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# QUANTITATIVE ENHANCEMENT OF ACTIVE CONTENT AND BIOMASS OF TWO ACONITUM SPECIES THROUGH SUITABLE CULTIVATION TECHNOLOGY

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***Abstract***

*High altitude medicinal plants are facing problem due to their unsustainable utilization. So, the cultivation of these plants with appropriate technology may fulfill the demand of pharmaceutical industry and may also promote the conservation at their natural habitat. Highly important two species of Aconitum were studied for the enhancement of net profit by applying protected farming system. Both the experimental species were grown under natural and greenhouse conditions. Very positive results were obtained in plants of both Aconitum species grown under greenhouse as compared to natural conditions. Enhancement in yield was 12 and 9 times high in Aconitum heterophyllum Wall. ex Royle and Aconitum balfourii Stapf. respectively grown under greenhouse as compared to natural condition. Pseudoaconitine and aconitine were also observed high in greenhouse grown (0.51% and 0.42% respectively) than naturally grown plants (0.49% and 0.40% respectively). The quantity of atisine and aconitine was also found high (0.35% & 0.27% respectively) in greenhouse than naturally grown plants (0.19% & 0.16% respectively). It was noticed that plant height, leaf number, and average length of tubers were high in plants grown inside greenhouse in contrast to natural habitat. Almost five and two folds more tubers were found in A. balfourii and A. heterophyllum respectively in plants grown under greenhouse in comparison to natural conditions.*

***Keywords:*** *Aconitum balfourii; Aconitum heterophyllum; greenhouse; natural condition; active content; pseudoaconitine; aconitine; atisine.*

## Introduction

Presence of medicinal plants supports the existence of life on earth. These medicinal plants are generally occurring in wild and most of them propagate vegetatively [1]. Approximately 250.000 higher plant species on earth are medicinal [2]. In India, Nepal and Srilanka millions of people currently used Indian health care system known as Ayurveda [3].

Herbal medicine is used by so many people around the world due to the unpleasant side effects of the other drugs [4]. Data on herbal medicine for their quality and quantity are not sufficient to promote their use worldwide. Lack of appropriate research methods may be one of

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the reasons for this. Therapeutic effect of herbal medicines can be attributed to the interactions of multiple phytochemicals [5]. Modern medicines are formulated by these medicinal plants with various plant derived therapeutic agents [6]. Physiological behavior of plants is directly affected by the climate of a specific region. More biomass in plants reflects the compatibility of plants to their physical environment. The effect of environmental factors on plant's biological system recognized as an effective factor in plant growth and development [7]. Growth in plants may affect by different factors like physiographical factor, climatic agents and soil agents and they are very variable due to the variability of the region [8].

Plants are rich sources to search new active compounds that become a challenge to modern pharmaceutical industry and many synthetic modern medicines are originated from plant [9]. Plants have secondary metabolites that suppress the growth and development of surrounding biological systems named as allelochemicals [10]. Plant extract which contain phenolic and flavanoid compounds have antioxidant and antibacterial effects [11, 12].

Sufficient knowledge regarding herbs is very important to know their uses and potential of drugs [13]. Demand of the herbal drugs are increasing day by day but the supply is decreasing, so there is an urgent need to cultivate and conserve these valuable plant species for their sustainable utilization [14, 15]. Unscientific extraction of the medicinal plant from nature at commercial scale has pushed some species to extinct and some others are at an endangered stage. Taking into account the medicinal importance of the species and the lack of adequate cultivation practice, the study was carried out to get the maximum profit by enhancing the productivity of the species during the cultivation.

## Materials and Methods

### *Growing conditions*

Fifty plants each of *Aconitum balfourii* and *Aconitum heterophyllum* having uniform shape and size or same age group were transplanted with 1 feet distance from plant to plant and row to row in field (natural condition) and inside greenhouse at Tungnath (3400m asl) situated in district Rudraprayag, Uttarakhand, India. The greenhouse was 20 X 10 feet in area with 10 feet heights at middle and 5.5 feet at corner. Tuberous roots were harvested after one year growth and were brought to laboratory for the estimation of active contents (Fig. 1).

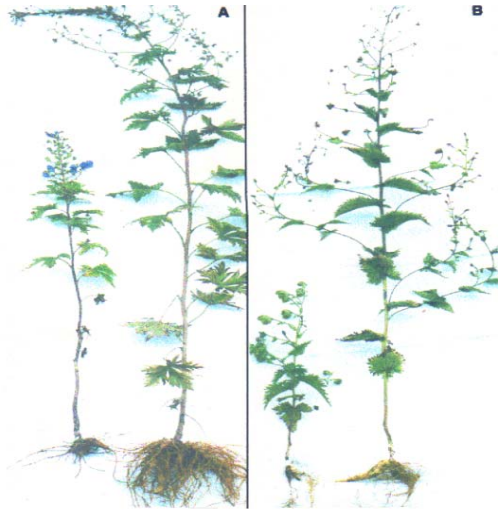


**Fig. 1.** Nursery (left) and greenhouse (right) grown plants of:
**A** - *Aconitum balfourii* and **B** - *Aconitum heterophyllum*

***Extraction***

Plant material (tuberous roots) was collected from both the growing conditions and was dried in shade and then the material was powdered and extracted at room temperature with 70% ethanol for 7 days. The extract was evaporated under reduced pressure to give a dark colored residue. This residue was shaken with hexane. The hexane extract was evaporated to collect residue. The defatted residue was suspended in $CHCl_3$ and extracted several times with 2% $H_2SO_4$. The neutral fraction was collected in the $CHCl_3$ layer. Crude alkaloidal fraction was obtained after basification of the acidic extract by $Na_2CO_3$ (upto pH 5.0) and extraction with $CHCl_3$.

***Chromatographic Technique***

The high performance liquid chromatography (HPLC) was used for separation and qualitative and quantitative determination of the major compounds.

***Preparation of Standard Solution***

Crude alkaloidal extract (0.01mg) was dissolved in 10ml of HPLC mobile phase [0.05M phosphate buffer: acetonitrile: tetrahydrofuran (80:15:5)] and from this stock solution 1, 2, 4, 8, 10 and 20 ppm solutions were prepared with standard solution of reference compound [aconitine, pseudoaconitine, mesaconitine, hepaconitine, atisine (sigma)]. Solutions were filtered through a milipore filter (0.45µm) and used for HPLC analysis.

***HPLC Condition***

The standard HPLC system consisted 125 Beckman System Gold, two liquid pressure pumps, a high valve equipped with a 20µL loop. As a control on retention behavior of standard a normal phase ODS Bondapack column (4.5 X 250mm) was used. 0.05M phosphate buffer: acetonitrile: tetrahydrofuran (80:15:5) being the mobile phase at a flow rate of 1mL/min. Sample solution (20µL) was injected and alkaloids were detected at λ max 240 nm using 166 Beckman System Gold variable wavelength UV detector. Compounds were identified by simultaneous run of standard with its retention time.

## Results and Discussion

A rapid, specific and precise method using HPLC was applied to separate and quantify *Aconitum* alkaloids in their tuberous roots. Well defined peaks for each alkaloid were resolved on HPLC monitor. Good linear response over the range of 0.08% to 0.62% was demonstrated for each alkaloid. Quantitative variation of alkaloids i.e. aconitine, pseudoaconitine, atisine in the tuber of *Aconitum* species were observed in tubers grown in natural and inside greenhouse conditions.

For the treatments and prevention of diseases, natural product of the plants plays a major role in the development of effective and new drug [16]. Quantitative estimation of pharmaceutical preparations by high performance liquid chromatography was used by B.L. Bhaskara [17].

In an attempt to estimate the productivity of tubers and total alkaloids, fresh tuberous roots of two species of *Aconitum* was collected from both the growing conditions. These plants were harvested in the month of September. About 12 and 9 fold enhancement in yield was recorded in *Aconitum heterophyllum* and *Aconitum balfourii* respectively in plants grown under greenhouse as compared to natural condition. It is interesting to note that all morphological character such as height, leaf number, and average length of tubers also high in plants grown under greenhouse in contrast to natural condition. The number of tubers was almost five and two folds high in *Aconitum balfourii* and in *Aconitum heterophyllum* respectively. The quantity of total alkaloid was recorded high (1.99%) in greenhouse grown plants of *Aconitum balfourii* than those of natural condition (1.72%). The percent value of active content i.e. pseudoaconitine and aconitine were also observed high in greenhouse grown plants (0.51% and 0.42% respectively) than naturally grown plants (0.49% and 0.40% respectively) (Fig. 1 and Table 1).

Similarly the total quantity of alkaloids of *Aconitum heterophyllum* was found high (2.1%) in greenhouse grown plants than naturally grown (1.8%). The quantity of its active ingredient i.e. atisine and aconitine was also found high (0.35% & 0.27% respectively) in greenhouse grown plants than naturally grown (0.19% & 0.16% respectively) (Fig. 2 & Table 1).

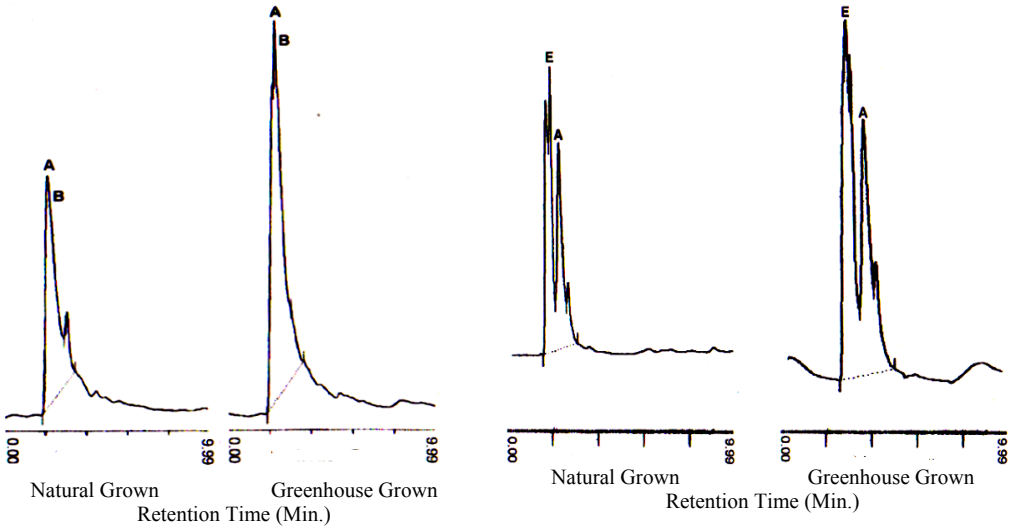


**Fig. 2.** Variation in the active contents of a - *Aconitum balfourii* and b - *Aconitum heterophyllum* grown at Natural and Greenhouse condition: A – Aconitine, B – Pseudoaconitine, E – Atisine

**Table 1.** Active content in greenhouse and natural grown plants of *Aconitum balfourii* and *Aconitum heterophyllum*

| Conditions | *Aconitum balfourii* | | *Aconitum heterophyllum* | |
|---|---|---|---|---|
| | Aconitine | Pseudoaconitine | Aconitine | Atisine |
| Natural Grown | 0.40% | 0.49% | 0.16% | 0.19% |
| Greenhouse Grown | 0.42% | 0.51% | 0.27% | 0.35% |

Varying growth condition arising from seasonal, climatic and soil difference have marked effects on alkaloidal production. It is hardly surprising that compounds so closely connected with amino acid metabolism should exhibit such variation. The alkaloidal content of plants changes as they mature and aged.

Significant enhancement of active content was reported in greenhouse grown plants in contrast to naturally grown plants of *Picrorhiza kurrooa* [18]. Physiological development of plants are directly related to the environmental conditions such as water and nutrient availability [19- 21] and growing habitat [20, 22, 23] which affect the synthesis of secondary metabolites [24].

## Conclusions

Lack of appropriate cultivation package and over exploitation of high altitude medicinal plants put these species under extinct and endangered stage. Present study reveals that the plants grown under control or protected system (inside greenhouse) instead of natural conditions is like a revelation to get the maximum plants yield in respect of their biomass and concentration of active contents of both the *Aconitum* species. So, the cultivation of these valuable plant species with suitable cultivation practices may protect these species in their natural habitat and also fulfill the demand of herbal drug industry.

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