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Full Length Research Paper

Studies on ecology, habitats diversification and seed germination behavior of *Valeriana jatamansi* Jones: A critical endangered plant

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Valeriana jatamansi **is high value threatened medicinal plant of Himalayan region. In present study, ecology, phenology and seed germination of this species domesticated at different altitudes, viz., 1290 m, 1550 m, 1800 m and 2000 m in district Darjeeling, West Bengal, India, were observed.** ***Our study revealed that Valeriana jatamansi*** **was a glabrous and more or less pubescent herb, nearly 15 – 45 cm tall herb. The surrounding major associated tree species are Bedulo** ***(Ficus*** **spp*),* Dhale Katus *(Castonopsis indica),* Dudhilo (***Ficus nemoralis)***, Khanyu (***Ficus cordata***), *and* Laligurans *(Rhododendron arboreum).* Optimum survival of *V. jatamansi* across 1290 to 2000 m indicated their adaptability at a wider range of altitudinal zones. However, low seed germination percentage of *jatamansi* above 1800 m restricted their cultivation possibilities up to low altitudinal zones only. Further, flowering and fruiting periods in most of the species varied greatly amongst the domestication sites. On altitude wise, UBKV, Kalimpong was registered more percentage of germination throughout the experiment and was followed by Algirah, Lava Sherpa Gaon and Lava. Work at UBKV, Kalimpong revealed that highest seed germination was registered with the seed soaked with cow urine and showed parity with the pre chilled seed treatment, and significantly better than other treatments. Seed soaked with cow urine enhance 50.3 % more germination percentage over the normal seed sown. These preliminary results provide baseline information for selection of suitable cultivation sites, developing agro techniques and conservation and management strategy of *V. jatamansi*.**

Key words: Altitude, ecology, habitat diversification, medicinal plants, low temperature.

INTRODUCTION

Himalayan region is well known for high diversity of native, endemic, rare and endangered medicinal plants which are valued worldwide due to their unique active compounds of therapeutic use. Despite the fact, a very few medicinal plants have been adopted under commercial level farming. Among important parameters, study of phonological behaviour of any wild plant species, which is being targeted for cultivation, is a pre-requisite exercise; it is helpful in developing and standardizing agro-techniques of targeted species. Identification of phonological stages is very crucial (Sanz Cortes et al., 2002). *Valeriana* is distributed through temperate and cold regions of Northern Hemisphere (Bell, 2004). *Valeriana jatamansi* is one of the most important medicinal herb of the western to eastern Himalayan grown at an altitude of 1200 - 2000 m. It belongs to family Valerianaceae, which is distributed in all the temperate ranges except Australia (Bennet, 1987) at an altitude of 1500 - 3000 m above sea level. It is an important medicinal herb of eastern himalayas 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. *V. jatamansi* Jones syn. *V. wallichi* Bennet1 (987), popularly known as Indian Valerian (English), Mushkibala (Kashmiri), Sughanthdawal or Tagar in Sanskrit (Raina and Srivastava, 1992). The species is being labeled as critically endangered due to over-exploitation of rhizomes for its medicinal value habi-

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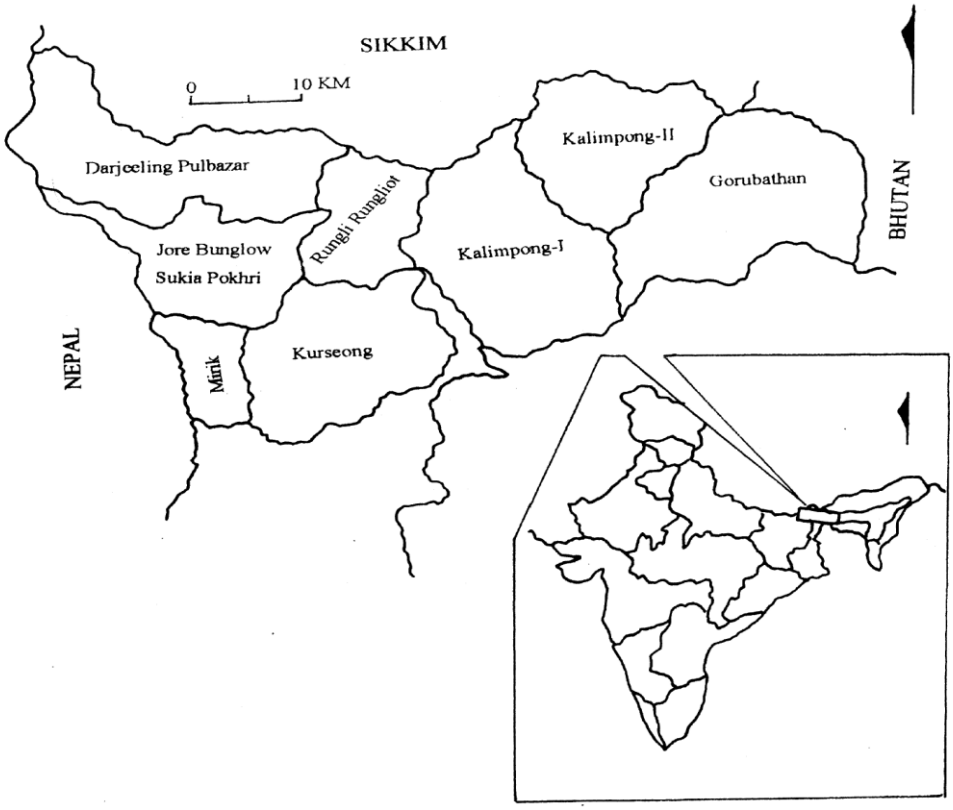

Figure 1. Area of study.

tat degradation and other biotic interferences in its distribution ranges (Polunin and Stainton, 1987). Like many other non-timber forest products (NTFPs), this critically endangered plant is taken as forest gift and hence there is neither any control system in its harvest nor its domestication. Locally it is being used for medicinal purpose especially for headache and eye trouble (Mukherjee, 2009). 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 *V. jatamansi* in its schedule for conservation and additionally, it has been listed an endangered species on the list of National Medicinal Plant Board, New Delhi, India (www.nmpb.nic.in).

Morphology in *V. jatamansi* is of great interest for its impressive diversity of forms, mainly resulting in adaptation to wide range of ecological conditions, concerning both vegetative and reproductive forms. This diversity has been well studied from the inflorescence structural perspective, with different forms and levels of complexity by (Weberling, 1989 and Hidalgo, 2004). The unique character of family is the pattern of four different stamen numbers in a series wherein mainly four are found in *Patrinia* and *Nardostachys*, three in *Plectritis*, *Valerianella* and *Valeriana*, two in *Fedia* while in *Centranthus* have only one stamen (Donoghue, 2003). Valerianaceae exhibits a considerable diversity in flower and fruit morphology (Erickson, 1989). This implicitly assumes particularly habitat diversification as well as prevailing climatic conditions have prominent effect on plant morphological characters and seed germination behavior, and its progeny in response to altered environmental conditions. The species *jatamansi* inhabit diverse habitats of Darjeeling - Sikkim Himalaya, so it was thought worthwhile to undertake detailed ecology and enable the species to survive in these varied habitats. 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). The relative response of the *V. jatamansi* for sprouting duration and sprouting percentage was in most cases different both between and among locations. Review of literature indicates that our understanding on phonological behavior of medicinal plants under cultivation is very poor (Butola, 2009; Vashistha et al., 2010). The species *V. jatamansi* inhabit diverse habitats of Darjeeling – Sikkim Himalaya, so it was thought worthwhile to undertake detailed plant behavior that enables the species to survive in these varied habitats. Therefore, the present study was undertaken to examine the ecology, survival and pheno-

Table 1. Physico-chemical status of soil sample collected at different altitude level of study.

Different location	*pH*	*ECE (mho/cm)*	*Available (kg/ha)*			*Total N (%)*	*Organic C (%)*	*Organic matter (%)*	*C/N ratio*
			N	**P_2O_5**	**K_2O**				
UBKV, Kalimpong	5.7	0.21	368	24.9	231	11.25	2.03	3.69	13.25
Algirath	5.4	0.26	301	22.36	281	12.36	1.98	3.22	12.36
Lava Sherpa Gaon	5.1	0.33	258	16.96	284	10.25	1.98	3.56	11.36
Lava region	4.7	0.36	265	18.25	258	12.25	2.65	4.01	10.56

phases of *V. jatamansi* domesticated at different altitudes, along with seed germination pattern under different location.

MATERIALS AND METHODS

Study sites

An experiment was carried out during 2009 to 2012 at R.R.S (HZ, under the aegis of Uttar Banga Krishi Viswavidyalaya, Kalimpong I (1290 m asl), West Bengal, India was carried out, along with three other different location of Kalimpong II (viz., Algarah, Lava Sherpa Gao and Lava).

An extensive exploration of different Darjeeling Himalayan habitats was carried out to identify the specific above areas across different geological conditions covering a wide range of habitats (Figure. 1). Among various populations four sites were selected for further studies on the basis of their accessibility of the site, habitat structure and plant density. All the area has different forest land-use types are available consisting of old-growth forests (with little to high human interference), forests degraded by logging, derived woodlands (grazing areas), agro forests, degraded areas, naturally re-growth forests and plantation forests (reforestation areas). Duplicate soil samples were collected from each site up to a depth of 5 cm. The air-dried samples were analyzed for physical and chemical characteristics. The soil of the experimental site was sandy loam having variable pH and other physicochemical properties (Table 1).

Biology and Phenological behaviour

Biology and phenological behavior of plant species provides information about morphological and functional attributes, which are useful to understand adaptation features (Nautiyal et al., 2001b). Flowering and fruiting are most critical stages of life of a plant species. From Darjeeling himalaya *V. jatamansi* was sampled from its natural habitats and all the prerequisite parameters were assessed for three years and were compared with plants growing at Uttar Banga Krishi Viswavidayalaya, Kalimpong (*ex situ* conditions). Two experiments were conducted first one was to study the comparative features of *V. jatamansi* at four different location and second one on seed germination behavior with three treatments [viz. normal seed, pre-chilled treated seed (- 4 $^{\circ}$C) and cow urine treated seed (soaked 12 hr)] at various altitude level under different agroclimatic condition. Morphological characters along with other parameters were analyzed under *ex situ* (UBKV) conditions with a mean temperature of 33± 3 $^{\circ}$C and an altitude of 1290 m amsl. These characters were compared with natural populations of Algirah (mean temperature of 18 ± 3 $^{\circ}$C and an altitude of 1550 m amsl), Lava Sherpa Gaon (mean temperature of 14 ± 3 $^{\circ}$C and an of altitude 1800 m amsl) and Lava (mean temperature of 10 ± 3 $^{\circ}$C and an of altitude 2000 m amsl). From these respective populations 20 individuals were tagged at each study site and were examined. Flower structure from all study areas were analyzed under zoom stereo-microscope. The stigma receptivity and flower anthesis was also analyzed in all the studied sites. Stigma receptivity was analyzed by using lactophenol and cotton blue. Stigmas with germinated pollen grains were labeled as receptive. Flower anthesis was checked out with respect to altitude and temperature. Flowers morphotype with unique adaptability in each population was analyzed with respect to temperature.

Seed germination

For seed germination study mature seeds were collected during Feb - March 2009 and kept in paper bags of after proper labeling. The fruits were shade dried in open air and gently hand shaken while inside the paper bag. The minute clean seeds fall and settle in the bottom of paper bags. These fallen seeds were collected and stored in an open container. This process was repeated till the seed were almost fully collected. Finally the remaining fruits (some left over seeds) were hand crushed and passed through a fine nylone siever and stored in a separate but

similar container. This contains impurities like vegetative, clay particles and other foreign materials of almost similar size. This seed material was kept in desiccators with silica gel to avoid moisture absorption. Each year fresh seeds were collected and sown into seed bed, as per treatment at different altitude levels. During the investigation, seed were surface sterilized by dipping them in 0.5% aqueous solution of $HgCl_2$ for 2 min to kill bacterial and fungal contamination (Bhatt et al., 2009) and then rinsed thoroughly (four times) with distilled water and then proceed as per treatment. The experiments were laid out in randomized block design with three treatments (normal seed sown, pre-chilled seed and seed soaked with cow urine) and replicated seven time. Sowing of seed in a nursery bed was done 25th April each year under careful observation with finally sieved and well sterilized (in autoclave) clay soil. For seed germination investigations 200 seeds were taken with triplicate in 0.50 x 0.50 m bed size (open field condition) to standardize best treatments for maximum germination. Seed germination potential under different treatments set were tested using randomized block design (Gomez and Gomez, 1984).

Data collection and analysis

Data were collected at 15 days after seed sowing. Observation mainly confined on seed germination percentage, days require for onset of germination and days required for completion of germination. These data were subjected to tests of analysis of variance. After rejecting null hypothesis of equal means using ANOVA F-test. Fisher's LSD (Least Significant Difference) was used for comparing treatment group means at 0.05. Ag Res (version 3.01) statistical software packages was used for study purpose. Values were means ± standard deviation.

RESULTS AND DISCUSSION

Biology and Ecology

During the present investigation the species was found sporadically distributed in the mountain ranges of Darjeeling Himalayas confined to sub-temperate and temperate regions, thriving in moist shady slopes, rocky slopes, land slide areas ranging in an altitude of 1200-3000m asl. 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. The species produce two types of leaves- basal (radical) leaves which arise from rhizomatous portion and cauline (middle and apical) leaves which arise from the stem. Among this shade inhabiting plants showed maximum leaf dimensions. 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. 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 showed better plant development.

The Darjeeling Himalay, extending amongst Khurseong, Kalimpong and Darjeeling subdivision. This part of Himalaya is credited all over the world as a treasure of medicinal plants (Mukherjee, 2009). Himalaya is ranked as one of the bio-diversity hot spots owing to its considerable abundance of medicinal plants. Among these medicinal gems *V. jatamansi* (Valerianaceae) is a species with tremendous medicinal importance adding color to the crown of Himalayas. Our study revealed that *V. jatamansi* was a glabrous and more or less pubescent herb, nearly 15 - 45 cm tall herb. This was perennial a tufted, hairy herbaceous perennial, gynodioecious herb with hermaphrodite plants ranging from 13 - 35.70 cm in height and female plants found usually dwarf than hermaphrodite with heights from 10 - 30 cm. The plant was characterized by thick horizontal rhizome with diameter ranging from 2 - 4 cm with roots ranging from 10 - 37 per stock. Basal radical leaves were long stalked, deeply cordate-ovate, usually toothed or sinuate up to 3 - 8 cm long and 2.45 - 8.50 cm broad. Radical leaves were persistent, long petioled, deeply cordate-ovate, usually toothed or sinuate. There were only a few cauline leaves which are much smaller, entire or pinnate. Flowers were often dioecious, white or tinged with pink, in a terminal corymb. Fruits were oblong, compressed, hairy or glabrous. Rootstocks were thick and horizontal, several erect stems of 15 - 45 cm. Long petiole bears white or pinkish flowers in a terminal comb. Cauline leaves were only a few, much smaller, entire or sometimes pinnate of 1.70 - 2.32 cm in length and 1.40 - 2.50 cm in breadth. Flowers were white or tinged with pink in terminal corymbs with 8 - 13 female flowers per inflorescence and 8 - 14 hermaphrodite flowers per inflorescence which were larger and broader than female flowers and ranging from 0.20 - 0.50 cm across. Calyx was represented by inwardly curved ring which opens into plumose pappus at fruit setting stage. However, corolla was five lobed with rotate, white or pinkish depending on the availability of light. Hermaphrodite and female plants do not differ much in vegetative characters but can only be segregated during flowering phase, as flowering axis was seasonal which dries up during senescence. These female and hermaphrodite plants can be segregated by quantitative flower morphological features. This plant was found mostly in the edge of bariland (un-irrigated agricultural field in upland). Flowering time of this herb is March - June. The surrounding major associated tree species are Bedulo (*Ficus* spp), Dhale Katus (*Castonopsis indica*), Dudhilo (*Ficus nemoralis*), Khanyu (*Ficus cordata*), and

Table 2. Comparative features of *Valeriana jatamansi* at four studied sites.

Sl. No.	Parameters	UBKV, Kalimpong	Algirath	Lava Sherpa Gaon	Lava region
1	Flowering period	25th February -15th April	15th March – 30th April	15th May-15th June	15th May - 15th July
2.	Flower anthesis	Female flowers anthesize 9-12 days earlier than hermaphrodite flowers	Female flowers anthesize 7- 9 days earlier than hermaphrodite	Female flowers anthesize 2 - 3 days earlier than hermaphrodite	Usually female and hermaphrodite flowers open at same times
3.	Mean temperature	33 ± 3°C	18 ± 3°C	14 ± 3°C	10 ± 3 °C
4.	Altitude	1290 m	1550 m	1800 m	2000 m
5.	Slope	Moderate	Moderate	Moderate to steep	Steep
6.	Stigma receptivity	6 - 7 day earlier	4 - 5 day earlier	2 - 3 days earlier	3 - 4 hour earlier
7.	Flower morphotypes	Much prevalent	Much prevalent	Prevalent	Less prevalent
8.	Habitat	Open plain	Open, plain with partial shade	Shady rocky slopes	Shady moist slopes
9.	Threat factor	No any threat factor, but lack of knowledge leads to its poor adaptation	Grazing, extraction, Habitat degradation and fragmentation	Grazing by animal etc and deforestation	Grazing, extraction, Habitat degradation and fragmentation

Laligurans *(Rhododendron arboreum).* Mostly found in north-facing slopes of *Rhododendron* forests. 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 tax on 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. 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).

Comparative features study under different location

During the present investigation the species was found sporadically distributed in the mountain ranges of Darjeeling 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 - 2200 m asl. Phenological behavior of plant species provides information about morphological and functional attributes, which are useful to understand adaptation features (Nautiyal et al., 2001). Flowering and fruiting are most critical stages of life of a plant species. In present study, flowering and fruiting in *V. jatamansi at* 1290 m asl took place two and half months earlier as compared to that at 2000 m. Such variation attributed to varied environmental conditions (temperature, humidity, rainfall, light, etc.) among domestication sites. Along altitudinal difference, the temperature is a major factor which is the main determinant of phenological plant development (Worral, 1993). Phenological and phenomenological variations of the plants are the product of interaction between genotype and environment. However, these modifications in plants may be reversible when plants are grown under diverse climatic conditions (Bhatt and Purohit, 1984). *V. jatamansi* is highly adaptable to environmental conditions and occurs in pockets with 8 - 12 individuals per pocket. In UBKV, out of 50 female individuals, 15 - 20 % anthesize earlier than hermaphrodite ones, while at Algirah out of 50 female individuals only 8 - 10% female individuals show prior anthesis (7 - 9 days earlier) and at Lava Sherpa Gaon population 4 - 5 % female individuals anthesize earlier (2 - 3 days earlier) and at Lava population < 1 % female individuals anthesize earlier. The anthesis of female flowers prior to hermaphrodites in four different sites seems to be temperature dependent.

Table 3. Effect of different hormonal treatments on seed germination.

Different location	*Treatments*	*Germination (%)*	*Days require for onset of germination*	*Days required for completion of germination*
Lava region	Normal seed sown	12.36 ± 1.02	62.66	76.91
	Pre-chilled seed	21.00 ± 3.12	71.00	77.00
	Seed soaked with cow urine	26.66 ± 2.06	60.33	74.33
LSD (*P* = 0.05)		7.73	3.98	1.33
Lava Sherpa Gaon	Normal seed sown	13.65 ± 1.36	68.33	78.95
	Pre-chilled seed	12.00 ± 2.33	69.00	74.33
	Seed soaked with cow urine	19.66 ± 3.02	65.00	72.66
LSD (*P* = 0.05)		4.32	2.91	2.56
Algirath	Normal seed sown	40.00 ± 2.11	51.23	77.33
	Pre-chilled seed	50.00 ± 4.10	38.66	44.00
	Seed soaked with cow urine	53.66 ± 3.21	23.00	35.00
LSD (*P* = 0.05)		8.11	4.33	5.98
UBKV, Kalimpng	Normal seed sown	42.00 ± 4.10	18.66	34.00
	Pre-chilled seed	59.66 ± 3.01	64.33	74.66
	Seed soaked with cow urine	63.15 ± 4.12	41.66	63.33
LSD (*P* = 0.05)		8.91	6.08	6.69

The individuals growing at UBKV depicted stigma receptivity of female individuals on 6 - 7 day of anthesis, which usually anthesize 9 -12 days earlier than hermaphrodite flowers. At Algirath the female flowers anthesize 4 - 5days earlier and were observed receptive on 7th days after anthesis. Further, at Lava Sherpa Gaon stigma receptivity of female individual on 2-3 days of anthesis, which usually anthesize 2 - 3 days earlier. However, at Lava usually both hermaphrodite and female flowers anthesize at same time (Table 2). Present study revealed that temperature has a prominent effect on anthesis and breeding behavior in the species.

Seed Germination

Seed germination study revealed significant improvement in germination percentage with cow urine treated seed compared to rest other two treatments at different altitude and temperature variation (Table 3). Amongst various treatments at lava region application of seed soaked with cow urine gave maximum seed germination percentage compared to normal seed sowing practices, and was at par with the application of pre-chilled seed treatments. This treatment enhances 115.6 % more germination percentage over the normal seed sown. Further, our observation revealed that seed soaked with cow urine took least time for onset of germination compared to rest of the treatment, and showed parity with the normal seed sowing. Completion of germination was faster registered with the seed treatment with cow urine (74.33 days), and took least time compared to other two set of practices. However, no significant statistical difference was observed amongst these treatments. In the present study, percentage of seed germination, days required for onset and final germination showed difference under different treatments. Such difference was common for many mountain species (Nautiyal et al., 2001).

Amongst various treatments at Lava Sherpa Gaon, seed soaked with cow urine gave maximum seed germination percentage, and was significantly superior to other two set of practices. However least seed germination was registered with the pre-chilled seed treatments. This treatment enhances 63.8 % more germination percentage over the pre chilled seed treatment. Seed soaked with cow urine took least time for onset of germination compared to rest of the treatment and statistically superior to other set of treatments. Days require for completion of germination was faster registered with the seed treatment with cow urine (72.66 days), and took least time compared to other treatments. Further, observation revealed that this treatment was at par with pre chilled seed treatment.

Days require for onset of germination was quite long in case of Lava Sherpa Gaon and Lava, this was due to extreme low temperature delay the sprouting, and this ultimately delay the whole process of germination; this corroborate with the finding of (Sharma et al., 2005).

Under Algirath condition, seed soaked with cow urine gave maximum seed germination percentage compared to normal seed sowing practices, and was significantly superior to other treatments. However least seed germination was registered with the application of normal seed sown. Seed soaked with cow urine enhance 34.1 %

more germination percentage over the normal seed sown. Further, our observation revealed that seed soaked with cow urine took least time for onset of germination (23 days) compared to rest of the treatment, and was significantly better than other treatments. Completion of germination was faster registered with the seed treatment with cow urine (35 days), and took least time compared to other two set of practices, and significantly better to other treatments.

Work at UBKV, Kalimpong revealed that highest seed germination was registered with the seed soaked with cow urine and showed parity with the pre chilled seed treatment, and significantly better than other treatments. Seed soaked with cow urine enhance 50.3 % more germination percentage over the normal seed sown. Seed treatment always favors good germination with short span of time (Choudhary et al., 1996). Further, our study revealed that normal seed sown took least time for onset of germination (18.66 days) and completion of germination (34 days) compared to rest of the treatment, and significantly least compared to other two set of practices, this corroborate with the finding of (Prakash et al., 2011).

Amongst all altitude range, maximum germination percentage was reported at UBKV, Kalimpong, this might be high temperature at the time of germination period, this will ultimately help to more sprouting and enhance germination percentage to 63.15%. However, further study revealed that on altitude wise, UBKV, Kalimpong was registered more percentage of germination throughout the experiment and was followed by Algirah, Lava Sherpa Gaon and Lava.

This treatment registered improved percentage of 136, 68.86, 15 % more compared to seed treatment with cow urine at Lava, Lava Sherpa Gao and Algirah situation, respectively. Our observation at different location revealed that days require for onset of germination was quite long in case of Lava Sherpa Gaon and Lava, this was due to extreme low temperature delay the sprouting, and this ultimately delay the whole process of germination; this corroborate with the finding of (Sharma et al., 2005).

CONCLUSION

Valeriana jatamansi was a glabrous and more or less pubescent herb, nearly 15 – 45 cm tall herb. Optimum survival of *V. jatamansi* across 1290 to 2000 m indicated their adaptability at a wider range of altitudinal zones. However, low seed germination percentage of *V. jatamansi* above 1800 m restricted their cultivation possibilities up to low altitudinal zones only. Further, flowering and fruiting periods in most of the species varied greatly amongst the domestication sites.

Medium altitude favors more percentage of germination and early onset of germination.

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