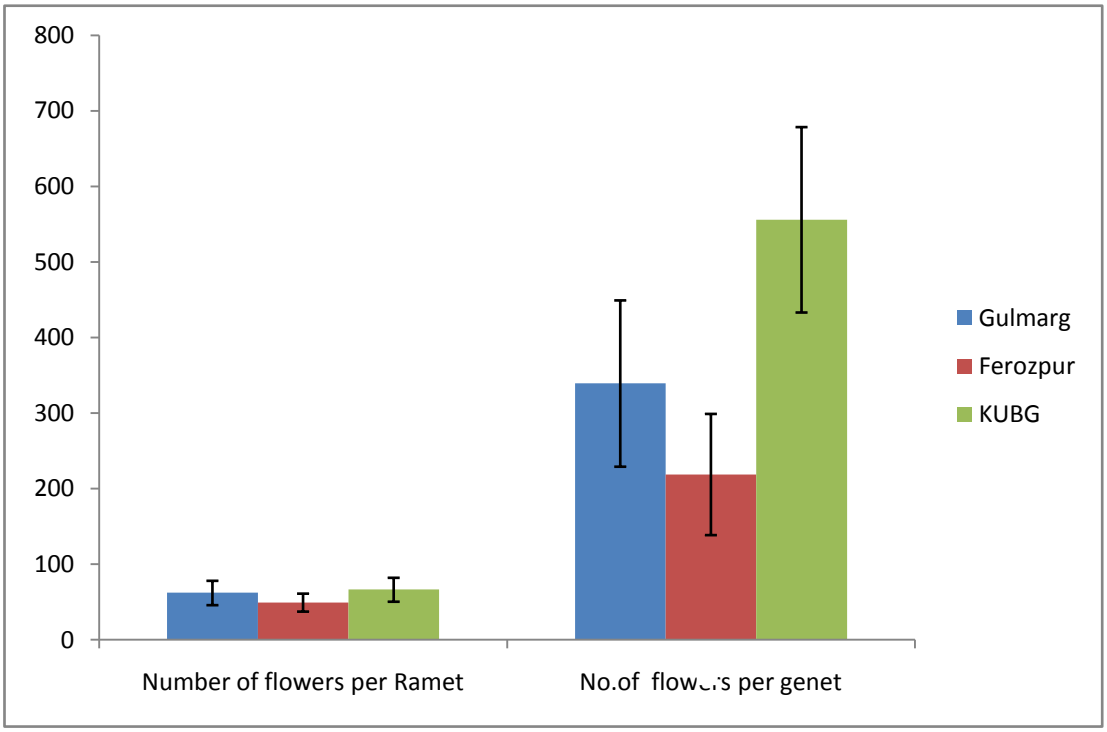


Fig. 2.2: Depicting flower number of female individual across three different sites

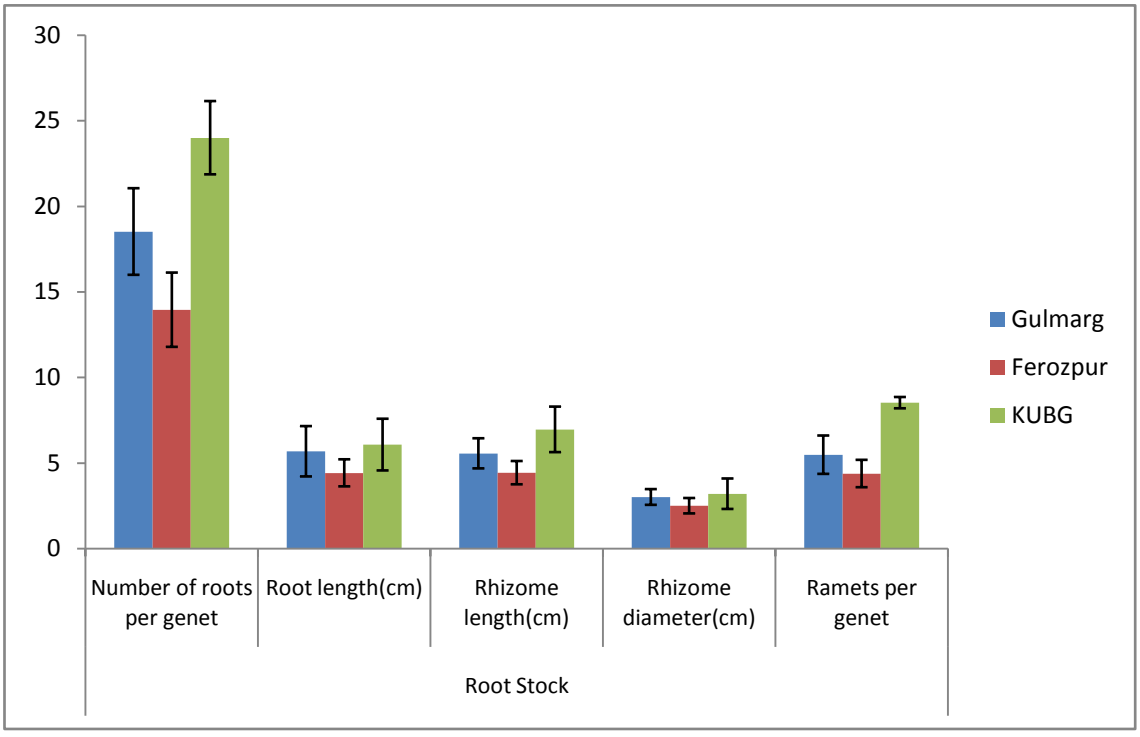


Fig. 2.3: Depicting root stock dimensions of female individual across three different sites

4.2.5. Flower characteristics

Flowers are actinomorphic exhibiting dimorphism in color, white (under shade) and tinged with pink (under open sun), with female and hermaphrodite flowers occurring on separate plants. The female flowers are markedly smaller wherein their flowers are colored as well as the whole plants are relatively bright in color (pinkish) than the hermaphrodite plants. The differential hermaphrodite and female flower dimensions are summarized in Table 4.

Table 4: Quantitative flower characters of hermaphrodite and female plant

Traits	Hermaphrodite plant			Female plant		
	Mean±S.D	**F**	**P**	**Mean±S.D.**	**F**	**P**
Petal length(mm)	4.27± .06	3.20	.14	2.13±.12	3.20	.15
Petal breadth(mm)	2.43±.06	.00	1.00	1.57±.06	.00	1.00
Sepal length(mm)	5.53±.12	.00	1.00	2.73±.12	.00	1.00
Sepal breadth(mm)	1.23±.06	.00	1.00	1.27±.06	.00	1.00
Stamen length(mm)	4.40±.17	1.23	0.32	-	-	-
Anther length(mm)	.57±.06	.00	1.00	-	-	-
Anther breadth(mm)	.37±.06	.00	1.00	-	-	-
Style length(mm)	4.83±.21	5.24	0.08	3.17±.15	.73	.44
Wholeflower length(mm)	**6.87±.12**	.00	1.00	**3.10±.10**	.73	.44

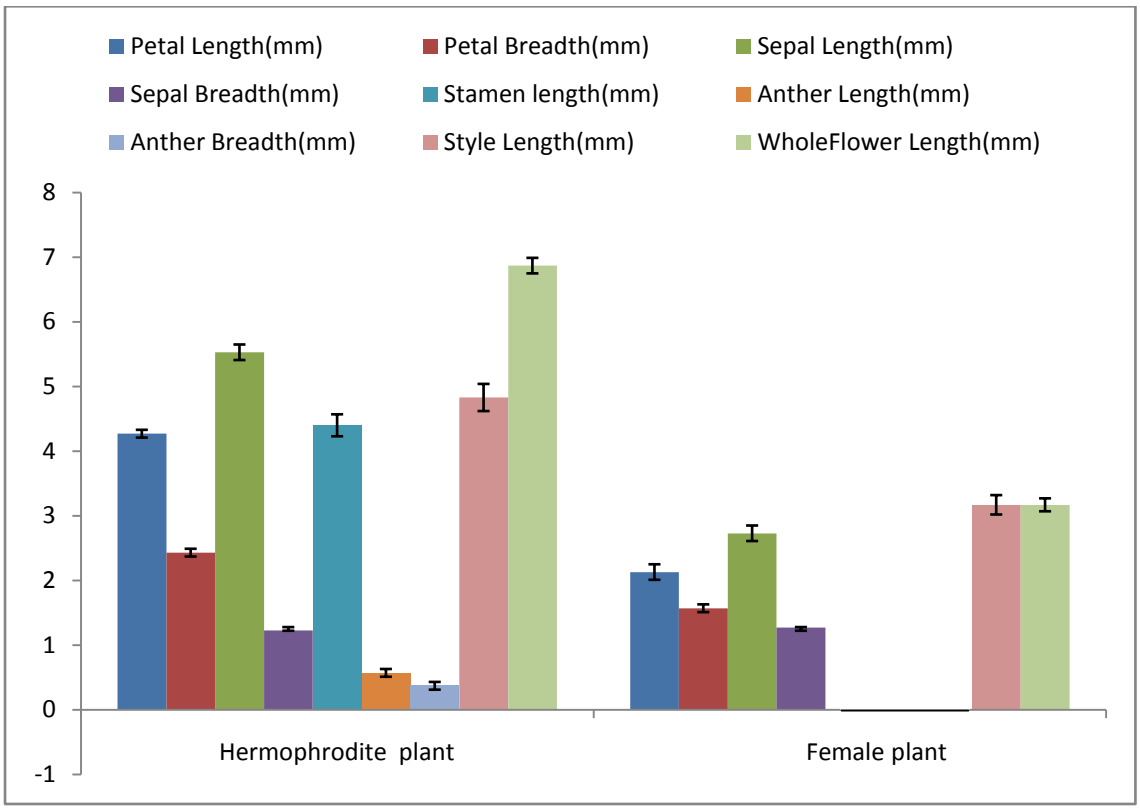
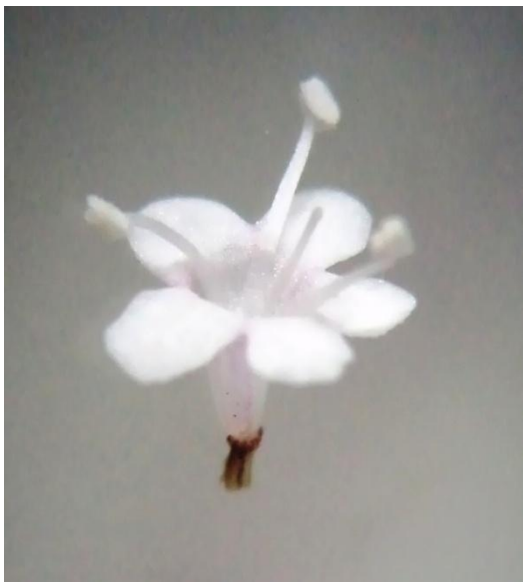


Fig. 3: Showing comparison of quantitative flower characters of hermaphrodite and female plant



(a)

(b)

(c)

(d)

Plate 3

a) Hermaphrodite flower
b) Female flower
c) Colour variation in female flowers
d) Colour variation in hermaphrodite flowers

4.3. PHENOLOGY

The phenological behavior of the species was monitored in natural as well as transplant populations. The altitude and eco edaphic conditions seem to play an important role in the phenological behavior of the species. It is evident from the data (Table 5) that at higher altitudes the plants enter into vegetative and reproductive phases of the life cycle relatively latter than the plants growing at lower altitudes. Under transplant conditions the total life span (aerial shoot) is considerably of a longer duration as compared to the life span in natural habitats. The data raised on phenological behavior of the species during the year 2008-2010 is described below and summarized in Table 5.

4.3.1. Sprouting of the rhizomes

The plants over-winter in the form of underground rhizomes, which remain dormant throughout the chilling winter months (November to March). With the advent of spring, the rhizomes start to sprout and give rise to a young shoot. Onset of sprouting starts in the 2nd week of March and continues till 2nd week of April in the natural populations while in the transplant population the sprouting of the plant starts in 1st week of February and continues up to 3rd week of February.

4.3.2. Initiation of the sexual phase

The plants remain in vegetative phase up to 1st week of April. Subsequently, a stem with flower buds in the axils of basal leaves marks the initiation of the sexual phase. The floral axis is seasonal, its development begins with the formation of a bud at apex which grows and elongates, reaching maximum and then produces flowers in terminal corymbs. The sexual phase starts in the 1st week of April and continues to produce sexual buds up to 4th week of April in natural populations while in transplant population the initiation of sexual phase starts from 3rd week of February and continues up to 3rd week of March.

4.3.3. Anthesis of flower

The flowers begin to open in the 3rd week of May and continue up to 2nd week of July in natural populations while in transplants it starts from 4th week of March and continues up to 4th week of May. Thus it is clear from the data that process of anthesis continues up to initiation of seed development which is marked by dryness of petals and stigmas. The flower anthesis is found to be highly asynchronous both within and across population.

4.3.4. Seed maturation

After the process of pollen transfer to the stigmas is complete, the initiation of seed development starts from 2nd week of July and continues up to 2nd week of August in natural population. However, in case of transplants, the maturation starts in 1st week of June and continues up to 2nd week of July. As is true of anthesis, the seed development is also asynchronous within population and across population as well with respect to two different plants i.e., hermaphrodite and female plants with female plants depicting early seed development than hermaphrodite plants. As and when seeds mature the entire lots of seeds comes out and disperse with the help of parachute mechanism. Aerial shoot of the plant remains vegetative (1-2 months) after seed dispersal till the dawn of complete above ground senescence.

4.3.5. Senescence of flowering shoot

The process of senescence is also asynchronous. Subsequent to the seed dispersal the floral axis dries up as it is seasonal. After flowering shoot senescence the plants remain in vegetative phase for at least 1 or 2 months which is then followed by complete above ground senescence. The senescence of the seasonal flowering shoot starts from 3rd week of August and continues up to 2nd week of September in natural population. The plants grown in KUBG, show signs of senescence of the flowering shoot from 1st week of June and continues up to 2nd week of July. Thus it takes 5 or 5½ months for the species

to complete its life cycle (from sprouting up to senescence of flowering shoot) in its natural home, however at lower altitudes (1595m) in KUBG the transplants completed their life cycle in 6½ months.

Table 5: The phenological behavior of select populations of *Valeriana jatamansi*

Phenophase		Ferozpora(2150m)	Gulmarg(2650m)	KUBG(1595m)
	I	2(3)*	4(3)	1(2)
Sprouting	C	1(4)	2(4)	3(2)
	D	20	19	17
	I	1(4)	2(4)	3(2)
Bud formation	C	4(4)	4(4)	3(3)
	D	20	18	23
	I	3(5)	3(5)	4(3)
Anthesis	C	1(7)	2(7)	4(5)
	D	49	47	53
	I	2(7)	3(7)	1(6)
Seed maturation	C	1(8)	2(8)	2(7)
	D	17	19	24
	I	3(8)	4(8)	4(7)
Senescence	C	1(9)	3(9)	4(8)
	D	13	10	28
Duration of aerial shoot (in days)		**174**	**160**	**212**

I- Initiation of phase

C- Completion of phase

D- Total number of days of a particular phase

*-Digit outside parenthesis is the week and inside the month

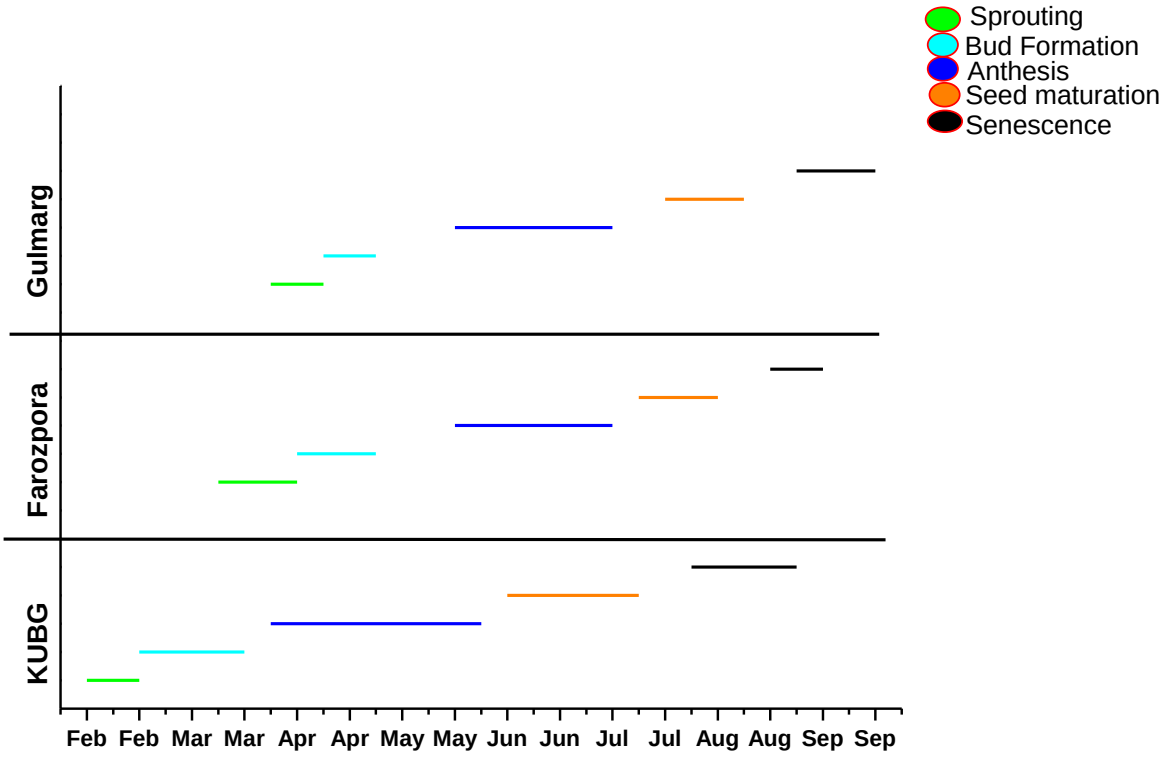


Fig. 4: Phenogram showing phenological behavior of select populations of *Valeriana jatamansi*

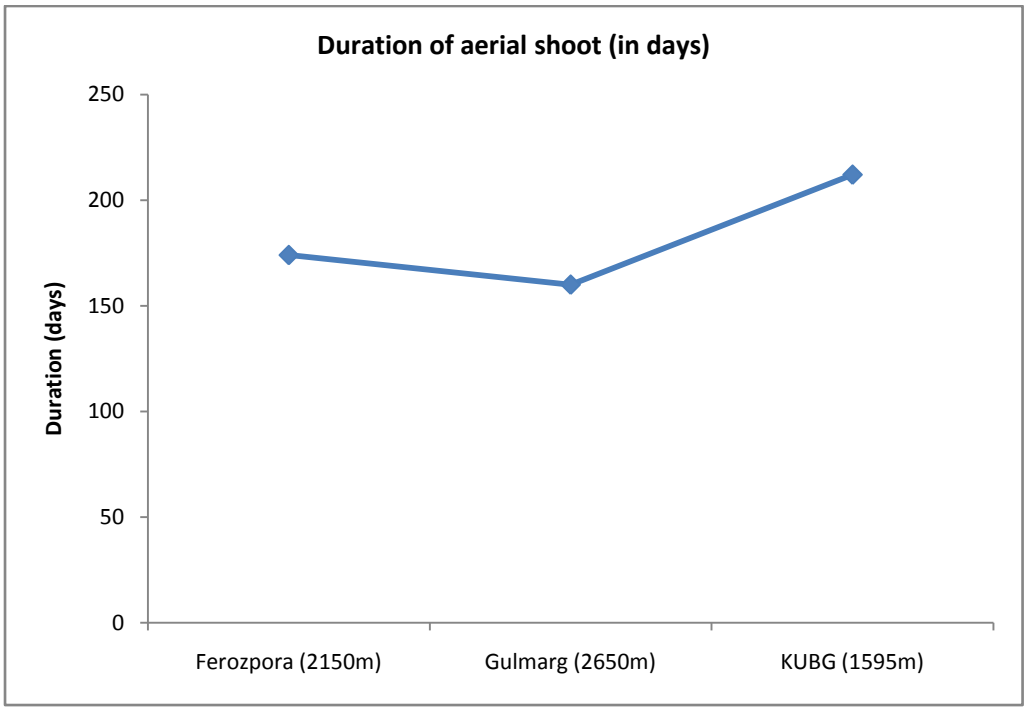


Fig. 5: Showing duration of aerial shoot with respect to altitude

(a)

(b)

(c)

(d)

(e)

(f)

(g)

(h)

Plate 4

a) Sprouting
b) Flower bud formation
c) Flower bud formation
d) Floral bud maturation
e) Flower axis elongation
f) Anthesis
g) Seed Formation
h) Senescence of floral axis

4.4. REPRODUCTIVE BIOLOGY

To assess the nature of breeding system operative in *Valeriana jatamansi*, the following aspects were analyzed

1. Pollen emission and stigma receptivity
2. Pollen viability
3. Pollen germination.
4. Pollen-ovule ratio.
5. Pollination system.
6. Breeding behavior.
7. Modes of propagation
8. Seed production.

4.4.1. Pollen emission and stigma receptivity

In hermaphrodite individuals of *Valeriana jatamansi* temporal separation of opposite sexes was observed (dichogamy), which prevents hermaphrodite from inbreeding as anthers mature early (protandry) and after their dehiscence; the concealed stigmas emerge out and become receptive within 2-3days. Anthesis of flower is highly asynchronous ensuring the availability of pollen for longer durations to both female and hermaphrodite plants. This species exhibit both xenogamy and geitenogamy (mixed) as modes of pollination. However, anthesis of female flowers occurs 3-4 days after anthesis of hermaphrodite flowers in a particular population making the maximum number of flowers (ovules) from female plants available for fertilization. As it is evident from the insect visiting efficiency that pollinators are more attracted towards female flowers and less visitation rates were found in hermaphrodites, as female flowers are brightly colored (pinkish) thus are easily visualized by insects. On stigmas, maximum number of germinated pollen grains was recorded on 2 days (hermaphrodites) and 3 days old stigmas (females), indicating the peak

receptivity of stigmas on that particular day when pollens received are found acuminated with long pollen tubes. Female stigmas are trifid which makes them advantageous over hermaphrodites having unifid stigmas to get every opportunity to receive pollens through any means by increasing their surface area. After 5 to 6 days of flower anthesis the stigmatic surface dry up which marks the end of receptivity. It was also observed that whole anthers are detached from their filaments after providing sufficient pollination services in the form of reward (pollen). (Table 6)

Table 6: Comparison of stigma receptivity at different developmental stages in plants bearing female and hermaphrodite flowers

	Female plant			**Hermaphrodite plant**		
Stigma receptivity	**Pollen load on stigma surface**	**Pollen germinated**	**%age germination**	**Pollen load on stigma surface**	**Pollen germinated**	**%age germination**
2DBA*	-	-	-	0	-	0
1DBA	-	-	-	0	-	0
ODA**	6.83±1.47	0	0	0	-	0
1DAA***	3.66±1.16	3.20±0.44	23.42	11.16±0.40	8.32±0.56	74.55
2DAA	2.83±0.98	9.60±0.54	74.82	15.67±0.51	5.00±1.23	**95.72**
3DAA	13.00±0.83	12.00±0.44	**92.30**	15.00±0.63	10.56±1.02	70.40
4DAA	4.67±1.03	9.60±0.54	65.43	10.50±0.83	2.86±0.78	27.2
5DAA	11.83±0.75	4.60±0.54	38.88	8.83±0.75	1.39±0.21	15.74
6DAA	14.66±1.03	2.20±0.45	15.00	4.33±0.51	0	0.00

***** day before anthesis
** on the day of anthesis
*** day after anthesis

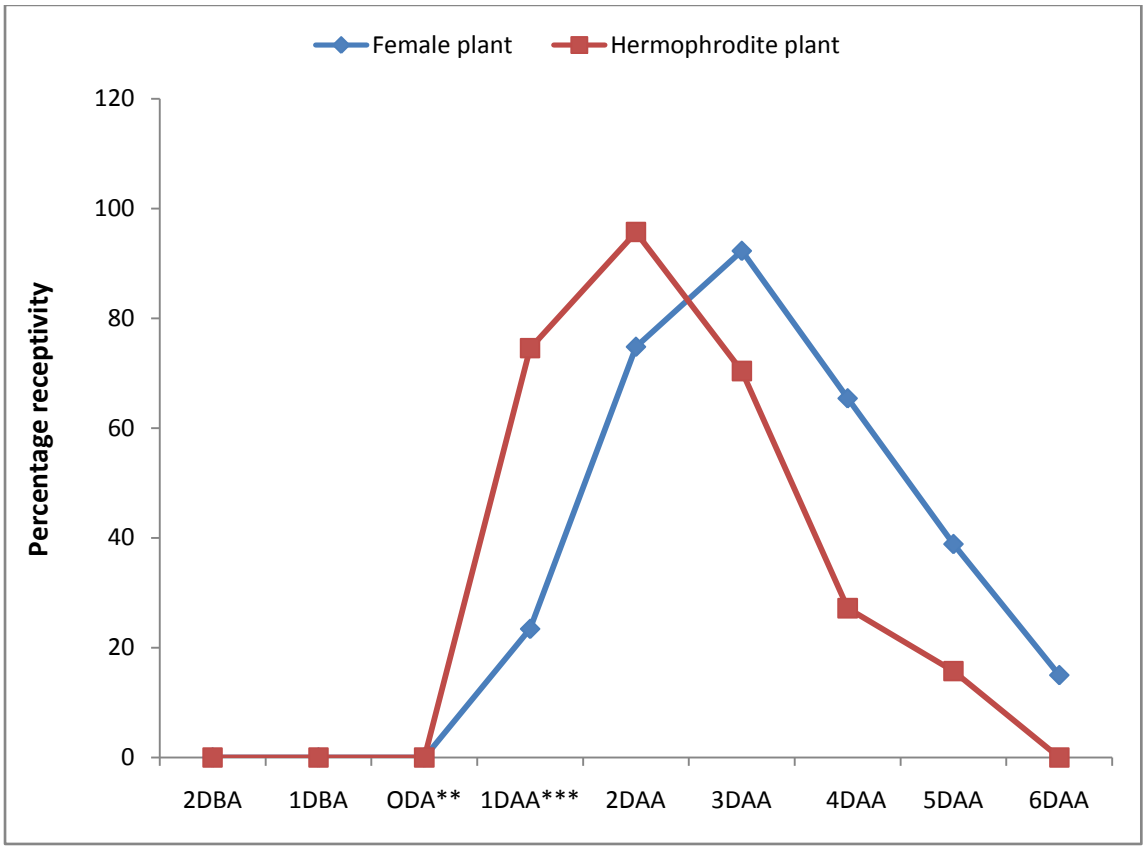
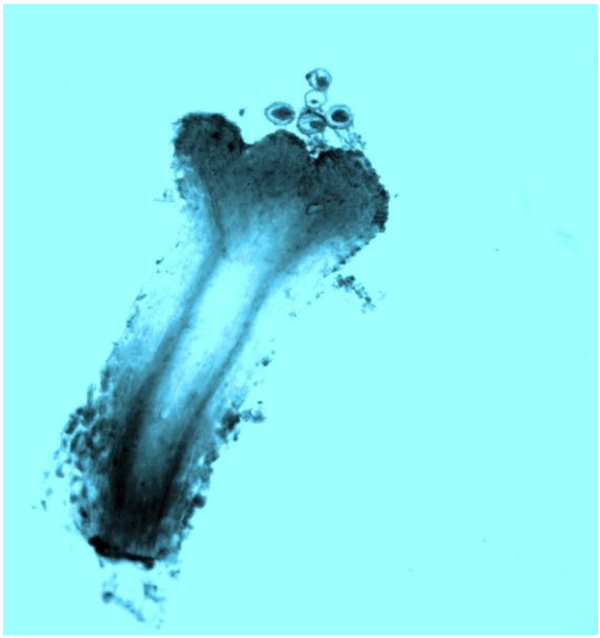
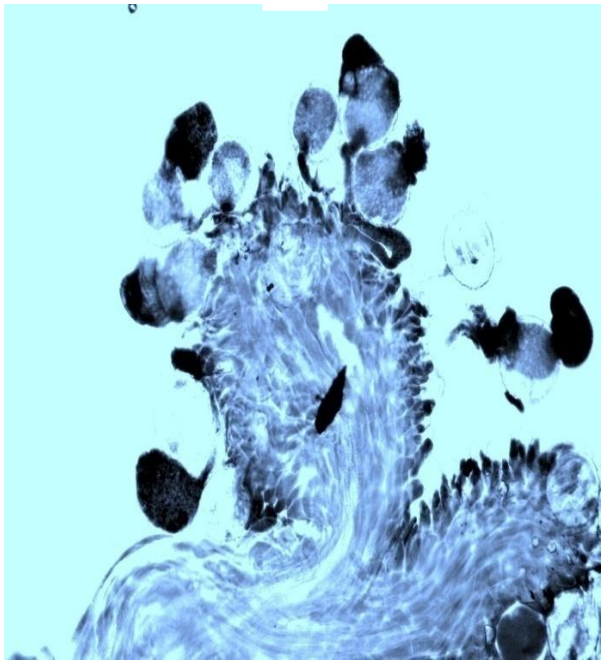


Fig. 6: Depicting comparison of stigma receptivity at different developmental stages in plants bearing female and hermaphrodite flowers



(a)



(b)

Plate 5

a) Pollen deposition on stigma
b) Stigma with germinated pollen grains

4.1.2. Pollen viability

Valeriana jatamansi produces large quantity of healthy, plump and stainable tricolpate pollen grains, showing 90% pollen viability. This serves as an indicator of a good seed set and the strategy to materialize maximum reproductive success. The data analyzed on pollen viability is summarized in Table 7.

4.4.3. Pollen-ovule ratio

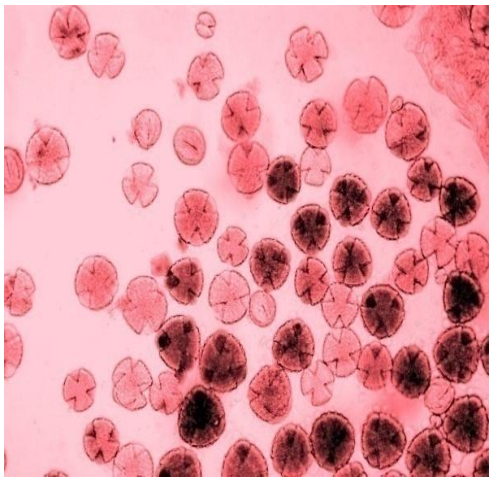
The flower of *Valeriana jatamansi* produces enormous number of pollen grains as compared to single basal ovule per flower. The pollen-ovule ratio has been worked out per flower, per ramet as well as in per genet. The data is summarized in Table 7.

Table 7: Pollen viability and pollen ovule ratio of *Valeriana jatamansi*

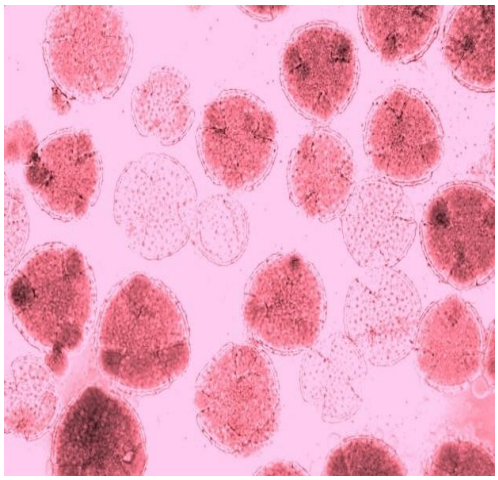
S. No.	Pollen ovule ratio and pollen viability		Mean± S.D.
I	**Pollen Number per**	Flower	2459.33 ±79.67
		Ramet	27404.0 ±887.8
		Genet	230976.5 ±7483.2
II.	**Ovule Number per**	Flower	1.00± 0.00
		Ramet	84.23±12.23
		Genet	670.23 ± 14.34
III	**Viable pollen per flower**		2379.06 ±84.43
IV	**Percentage viability**		95.29 ±.898
V	**P/O ratio**	Flower	2459.33

Ovule number per ramet (84.24±12.23) as well as in per genet (670.23±14.34) varies with respect to number of flowers per ramet and per genet as there is single basal ovule per flower.

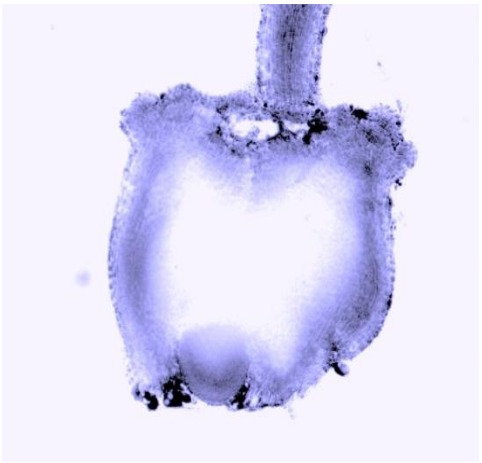
The pollen grains per anther, per flower, per ramet, per genet on an average are 840±25.105, 2459.33±79.678, 27404.00±887.846, 230976.57±7483.280 respectively. This shows that *Valeriana jatamansi* invests maximum amount of energy for the development and production of pollen grains as compared to ovule formation



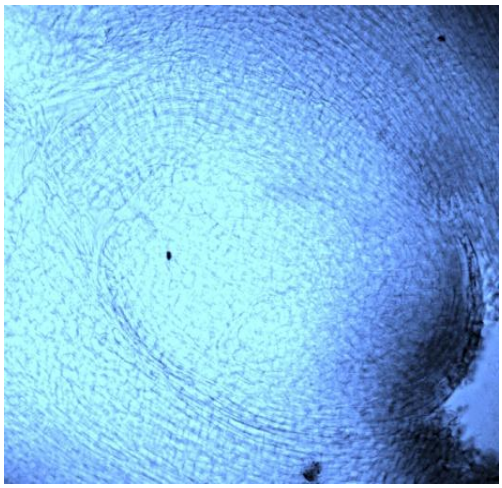
(a)



(b)



(c)



(d)

Plate 6

a) Pollen grains of *Valeriana jatamansi*
b) Pollen viability
c) Ovary with single basipetal ovule
d) Structure of ovule

4.4.4. *In vitro* pollen germination

The ability of a pollen grain to successfully fertilize an ovule depends upon number of performance factors, including pollen grain germinability rate, growth rate of pollen tube through the style and ability of pollen tube to reach and fertilize an ovule. In order to observe the percentage germination, the number of germinated pollen grains and pollen grains placed on each stigma were determined and percentage was calculated .The effect of time duration and proper concentration for successful germination of pollens of *Valeriana jatamansi* was statistically analyzed. It was found that the time duration and concentration are important features for successful in *vitro* pollen germination. (Table 8)

Table 8: *In vitro* pollen germination chart of *Valeriana jatamansi*

S.NO.	Treatment			Pollen count	Pollen germinated	%age germination	ANOVA			
							Source	df	F	P
1	**S:B:C***	**1:1:1**	**6h**	46.71±4.44	11.23±1.43	23.91	Conc.	6	3.26	0.01
2	**S:B:C**	**1:1:2**	**6h**	81.42±6.28	30.45±2.67	**37.03**	Time	1	14.92	0.00
3	**S:B:C**	**1:2:1**	**6h**	72.00±5.88	18.67±0.32	25.39	Error	88		
4	**S:B:C**	**1:2:2**	**6h**	44.71±1.57	14.89±2.43	**31.18**	Total	95		
5	**S:B:C**	**2:1:1**	**6h**	75.28±5.98	14.78±1.56	18.16				
6	**SS:B:C**	**2:2:1**	**6h**	34.67±3.56	6.56±0.30	17.64				
1	**S:B:C**	**1:1:1**	**3h**	44.00±4.43	9.21±0.23	20.45				
2	**S:B:C**	**1:1:2**	**3h**	55.42±3.34	18.51±0.43	32.72				
3	**S:B:C**	**1:2:1**	**3h**	39.57±2.92	8.71±1.05	20.51				
4	**S:B:C**	**1:2:2**	**3h**	40.71±2.05	11.32±1.51	27.05				
5	**S:B:C**	**2:1:1**	**3h**	56.23±5.65	7.25±0.87	12.05				
6	**S:B:C**	**2:2:1**	**3h**	55.14±3.88	7.34±0.43	12.72				

* Sucrose-15%, Boric acid (100mg/l), Calcium nitrate (300mg/l)

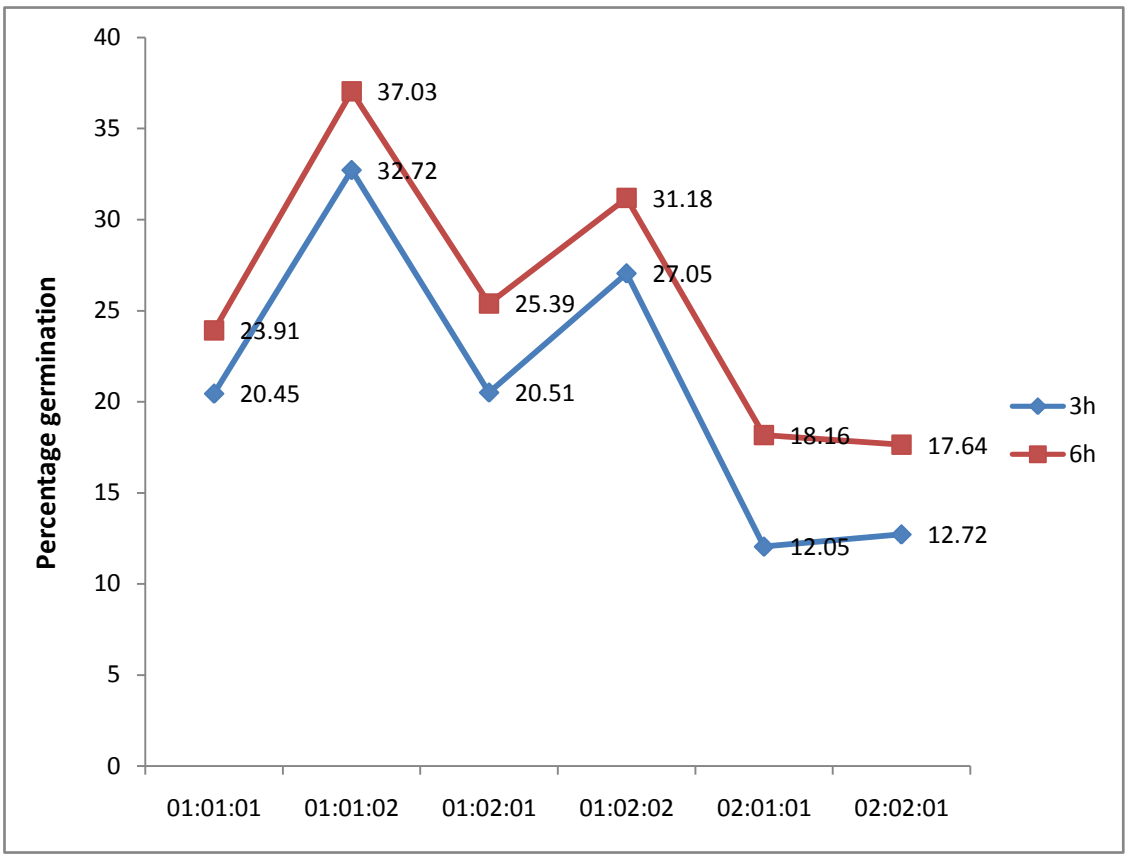
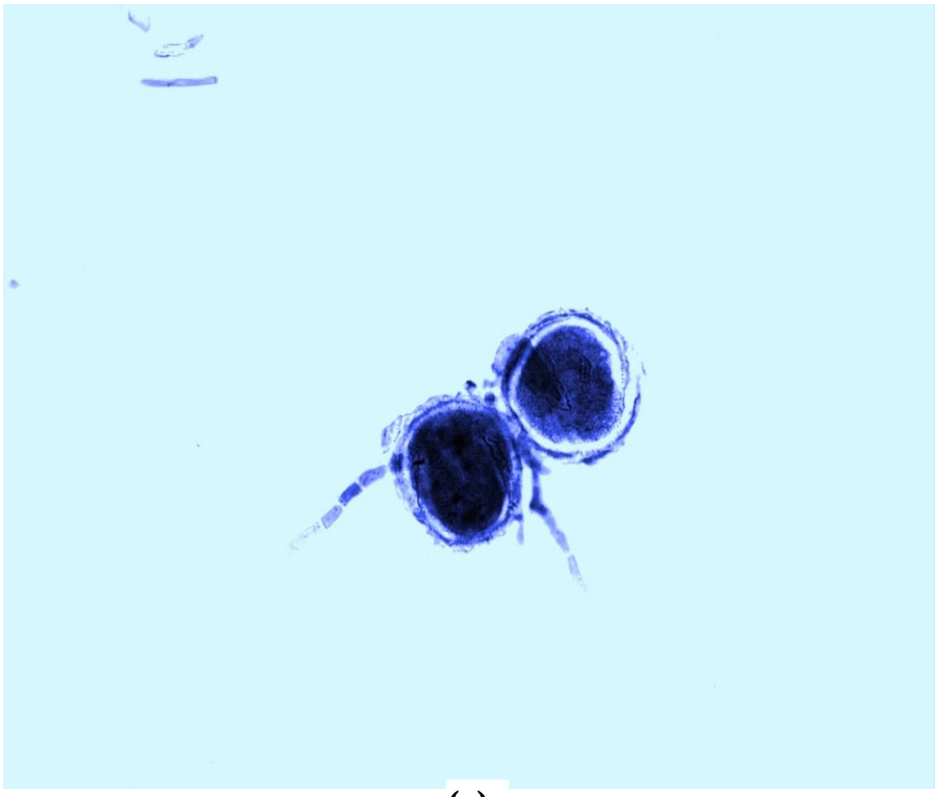


Fig. 7: Depicting *in vitro* pollen germination of *Valeriana jatamansi*



(a)

Plate 7

a) *In vitro* pollen germination

4.4.5. Pollination system

The species produces two types of individuals; female and hermaphrodite (gynodioecious). The enormous quantities of pollen grains by the species in the form of white mass attract the insects to visit the flowers frequently. As mentioned early, anthesis in *Valeriana jatamansi* is asynchronous. The species ensures the long term availability of flowers and pollen for visitors thus attracting the insects again and again to pollinate number of flowers. It was observed that pollinators visit more frequently to female flowers as compared to hermaphrodite flowers as they have brightly colored inflorescences. The foraging starts early in the morning on a normal sunny day and the process of visitation are at its peak during mid-day hours 11:00-14:00 hr and gradually decreases in the late hours. The insect visitation slows down or ops altogether on rainy days. The visitors (insects) displaying maximum pollen load were expected as the major pollinators which were identified in Department of Zoology, Kashmir University. However, in natural populations, the major pollinators belong to the genus *Syrphus*, even though these plants are visited by several other insects of the genus *Apis* etc. The visitation rate or visitors of *Valeriana jatamansi* are summarized in Table 9.

Table 9: Insect visiting efficiency of *Valeriana jatamansi*

Order	Family	Genus	Duration of stay on single female flower (seconds)	Duration of stay on single hermaphrodite flower (seconds)	Duration of one visit per population (seconds)	Visiting efficiency
Hymnoptera	**Sypridae**	**Syrphus spp***	5.65±2.52	5.45±2.34	47.56±14.32	0.09±0.01
Diptera	**Apidae**	**Apis spp***	4.32±2.04	4.12±1.89	37.45±23.65	0.07±0.01

The data reveal that the *Syrphus* sp. stay for maximum duration per flower as well as per bout followed by *Apis* sp. The visiting efficiency of *Syrphus* sp. is higher than *Apis* species. On the basis of visiting efficiency of 0.09±0.01 the *Syrphus* sp.seems to be major pollinator, and the *Apis* sp. an infrequent visitor (0.07±0.01). In Natural populations, where due to intense cold and weather inclemency (as in Gulmarg population), continuous visitation of pollinators on a particular day was not observed. In addition, wind has been found to be another option as pollinating agent in this species especially in cold areas indicating both anemophily/entemophily in this species.

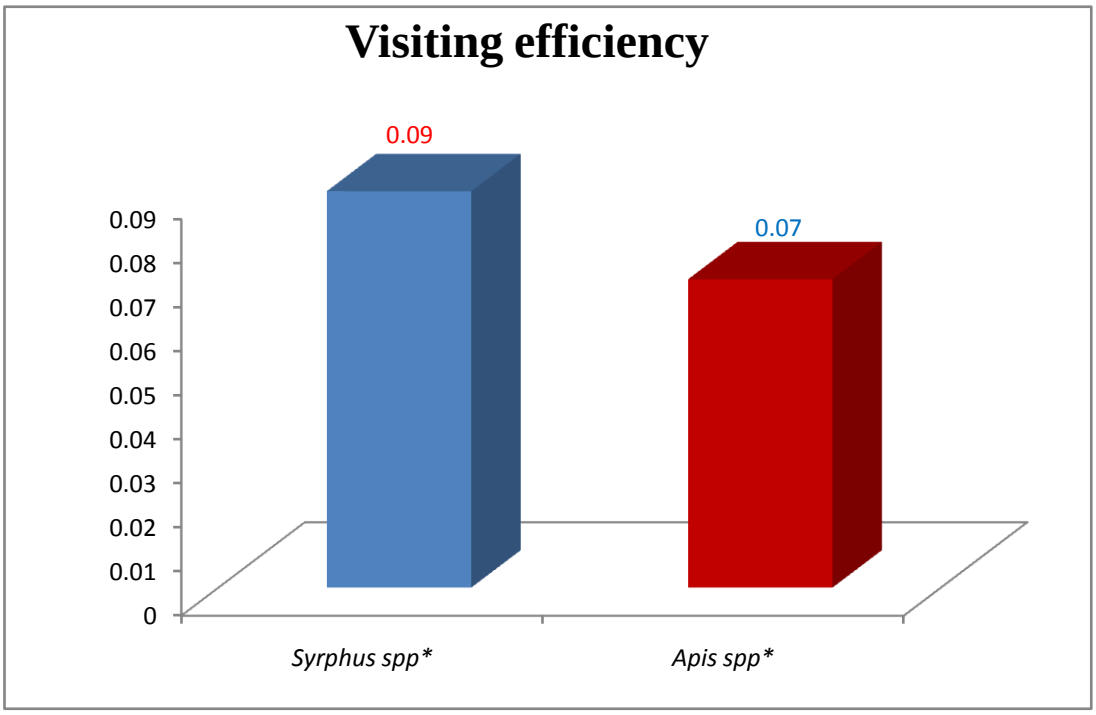
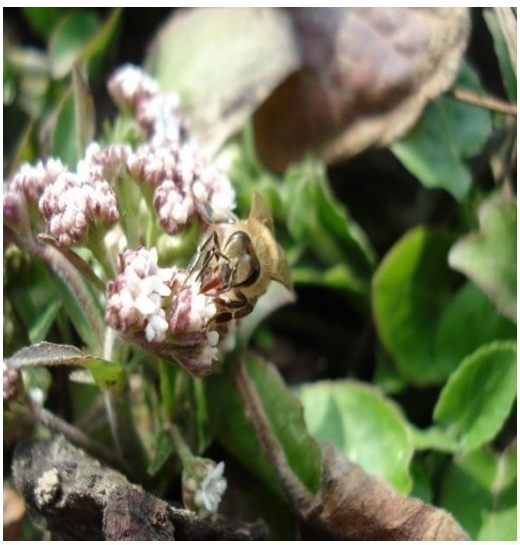


Fig. 8: Showing comparative insect visiting efficiency in *Valeriana jatamansi*

(a)

(b)

(c)



(d)

Plate 8

a) Pollinators of *Valeriana jatamansi*
b) *Syrphus sp.* visiting flowers
c,d) *Apis sp*. visiting flowers

(a)

(b)

(c)

(d)

Plate 9

a) Female inflorescence color in open sun
b) Female inflorescence color under shade
c) Hermaphrodite inflorescence color in open sun
d) Hermaphrodite inflorescence color under shade

4.4.6. Nature of the breeding system

In order to unravel the nature of breeding system operative in the species, the following bagging experiments were carried out during year 2009-2010 and the results are summarized in Table 10.

Exp. 1. Unemasculated flower were tagged and allowed to open pollinate.

Exp.2. Unemasculated flowers were bagged with butter paper to avoid foreign pollen and allow selfing.

Exp.3. Stigmas of some flowers were removed at regular intervals in case of female flowers (because of asynchronous anthesis) by a fine forcep and the flowers bagged to check whether the seed development occurs without pollination.

Table 10: Seed set per plant under various bagging experiments in *Valeriana jatamansi*

S.No.	Experiment (Pollination method)	Seeds/genet in Hermaphrodite plant	%age seed set in Hermaphrodite plant	Seeds/genet in Female plant	%age seed set in female plant
1.	**Open pollination**	365±13.32(500)*	73%	380±10.43(500)*	76%
2.	**Forced self pollination**	08±3.23(50)*	16%	Nil	0%
3.	**Apomictic seed development**	Nil	-	Nil	-

* Number of flowers used for each experiment.

The results obtained showed that the unemasculated hermaphrodites and female flowers allowed to cross pollinate (Exp.1) produce maximum number of seeds i.e.; 380±10.43 per genet in case of females and 365±13.32 per genet in case of hermaphrodites. While female plants forced to self pollinate (Exp.2) did

not set any seed. However, in order to check for apomictic seed development in case of females, their stigmas were removed at regular intervals and were covered by butter paper to block the foreign pollen reception from any source (Exp.3). It was observed that 73% and 76% seed set was obtained in hermaphrodite and female flowered plants under open pollination conditions respectively. However, hermaphrodite plants set more seeds under open pollination condition as compared to forced self pollination condition (16% seed set). No seed set was observed in case of female plants under forced self pollination conditions. Thus it is evident from the data that seed formation in female plants is result of obligate out crossing. While hermaphrodites can form seeds under both conditions i.e.; selfing (alternate way) and outcrossing (usual way).

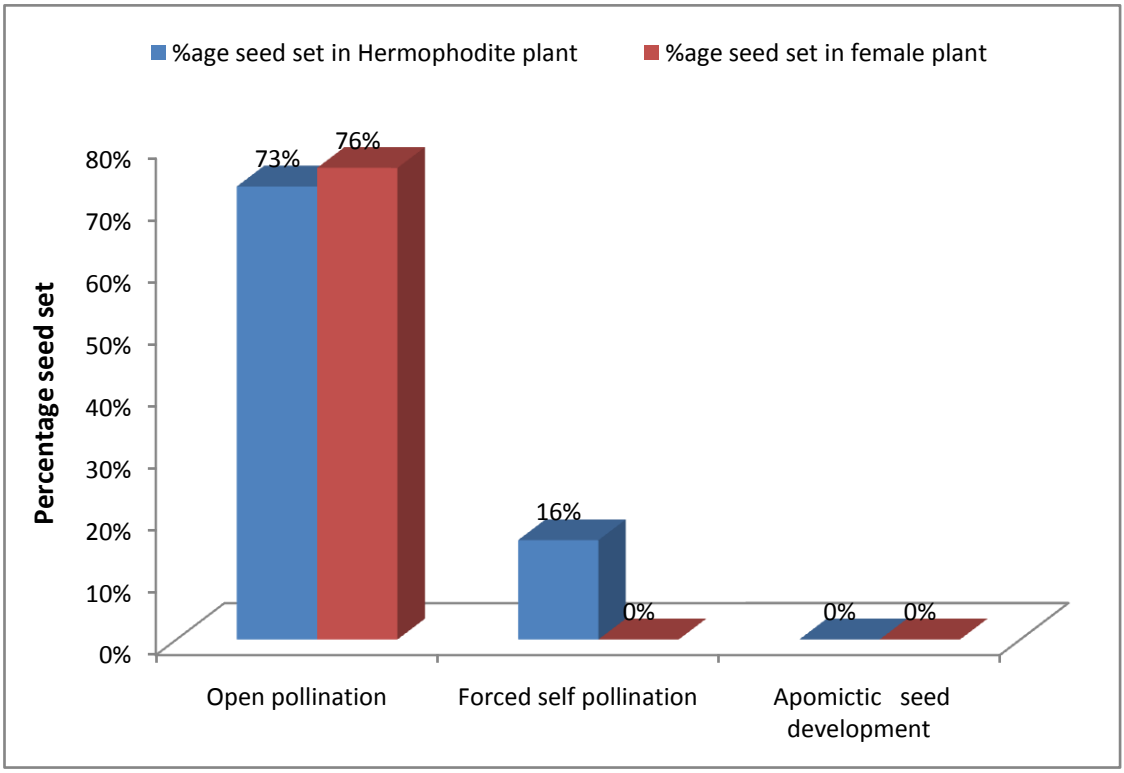


Fig. 9: Showing percentage seed set per plant under various bagging experiments in *Valeriana jatamansi*

(a)

(b)

(c)

(d)

Plate 10

a) Bagging in female plant
b) Bagging in hermaphrodite plant
c,d) Bagging experiment under *ex situ* condition

4.4.7. Modes of propagation

Reproduction in *Valeriana jatamansi* occurs by both vegetative and sexual means. The vegetative reproduction occurs through the underground rhizome, while the sexual reproduction takes place through seeds. This feature not only helps the plant to establish at a location but also ensures perpetuation even in absence of the seed set, which is especially critical to female flowered plants growing in isolation from hermaphrodite plants, which do not set seeds independently.

(a)Vegetative reproduction

By the end of each growing season the underground rhizome produce the vegetative buds which remain dormant during the winter. In the next growing season these buds develop- into leafy shoots. The simple rhizome produces 7-9 vegetative buds, which mostly form the leafy shoots. The studies reveal that several shoot apices from the nodes of rhizome form rosette of leaves with the inflorescence emerging either directly or from the centre of the rosette.

In vivo propagation studies reveal that rhizome cuttings of this plant (4-8 per plant) respond well to different treatments with IAA (1mM) and IBA (1mM). These chemicals were found to be most effective in increasing %age rooting and %age shooting with successful regeneration of each cutting into a new plant (Table 14).

Table 14: *In vivo* propagation of *Valeriana jatamansi*

S. No.	Treatment (mM)		Leaf shoot emergence after shifting to trays*	Percentage survival
1	**IAA**	**0.25**	4	33.33
2	**IAA**	**0.50**	9	75.00
3	**IAA**	**1.00**	10	83.33
5	**IBA**	**0.25**	3	25.00
6	**IBA**	**0.50**	9	75.00
7	**IBA**	**1.00**	10	83.33
9	**GA_3**	**0.25**	3	25.00
10	**GA_3**	**0.50**	6	50.00
11	**GA3**	**1.00**	8	66.66
12	**Control**	**-**	3	25.00

***Total number of cutting used for each replicate -12

**Total time duration to observe the propagation rate -48 hours

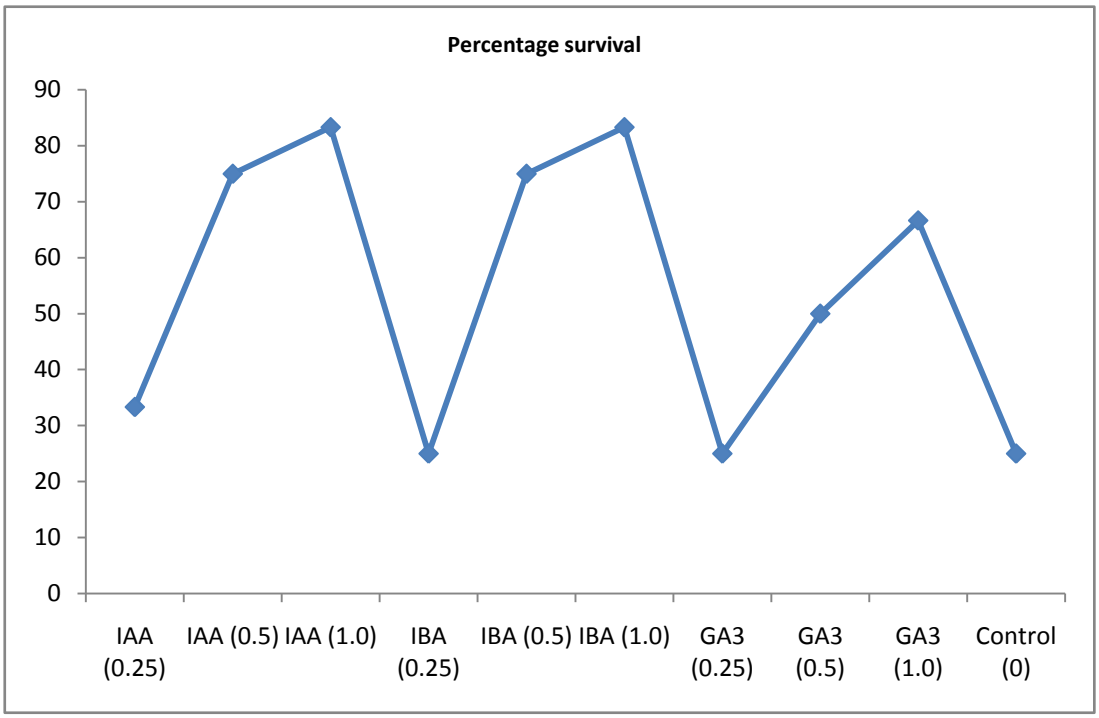


Fig. 10: Showing effect of different treatments on percentage survival of *Valeriana jatamansi*

(a)

(b)

(c)

(d)

(e)

(f)

Plate 11

a, b) Treated rhizome cuttings
c,d) Leafy shoot emergence from treated cuttings
e,f) Successful establishment and regeneration of plants from individual cuttings

(b) Sexual reproduction

After long distance dispersal by parachute mechanism, the seeds of *Valeriana jatamansi* germinate when subjected to the availability of suitable micro site. But they have to face onslaught of winter which renders them to over-winter by remaining dormant until suitable conditions prevail. The formation of seeds is important as they are the outcome of recombination (sexual reproduction), which guarantees the genetic variability and which in turn is especially critical to female plants growing in isolation from hermaphrodite plants.

4.5. SEED BIOLOGY

4.5.1. Seed size

Seed size was determined in both hermaphrodite and female plants to observe quality seed lot from the two individuals. The seed size was calculated by weighing 100 seeds from each lot (female and hermaphrodite). It was observed that seed size vary to a significant extent with female seeds weighing more than hermaphrodite seeds as shown in Table 11.

Table 11: Average seed size of female and hermaphrodite individuals (using t- test)

Plant type	Weight (mg)
Female	32.50±3.87*
Hermaphrodite	27.50±2.08*

*-Mean ± S.D.

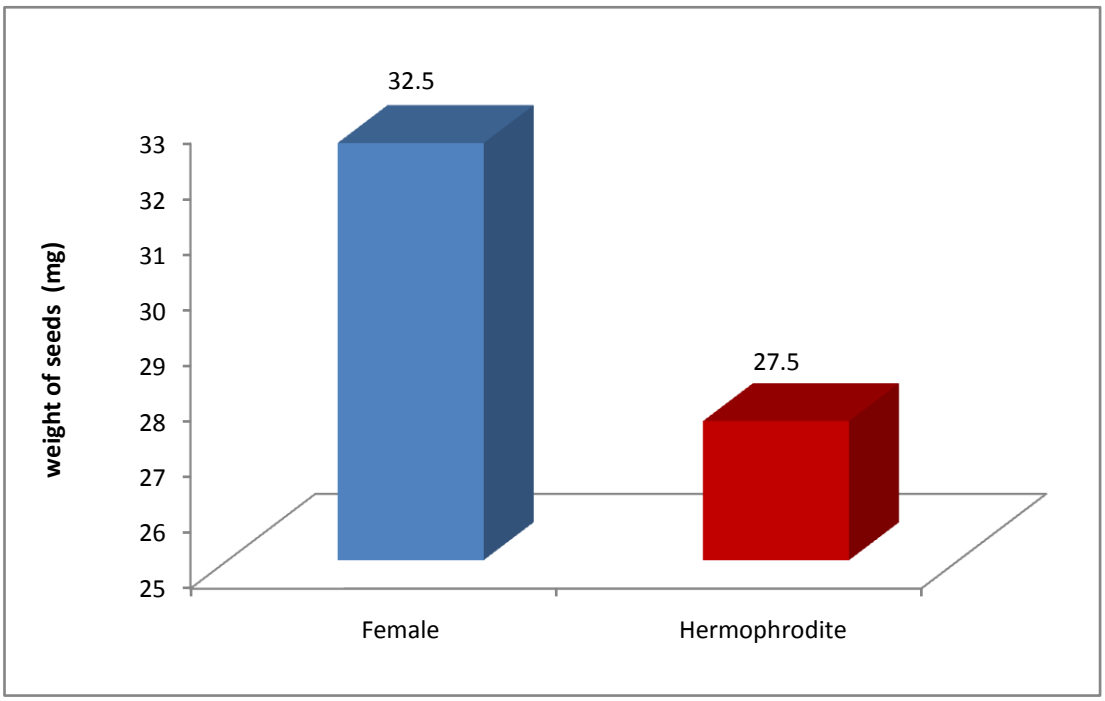


Fig. 11: Depicting average seed size of female and hermaphrodite individuals

4.5.2. Seed viability

Seed viability was checked in both hermaphrodite and female individuals and it was observed that female seeds showed more viability (89.5%) than hermaphrodite ones (84.25%)

Table 12: Percentage seed viability in *Valeriana jatamansi*

S.NO.	Seed type	No.of seeds incubated	Viable seeds	%age viability
1.	**Female**	20.00±0.00*	17.33±0.57	89.50
2.	**Hermaphrodite**	20.00±0.00	15.33±1.52	84.25

*Mean±S.D.

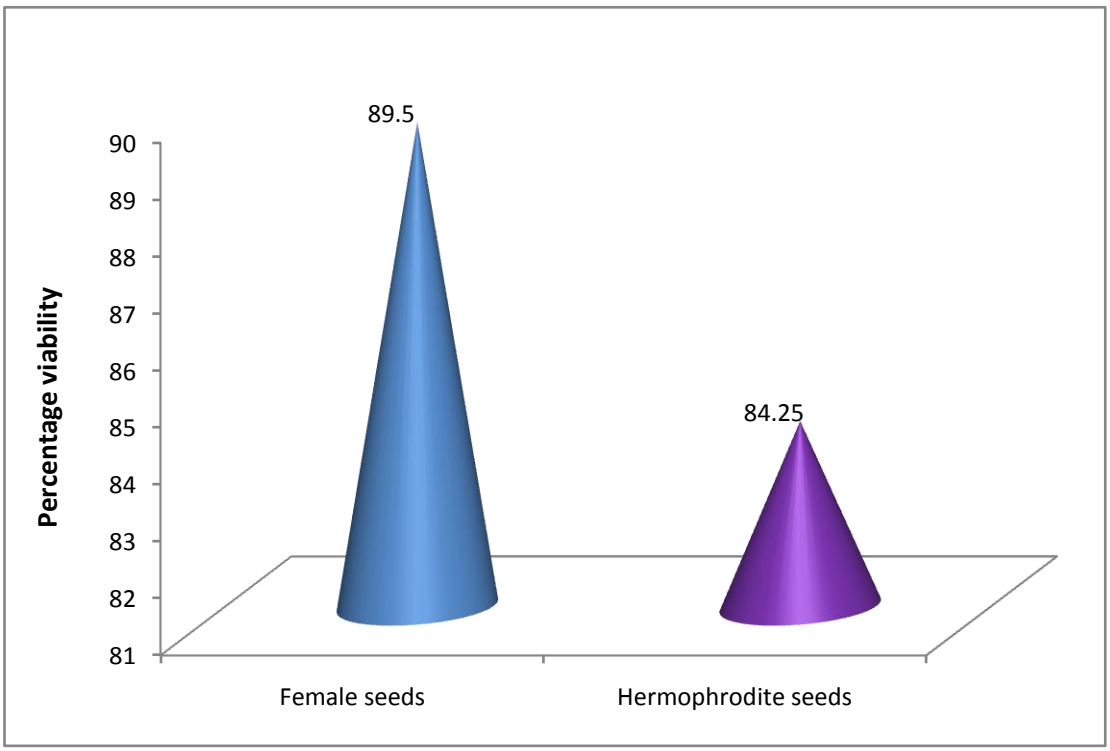


Fig. 12: Percentage seed viability in *Valeriana jatamansi*

4.5.3. In *vitro* seed germination

Germination of seeds is the most common method of multiplication of flowering plants and is a well programmed process controlled by both internal and external factors. Mayer and Poljakoff (1982) defined it as, "group of processes which cause a sudden transformation of dry seeds into young seedlings." The physiological and chemical treatments are well known to influence the phenomena of seed germination, seedling growth and nitrogen metabolism in plants.

The seeds of *Valeriana jatamansi* usually over-winter in resting phase, exhibiting physiological dormancy. However, few seeds as and when get dispersed by parachute mechanism at relatively immature stage do germinate immediately subjected to the availability of micro site. The seedlings of such seeds hardly survive in nature because they have to face the onslaught of chill in winter. With the onset of favorable conditions (March-April) the least

percentage of dormant seeds starts to germinate accounting for meager addition to the population in terms of new recruitments. Hence, in order to boost the seed germination various physiological, chemical and hormonal treatments were given to combat the bottlnecks if any present in seeds.

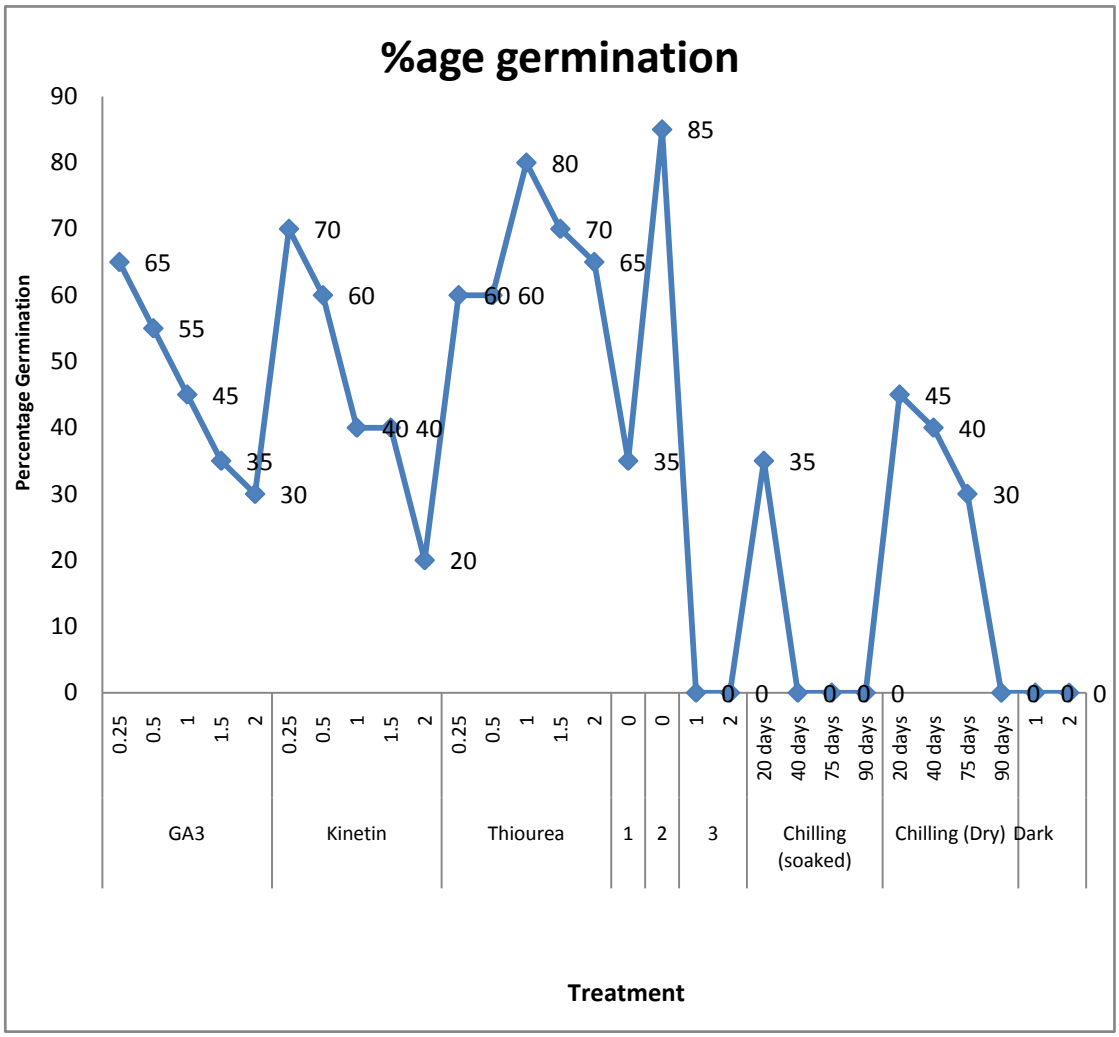
It was observed that the seeds of *Valeriana jatamansi* responded best to the nitrogenous compounds apart from scarification (85% with MGT 4.25 days), showing 80% seed germination in 1mM of thiourea with mean germination time of 3.75 days followed by 70% germination in 0.25mM of Kinetin with MGT of 6.30 days, as against the control (35%). The prolonged chilling has been observed to be ineffective with respect to germination of such seeds. The results of seed germination are summarized in Table 13.

Table 13: Effect of different physical and chemical treatments on seed germination of *Valeriana jatamansi*

S. No	Treatment (mM)		Seeds germinated	Days taken for first seed to germinate	Total days taken for completion	%age germination	% survival	Mean germination time (Days)
1	GA_3	**0.25**	13.50±1.29*	6	13	**65**	66	5.11
		0.5	11.50±0.88	7	16	55	60	7.45
		1.0	9.45±0.50	7	16	45	45	4.76
		1.5	7.50±0.57	7	12	35	50	6.92
		2.0	6.50±0.58	8	15	30	60	9
2	**Kinetin**	**0.25**	14.25±1.32	8	12	**70**	72.7	6.3
		0.5	12.50±0.58	9	14	**60**	66.6	7.8
		1.0	8.50±0.67	12	14	40	50	5.3
		1.5	8.75±0.80	7	12	40	65	6.28
		2.0	4.75±0.50	6	14	20	65	8.8
3	**Thiourea**	0.25	12.25±0.50	5	9	60	75	4
		0.5	12.75±1.30	5	8	60	66.6	5
		1.0	16.75±1.36	5	9	**80**	75	**3.75**
		1.5	14.25±0.90	5	9	**70**	70	5.14
		2.0	13.50±0.58	5	9	65	54.5	5.38
4	**Control**	-	7.75±0.80	6	18	**35**	52.5	6.15
5	**Scarification**	-	17.25±1.54	5	9	**85**	60	4.25
6	**Conc.H2S04 dip(for 10 seconds)**	1	0	0	0	0	0	0
		2	0	0	0	0	0	0
7	**Chilling(soaked)**	20 days	7.50±0.58	26	29	35	38	8.00
		40 days	0	0	0	0	0	0
		75 days	0	0	0	0	0	0
		90 days	0	0	0	0	0	0
8	**Chilling (Dry)**	20 days	9.25±0.52	6	19	45	55	9.14
		40 days	8.75±0.76	9	8	40	40	9.6
		75 days	6.75±0.58	11	6	30	33.3	9.33
		90 days	0	0	0	0	0	0
9	**Dark (1)**		0	0	0	0	0	0
	(2)		0	0	0	0	0	0

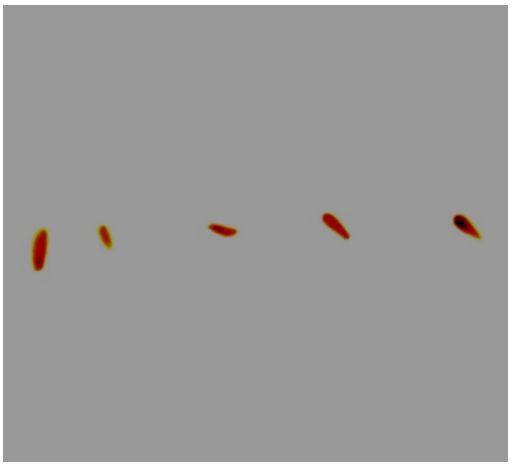
*Seed sample for each replica-20, Temperature range 15-20^{0}c,

Source	ANOVA	
	F	P
Number of seeds germinated in light	3.476	.017
%age germination	3.476	.017

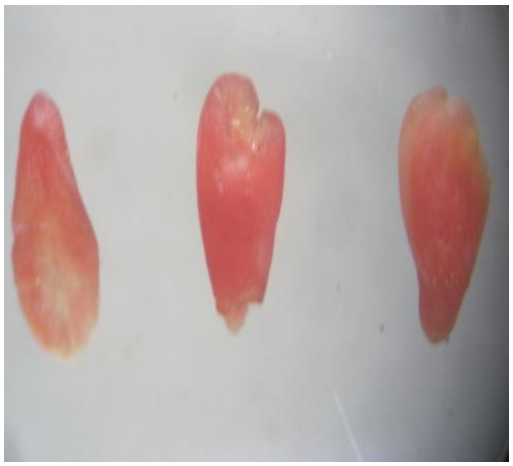


Note: 1-Control, 2-Scarification 3-Conc. H2SO4

Fig. 13: Depicting effect of different physical and chemical treatments on seed germination of *Valeriana jatamansi*



(a)



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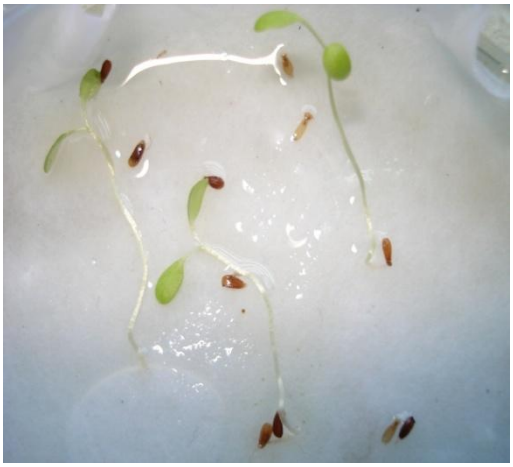
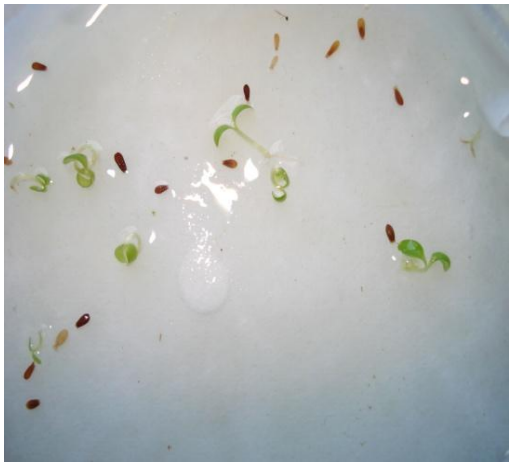


(c)



(d)

Plate 12

a) Seeds of *Valeriana jatamansi*
b, Seed viability
c,d) Germination of Seeds

4.5.4. *In vivo* seed germination

The *in vivo* seed germination was worked out in KUBG to see how seeds of *Valeriana jatamansi* behave to different habitat conditions and to successfully investigate the effect of open conditions and dappled or partial shade on germination of seeds in natural soils. It was observed that under dappled shade the %age seed germination was remarkably on the higher side (84.7%) as against the open sun conditions wherein only 48.73% germination was registered. The data deciphers that availability of moisture is important for seed germination .The details of *in vivo* seed germination are listed in Table 14.

Table 14: *In vivo* seed germination chart

S. No.	Condition of pots	Number of seeds sown	Days taken for first seed to germinate	Number of days taken by all the seeds to germinate	Total number of seeds germinated	%age germination
1	Open sun	30.00±0.00	9	19	12.50±2.12	48.73
2	Complete shade	30.00±0.00	4	12	24.00±1.41	84.7

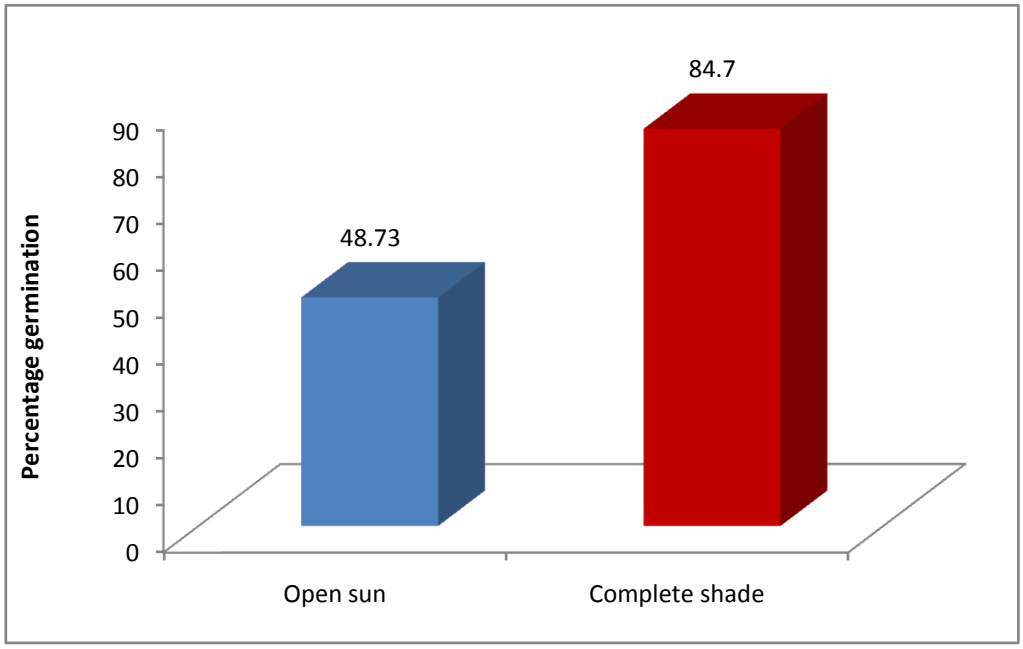


Fig. 14: Depicting comparative seed germination under two different condition

(a)

(b)

(c)

(d)

(e)

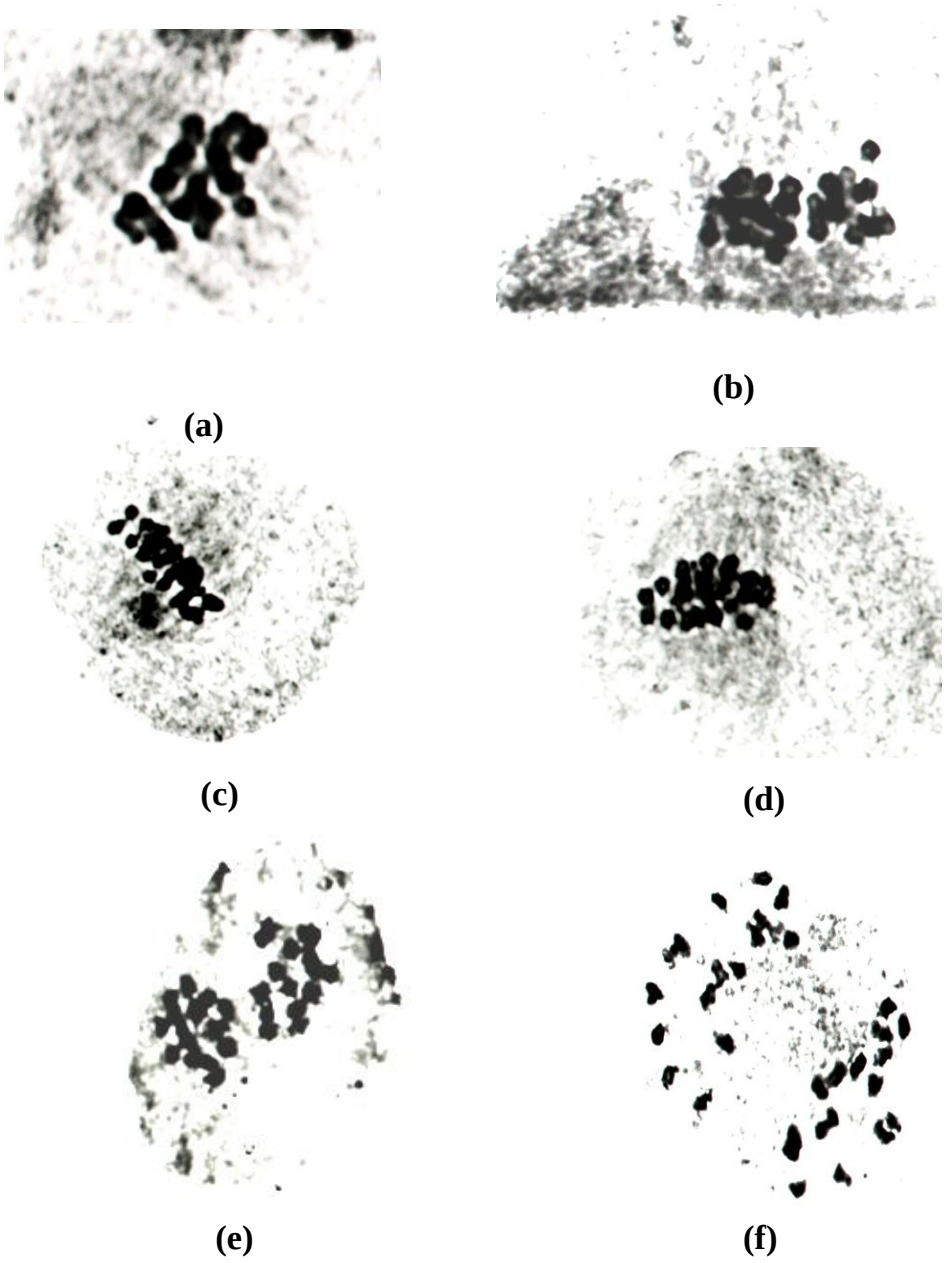
(f)

Plate 13

a,b) Seed germination under shade
c,d) Seed germination under open sun
e,f) Establishment of seedlings

4.6. POLLEN MOTHER CELL MEIOSIS

Pollen mother cell meiosis in *Valeriana jatamansi* was found regular with small chromosomes which are suitably countable at Metaphase-I and Anaphase-I. Segregation of chromosomes was normal with16 chromosomes clearly seen at two opposite poles of pollen mother cell at anaphase. Regular 16 bivalents at metaphase-I were observed in all populations of *Valeriana jatamansi* studied indicating the chromosome number to be 2n=32. Meiosis was completely normal and no abnormalities like laggards, bridges, micronuclei etc. were observed. This is also supported by the fact that seed germination and pollen viability were quite normal in the present studies.



(a) **(b)** **(c)** **(d)** **(e)** **(f)**

Plate 14:

a,b,c,d) Metaphase with 16 bivalents

d,e,f,).Anaphase showing normal segregation

4.7. RESOURCE ALLOCATION AND RESPONSE OF PLANTS

Present investigation reveal that more resources have being allocated to below ground parts like rhizomes whose dry weight in natural population was 1.17±0.80g (hermaphrodite plants), 1.22±0.76g (female plants) followed by roots , 0.37±0.26g (hermaphrodite), 0.14±0.02g (females) .Thus the total below ground dry biomass allocation in natural population is 3.54±1.05g (hermaphrodite plants) and 3.36±0.78g (female plants). The total above ground dry biomass allocation of hermaphrodite plant in natural population is 2.53±0.94g which is statistically at par with female above ground dry biomass of 2.50±0.71g. In the aerial shoots the maximum resources were utilized in the development of leaves with dry biomass of 0.27±0.22g followed by biomass of stem of 0.17±0.11g (hermaphrodite). Stem dry biomass of 0.19±0.11g, leaves dry biomass of 0.15±0.13g was however reported in case of female plants. This species also utilized a good resource towards reproductive part by consolidating an inflorescence dry biomass of 0.14±0.05g in case of hermaphrodite and which is more towards females by allocating more resources whose dry biomass value comes as 0.16±0.05g.

The plants of *Valeriana jatamansi* were given different nutrient treatments along with different conditions in order to observe the response of plants viz- a-viz. biomass allocation to these treatments and conditions .The data revealed that plants which thrive in dappled shade showed better growth followed by partial shade. However plant growth got affected under open sunny conditions which confirms its adaptability towards shade. The different fertilizers were given to all the three conditions (shade, partial shade, open sun) to observe the response of plant to fertilizer treatments. The data reveals that the maximum dry biomass in the species was seen in plants growing under dappled shade supplemented with NPK (combined effect) wherein total biomass allocation was recorded to be 5.41±0.47g followed by plants growing under shade supplemented with DAP (Diammonium phosphate) with 3.72±0.81g. However, least response of this plant is shown by plants under open sun supplemented by urea (2.06±0.06) g.

The data reveals that plants are best suited for dappled shade and partial shade, showing profuse growth under combined effect of fertilizers.

Table 15: Resource allocation of *Valeriana jatamansi*

Hermaphrodite plant	**Mean ± S.D.**
Roots dw	0.37 ± 0.26
Rhizome dw	1.17 ±0 .80
Leaves Dw	0.27 ± 0.22
Stem dw	0.17 ±0.11
Infloresence dw	0.14± 0.05
Female Plant	
Roots dw	0.14 ± 0.02
Rhizome dw	1.22 ± 0.76
Leaves Dw	0.15± 0.13
Stem dw	0.19 ± 0.11
Infloresence dw	0.16 ± 0.05
Supplemented ones	
Osp*1	2.14 ±0.16
osd1	2.16 ±0.11
osu1	2.06 ±0.06
psp1	3.35 ±0.37
psd1	3.17 ±0.11
psu1	3.35 ±0.38
dsp1	3.41 ±0.43
dsd1	3.72 ±0.81
dsu1	3.60 ± 0.46
Npk	5.41 ±0.47

*-os-open sun, ps-partial shade, ds-Dappled shade,P1-phosphorous (as DAP), potassium (as potassium

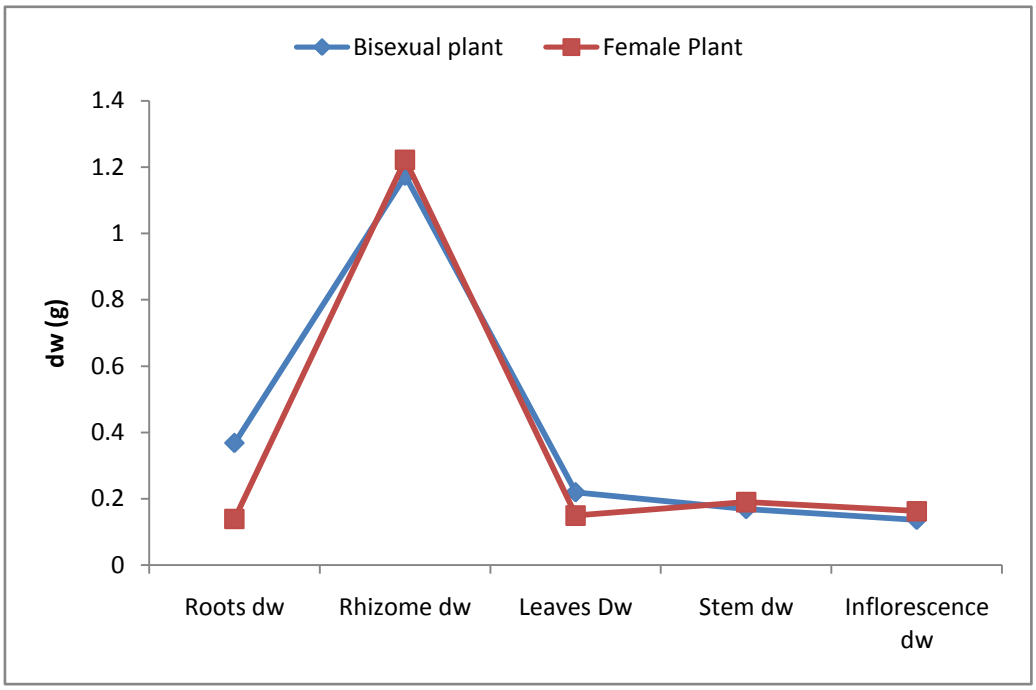


Fig. 15: Showing comparative resource allocation between two individuals of *Valeriana jatamansi*

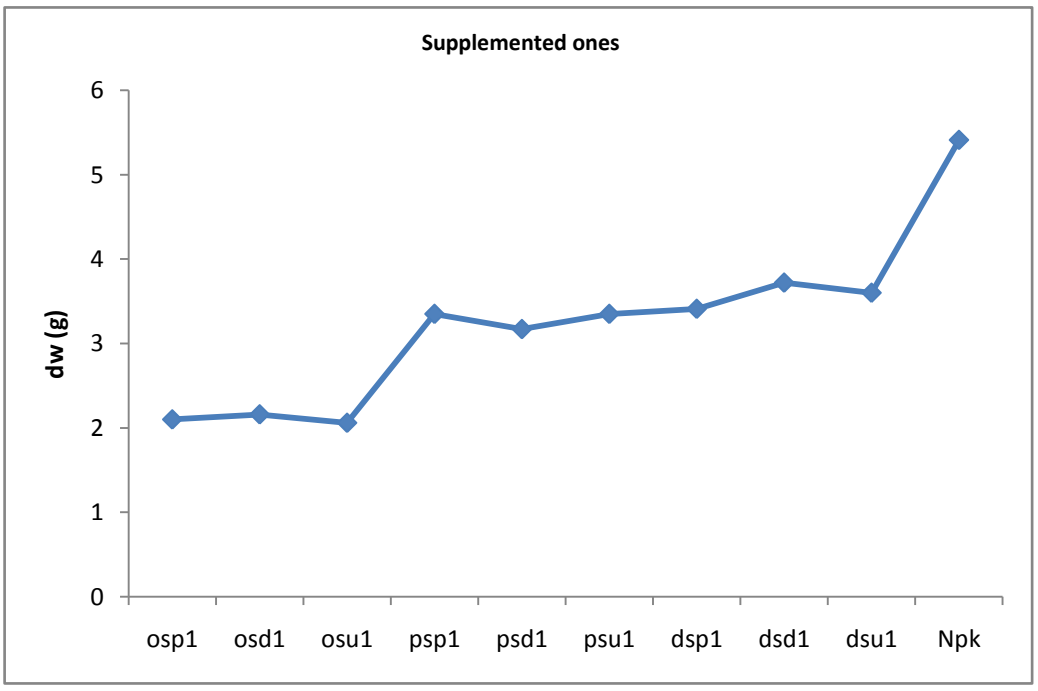


Fig. 16: Depicting effect of fertilizer treatment on plant growth

Table 16: Response of plants to different conditions

Type	Under open sun		Under shade		Combined effect(NPK)	
	Aerial dry weight(g)	Rootstock dry weight(g)	Aerial dry weight(g)	Rootstock dry weight(g)	Aerial dry weight(g)	Rootstock dry weight(g)
Female	11.6 (4.5-18.7)*	6.9 (3.2-10.6)	14.8 (7.2-22.4)*	13.65 (5.7-21.6)	16.85 (8.3-25.4)*	14.35 (6.2-22.5)
Hermaphrodite	12.8 (5.2-20.5)	7.2 (3.4-11)	15.6 (7.4-23.8)	14.00 (5.8-22.2)	17.2 (8.5-25.9)	14.65 (6.2-23.1)

*values in parenthesis are ranges

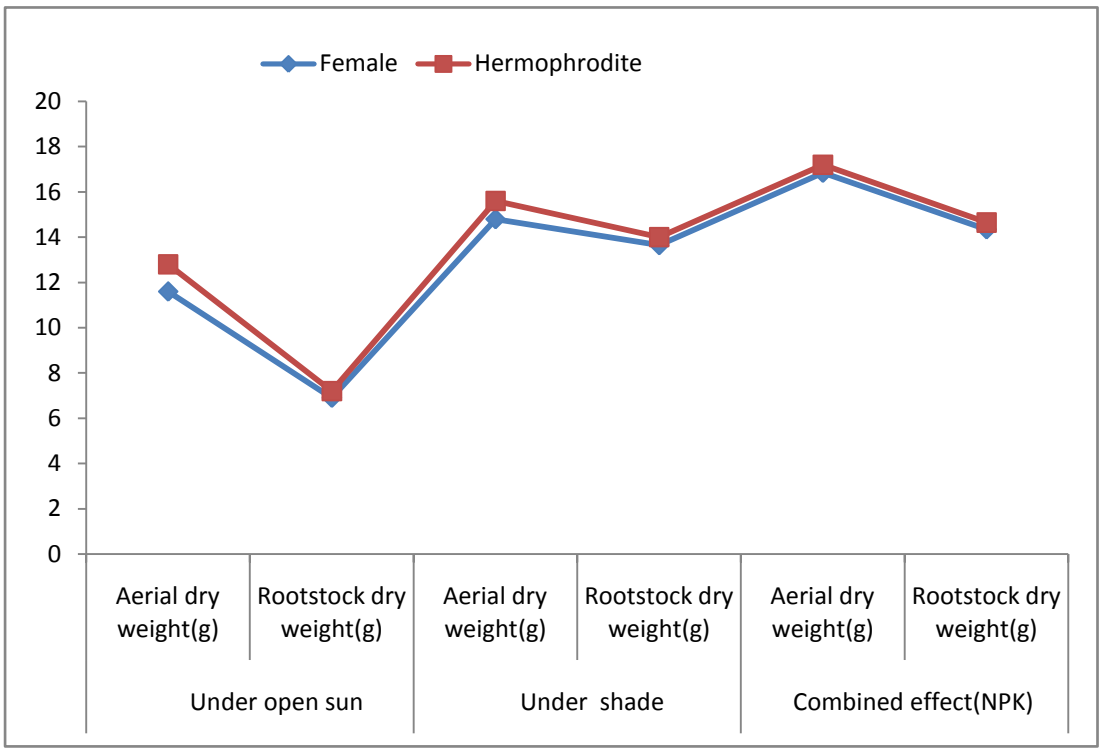


Fig. 17: Showing response of plants to different conditions as against combined fertilizer treatment

(a)

(b)

(c)

(d)

Plate 15:

a) Plant growth response under dappled shade
b) Plant growth response under partial shade
c) Plant growth response under open sun
d) Plant growth response to NPK

Maximum dry weight per plant were recorded in hermaphrodite plants which were statistically at par from dry weight of females. Similarly, dry weight of below ground parts are also at par with each other. However, plants under shade showed profuse growth with more dry weight than plants under open sun where values vary to a significant extent. The data (Table16) revealed that these plants thrive best in shady conditions and responds well to fertilizers when given in proper combination.

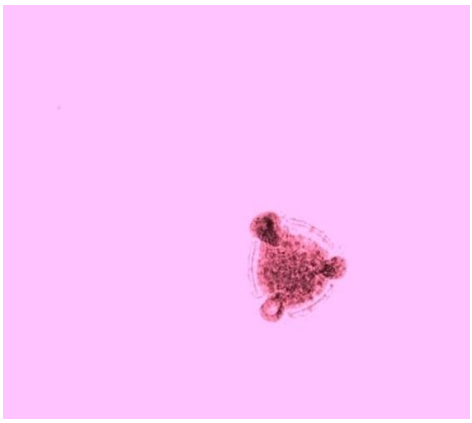
4.8. SEM STUDIES

4.8.1. Pollen shape and size

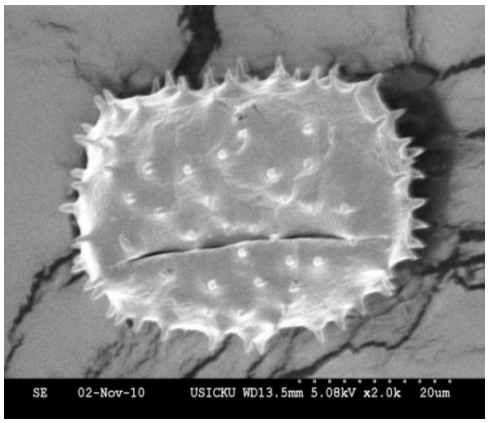
Pollen grains of *Valeriana jatamansi* are oblate-spheroidal, tri-colpate bearing echines with cushions (ornamentation) .The apertures are long and rounded with acute ends having length, 39.05±0.52µm and breadth 41.55±0.59µm. These echinate type of pollen grains are contrivance for reproductive assurance of *Valeriana jatamansi* as well its survival (especially critical to isolated female plants) by providing advantage to these isolated female plants to accommodate the dispersed pollen through every possible way to ensure successful fertilization.

4.8.2. Seed shape and size

Seeds of *Valeriana jatamansi* are 3-4mm in length characterized by papery seed coat with plumose pappus attached with spine like structures which helps in its long dispersal and retention of moisture needed at the time of germination.



(a)



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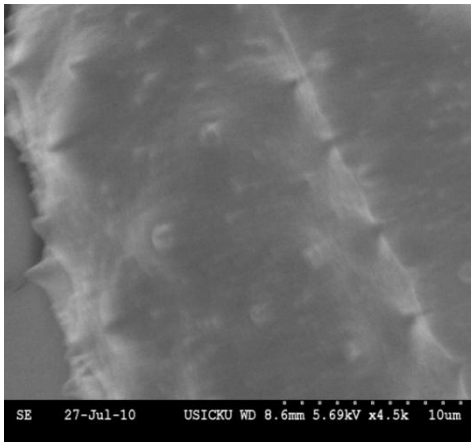
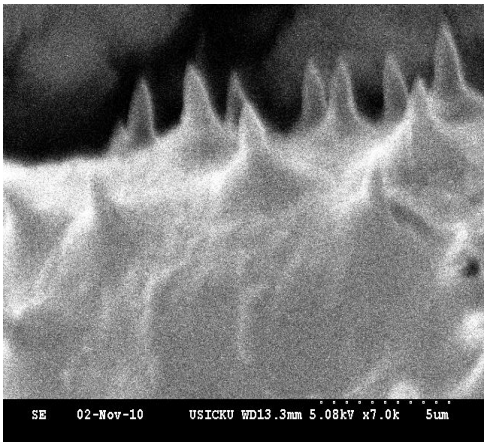


(c)

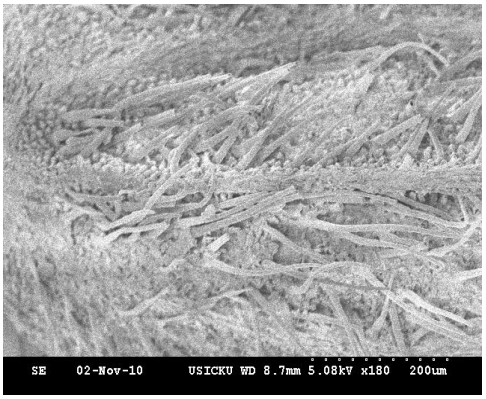


(d)

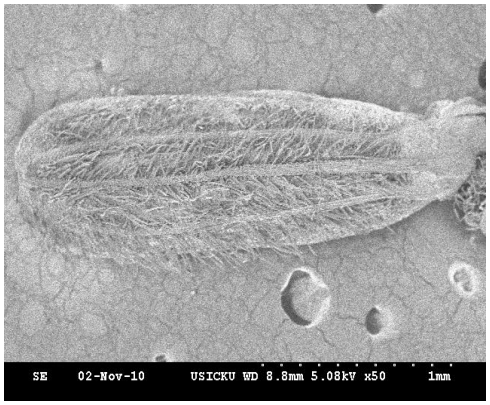
Plate 16

a- Tricolpate pollen grain, b- pollen ornamentation, c- showing pollen surface with deep furrow, e- showing echinate surface of pollen

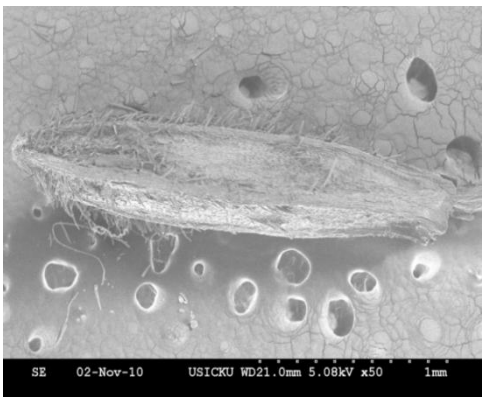
(a)



(b)



(c)



(d)

Plate 17

a- Surface with pointed spines, b- showing rough surface, c- seed with various furrows, d- showing papery seed coat

Discussion and Conclusion

The Present study is based on "Reproductive biology of *Valeriana jatamansi* Jones" belonging to the family Valerianaceae. A variety of approaches, both *in situ* and *ex situ,* have been proposed and implemented for conservation of plant resources but any conservation approach has to be based on in-depth study of plant reproductive biology. Reproductive characteristics such as seed dispersal, germination capacity, survival rate of seedlings and adults, age of flowering, reproductive life span and number of flowers and seeds refer to a set of responses that allow a species to adapt to a particular environment. Besides these, the processes of gamete development, pollination, endosperm and embryo development and other reproductive features can provide important clues regarding the reproductive constraints of plants that need conservation. The studies can also help in developing certain protocols to combat the problems that impede regeneration, (Moza and Bhatnagar, 2007).

5.1. Distribution

The valley of Kashmir, extending from Banihal to Baramulla is situated in the lap of the Himalaya. Himalaya is credited all over the world as a treasure of medicinal plants. Himalaya is ranked as one of the bio-diversity hot spots owing to its considerable abundance of medicinal plants. Among these medicinal gems *Valeriana jatamansi* (Valerianaceae) is a species with tremendous medicinal importance adding color to the crown of Himalayas. The genus *Valeriana* represents about 210 species mainly confined to temperate to alpine regions of the Himalayas (Raina and Srivastava, 1992). The main areas of distribution include Afghanistan, Bhutan, South West China, Burma and Nepal (Polunin and Stainton, 1987). Of these 12 species of this genus occur in India (Anonymous, 1976). In India, *Valeriana* is localized mainly to temperate Himalayas and found growing at an altitude of 1200 to 3000m asl (Kirtikar and

Basu, 1975). In India the species is found in Jammu & Kashmir, Sikkim, Uttaranchal and Himachal Pradesh (Raina and Srivastava, 1992).

In Kashmir Himalayas *Valeriana jatamansi* inhabits sub-temperate and temperate habitats ranging from 1500 to 3000m asl. This species was found sporadically distributed over various sites of Kashmir Himalayas which include Shajnar, Dara, Harwan, Gulmarg, Yusmarg, Ferozpur, Sonamarg and Pahalgam (Naqashi and Dar, 1982-1986- KASH Herbarium collection). These sites experience severe climatic conditions (low temperate, extreme variability in rainfall, fast winds, frequent clouds and high cosmic fallout etc.) and are too inaccessible. Within these specific natural habitats the individuals are sporadically distributed in a population, that too much less in number. This taxon has a greater endurance to extreme environments which are ecologically specific and unique in terms of habit, altitude, plant associations, edaphic conditions. These ecological preferences act as barriers preventing them from further spread. The alteration of these habitats for various developmental programs such as tourism or defense has also declined the population size of this herb. This resultant low density within and across these populations and localized distributions in small pockets reflects their critically rare status. It needs to be borne in mind that species with highly stringent and specific habitat requirements have greater possibilities of extinction than species with a broad habit range (Samant *et al*., 1996). Due to high demand of its rootstock, it is being extracted from wild sources without any concerted efforts to grow them that also leads to extinction of the species from natural habitats, (Gupta *et al*., 2004). The population assessment of *Valeriana* has revealed that on an average there is a decrease of about 30-40 plants per 100sq mts (Wyatt, 1981) which is increasing every passing year. At some localities this species is locally wiped out from some lower altitudes thus its distribution has dissected and shrunken. Thus *in situ* and *ex situ* strategies have been suggested to protect this species from extinction. This situation calls for an in-depth study of plant reproductive

biology of this taxon so as to derive any meaningful conclusion for its conservation.

5.2. Phenotypic Variability

Valeriana jatamansi is displaying variability in various phenotypic traits at various stages in its populations. This wide distribution in trait values is the raw material for operative evolutionary forces and natural selection. This phenotypic variability speaks the language of a typical meiotic and breeding system, which generate the variability in *Valeriana jatamansi*. The present study reveal that in response to their highly specific ecological environments this species have developed a spectacular diversity in their morphological characters viz., plant height, leaf number and dimensions, number of ramets, floral density, root length and number, rhizome dimensions etc. detailed morphological studies not only give specific botanical identity to a species but such studies reveal interesting features which are helpful in understanding the range of morphological variations present across different ecological zones. (Anonymous, 1976). The diversity across various ecological zones provides a strong edifice at which an ambitious plan for domestication and genetic improvement for commercial exploitation can be built. The details are as under;

Plant height is highly plastic and varies among different populations. The plants growing in complete shady or dappled shade environments show maximum variability in this trait while the individuals growing in open or exposed conditions show least variation and are by and large uniform. Increase in plant height in shady environments seems advantageous for the species as the shady environments provide the conditions where plants have to compete for light (Abrahamson and Gadgil, 1973). The plants of Gulmarg which are under complete shade or dappled shade shows maximum height than plants growing in exposed condition of Ferozpur where plants shows minimum height than Gulmarg ones. The two populations do not differ much significantly as they inhabit the similar climatic conditions. However plant height in

transplanted populations showed maximum height as compared to natural ones, because phenotypic response to environmental changes seems to be related to the severe climatic conditions at higher altitudes which have a negative impact on the overall growth of a plant. (Siddique, 1991; Gurvevitch, 1992).

The species also exhibit enormous variability in leaf number per genet and leaf dimensions at inter-population level. Plants growing at KUBG show maximum leaf number and leaf dimensions than the natural populations. Because these plants do not encompasses the severe environmental conditions which has negative impact on overall growth of plants including leaf size and number. Similar results were also observed by (Siddique, 1991 and Shah and Yadav, 1970) indicating that altitude registers a profuse influence on overall plant vigor in *Valeriana jatamansi*. However, it was also observed that plants growing under shade respond to shade by allocating more biomass to leaves and hence registers maximum leaf number and dimensions as well as plant height to compete for light, as holds true of many other plant species (Abrahmson and Gadgil, 1973). The plants growing at KUBG have largest leaves followed by those inhabiting Gulmarg. While the plants comprising Ferozpora population produce small sized leaves. It may be pointed out here that the shady populations develop largest leaves as compared to the open and exposed populations as shade plants maximizes the leaf surface area to encompass more light.

The plants inhabiting shady, moist and humus rich soils produce maximum number of flowers per ramet as well as per genet than plants facing direct sun or exposed conditions. This is because plants in shade have dense ramets per genet to compete for light and also these shade inhabiting plants can allocate more resources towards reproductive structures (flowers) than that of plants facing directly to sunlight (Abrahamson,1979) which have least number of ramets per genet. As maximum ramets bearing genets produce maximum number of flowers.

The data generated on the phenotypic quantitative characters reveal that the species exhibits vigorous growth, rich biomass and high reproductive potential in shady populations as compared to the populations under open sun. It thus becomes apparent that the species is well adapted to shady, moist habitats in its natural home. The species delimits the shade stress not only by increasing the plant height but also by the enhancement of the total photo synthetically active surface.

It is evident from the information account that plants growing at lower altitudes viz., KUBG, and that of plants inhabiting complete shade (Gulmarg) as against to direct exposure to sun (Ferozpur) are more vigorous, taller and bear larger number of leaves and inflorescences, this finding is in quite conformity with the observation of Billings and Money, (1968); Johnson and Cook, (1968); Hickman (1975); Siddique, (1991), and Siddique, *et al.* (1997). The phenotypic variability as observed in the present study helps the species to adapt in various eco-edaphic conditions.

5.3. Flowering Phenology

The plants of *Valeriana jatamansi* over-winter in the form of seeds and rhizomes. Soon after snow melts the seasonal dawn of sprouting is set in March. Natural homes of this species are characterized by low temperatures, snow cover and short growing seasons which compel the species to complete their life cycles in the short photosynthetic periods. Phenology, the seasonal timing of life history events, comprises a set of traits that may critically affect the reproductive success of a species (Rathcke and Lacey, 1985). The phenology of a species would constitute all the events from seed germination upto senescence of the individual. The phenological studies prove useful in planning out the conservation strategies as well as formulating measures for cultivating them on a large scale (Wafai *et al*., 1996; Beigh *et al*., 1998). Flowering phenology is particularly important because it determines the reproductive synchrony with potential mates (Marquis, 1988) and synchronizes

attractiveness of pollinators (Gross and Werner, 1983). Flowering time may also strongly affect reproductive success by determining synchrony with, and thus vulnerability to floral herbivores and seed predators (Loeb and Arban, 1992).

The floral community structure determines pollinator services among plants through competitive exclusion and differentiation of floral forms or phenologies (Campbell, 1985; Fishman and Wyatt, 1999; Brown *et al.*, 2002). Some plant species within different communities have over- lapping flowering periods and share generalist pollinators (Ramirez *et al*., 1998; Gross *et al.*, 2000) suggesting that plant reproduction is not limited by pollinator availability. Thus evidently, an understanding of phenological events is quite useful particularly in respect of endangered taxa, in planning their conservation strategies as well as formulating measures for commercial cultivation (Gross and Werner, 1983; Anderson, 1999; Ollerton and Lack, 1998; Wafai *et al*., 2005).

The hermaphrodite and female flowers however show asynchrony from flower to flower in a ramet or in different flowers of a genet in a population. This protracted asynchronous pollen presentation assures pollen availability for long periods to ensure effective pollination and also ensures survival of female plants. The asynchronous anthesis is quite advantageous for the species since it not only makes pollen available for long periods but also is a means to attract pollinators for long durations thereby boosting the out breeding potential of the species (Wyatt, 1982; Siddique, 1991).

Valeriana jatamansi over-winters in the form of rhizome (vegetative propagule) and seeds (sexual propagules). Soon after snow melts, sprouting begins in the month of February and continues upto April in all prudently studied populations. This phenophase is followed by floral bud formation which experiences a partial overlap with the process of sprouting .Floral bud formation is followed by anthesis which also shows the overlapping trend with

last phase of plant i.e., seed setting. Among phenophases anthesis and full bloom attract considerable interest and attention, because of the extra significance attached with this phase-the phase where from the core process of reproduction, pollination, pollen tube formation, fertilization etc., set the stage for successful reproduction. The female set seeds as early than hermaphrodites because these are hermaphrodites flowers which makes pollen available for long durations in order to ensure that atleast all female flowers have been in a way to receive the possible pollen source to fertilize all their ovules in order to produce a sufficient seeds to maintain diversity and pass on its progeny. The temporal differences in the development of maleness and femaleness enhance the out-crossing rates as also supported by Proctor and Yeo (1972); Wyatt (1982); Griffin *et al*. (2000) and Wafai *et al*. (2005). Also the extended blooming period increases the chances of individual having a large number of mates both as pollen donors and recipients (Torres *et al*., 2002).

At inter-population level the species operates long and short duration phenological events at lower and higher altitudes respectively. This is evident from the fact that the plants under cold conditions have short life span than plants growing under normal range of temperature regions. Temperature has a deterministic role in inducing sprouting and blooming irrespective of location or altitude. Franks *et al*. (2007) proposed that within population variation in phenological responses might be due to genetic diversity of species, because it increases the diversity of physiological response to temperature. Changes in phenological timing may increase as temperature increases (IPCC, 2007). The occurrence of specific phenophase at specific time and specific place/ location being fixed probably at gene level, where this spatio-temporal specificity of occurrence of phenophase may be the result of natural selection. Because early flowering by this plant is due to increasing temperatures which led the mismatch of pollinators, so that other pollinators of different plants also approach to this plant to enhance fertilization process hence renders lower

altitude plants ahead in completing reproductive life span than higher altitude ones where such temperatures meet after 2-3 months. In recent years, mismatch is plant pollinator interaction have increased, owing to climate change because of differences of phenological responses between species (Gorodo and Sanz, 2005; Doi and Takashi, 2008). Another reason may be to assured pollinator availability across different ecological zones because pollinator availability is believed to be the selection pressure/stimuli, as manifestation of the underlying principle (temperature), dictating the expression of gene or cascade of genes controlling flowering and flowering date. Thus *Valeriana jatamansi* has fixed its clock with biotic and abiotic factors, which act as agents or signals with phenophase occurrence as their manifestation.

5.4. Breeding System

The meiotic behavior and breeding system of a species, together constituting the genetic system are the approved systems holding keys for generation of variations and variability, out breeding out radiating flow of variations and inbreeding subtracting the sums.

Knowledge of reproduction is crucial to understand the causes of rarity and for conservation of rare plant taxa (Harper, 1979; Raina *et al*., 2003) and the study of factors that critically affect reproduction need to be considered in the design of conservation strategies (Godt and Hamrick, 1995). Studies on reproductive biology have revealed that the species operates a very efficient breeding system combining the advantages of both vegetative as well as sexual means.

The breeding system of an individual constitutes the mechanism of gamete differentiation, pollen transfer, fertilization and the modes of propagation. Differentiation of the sex organs determines the nature of the breeding system. While the homogenous condition favors selfing, dichogamy, herkogamy, self incompatibility and the male sterility favor out breeding (Endress, 1994). There is definite balance in operation between asexual and sexual reproduction

(Weberling, 1989). On one hand this stability guarantees genetic stability, which permits a population to survive in a particular habitat and on the other hand, a certain degree of genetic plasticity maintains adaptability to change in the environmental conditions through a large number of possible recombination's of old and new characters via sexual reproduction.

The presently investigated taxon shows gynodioecy i.e. co-occurrence of females and hermaphrodites in a species and is the most common sexual polymorphism in plants (Richards, 1997). The temporal separation of sex organs in this plant avoids inbreeding in hermaphrodites and enforces out breeding phenomena which comes out with a different mating strategy i.e., ecological xenogamy. However, females set seeds under obligate out crossing. Gynodioecy usually develops when some individuals produce flowers with sterile or aborted pollen, thus becoming functionally females (Stevens and Kay, 1991). Gynodioecy has been attributed as potential intermediate step in the evolution of dioecy by Charlesworth and Charlesworth (1978) and they are of the view that gynodioecy should only spread when there is a considerable advantage to the females in terms of seed set or vegetative growth. Females of gynodioecy taxa often have smaller floral size (Delph *et al*., 1996; Eckhart, 1999). This sexual dimorphism is flower size has been hypothesized to be advantageous because the smaller size of female flowers allow reallocation to greater seed production, or because greater size in hermaphroditic flowers may result in better pollinator attraction and pollen dispersal (Miller and Venable, 2003) and hence keeps the pollens available for longer durations for females. This notion is also supported by Mani (1962) whose view was that low temperature stress with altitude affects distribution and abundance of insects. Thus such conditions adapts this plant for wind pollination to successfully meet the demands of female plants in seed set which credits this plant to opt both entemophilous and anemophilous approach. Another important paradigm regarding more seed production in females and their perpetuation is because of

having an advantage over hermaphrodite plants by displaying their bright and colored flowers which are easily visualized by pollinators resulting in increased insect visiting efficiency than hermaphrodites.

Valeriana jatamansi is adapted both to vegetative as well as sexual reproduction with the former providing stability to the genotype at a particular site and the later contributing to generation of variability through sexual recombination of genes. The extent of variability and consequent stability to different ecological niches can be guazed by the wide area of distribution of this species not only in terms of altitude but also in aspect. This species has a wide distribution zone ranging from 1500 to 3000m asl (Chauhan, 1998; Anonymous, 1976). During the present study, female flowered plants did not set any seed when isolated from bisexual flowered plants. However, bisexual flowered plants set seeds under both open and self pollination conditions, thus bi-sexual plants of *Valeriana jatamansi* are both self as well as cross compatible while the female are only cross compatible. This view is also supported by Charlesworth and Charlesworth (1978), Ganders (1978) and Kesseli and Jain (1984) that females are at an advantage because seeds produced by female are product of obligate out crossing while the seeds produced by hermaphrodites are at least partly likely to be the product of self fertilization.

In *Valeriana jatamansi*, just after anthesis, the anthers start to dehisce pollen grains, while the stigma remains concealed within the flower. Once the anthers dehisce completely, the stigma starts to emerge out and becomes receptive within 3 days. After frequent pollinator visitation to both hermaphrodite and female flowers, the female flowers became receptive after 2-3 days after anthesis. Dichogamy (protandry) is the possible mechanism in *Valeriana jatamansi* that prevents inbreeding in hermaphrodite flowers and ecological xenogamy may thus be operative phenomena in seed formation incase of these hermaphrodite plants.

The pollen viability in this species is very high ranging from 86% to 98% which serves as a litmus test confirming normal meiotic behavior. The high pollen viability can be perceived by very good seed set and the strategy of the species to materialize maximum reproductive success. The *in vitro* pollen germination studies reveal that pollen grains germinate with ease in S:B:C and the pollen germination was highest for 6 hour time period and it decreases significantly with decreasing time duration. However, when calcium nitrate and boric acid are added the %age germination increases. Similar response of the pollen grains has been observed in many other species such as *Digitalis purpurea, D. grandiflora and D. lanata* (Brewbaker and Kwach, 1963; Romaisa, 2004).

The pollen ovule ratio serves as an conservative index of breeding system (Cruden, 1977). He demonstrated that outcrossing species had higher pollen ovule ratio than the predominant selfers. The above is best explained interms of pollination efficiency. In autogamous taxa, the pollen production is less, numerically compatible with ovule number-requiring few pollen grains per ovule because pollination is easily realized in these taxa i.e., selfers. Whereas in xenogamy, out crossing involves the uncertainties that accompany reliance on pollen vector and necessities greater pollen production. As observed in the present study, *Valeriana jatamansi* displays a very high P/O ratio, indicating its out breeding nature. The pollen ovule ratio on an average ranges between 2459±79.67:1. The higher pollen ovule ratio suggests out-breeding nature of the species. In populations, pollen grain load may vary greatly and have the potential to effect the intensity of pollen competition for ovules. Pollen competition can in turn, affect seed production, fruit set, and progeny vigor, with important ecological and evolutionary consequences (Barbara and Nancy, 2003).

Floral color change in *Valeriana jatamansi* is thought to be have evolved to capture the visual attention of pollinators, thus enhancing pollination success

especially critical to female plants growing in isolation from hermaphrodite ones. Such similar observations were achieved by various researchers and they believe that retention of color by flowers enhanced the attractiveness of individual plants to increase the approach frequency of pollinators to the plants. (Gori, 1983, 1989; Delph and Lively, 1989). In open sun these plants develop pink flowers which are because that maximum carbohydrate metabolism occurs in these conditions resulting in increase in sucrose concentration which enhances pigmentation (anthocynin) deposition.

5.5. Pollination system

Pollination may represent a biological market with particularly simple trading relations. The commodities exchanged are primarily sex (pollen) and food (nectar and other rewards).

The estimates of pollinator visitations are critically important to address a variety of ecological questions, including the impact of pollinators on selection, floral trait and role of inter specific plant competition on selection, on pollinator visitation and pollination success (Engel and Irwin, 2003). The pollination failure in small, isolated populations has been identified as a potential threat to the long term persistence of declining plant species (Rathcke and Jules 1993; Lennartsson, 2002). The size and density of a plant population may affect interactions with pollinators and pollen transfer in several ways. With the increasing size and density the attractiveness of a population to pollinators should increase, which may increase the number of pollinator visits per plant (Powell and Powell, 1987) and the amount of pollen received per flower. Second, pollinator foraging behavior may change with the size and density of the population (Goulson, 2000) thereby, affecting the composition of the pollen deposited.

Valeriana jatamansi has a relative advantage due to phenomena of gynodioecism by which the female plant set seed is always the result of cross

fertilization as they lack pollen. During the present studies, as expected the female flowered plants did not set any seed when isolated from hermaphrodite flowered plants. The hermaphrodite flowered plants on the other hand set seed under both self as well as open pollination conditions. Although self pollinated plants produced significantly less seed in comparison to open pollinated ones. This higher seed set in open pollinated plants appears to be result of combined effect of self as well as cross pollination as otherwise had this species been cross incompatible, there would have been no difference in seed set between self and open pollinated ones. Females of gynodioecious populations are intrinsically disadvantaged because they contribute genes only through ovules, while hermaphrodites can reproduce via both ovules and pollen (Lloyd; 1975). Females may attain the necessary compensatory advantage by achieving greater life time seed production. When seeds number cannot explain the persistence of females it is necessary to look for a female advantage in term of seed quality. Females plants are expected to produce better quality of seeds than hermaphrodites for atleast by a reason that, seed from females are obligate out crossed than hermaphrodites seeds which involves partial involvement of self fertilization, (Richards, 1986; Gouyon and Couvet, 1987). The best reason is that hermaphrodite plants has to allocate resources both towards male and female sex organs while female plants allocate resources only with perfection to female sex organs (Van Damme, 1984; Shykoff, 1988).

Valeriana jatamansi is observed to be pollinated by few insect visitors belonging to Apis and Syrphus. Among these Apis is the major pollinator as it registers highest visiting frequency. However at higher altitudes due to severe climatic conditions and sporadic distribution of plants less pollinator visitation was observed. Thus as per this individuals which are separated from hermaphrodites plants and having less pollinator availability can opt vegetative propagation which not only help the female plants to establish but also ensures perpetuation even in absence of hermaphrodite plants. This was observed as per

the notion that increased low temperate stress with altitude effects the distribution and abundance of insects (Mani, 1962). These plants pollinator relationships might change both qualitatively and quantitatively with altitude.

Also strong winds and inclement weather conditions at higher attitudes favor wind pollination which is more effective than animal pollination is promoting pollen dispersal and associated benefits of our crossing in this plant also supported by same statements of Carlquist (1974) and Ehrendorfer (1979).

During, the present study, as expected, the female flowered plants did not set any seed when isolated from hermaphrodite flowered plants. The hermaphrodite flowered plants on the other hand set seed under both self as well as open pollination conditions. Although self pollinated plants produced significantly less seeds in comparison to open pollinated ones. The higher seed set in open pollinated plants appears to be the result of combined effect of self as well as cross pollination, as otherwise had this species been cross incompatible, there would have been no difference in seed set in open pollinated and self pollinated plants.

5.6. Modes of propagation

Valeriana jatamansi produce small sized seeds which disperse during September-October and remain under snow for pretty long period's upto March or April of next growing season. The seeds of *Valeriana jatamansi* usually over-winter in resting phase, exhibiting physiological non-deep dormancy. However, few seeds as and when get dispersed by parachute mechanism at relatively immature stage do germinate immediately subjected to the availability of micro site. The seedlings of such seeds hardly survive in nature because they have to face the onslaught of chill in winter. With the onset of favorable conditions (March-April) the least %age of dormant seeds starts to germinate accounting for meager addition to the population in terms of new recruitments.

In order to overcome this bottleneck, the species has established an efficient method of vegetative propagation. The vegetative reproduction is accomplished through the underground perrenating buds borne on the underground rhizome. However, in next growing season the fresh rhizomes usually produces 5-8 perrenating buds, which develop into leafy shoots.

Thus *Valeriana jatamonsi* is adapted both to vegetative as well as sexual reproduction with the former providing stability to the genotype at a particular site and the later contributing to generation of variability through sexual recombination of genes. As the species perrenates through rhizomes which give rise to numerous flowering shoots in each flowering season, this feature not only helps the plant to establish at a location but also ensures perpetuation even in absence of seed setting ((Dhami and Mahindru, 1998).This is especially critical to female flowered plants growing in isolation from bisexual flowered plants, as female plants cannot set seeds independently. However, vegetative propagules also have some advantage over the seeds in that they have a tremendous head start development and growth, often mature earlier and may have better juvenile survivorship and less mortality (Amor, 1974; Abrahamson, 1980).

5.7. Seed Production

The species exhibit remarkable differences in the production of seeds or flowers from plant to plant and population to population and it is evident from the data that high altitude populations produce less seeds as compared to low altitude ones because of less availability of pollinators at high altitudes (Bingham and Orthner, 1998; Mohi-ud-din *et al.,* 2006). Therefore, seed production at high altitude may be pollinator limited as also argued by Lubbers and Chistensen (1986) in several other cases. This species also exhibit considerable difference in seed setting from hermaphrodites to female plants because Stevans and Van Damme (1988) are of the view that gynodioecy should only spread when there is considerable advantage to the females in

terms of seed set or vegetative growth. While in few species hermaphrodites produce more seeds as compared to females (Vaarama and Jaaskelainen, 1967). The same trend of more seed production in females (76%) a as compared to hermaphrodite (73%) was also observed in *Valeriana jatamansi*. This is because of the reason that smaller flower size and allocation of resources only towards femaleness helps female individuals to set more seeds than bisexual ones. However, this trend sometimes can be reversed at high altitude populations where bisexual plants can establish more seeds than female plants .The possible reason being the less availability of pollinators or infrequent pollinator visitation. This view is also supported by Charlesworth and Charlesworth (1978).

5.8. In vitro seed germination

The ultimate outcome of the sexual ceremony in plants is the seed which besides multiplication governs some fundamental processes-serving as an agent of perrenation, generation of variation and dispersal.

Seed germination is basically a reflection on the reproductive fitness, pollination efficiency, successful fertilization and proper physiological development of the seed in a species. Seed germination is the essential part of reproductive cycle of any plant species. It is a process, which transforms a day seed into a growing plant (Meyer and Carlson, 2004). Seeds of many plants fail to germinate immediately and pass through a phase of dormancy that may be caused by sexual factors thus delaying the life cycle of these plants. Some plants display a wide range of dormancy breaking and germination requirements (Leon, 1985). Besides, germination at the right time and in the right place largely determines the probability of seedling survival (Thompson, 1974). The dormancy characteristics and germination responses are considered to be the key elements in plant life history strategies (Meyer *et al.,* 1990). Conditionally, dormant seeds germinate over only a portion of the range of conditions possible for the species (Vegis, 1964; Baskin and Baskin, 1985).

The physiological and chemical treatments have been found to improve the seed germination, seedling growth and nitrogen metabolizing in plants (Joshi and Dhar, 2003; Mohi-ud-din *et al.,* 2005). Among many treatments used to increase percentage germination and reducing MGT scarification (seed coat removal) ranks 1st with %age germination enhanced to 85% followed by 1mM thiourea and 0.25 mM Kinetin. In *Valeriana jatamansi* the seed coat which is thin and papery interferes with seed germination (showing non-deep physiological dormancy) because of inhibitors contained in seed coat.

To understand the occurrence and distribution of a species in certain habitats and its life history strategy, the timing of germination and the nature of dormancy breaking mechanism assume great significance. The development of seed germination protocols is therefore, an important step (especially for threatened plants) to overcome the increasing demand of the plant material in pharmaceutical industry (Joshi and Dhar, 2003). Such studies will also help in developing conservation strategies for the proper management and the maintenance of the bioresources especially economically important plants in general and medicinal plants in particular at lower and easy-to-approach habitats (Mohi-ud-din *et al.,* 2005).

5.9. *In vivo* Propagation

The increasing demand for herbal drugs in general and Himalayan medicinal plants in particular and the depletion of important species from natural resources make it imperative to develop proper methods and strategies for their conservation and commercial production (Raina *et al.,* 2003).

The *in vivo* propagation technique for the species through rhizome cuttings was successfully developed for the first time. The experimental manipulations (Chapter 4, Table 13) reveal that the species has a potential to propagate through rhizome cuttings in sandy loam soils. Treatments of cuttings with IAA, IBA and GA_3, however, increased survival potential and decreased the days

required for shoot regeneration under *ex-situ* conditions. The cuttings without treatment (control) showed low survival rate (16-35%) as against the treated cuttings with survival rate upto (83%). The difference even though marginal clearly demonstrates that rhizome cuttings with an apical shoot have the capability to regenerate into new plants under *ex-situ* conditions. Utilizing this cost effective method of propagation, the species can be multiplied in lesser time with good survival rates. Similar kind of trend has been earlier been reported by Nautiyal, *et al.,* 2001).

5.10. Pollen Mother Cell Meiosis

A persual of literature reveals that out of 47 species of *Valeriana* for which chromosome number are known (Federov, 1974), 19 species are based on X = 8 and 13 species on X = 7. Diploid chromosome number in these 47 species range from 14 to5 6 with 2n = 32 reported in seven species and 2n = 28 reported in nine species of Valeriana (Federov, 1974). The present observation of 2n = 32 in *Valeriana jatamansi* confirms the earlier reports of Mehra and Sobti (1955). During present studies no cytotype based on X = 7 (2n = 28) was observed. Thus the probable base number of *Valeriana jatamansi* is 8 as base number above 12 are considered as secondary base number (Stebbins, 1971), which arise due to cross between forms with different chromosome numbers followed by chromosome doubling. The high percentage of pollen viability and normal seed germination also supports normal meiotic behavior in this species as observed in the present study.

5.11. Resource allocation and fertilizer application

The temperate and sub-temperate medicinal plants species constitute an important natural bio resource. This wealth has been exploited extensively as a source of traditional herbal drugs and also in the manufacture of many allopathic drugs. The over-exploitation of important species is the basic reason of their extinction. This has necessitated the development and standardization

of agro techniques for their cultivation and consequent conservation (Joshi *et al.*, 1990). The major conservation strategies recommended are development of germplasm centers for *in situ* and *ex situ* conservation of critically endangered species, establishment of high altitude nurseries, systematic collection and domestication etc. (Joshi and Rawat, 1997; Dwivedi, 1999).

In view of large-scale exploitation, biotic interferences and increasing demand for *Valeriana jatamansi* as herbal drugs, their assessment as threatened species in nature, and consequently the need for their cultivation to salvage it from loss and also raise the germplasm and provide economic avenues for the poor, it becomes obligatory to initiate steps for its large scale cultivation and development of elementary agro- techniques at lower attitudes under *ex-situ* conditions.

As the principle of allocation advocates that every organism allocates its resources to three essential activities, i.e.

(1) Maintenance and survival
(2) Growth-increase in biomass
(3) Reproduction

The resource allocation pattern of a species is programmed in such a way as to achieve maximum evolutionary fitness. This partitioning of the resources may vary according to the habitat and community characteristics of a species. The differences in the resources may be environmentally induced. The environmental factors include plant density, soil fertility, habitat characteristics, stability of the plant communities and altitude etc. (Abrahmson, 1979 and Kawano and Masuda, 1980). The available literature reveals that analysis of the energy allocation pattern can provide individual information regarding the reproductive status of a species.

The data obtained on the resource allocation in *Valeriana jatamansi* is summarized in Table 16 and reveal that more dry biomass was found in plants

growing in shady environments than at exposed sites. It is evident that the population growing in shady conditions is rich in soil nutrients and moisture availability compared to the exposed population. The available nutrients present in soil are properly utilized by the plant resulting in enhanced growth by better accommodation of resources. Similar observations have also been reported in various plants by Kawano and Masuda (1980). The biomass partitioning in the species reveals that the partitioning among the different organs of the plant is not even. Maximum amount of biomass is allocated to the organs of support i.e., rhizome and stem followed by leaves and inflorescences which is more in plants grown is shady habitat as holds true of other plants growing in shady environments of the forests (Abrahmson and Gadgil, 1973; Abrahmson, 1979). *Valeriana jatamansi* grows in densely forested areas where light is one of the limiting factors and thus the plants of this species have adapted accordingly.

During the course of present investigation on agro techniques plants of this species performed well and gave excellent adaptability when grown at an altitude of 1595m in Botanical Garden of Kashmir University, Srinagar. The plants were also grown in different soil textural classes and various nutrient trials were applied to assess the performance of the plants. Although the plants grew nicely on various textural soils, maximum plant survival and vigorous growth was obtained in loamy textural soils, possibly because this texture offers little or no resistance for rooting and less leaching of nutrients than clayey (difficult to root) and sandy (maximum nutrient leaching) soils. However, the plants grown in natural soils but under complete shade also registered good dry mass production per individual as compared to exposed sited individuals. However, NPK (combined effect) was found to be more suitable by registering maximum dry biomass. From the data it was revealed that maximum dry weight per plant were recorded in hermaphrodite plants which were statistically at par from dry weight of females .Similarly, dry

weight of their below ground parts are also at par with each other..This revealed that these plants thrive best in shady conditions. Moreover, it was also observed that these plants show positive response to fertilizers with tremendous growth on combined effect (NPK). Similar kind of results was obtained by Ramesh *et al.* (1989) while working on *Plantago ovata* using NPK.

Bibliography

Abrahamson*, W.G. 1979. Patterns of resource allocation in wild flower populations of fields and woods. *Amer. J. Bot*., **66**: 71-79.

Abrahamson, W.G. 1980. Demography and vegetative reproduction. *Bot. Monog*r., **15**: 89-106.

Abrahamson, W.G. and Gadgil, M. 1973. Growth form and reproductive effort in golden rods (*Solidego*, Compositae): *American Naturalist*, **107**: 651-661.

Agarwal, P. K. and Dadlani, M. (eds.) 1992.Techniques in seed science and technology, South Asian Publication.

Allard, R.W., Jain, S. K. and Workman, P.L.1968. The genetics of inbreeding populations, *Advances in Genetics* **14**: 55-131.

Amor, R.L. 1974. Ecology and control of black berry (*Rubus fruticosus* L. agg.), II: Reproduction. *Weed Res*., **14**: 231-238.

Anderson, S. 1999. The cost of floral attractants in *Achillea ptarmica* (Asteraceae): evidence from a ray removal experiment. *Plant biology*, **1**: 569-572.

Anonymous*, 1976. *The Wealth of India*, vol. x: Sp-D (Raw material), CSIR, publication, New Delhi, India, pp. 424-426.

Argen, J., Willson, M.F. 1991. Gender variation and sexual differences in reproduction characters and seed production in gynodioecious *Geranium maculatum*. *Amer. J. Bot***. 78**: 470-480.

Ashman, T. L., Knight T. M., Steets, J. A., Amarasekare, P., Burd, M., Campbell, D. R., Dudash, M. R., Johnston, M. O., Mazer, S. J., Mitchell, R. J., Morgan, M. T., and Wilson, W. G. 2004. Pollen

limitation of plant reproduction: Ecological and Evolutionary causes and consequences. *Ecology*, **85**(9): 2408-2421.

Aswanthaiah*, B., Gupta, D. G., and Reddy, M. V. 1993. *Germination testing*. In: Hand book of seed testing, P.K. Agarwal (ed.) Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India, New Delhi, 45p.

Augspurger, C. K. and Franson, S. E. 1986. Wind dispersal of artificial fruits varing in mass, area and morphology. *Ecology*, **68**: 27-42.

Backlund, A. A. and Nilsson. 1997. Pollen morphology and systematic position of *Triplostegia* (Dispsacales).*Taxon*, **46**: 21-31.

Backlund, A. A., Moritz, T. 1998. Phylogenetic implications of an expanded valepotriate distribution in the Valerianaceae. *Biochem. Syst. Ecol*. **26**: 309-355.

Barbara, M. N. and Nancy, L. 2003. Pollen deposition, Pollen tube growth, seed production and seedling performance in Natural populations of *Clarkia unguiculata* (Onagraceae) *Int. J. Plant. Sci*. **164**(1): 153-164.

Barret, S. C. H. 1998. The evolution of mating strategies in flowering plants. *Elsevier Science;* vol. **3**(9): 1240-1253

Baskin, C. C., Baskin, J. M. 1985. The annual dormancy life cycle in buried weed seeds: *A continuum. Bioscience*, **35**: 492-498.

Beigh, S. Y., Siddique, M. A. A. and Wafai, B. A. 1998. Patris (*Aconitum heterophyllum*, Ranunculaceae), an important endangered N.W. Himalayan drug plant-problems and prospects. 68th Annu. Session. *Nat. Acad. Sci. India*, C. D. R. I. Lucknow, Biosis, Abst-**17**: 11-12.

Bell, C. D. 2004. Phylogeny and biogeography of *Valeriana* (Dipsacales). Ph.D. Thesis, Yale University, USA.

Bell, C. D. 2004. Preliminary phylogeny of Valerianaceae (Dipsacales) inferred from nuclear and chloroplast DNA sequences data. Mol. Phylogen. *Evolution.* **31:** 3410-350.

Bell, C. D. and Donoghue, M. J. 2005. Phylogeny and biogeography of Valerianaceae (Dipsacales) with special reference to the South American Valerians. *Organisms Diversity and Evolution*, **5**: 147-159.

Bennet, S. S. K. 1987. *Name changes in flowering plants of India and adjacent regions*. Treseas Publishers. Dehradun, India: 583pp.

Bernath, J., Foldesi, D., Lassanyi, Z. 1973. The effect of nutrient supply and soil type on the root system and yield of *Valeriana officinalis. In orzagos Gynovenykonferencia Eloadasainak osszefoglalojia Budapast,Hungry*, **23***:* 1222-1228.

Billings, W. D. and Mooney, B. A. 1968. The ecology of arctic and alpine plants. *Biol. Rev*., **43**: 481–529.

Bingham, R. A. and Orthner, A. R. 1998. Efficient pollination of alpine plants. *Nature*, **391**: 238-239.

Bink, E. 1980. The influence of fertilization on the root yield of *Centranthus ruber* Dc and valtrate content. *Acta Hort*., **96**(2): 201-205.

Bounthanh, C., Bergmann, C., Beek, J. P., Berrurier, M. and Anton, R. 1981. Valepotriates, a new class of cytotonic and antitumor agents, *Planta Medica* **41**: 21-28.

Brewbaker, J. L. and Kwach, B. H. 1963. The essential role of calcium ion in Pollen germination and pollen tube growth. *Amer. J. Bot*., **50**: 859-865.

Bronstein, J. L., Gouyon, C., Gliddon, F., Kjellberg and Michaloud, G. 1990. The ecological consequences of flowering asynchrony in monoecious frogs: A simulation study. *Ecology*, **71**: 2145-2156.

Brown, B. J., Mitchell, J. and Graham, S. A. 2002. Competition for pollination between an invasive species (purple loosestrife) and a native *congener*. *Ecology*, **83**: 2328-2336.

Burd, M. 1994. Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. *Botanical Review*, **60**: 83-139.

Campbell, D. R. 1985. Pollinator sharing and seed set of *Sttelaria Pubera*: Competition for pollination. *Ecology*, **66**: 544-553.

Carlquist, S. 1974. *Island Biology*. New York: Columbia University Press.155-172pp.

Caruso, C.M. 2000. Competition for pollination influences selection on floral traits of *Ipomopsis aggregata*. *Evolution*, **54**: 1546-1557.

Charlesworth, B. and Charlesworth, D. 1978. A model for the evolution of dioecy and gynodioecy. *American Naturalist*, **112**: 975-997.

Charlesworth, D. 1981. A further study of problem of the maintenance of females in gynodiocious species. *Heredity*, **46**: 27-39.

Chauhan*, N. S. 1998. Medicinal and Aromatic plants of Himachal Pradesh. Indus publishing company, tagore Garden, New Delhi, 426-428.

Chauhan, N. S. and Khosla, P. K. 1988. Commercial important medicinal plants of Himachal Pradesh. In: PK Khosla (Ed) *Trends in Tree Sciences*, ISTS Publication, Solan, 81-89.

Chauhan, R. S. and Nautiyal, M. C. 2005. Commercial viability of cultivation of an endangered medicinal herb *Nardostachys jatamansi* at three different agroclimatic zones. *Current Science* **89**(9): 1481-1487.

Chauhan, R. S., Kaul, M. K., Kumar, A. and Nautiyal, M. C. 2008. Pollination behaviour of *Nardostachys jatamansi* D. C., an endangered medicinal and aromatic herb, Ph.D Thesis. Biodiversity and Applied Botany division, Indian Institute of Integrative Medicine (CSIR) Jammu.

Chopra, R. N., Nayyar, S. O. and Chopra, I. C. 1956. *Glossary of Indian Medicinal Plants*. CSIR Publication, New Delhi. 251 pp.

Clarke, G. 1978. Pollen morphology and generic relationships in the Valerianceae. *Grana*., **17**: 61-75.

Couvet, D. Atlan, A., Belhassen, E., Giddon, C., Gouyon, P. H. and Kjellberg, F. 1990. Co-evolution between two symbionts. The case of cytoplasmic male sterility in higher plants. *Oxford Surveys in Evolutionary Biology* **6**: 225-249.

Cronquist, A. 1981. *An integrated system of classification of flowering plants*. Columbia University Press, New York.

Cruden, R. W. 1977. Pollen-ovule ratio: A conservative indicator of breeding system in flowing plants. *Evolution*, **31**: 32-46.

Cruden, R. W. 2000. Pollen grains: why so many? *Plant Systematic and Evolution*, **222**: 143-165.

Darwin*, C. 1877 *Different forms of flowers on plants of same species*, John Murray, London.

Delannay, X., Gouyon, P. H. and Valdeyron, G. 1981. Mathematical study of evolution of gynodioecy with cytoplasmic inheritance under the effect of nuclear restorer gene. *Genetics* **99**: 169-181.

Delph, L. F. and Lively, M. S. 1989. Flower color changes in response to pollinator visitation. *Nature*, **45**: 212-223.

Delph, L. F., Galloway, L. F. and Stanton, M. L. 1996. Sexual dimorphism in flower size. *Amer. Nat*., **148**: 229-320.

Dhami, P. S. and Mahindru, R. C. 1998. *A text book of biology*. Pardeep, Publisher, Jalandhar 28 p.

Diapher, A. and Hindwarch, I. 2004. A double blind placebo controlled investigation of the effects of two doses of valerian preparation on the sleep, cognitive and psychomotor function of sleep disturbed order adults. *Phtotherapy Research,***18**: 831-836.

Dieringer, G. 1991. Variation in individual flowering time and reproductive success of *Agalinis Strictforia* (Scrophulariaceae). *Amer. J. Bot*., **78**: 479-503.

Doi, H., Takashi, M. 2008. Latitudinal patterns in phenological responses of leaf coloring and fall to climate change in Japan. *Global Ecology and Biogeography*, **17**: 556-561.

Dwivedi, S. W. 1999. Traditional health care among tribal's of Rewa district of M.P. with special reference to conservation of endangered and vulnerable species. *Journal of Economic and Taxonomic Botany*, **23**: 315-320.

Eckhart, V. M. 1999. Sexual dimorphism in flowers and inflorescences. In: Gender and sexual dimorphism in flowering plants (M. A. Geber, T. E. Dawson and L. E. Delph, eds.), *Spinger*, Berlin, 123-148.

Ehrendorfer, F. 1979. *Reproductive biology in island plants*. In Plants and Islands. (Rd. D. Bram well,).London. Academic Press, 293-306.

Endress, P. K. 1994. *Diversity and Evolutionary Biology of Tropical Flowers.* Cambridge University Press, New York, USA.

Engel, E. C. and Irwin, R. E. 2003. Linking pollinator visitation rate and pollen receipt. *Amer. J. Bot*., **90**: 1612-1618.

Engel*, K. 1976. *Beitrage Zur Systematic Der Valerianaceae a Unter Besenderes.***76**: 1290-1298.

Eriksen, B. 1989. Notes on generic and infrageneric delimitation in the Valerianceae. *Nordic Journal of Botany*, **9**: 179-187.

Faivre, A. E. and Windus, J. L. 2002. Genetic variation among populations of *Valeriana ciliata* T. and G. (Prairie valerian) in Midwestern Prairie Fens. *Journal of Torrey Botanical Society*, **129**(1): 39-47

Federov, 1974. *Chromosome Numbers of Flowering Plants*. Otto Koetz Science Publishers, West Germany, 713-714.

Fenner, 1998. The growth and reproduction in plants. Perspectives in Plant Ecology, *Evolution and Systematic*, **1**: 78-91.

Fishman, L. and Wyatt, R. 1999. Pollinator-mediated competition; reproductive character displacment; and the evolution of selfing in *Arenaria uniflora* (Caryophyllacae). *Evolution*, **53**: 723-1733.

Franks, S. J., Sim, S., Weis, A. E. 2007. Rapid Evolution of flowering time by an annual plant in response to a climate fluctuation. *Proceedings of National Academy of Science USA*, **104**: 1278-1282.

Ganders, F.R. 1978. The genetic and evolution of gynodioecy in *Nemophila menziesii* (Hydrophyllaceae). *Can J. Bot*., **56**: 1400-1408.

Ganders, F. R., Carey, K. and Griffiths, A. G. F. 1977a. Natural selection for a fruit dimorphism in *Plectris congesta* (Valerianaceae). *Evolution,* **31**: 873-881.

Ganders, F. R., Carey, K. and Griffiths, A. G. F. 1977b. Out crossing rates in natural populations of *Plectritis brachyestemon* (Valerianaceae). *Can. J. Bot.*, **55**: 2070-2074.

Godt, M. J. W. and Hamrick, J. C. 1995. The mating system of *Liatris halleri* (Asteraceae), a threatened plant species. *Heredity*, **75**: 398-404.

Gorbunov, Y. U. N. 1979. The biomorphology of certain Caucasian *Valeriana* species in relation to their introduction in to cultivation. B*ulleten Glavnogo Botanicheskogo Sada*, **113**: 25-33.

Gori, D. F. 1989 Floral color change in *Lupinus arenteus* (Fabaceae): Why should plants advertise the location of unrewarding flowers to pollinators. *Evolution*, **43**:870-882.

Gori, D. F. 1983. Post pollination phenomena and adaptive floral changes. In C. E. Jones and R. J. Little (eds.). Handbook of experimental pollination biology. Van Nostrand Reinhold, New York. 31-49.

Gorodo, D., Sanz, J. J. 2005. Phenology and climate change a long term study in a Mediterranean locality. *Oecologia*, **146**: 484-495.

Goulson, D. 2000. Why do pollinators visit proportionally few flowers in large patches? *Oikos*, **91**: 485-492.

Gouyon, P. H. and Couvet, D. 1987. A conflict between two sexes, females and hermaphrodites. In S. C. Stearns (ed.). The evolution of sex and its consequences, 245-262.

Griffin, S. R.., Mavranganis, L. and Eckert, C. G. 2000. Experimental analysis of protogyony in *Aquilegia* c*anadensis* (Ranunculaceae*). Amer. J. Bot*., **87**: 1246-1256.

Gross, C. L., Mackay, D. A. and Whalen, M. A. 2000. Aggregated flowering phenologies among three sympatric legumes: the degree of non-randomness and the effect of overlap on fruit set. *Plant Ecology*, **148**: 13-21.

Gross, R. S. and Werner, P. A. 1983. Relationships among flowering phenology, insect visitors and seed set of individuals: experimental studies on for co-occurring species of goldenrod (*Solidago*: Compositae). *Ecological monographs*, **53**: 95-117.

Gunn, C. R., Wiersema, C. A., Ritchie, C. A., Kirkbridge, J. R. 1992. Families and genera of spermatophytes recognized by the agriculture research service, U. S. D. A. *Techn. Bull*.**1796**:1-499

Gupta*, B. K., Suri, J. L. and Gupta, G. K. 1996. Isolation and evaluation of Valepotriates from Indian Valerian. In: Supplement to cultivation and utilization of Medicinal Plants: S. S. Handa and B. K. Kaul (eds.) Jammu Tawi, RRL, CSIR, 373-379.

Gupta, L. M., Rana, R. C. and Menakashi, G. 2004. Cultivation and importance of medicinal plants, *Journal of Medicinal and Aromatic Plant Sciences* **26**: 700-706.

Gurvevitch, J. 1992. Sources of variation in leaf shape among two populations of *Achillea lanulosa. Genetics*, **130**(2): 385 – 394.

Harder, L. D. 1990. Behavioral responses in bumble bees to variation in pollen availability .*Oecologia*, **85**: 41-47.

Harder, L. D. and Barret, S. C. H. 1996. Pollen dispersal and mating patterns in animal pollinated plants. In D.G. Lloyd and S.C.H. Barret (eds.), floral biology: Studies on floral evolution in animal pollinated plants, Chapman and Hall, New York, USA, 140-190.

Harper, K.T. 1979. Some reproductive and life history characteristics of rare plants and implications for management. The endangered species: a symposium. *Great Basin Naturalist Menioirs***, 3**: 129-137.

Hauser, E. J. P. and Morrison, J. H. 1964. Cytochemical reduction of nitro blue- tetrazolium as an index of pollen viability. *Am. J. Bot*. **51**:748-753.

Heywood, V. H. 1995. *Global Biodiversity Assessment*, Cambridge University Press, Cambridge USA.

Hickman, J. C. 1975. Environmental unpredictability and the energy allocation strategies in the annual *Polygonum cascadense* (Polygonaceae). *J. Ecol*., **63**: 689 – 702.

Hooker, J. D. 1881. *Flora of British India*. vol. III. pp. 213.

Horn, W. A. H. 1992. Micropropagation of rose (Rose. L) in Bejaj YPS. Editor. Biotechnology in Agriculture and Forestry. *Spinger*, **20**: 320-42.

Houssard, C. and Escarre, J. 1991. The effects of seed weight on growth and competitive ability of *Rumex acetosella* from two successional old fields. *Oecologia*, **86**: 236-242.

IPCC, 2007. Contribution of working group II to the fourth assessment report of the intergovernmental panel on climate change. In climate change 2007. impacts, Adaptations, and Vulnerability. Cambridge University Press, New York.

Jaimie, A., Moise, S. H., Loreta, G. S., Doglas, A. J. and Brian, L. A., Miki. 2005. Seed coats, structure, development, composition and biotechnology, in vitro cell, *Dev. Biol*., **41**: 620-644

Jain, S. K. 1968. *Medicinal Plants*. National Book Trust, India, New Delhi. 154 pp.

Johnson, M. P., and Cook, S. A 1986. Clutch size in butter cups. *Amer. Nature*, **120**: 45-411.

Joshi, D. N. and Rawat, G. S. 1997. Need for conservation and propagation of alpine and sub-alpine medicinal plants of worth west Himalaya. *Indian Forester*, **123**: 811-814.

Joshi, G. C., Pandey, N. K., Tiwari, K. C. and Tiwari, R. W. 1990. A preliminary synecological approach for the study of herbal wealth of high altitude Himalaya and their conservation strategy. *Bulletin of Medico-Ethnobotonical Research.* **11**(14): 96-102.

Joshi, M. and Dhar, U. 2003. Effect of various presuming treatments on seed germination of *Heracleum condicans* wall. Ex Dc: a high value medicinal plant. *Seed Science and Technol*, **31**: 737-743.

Judd, W. S., Sanders, R. W. and Donoghue, M. J. 1994. Angiosperm family pairs: preliminary phylogenetic analyses. *Harv. Pap. Bot.*, **5**: 1-51.

Kaul, M. K., Handa, S.S. 2000. Response of Medicinal Plants to changed habitats and altitudes. *J. trop. Med. Plants.* **1**:125-137.

Kanon*, N. T. 1978. Biology of flowering and pollination of *Valeriana officinalis* in the Mascow region, *Rastitel Nye-Resursy,* **14**(1): 73-77.

Kaufman, P. B., Carlson, T. F., Dayanandan, P., Evaus, M. L., Fisher, J. B., Parks, C. and Wells, J. R. 1989. *Plants their Biology and Importance*, Hoppe and raw Publishers, New York, 714-730.

Kaur, R., Sood, M., Chander, S. D., Mahajan, R., Kumar, V., Sharma, D. R. 1999. *In vitro* propagation of *Valeriana jatamansi. Plant Cell, Tissue, Organ Culture,* **59**(3): 227-229

Kawano, S. and Masuda, I. 1980. The productive and reproductive biology of flowering plants VII. Resource allocation and reproductive capacity in wild populations of *Heloniopsvi orientalis* (thumb). C. Tanaka (Liliaceae). *Oecologia* (Berl.), **45**: 307-317.

Kelly, M. G., and Levin, D. A. 2000. Directional selection an initial flowering date in *Phlox drummondi* (Polemoniaceae). *Amer J. Bot*., **87**: 382-391.

Kesseli, R. and Jain, S. K. 1984. An ecological genetic study of gynodioecy in *Limnathes dauglassii* (Limnathaceae). *Amer. J. Bot*. **71**: 775-786.

Kritikar, K. R. and Basu, B. D. 1975. *Indian Medicinal Plants*. VII. M/s Bishen Singh Mahendra Pal Singh, Dehra Dun. 311-312.

Kokwar, J. O. 1968. Flora of Tropical East Africa, Herbarium; Royal Botanic Garden Kew (k), 1-7.

Larson, B. M. H., and Barret, S. C. H. 2000. A comparative analysis of pollen limitation in flowers plants. *Biological Journal of the Linnean Society* **69**: 503-520.

Lawrance, H. M. 1951. *Taxonomy of Vascular Plants*. The Macmillan Company, New York. 670-672.

Layton, C. L., and Ganders, F. R. 1984. The genetic consequences of contrasting breeding systems in *Plectritis* (Valerianaceae). *Evolution*, **38**: 1308-1325.

Lenartsson, T. 2002. Extinction threshold, and disrupted plant pollinator interactions in pigmented plant populations. *Ecology*, **83:** 3060-3072.

Leon, J. 1985. *Germination strategies*. In: P. Green wood, P. H. Harvey and M. Slatkin (eds.). Evolution. Essays in hour of John Maynard Smith. Cambridge University Press, Cambridge, U.K.

Leppik, E. E. 1953. The ability of insects to distinguish number. *Am. Nat*., **87**: 229-36.

Lloyd, D. G. 1975. The maintenance of gynodioecy and androdioecy in angiosperms. *Genetica*, **45**: 325-339.

Loeb, G. M. and Arban, L. 1992. Consequences of variation on phenology for seed head herbivory and reproductive success in *Erigeron glaucus* (Compositae). *Oecologia*, **89**: 588-595.

Lubbers, A. E. and Christensen, N. L. 1986. Intraseasonal Variation in seed production among flowers and plants of *Thalictrum thalctroides* (Ranunculaceae). *Amer. J. Bot*., **73**: 190-203.

Luissa, A. S., Andea, L. S., Arthur, G.N., Gilsanelino, V. F. and Sander, B. R. 2002. *Valeriana glechomifolia*: *in vitro* propagation and production of valepotriates. *Acta Hort*. **426**: 525-536.

Mace, G. M. 2004. The role of taxonomy in species conservation. Philosophical Transactions: *Biological Sciences*, **359**(1444): 711-719.

Mani, M. S. 1962. *Introduction to high altitude entemology*: insect life above timberline in the north-western Himalayas. Methuen, London. Willey Publishers, 23-45.

Marquis, R. J. 1988. Phenological variation in the neotropical understory shrub *Piper arieianum*: causes and consequences. *Ecology*, **69**: 1552-1565.

Marshall, D. L.,1987. Effects of seed size on seedling success in three species of *Susbania* (Fabaceae). *Am. J. Bot*., **73**: 457-464.

Mather, K. 1940. Out breeding and separation of the sexes. *Nature* **145**: 485-486.

Mattana, E., Mattew, I. D. and Gianluigi, B. 2010. Comparative germination ecology of the endemic *Centranthus amazonum* (Valerianaceae) and its widespread *C*ongener *Centranthus ruber*.

Mehra, P. N. and Sobti, S. N. 1955. *Cytology of Indian Medicinal Plants*. II. Aconites, Valeriana and Senna. Res. Bull. Punjab. University, **80**:159-171.

Meyer, S. E and Carlson, S. L. 2004.Comparative seed germination biology and seed propagation of light intermountain species of Indian paint brush. USDA Forest Service Proceedings, RM RS, p-31, 125-130.

Meyer, S. E., Monsen, S.B. and Author, E. D. 1990. Germination response of *Artemiria tridentata* (Asteracease) to light and cost benefit analysis of *Picrorhiza Kurroa* cultivated at lower altitudes. *Current Science,* **81**: 579-585.

Miller, J. S and Venable, D. L. 2003. Floral morphometrics and the evolution of sexual dimorphism in *Leum* (Solanaceae). *Evolution,* **57**: 74-86.

Mohi-Ud-Din, G. G. Nawchoo, I. A. and Wafai, B. A. 2006. Mating and pollination mechanisms in *Saussurea costus* (Falc.) Lipsch.- A critically

Endangered Medicinal Plant of N. W. Himalaya. *Phytomorphology*, **56**: 41-46.

Mohi-Ud-Din, G. G., Nawchoo, I. A. and Wafai, B.A. 2005. Seed germination studies on *Saursurea Costus* (Falc.) Litch-A threatened medicinal plant of N.W. Himalaya. *Indian. J. Plant Physical*, **10**: 394-396.

Moor, R. P. 1973. *Tetrazolium Staining for Assessing Seed Quality. In Seed Ecology*, ed. W. Heydecker, Butterworth, London, 347-366.

Moza, K. M. and Bhatnagar, A. K. 2007. Plant reproductive biology studies crucial for conservation, *Current Science*, vol. **92**(9)

Mukerji, B.1953. *Indegeneous Drugs*. Indian Pharmaceutical Codex, CSPR (Delhi). 431 p.

Naqashi and Dar, G. H. 1982-1986- Kashmir University Herbarium Collection (KASH), Centre of Plant Taxonomy, University of Kashmir.

Nath, R. 1996. *Comprehensive College Botany*. Kalyami Publisher, New Delhi, vol. **11:** 57-98.

Nautiyal, B. P., Prakash, V. Chauhan, R. S., Purohit, H. and Nautiyal, M. C. 2001. Assessment of germinability, productivity populations of fields and woods. *Amer. J. Bot*., **66**: 71-79.

Naylor, R. A. L., 1993. The effect of plant nutrition on seed size, viability and vigour and on germination of wheat and *triticale* at different temperatures. *Ann. Applied Biol*., **123**: 379-390.

Neal, R. P., Dafini, A. and Giurfa, M. 1998. Floral symmetry and its role in plant-pollinator systems: Terminology, Distribution, and Hypotheses. *Annu. Rev. Ecol. Syst*. **29**: 345-373.

Nishitani, S. and Masuzawa, T. 1996. Germination characteristics of two species of polygonum in relation to their altitudinal distribution on Mt. Fuji. Japan. *Arctic and Alpine Res*., **28**:104-110

Olesen, J. M. and Warncke, E. 1989. Flowering and seasonal changes in flower sex ratio and frequency of flower visitors in a population of *Saxifraga hirculus*. *Holaratic Ecology*, **12** (1): 21-30

Ollertan, J. and Lack, A. 1998. Relationships between flowering phenology, plant size and reproductive success in *Lotus comiculatus*. (Fabaceae). *Plant Ecology*, **139**: 35-47.

Pal, P., Maity, R. G. and Bose, T. K. 1984. Effects of nitrogen, phosphorus and plant density on growth and yield of flower in *Valeriana officinalis*. *South Indian Hort*., **32**(3): 146-150.

Pande, A., Uniyal, G. C. and Shukla, Y. N. 1994. Determination of chemical constituents of *Valeriana wallichi* by reverse phase HPLC. *Ind. J. of Pharm. Sc*., **56**(2): 56-58.

Parveen, A. and Qaiser, M. 2007. Pollen flora of Pakistan-LVI. Valerianaceae. *Pak. J. Bot*., **39** (6): 1889-1896.

Polunin, O. and Stainton, A. 1987. *Concise Flowers of the Himalaya*. Oxford University Press, London, 255pp.

Poot, P. 1997. Reproduction allocation and resource compensation in male-sterile and hermahroditic plants of *Plantago lanceolata*. *Amer. J. Bot*., **84**: 1256-1265.

Powell, A. H. and Powell, G. V. N. 1987. Population dynamics of male *Euglossine* bees in Amazonian forest fragments. *Biotropica*, **19**: 176-179.

Prakash, V. and Mahrotra, B. N. 1994. Attributes of utility and scarcity of Valerianaceae in India. Ethnobiology in human welfare: Abstracts of the Fourth International Congress of Ethnobiology, Lucknow, U .P. India. 17-2, Nov. 122p.

Preciak, W. 2002. Seed size, number and habitat of fleshy fruited plant. Consequences for seedling establishment. *Ecology,* **83**: 294-800.

Primack, R. B. 1985. Patterns of flowering phenology on communities, populations, individuals and single flowers. In: white J, ed. The population structure of vegetation dordrecht. Dr. W. Junle Publishers, 571-593.

Proctor, M and Yeo, P. 1972. *Pollination of Flowers*. Taplinger, New York.187pp.

Proctor, M., Yeo, P. and Lack, A. 1996. *The Natural History of Pollination.* Lodon: Collins. 479 PP.

Pyke, G. H. 1978. Optimal foraging movement patterns theory: a critical review. *Ann. Rev. of Ecology and Syst.,* **15**: 523-575.

Raina, R. and Srivastava, L. J. 1992. Floral polymorphism in *Valeriana jatamansi Indian J. of Plant Genetic Resources*, **5**(2): 93-94.

Raina, R., Behera, M.C., Chand, R. and Sharma, Y. 2003. Reproductive biology of *Gentiana Kurroo* Royle. *Current Science*, **85**: 667-670.

Ramesh, M. N., Farooqui, A. A. and Tilak, S. 1989. Influence of sowing date and nutrients on growth and yield on Isbagol (*Plantago ovata*). *Crop Research*, **2**: 169-174.

Ramirez, S. C., Armesto, J. J. and Figueroa, J. 1998. Flowering, fruiting and seed germination in Chilean rain forest Myrlaceae: Ecological and phylogenetic constraints. *Plant Ecology,* **136:** 119-131.

Rao, P. S., Sethi, H., Randhawa, G. S. and Badhwar, R. L. 1977. Aromatic plants of India. Part XVIII family LX Valerianceae. *Indian Journal of Horticulture,* **34**(3): 329-333

Rathcke, B. J. and Jules, E. S. 1993. Habitat fragmentation and plant pollinator interactions. SAS institute, SAS/STAT users guide, version 6.4 edu.

SAS Institute, Carry, North Carolina, USA., *Current Science*, **65**: 273-277.

Rathcke, B.J. and Lacey, E.P. 1985. Phenological patterns of terrestrial plants. *Ann. Rev. of Ecology and Syst*., **16**: 179-214.

Richards, A. J.1996. A. breeding systems in flowering plants and to control of variability folia Geobotanica and phytol axonomica, apomixis and taxonomy. *Proceedings of Symposium*. **31**(3): 283-293.

Richards, A. J. 1986. *Plant Breeding Systems*. Allen and Unwin, London, U.K.

Richards, A. J. 1997. *Plant Breeding Systems*. London: Chapman and Hall. London, UK.

Robertson*, 1924. Phenology of entemophilous flowers. *Ecology*, **5**: 393-407.

Romaisa, N. 2004. Reproductive strategies of some pharmaceutically important species of genus *Digitalis* grown in Kashmir Himalaya. M. Phil. Dissertation, University of Kashmir, Srinagar (J&K), India.

Ross, M. D. 1970. Breeding systems in *Plantago*. *Heredity,* **25**: 129-133.

Ross, M. D. and Gregorius, H. R. 1985. Selection with gene cytoplasmic interactions II. Maintenance of gynodioecy. *Genetics*, **109**: 427-439.

Routly, M. B. and Husband, B. C. 2003.The effect of Botany on siring success in *Chamsion augustifolium* (Onagraceae) with different inflorescence sizes. *Evolution*, **57**: 240-248.

Samant , S. S., Dhar, U. and Rawal, R.S. 1996.Natural Resource use by some natives within Nanda Devi biosphere Reserve in West Himalaya. *Ethno Botany*, **8**: 40-50.

Schmitt, J. 1983. Individual flowering phenology, plant size and reproductive success in *Linanthus androsaecus*, a California annual. *Oecologia*, **59**: 135-140.

Shah, N. C. and Yadav, B. B. L. 1970. Behaviour of *Aconitum hetrophyllum* wall. (Ativisha) at low altitude. *J. Res. Ind. Medicine,* **5**: 119-124.

Shivanna, K. R. and Rangaswamy, N. S. 1992. *Pollen Biology*- A laboratory Manual, Narosa Publishing House New Delhi, Madras, Bombay, Calcutta, 21-37.

Shykoff, J. A. 1988. Maintenance of gynodioecy in *Silence acaulis* (Caryophyllaceae) stage specific fecundity and viability selection. *Amr. J. Bot.,* **75**: 844-850.

Siddique, M. A. A. 1991. Germplasm Assessment of rare and threatened medicinal plants of Kashmir Himalayas. Ph. D. Thesis, University of Kashmir, Srinagar, (J&K) India.

Siddique, M. A. A., Dar, N. A., Wafai, B. A. and Beigh, Y. S. 1997. Reproductive biology of *Podophyllum hexandrum* Royle (Podophyllaceae) an important, rare and threatened Himalayan medicinal plant proceedings of 6th annual session. *Nat. Acad. Sci. India*, Bhubaneshwar. PP. 10-11.

Singh, R. D, Ahuja, P. S., Nagar, P. K., Kaul, V. K., Singh, V., Lal, B., Vats, S. K., Yadav, P. and Mishra, S. 2005. Effect of manuring and shades on yield and quality of V. *wallichi*. *Journal of Medicinal and Aromatic Plant Science,* **22**(1B): 669-670.

Singh,V.P. and Purohit, S. 2003. *Research Methodology in Plant Sciences*. Scientific Publishers (India), Jodhpur, 1pp.

Sobral, M. 2000. *Valeriana eupatoria* (Valerianaceae), a New Species from Rio Grande do Sul, Brazil. *Novon,* **10**(2): 149-152.

Sollabanks, R. S. 1992. Fruit fate, frugivory and fruit characteristics: a study of hawthorn, *Crataegus monogyna* (Rosaceae). *Oecologia,* **91**: 296-304

Sowing, P. 1989. Effects of flowering plants patch size on species composition of pollinator communities, foraging strategies and resource partitioning in bumblebees. (Hymenoptera: Apidae). *Oecologia*, **78**: 550-558

Stamp, N. E., 1990. Production and effect of seeds size in a grassland annual (*Erodium brachycarpum*, Geraniaceae). *Am. J. Bot*., **77**: 874-882.

Stebbins, G. L. 1951. Natural selection and differentiation of angiosperm families. *Evolution* **5**: 229-324

Stebbins, G. L. 1971. *Chromosome Evaluation in Higher Plants*. Arnold London.

Stefan, G. M. and Durka, M. 2007. Synchrony pulsed flowering; Analysis of the flowering phenology in *Juncus* (Juncaceae). *Annals of Botany* **100:** 1271-1285.

Steiner, K. E. 1985. Functional dioecism in the Malpighiaceae breeding system of *Spachea membranaceae* Cuatr. *Amr. J. Bot*. **72**: 1537-1543.

Stevens, D. P. and Damme, V. J. M. M. 1988. The evolution and maintenance of gynodioecy in sexually and vegetatively reproducing plants. *Heredity* **61**: 329-337.

Stevens, D. P. and Kay, Q. O. N. 1991. The distribution and reproductive biology of gynodioecy in the native flora of Britain and Ireland. Unpublished Manuscript. PP. 345-346.

Swanson, S. D. and Sohmer, S. H. 1976. The biology of *Podophyllum peltatum* L. (Berberidaceae), the May apple II. The transfer of pollen and success of sexual reproduction.*Bull Torrey. Bot. Club*, **103**: 223-226.

Thies*, P.W. 1966. Uber die Wirkostofee des Baldrians.2. Zur Konstitition der Isovaleriansaureester Valepotriat, Acetoxy Valepotriate Und Dihydro-Valepotriate.*Tetrahedron Letters* **11**: 1163-1170.

Thompson, P.A. 1974. Effects of fluctuating temperatures on germination. *J. Exp. Bot*., **25**: 164-175.

Titz, E. and Titz, W. 1981. Diploid and tetraploid medicinal *Valeriana* (Valerianaceae) in Switzerland and neighbouring areas. *Botanica J. D. Helvetica,* **91**: 169-188.

Torres, M. E., Iriondo, J. M. and Perez, C. 2002. Vulnerability and determinants of reproductive success in the narrow endemic *Antirrhinum microphyllum* (Scophulariaceae). *Amer. J. Bot*., **89**: 1171-1179.

Uniyal,M.R. and Issar,R.K.1967.Commercially important medicinal plants of Kanatal Forest Tehri-Garhwal. *Indian Forester* **93**(2): 107-114.

Vaarama, A. and Jaaskelainen, O. 1967. Studies on gynodioecism in the finish populations of G*eranium sylvaticum*. *Annales acedemiae Scientiarum fennicaee series Aiv*, *Biological* **108**: 1-39.

Valdeyron, G., Dominee, B. and Valdeynon, A. 1973. Gynodioecy: another computer simulation model. *Amr. Natr.* **107**: 454-459.

Van Damme, J.M.M. 1984. Gynodioecy in *Plantago lanceolata* L. IV. Fitness components of sex in different life cycle stages. *Evolution* **38**: 1326-1336.

Vashist and Kant, 1998. The physiology and biochemistry of seed development, dormancy and germination, *Elsevier,* **40**(4): 625-628.

Vats, S.K., Pandey, S. and Nagar, P.K. 2002. Photosynthetic response to irradiance in *V. jatamansi* Jones, a threatened under storey medicinal herb of Western Himalaya. *Photosynthetica* **40** (4): 625-628.

Vegis, A. 1964. Dormancy in higher plants. *Annu. Rev. Plant Physiol*, **15**: 185-215.

Verma, S.,Khajuria, A. and Sharma,P. 2011. Stylar movement in *Valeriana wallichi* DC. a contrivance for reproductive assurance and species survival. *Current Science,* **100**(8): 1143-1144.

Victor, R. 2010. Biodiversity Conservation in Oman. Current status and future options. *Oman Biodiversity,* 1-11.

Von Eickstedt, K. W. and Rehman, S. 1969. Psychopharmakologische Wirkungen von valeportriaten. *Arzeimeittel-Forshung* **19**: 316-319.

Wafai, B. A. and Nawchoo, I. A. 2001.Conservation strategies of some invaluable and endangered medicinal herbs of Kasmir. Proc. Golden Jubilee Symposium, University of Delhi, *Abstract*, 4pp.

Wafai, B. A. Siddique, M. A. A., Beigh, Y. S. and Dar, N. A. 1996. Reproductive biology of petris (*Aconitum heterophyllum* wall. ex. Royle; Ranunculaceae) an important rare and threatened Himalayan Drug Plants, Y.S. Parmar, University of Agricultural Science, Solan, H.P., India.

Wafai, B. A., Siddique, M. A. A., Nawhoo, I. A., Dar, N. A., Bhat, M. A. and Mohi-ud-din, G. G. 2005. Studies on reproductive biology of some endangered medicinal herbs as a prelude to their *ex situ* conservation. *J. Ind. Bot. Soc.*, **84**: 88-106.

Webb, C. J. 1984. Constraints on the evolution of plant breeding system and their relevance to systematics. In: grant W. F. (ed.) Plant Biosystematics. Academic Press, New York, NY, 249-270.

Weberling, F. 1970. In Hegig, Illustrate floravon mitterleuropa famillie Valerianaceae,. Carl Hansen, Munchen, Germany. 97-176

Weberling, F. 1989. *Morphology of Flowers and Inflorescences*. Cambridge University Press. New York. 1-2, 17-18, 319.

Weins, D. 1984. Ovule survivorship, brood size, life history, breeding systems and reproductive success in plant. *Oecologia* **64**: 47-53.

Widden, B. 1991b. Phenotypic selection in flowering phenology in *Senecio integrifolius*, a perennial herb. *Oikos* **61**: 205-215

Wilcock*, C. C. and Neiland, N. R. M. 2002. *Trends Plant Sci.*, **7**: 1779-1990.

Wilson, M. F. 1983. *Plant Reproductive Ecology*. A Wiley-inter Science Publication. John Wiley and Sons Inc. New York, 5-32.

Wyatt, J. 1981. A dictionary of economic products of India. *Oecologia*, **5**: 338-341.

Wyatt, R. 1982. Inflorescence architecture. How flower number, arrangement and phenology affect pollination and fruit set. *Amer. J. Bot*., **69**: 585 – 594.

Young, D. A. 1972. The Reproductive Biology of *Rhus integrifolia* and *Rhus ovata* (Anacardiaceae). *Evolution,* **26**: 406-414.

Zimmerman, M. 1981. Optimal foraging, plant density and the marginal value theorem. *Oecologia*, **49**: 148-153.

***-Not seen in original**