Development of Agro-technology for the Cultivation and Conservation of *Arnebia benthamii* - A Critically Endangered Medicinal Plant of North West Himalaya

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Abstract: The domestication of *Arnebia benthamii* was carried out successfully at low altitude. The species was exposed to a varied complex of conditions to screen conducive conditions for the cultivation and ex-situ conservation of the species. The species performed well under sunny conditions and grew nicely in soils of varied textures. The maximum survival was achieved on loamy soil followed by sandy and clayey textures. The species can be grown on acidic as well as alkaline soils within a PH range of 5-9. The species requires a good amount of moisture and therefore plants under exposed sunny conditions need to be irrigated twice a week. Frequent irrigations under shady conditions mar the survival of plants. Besides, waterlogging also proves detrimental for the species. The addition of fertilizers enhanced the herbage yield, with NPK mixture displaying maximum value in plants grown on loamy soil. Under sunny conditions in the hot summer input of potassium fertilizers reduced wilting and number of leaves showing wilting. Plants treated with potassium fertilizers showed luxuriant growth during hot summer. The cultivation time is from March to July with March to May being the ideal period of transplantation. For raising nursery, seeds should be sown to a depth of 0.5 cm in a 1:1 sand soil mixture.

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1. Introduction:

The Valley of Kashmir is situated in the lap of Western Himalayas. Himalaya is credited all over the world as a treasure of medicinal herbs providing refugee to these plants in its varied ecosystems (3, 13). The alpine and sub alpine zones of Himalaya host a remarkably rich wealth of medicinal plants viz. *Aconitum heterophyllum, Podophyllum hexandrum, Inula racemosa, Taxus baccata, Rheum emodi, Picrorhiza kurroa, Arnebia benthamii and Saussrea costus*, etc. These plants have proved of tremendous medicinal potential, serving as a source of wonder drugs and withstanding the test of times (14). However, this biodiversity hot spot is experiencing a tremendous anthropogenic pressure in terms of overexploitation, overgrazing, habitat destruction, habitat fragmentation and degradation (6, 13). These biotic interferences have resulted in the depletion, dwindle and decline of this medicinal wealth (13). As a result, a considerable fraction of this bio-wealth is threatened with extinction, therefore, demanding and deserving conservation (3, 4).

For the last two decades there is a growing trend of domesticating and cultivating medicinal plants(8). Domestication serves twin benefits of (a) conservation and (b) source of plant material for drug extraction, relieving the pressure of collection and exploitation from the wild populations. Cultivation of medicinal plants is encouraged all over the world in order to save them from extinction (8, 10).

Arnebia benthamii (Boraginaceae) commonly called as 'Kahzaban' is a perennial medicinal herb growing in the sub alpine and alpine zones of North West Himalaya. It finds use in the treatment of tongue and throat ailments, fevers, and heart ailments, and as antiseptic. The species witnesses tremendous decline in its populations (both in size and number) as a result of overexploitation and overgrazing (13). This biotic stress has brought the species to the brink of extinction, which calls for immediate conservation and cultivation. In order to domesticate the species at low altitudes, there is a need for the development of specific agro-techniques which aim at screening and selecting conducive conditions for their survival and growth. The present study was aimed at developing such an agro-technology for the cultivation and conservation of *Arnebia benthamii.*

2. Materials and methods

2.1 Collection and Domestication

For domesticating the species the propagules in terms of rhizomes and seeds were collected from many natural populations. They were transplanted into field and pots furnishing varied climato-edaphic conditions to screen the appropriate conditions for their survival and performance in terms of vigor and yield (Herbage). The seeds were germinated in 1:1 sand soil mixture. The seedlings were transferred into pots with varied soil texture and reaction. The pots were kept under different set of conditions. Each set of pots consisted of 11 pots, with replicates for each nutrient treatment and a control. The above said activity of domestication and establishment of Gene bank was carried out at the Botanical Garden of Kashmir University (1450 m amsl).

2.2 Soil Texture and Soil pH

The texture of soil in the field was determined by the finger feel method, besides different textures for the study were prepared by mixing sand, clay and silt classes in different desired proportions. The soil pH was determined by using pH meter.

2.3 Soil organic carbon

Soil organic carbon was determined by Walkly and Black Method.

2.4 Plant Performance

The plant performance was measured in terms of plant height, number of leaves and then the biomass. After complete annual growth, the plants under different set of conditions were harvested, dried and then weighed. The climato-edaphic conditions in which plants showed maximum survival, growth and multiplication were recorded as ideal and conducive ex-situ conditions for the cultivation and conservation of species.

3. Results

For domesticating *A. benthamii* a complete agro technology was developed. The various eco edaphic conditions were screened which could result conducive for the growth and survival of the plants. The domestication of the species was carried out along the following lines:

3.1 Soil texture-Pot trials

The plants of *A. benthamii* grow nicely on soils of different textures. The plants thrived well on sandy, loamy, clayey, sandy loam, clayey loam textures. Under open sunny conditions the maximum survival of plants, 90% is witnessed in loamy texture, followed by 70% and 60% in sandy and clayey soils respectively as depicted in the graph.

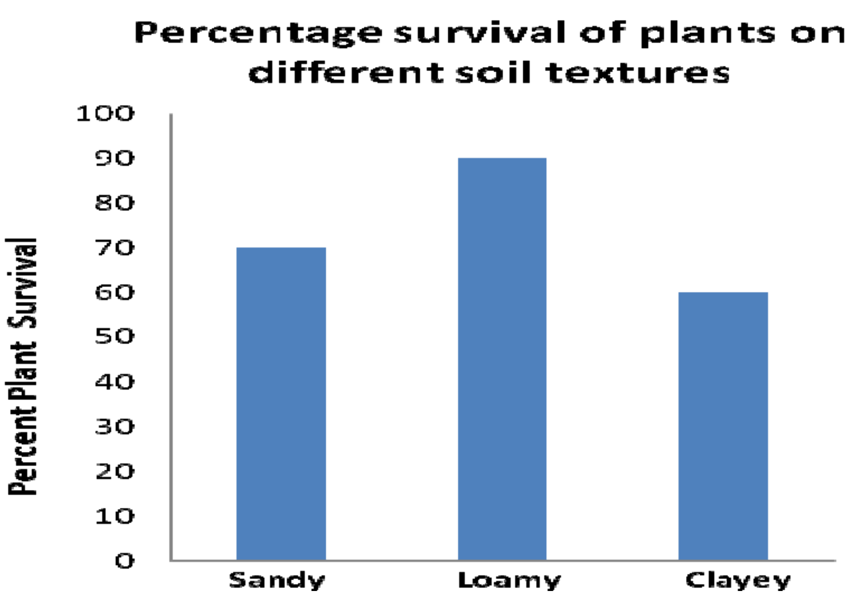

Figure 1: Survival of *A. benthamii* on different soil textures

In our present investigation, the plants of *Arnebia benthamii* show preference for sunny condition as compared to shady condition, as depicted by the survival percentage of plants.

3.2 Slope and Soil Reaction

The plants can be grown or cultivated on both sloppy as well as flat land with different soil textures and reactions as shown in Table

Table 1: Soil characters of different natural habits of *A. benthamii* in comparison to *ex situ* habitat

Characteristics	Natural habitat, Panchal, Nagabarn	Soil used for different treatments (pot trials).			Botanical Garden
Texture	Silt loam, sandy	Sandy	Loamy	Clayey	Clayey loam
PH	5.8-6.0	8.6	8	7.8	7
Organic C%	3.9%, 7.2%	0.3%	1.44%	1.68%	1.49%
Organic matter	6.72% -7.24%	0.517%	2.48%	2.89%	2.56%

Table 2: Soil characteristics of different sub alpine and alpine sites where *A. benthamii* is growing

S. No	Site	%Organic Carbon	P_H	Nitrogen
1	Panchal	7.20	5.80	0.53%
2	Munwarsar	10.13	5.67	0.81%
3	Gulmarg Aparwat	6.69	5.74	0.55%

3.3 Irrigation

The studies revealed that under ex situ conditions plants should receive irrigation at least twice in a week. However during summer months plants need to be irrigated on need basis, with irrigation incidence sometimes shooting up to 5 times a week. This holds true for plants growing under open sunny conditions. Under shady conditions frequent irrigation deters survival. However under shady conditions survival is jeopardized when irrigation is carried out daily. Under shady conditions irrigation after intervals of three days and one week gave good results of survival-70% and 90% respectively.

3.4 Water logging

Water logging is detrimental for the species resulting in rhizome rotting. The effects are reflected much earlier by plants grown in shade as compared to those growing under sunny open condition.

3.5 Time of Cultivation

The plants can be transplanted from mid- March to July. However the studies revealed that the period between March to May is best for the cultivation of the species.

3.6 Seed Sowing

For raising plants in nursery, the seeds can be sown from March to July. The seeds should not be kept deep inside the soil, a depth of 0.5 cm is appropriate. A 1:1 sand - soil mixture proved ideal for germination. Seeds sown along with fruit wall witness a delayed germination pattern (2-3 months to germinate) as compared to those sown without fruit wall which usually germinate in 25-45 days. However the germination time was reduced successfully to just 15 days by pre-treating seeds with 100 ppm Kinetin. Sowing seeds in March result in maximum percentage germination-75% with 66% seedlings surviving to mature stages.

3.7 Propagation

The plants can be grown from seeds as well as by using vegetative propagules. At maturity, in ex-situ conditions the plants produce about ten vegetative clums or buds which can be separated and transplanted to multiply the species.

3.8 Nutrient Inputs-Pot Trials

Each plant responded positively to the application of nutrients which include N, P, K, NPK and Farmyard manure. However, on sandy soils with PH 8.6, the application of nitrogen as urea resulted in death of plants. On applying N as KNO_3, plants survived and showed normal growth.Besides, maximum biomass of plants was observed on loamy soil with NPK treatment. On clayey soils the plants treated with farmyard manures showed maximum biomass and maximum number of leaves. In sandy texture, plants treated with P and K showed good performance.

L- Light (sunny condition)
L-Texture
N-Nitrogen
K-Potassium
P - phosphorus
F_{yM} - Farm yard manure
P_{NK} -Mixture of NPK

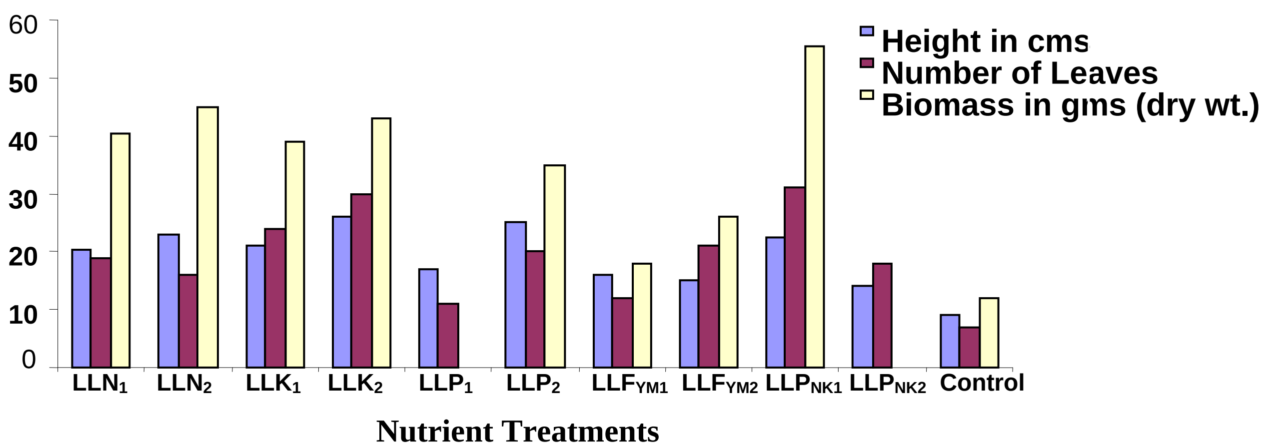

Figure 2: Response of the Plant to various nutrient treatments under Sunny conditions on Loamy soil.

L- Light (sunny condition)
C-Texture (Clayey)
N-Nitrogen
K-Potassium
P-phosphorus
F_{yM}-Farmyard
P_{NK}-Mixture of NPK

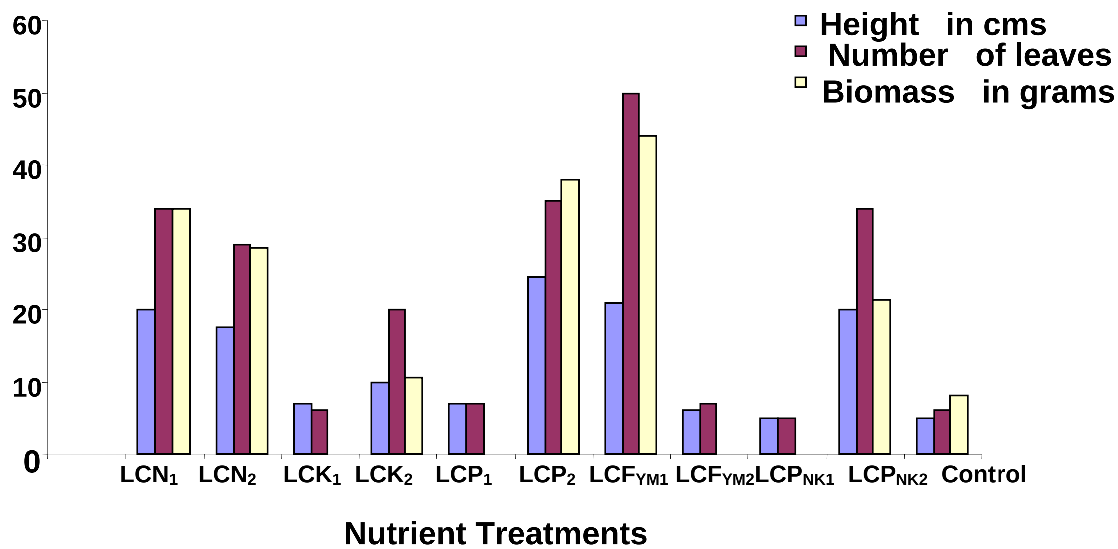

Figure 3: Graphic representation of various treatments and plant response under sunny conditions on clayey soils

L- Light (sunny condition)
S-Texture(Sandy)
N-Nitrogen
K-Potassium
P-phosphorus
F_{yM}-Farm yard manure
P_{NK}-Mixture of NPK

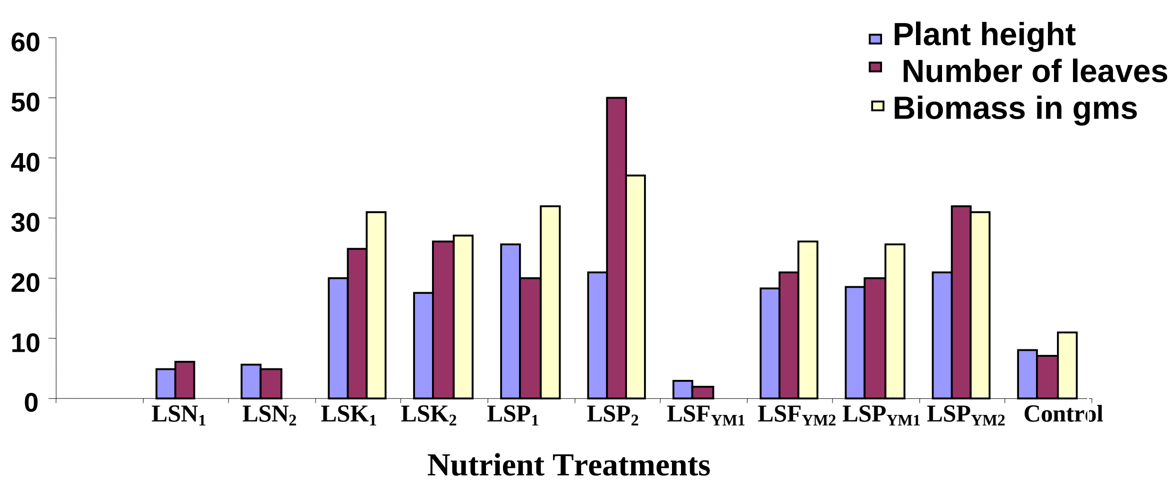

Figure 4: Effect of various nutrient treatments under sunny conditions on sandy soils

S- Shady
C- Clayey Texture
N-Nitrogen
K-Potassium

P-phosphorus
F_{yM}-Farm yard manure
P_{NK}-Mixture of NPK

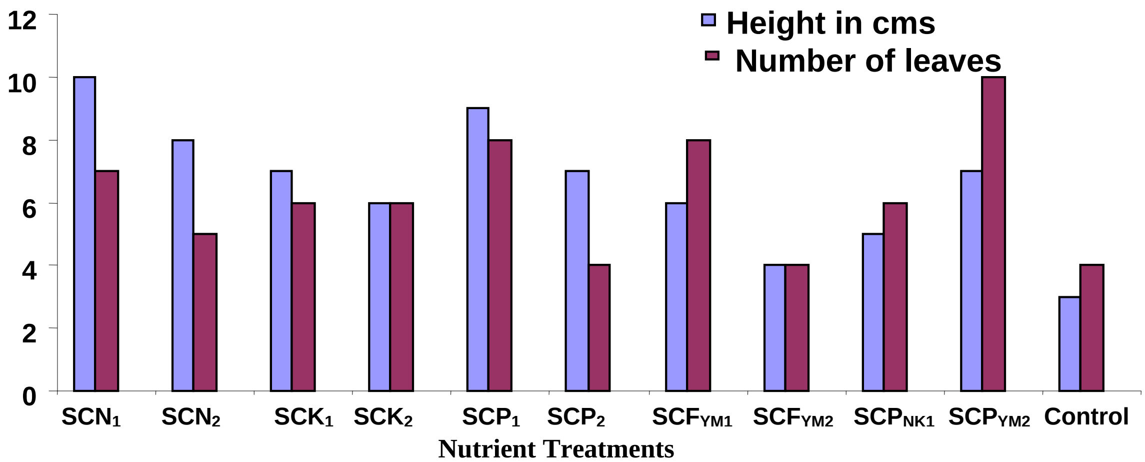

Figure 5: Graphic representation of various treatments and plant response under shady conditions on clayey soils. (Abbreviations:SCN-Plant grown under shady condition on clayey soil with Nitrogen inputs)

S- Shady
S-Sandy Texture
N-Nitrogen
K-Potassium

P-phosphorus
F_{yM}-Farm yard manure
P_{NK}-Mixture of NPK

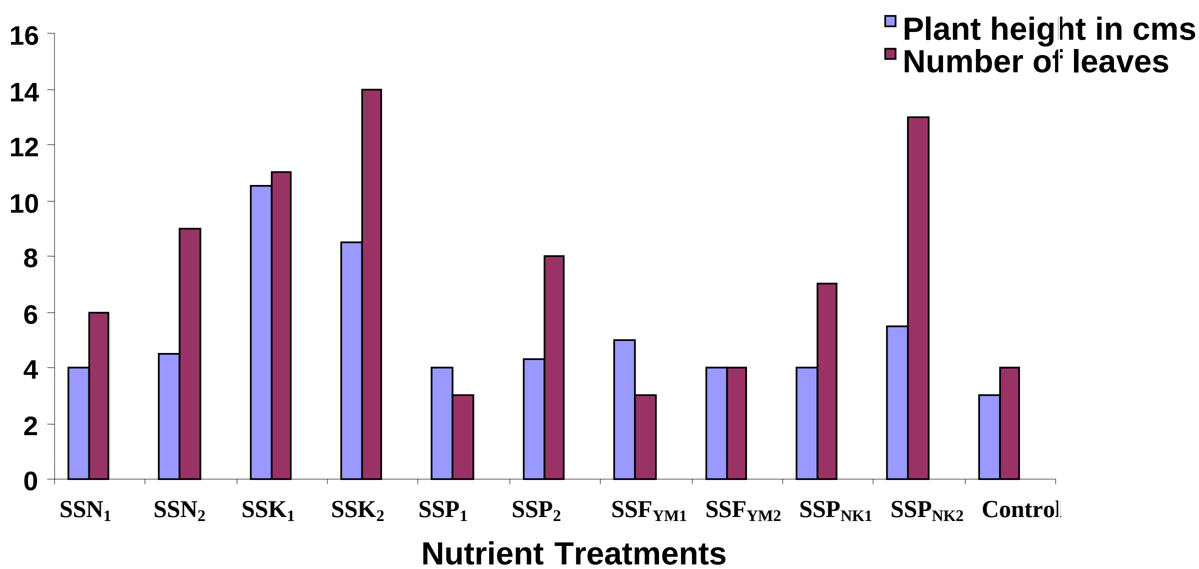

Figure 6: Response of plants grown under shady conditions on sandy soils to various nutrients treatments.

S- Shady
L- Loamy Texture
N-Nitrogen
K-Potassium
P-phosphorus
F_{yM}-Farm yard manure
P_{NK}-Mixture of NPK

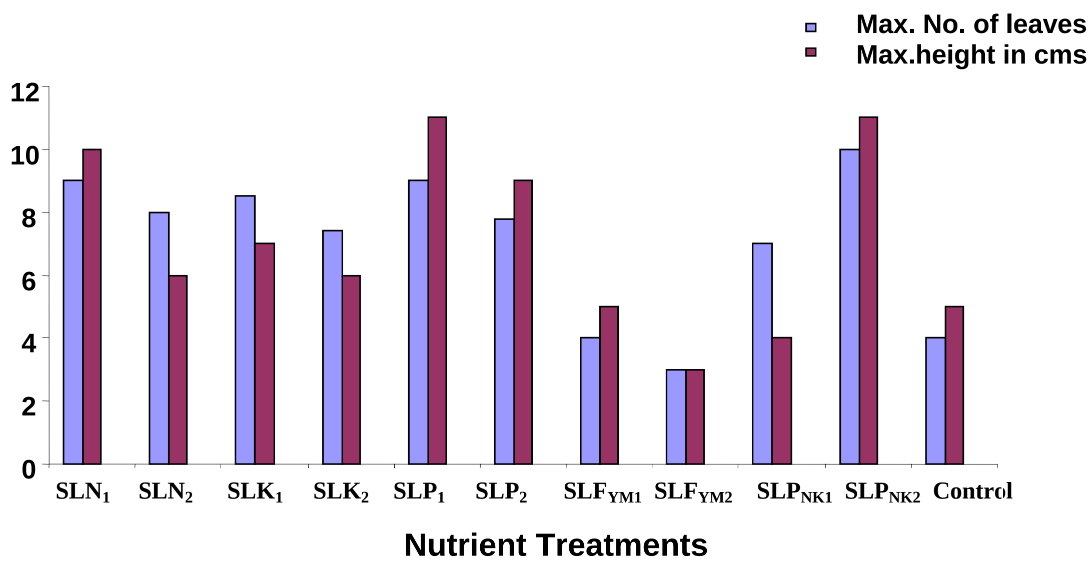

Figure 7: **Graphic representation of various treatments and plant response under shady conditions on loamy soils**

3.9 Potassium Fertilizers Inputs

Under sunny conditions in the hot summer K (potassium) fertilizers reduced wilting and number of leaves showing wilting. Plants treated with K fertilizers showed luxuriant growth during hot summer.

3.10 Pests

Under shady condition the green caterpillars of the insects order Lepidoptera were observed to feed voraciously on the leaves and stem axis of plants causing tremendous damage to them. However under sunny conditions no such attack was observed.

3.11 Spacing

For better growth of plants, a spacing of 40-50 centimetres is most appropriate.

4. Discussion

The successful introduction of a species and then its acclimatization are the two important aspects of a domestication process. The newly introduced plant species responds to the new environmental complex and its fate, survival or rejection (death) is determined by its ecological amplitude. The environmental complex (terrestrial) when analysed consists of edaphic factors (including soil texture, reaction, and nutrient conditions) light, temperature, precipitation, humidity, topography, biotic factors and a host of others. A plant species responds to these factors showing good amount of tolerance and demand for one factor and at the same time requiring the other factor in a narrow range. These specific requirements of a species showing temporal as well as spatial variation affect and are responsible for the distribution of the species. Thus a species growing on the alpines needs a specific set of environmental requirements (its ecological niche), which its habitat is fulfilling and making sure its survival and overall fitness. Such specific conditions are not met at low altitudes. Thus for cultivating such species growing on alpines at low altitudes, one needs creation of these conditions which should parallel those of its natural abode and falling within its tolerance limits. Therefore, agro techniques aim at screening, simulation and manipulation of conditions, which should prove conducive for the growth and survival of such species. Domestication of alpine plants like *Picrrhiza kurroa* has already been taken up (14) and according to him, these alpine plants pose a problem to get adapted at lower altitudes. A similar task of cultivating *Arnebia benthamii* (Kahzaban) an alpine herb, at low altitudes (1450m) was carried out and the results are encouraging.

The plants of this species performed well and showed excellent adaptability when grown at an altitude of 1450m (Botanical Garden of Kashmir University).This adaptability can be credited to its breeding/genetic system where tremendous variations spring out. Outbreeding species normally show a wide tolerance and variability range. *A.benthamii* being an out breeding species also displayed tremendous tolerance of varied eco-edaphic conditions. At low altitudes, the plants performed well under open sunny conditions, as compared to shady conditions. Besides the yield in terms of overall biomass is less under shady conditions, than in sunny open conditions. Similar results were obtained by Jayachandran *et al.,* (1991), while assessing the performance of *Zingiber officinale* under shade and open conditions.

Being a moisture loving plant, it requires good amount of moisture. The %age survival of plants under shady and sunny conditions is also related with the amount of moisture. Under shady conditions, irrigation after a week's interval results in highest survival rates of the plant, while in sunny open conditions during summer frequent irrigation(2-5 times in a week) is necessary for survival of plants. Under shady conditions daily irrigation drastically effected the survival of plants in sandy textured soils, possibly because of nutrient leaching and rhizome rotting. The impact of this daily irrigation under shady conditions, on the plants growing in other soils types was also negative resulting in mass death of plants, due to rotting of the underground rhizomes.

The plants under shady conditions are comparatively much more sensitive to water logging than their counterparts in the open sunny conditions. Thus water logging is detrimental for the survival of *A. benthamii* plants as that of several species of *Digitalis* as reported by Singh *et al.* (1991).

Of the different factors affecting plant life, edaphic factor is very important as far as plant recruitment and survival is concerned. In its natural habitat, the plants of *Arnebia benthamii* grow on sandy, loamy silt soils, with pH ranging from 5-6.5. However, at low altitudes (1450m Botanical Garden of Kashmir University) the plant was grown nicely on different textural classes with soil pH ranging from 6-9. Thus the plant enjoys and tolerates a wide range of soil texture and soil reaction. Similar results were obtained by Delabays (1990) in pot trials while working on *Arnica montana* in Switzerland.

Although the plant grows nicely on various textural classes, maximum plant survival and vigorous growth, (giving maximum herbage yield) of *A. benthamii* plants is witnessed in loamy textural class, under both sunny and shady conditions, possibly because this texture offers little or no resistance for rooting and less leaching of nutrients than clayey (difficult in rooting) and sandy soils (maximum nutrient leaching). Clayey and sandy soils showed survival rates of 70% and 60% respectively.

The application of fertilizers increases the herbage yield, plant height, number of leaves, overall biomass and longevity of growth period as compared to control. The plants of *A. benthamii* responded to each nutrient treatment. Of the different nutrients, K-fertilizer is very important during hot summer, conditions where plants show maximum wilting. K-fertilizer reduces the wilting of leaves, and the transpirational H_2O loss, keeping plants turgid for longer periods and thus resulting in luxuriant growth of these plants. On the other hand, N and P-fertilized plants also performed well but the plants provided with NPK mixture, attained maximum height, maximum herbage, maximum biomass and maximum number of leaves especially on loamy texture of soil. Similar results of NPK fertilizer on growth and yield attributes were observed by Ramesh *et al.*, (1989) on *Plantago ovata*, Sudeendra *et al.,* (1993) on Celery, Singh and Neoparly (1993) on *Zingiber officinale*.

Sandy textured soils with high pH (8.6) when treated with nitrogen in the form of urea, witnessed the death of plants (*A. benthamii*). After repeating the experiment several times same results were obtained. The possible reason causing the death of the plants is Nitrite toxicity. The added urea dissociates to form ammonium, which gets converted to Nitrite. Under these soil conditions nitrite gets accumulated proving toxic to the plants, thus causing their death. However, adding Nitrogen directly in the form of nitrates (KNO_3) the plants survive, grow nicely on these soil conditions (Sandy texture, high pH).

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