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Altitudinal Variation in Alkaloid Composition of *Hyocymus Niger*: A study with reference to Kashmir region of Himalayas

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Abstract

The present study has been undertaken to understand the impact of altitude on the synthesis of alkaloids in *Hyoscyamus niger* found in Kashmir valley of J&K state in India. Given its wide distributional range, the study aimed to quantify the alkaloids (hyoscyamine and scopolamine) in *Hyoscyamus niger* grown at various altitudes in Kashmir valley. The sampling of populations at varying altitudes allowed assessing the intraspecieac variation and ecological trends of accumulation of alkaloids in the plant. Furthermore the resource allocation in the parts of the plant has also been shown. The four different sites were Gulmarg, Pahalgam, Gurez and Qazigund in Kashmir valley. The study was able to identify the elite genotype and the best suited habitat (altitude) for commercial cultivation of the species with higher quantity of alkaloids.

Keywords: Medical Plants, *Hyoscyamus niger*, Kashmir, Alkaloids, Altitudinal variation.

1. Introduction

Secondary metabolites in plants have been reported to play significant role in growth, attracting pollinators, warding off pathogens and herbivores [1-5], enhancing tolerance against environmental stresses, regulating developmental stages and suppressing the growth of neighboring plants [6]. Most of the valuable secondary metabolites are of low content and are produced through complicated processes [7] and have been found to be induced by a wide range of biotic and abiotic factors [8,9].

Factors affecting quantity of secondary metabolites include age of the plant, season, microbial attack, grazing, radiation, competition, and nutritional statuses [10]. Besides, habitat conditions or environmental factors have been report to cause considerable qualitative and quantitative variations among plant species as well as across populations in the production of secondary compounds [11-13]. The habitat factors are light conditions [14], high/low temperature [15], altitude [16], drought [17] and soil fertilization [17-20]. Mineral nutrition is another important factor known to significantly influence the active principal synthesis [21,22]. Besides secondary metabolite production, accumulation of these is similarly affected by water availability, exposure to soil microorganisms and variations in soil pH and nutrients [23].

The effect of environmental conditions on the quality and quantity of these secondary metabolites actually serve as adaptations to environmental conditions such as temperature and light conditions (e.g. phenols and flavonoids as antioxidants), stress (e.g. proline), infection (e.g. flavanoids) or herbivory (e.g. alkaloids). For example, Caucasian-grown Atropa belladonna has an alkaloid content of 1.3%, compared to 0.3% in plants grown in Sweden Shade-grown. Mentha piperata has lower essential oil content (1.09% v 1.43%) and lower menthol content within the oil (57.5%v 61.8%) compared with light-grown Mentha piperata. Cool-grown Papaver somniferum (poppy) contains more morphine but has lower alkaloid content than warm-grown P. somniferum Similarly Carotenoid content is influenced by different environmental conditions like drought and temperature [23].

Hyoscyamus niger L. vernacular name Black Henbane, known as *Bazar Bhang* in local language Kashmiri, of family Solanaceae is a plant of high medicinal importance [24]. The specie grows wild in Kashmir and is considered as a significant herb in Ayurvedic, Unani and Chinese system of medicine [25]. Traditionally powder of its seeds is used for curing tooth ache [26]. *Hyoscyamus niger* is commercially cultivated in Europe for its alkaloid compounds. The plant has been used for the treatment of motion sickness, asthma and serves as anaesthetic agent. Detailed phytochemical studies have revealed the presence of hyoscine (scopolamine), hyoscyamine, anisodine, anisodamine [27,28], aesculetin, coumarin, kaempferol, quercetin, rutin, cuscohygrine, chlorogenic acid, linoleic acid, myristic acid, oleic acid, stearic acid, pyridine, trimethylamine [29] b-sitosterol, grossamide, cannabisin D & G, daucosterol, N-trans-feruloyltyramine, 1-O-octadecanoyl glycerol, 1-O-(9Z,12Z-octadecadienoyl) glycerol, 1-O-(9Z,12Z-octadecadienoyl)-3-O-(9Z-octadecenoyl), vanillic acid [30] calystegines [31] and with anolides [32]. However, the substances for which *Hyoscyamus niger* is considered highly valued are alkaloids present in almost all parts of the plant particularly leaves and seeds.

IUCN, 2011 lists *Hyoscyamus niger* L. in vulnerable category. *Being* rich sources of tropane alkaloids, mainly hyoscyamine and scopolamine, used for their mydriatic, antispasmodic, anti- cholinergic, analgesic and sedative properties, they are currently extracted industrially from various *Solanaceous* plants including *Hyoscyamus niger* as the synthetic production of these alkaloids is more expensive than their extraction from plant materials [33]. This has resulted in the reckless harvesting and over exploitation of these plants.

2. The Present Study

The present study has been undertaken to understand the impact of altitude on the synthesis of alkaloids in *Hyoscyamus niger* found in Kashmir valley of J&K state in India. Given its wide distributional range, the study aimed to quantify the alkaloids (hyoscyamine and scopolamine) in *Hyoscyamus niger* grown at various altitudes in Kashmir valley. The sampling of populations at varying altitudes allowed assessing the intraspecific variation and ecological trends of accumulation of alkaloid scopolamine in the plant. The study was able to identify the elite genotype and the best suited habitat (altitude) for commercial cultivation of the species with higher quantity of alkaloid hyoscymine and scopolamine. For the study the following four study sites were selected on the basis of altitudinal gradient and accessibility. These were named as Site I, Site II, Site III, Site IV.

Table 1: showing the characteristics of the selected sites

Study Site	Altitude (m asl)	District	Latitude and Longitude	Climatic zone	Habitat
Site I (Gulmarg)	2661	Baramulla	34˚03′N 074˚23′E	Sub alpine	Slope with partial shade
Site II (Pahalgam)	2144	Anantnag	34˚00′N 075˚19′E	Sub alpine	Slope with shade
Site III (Gurez)	1990	Bandipore	34˚68′N 074˚83′E	Sub alpine	Open meadow
Site IV (Qazigund)	1679	Anantnag	33˚42′N 075˚07′E	Temperate	Open slope

3. Methodology

3.1 Extraction and Preparation

Extraction of powdered plant samples (50 g/250 ml) was done with petroleum ether, chloroform, ethyl acetate, methanol and water using Soxhlet for 8-10 h at 55-85 °C. The powdered material was air dried and then used for each of these. These were then reduced under room temperature and the samples were stored at 4 °C. To get the solution of 10 mg/10 ml, the dried samples were redissolved in dimethyl sulfoxide [34].

3.2 Quantitative Estimation

A total of 2.5 g of powder was extracted using 100ml of 20% acetic acid in ethanol. The solution was almost covered for 4 hours. Filtrate was concentrated to 25 ml. and concentrated ammonium hydroxide was added stepwise to attain precipitation. The whole solution was kept as such so that precipitate will settle. Collected precipitate was washed with dilute ammonium hydroxide and finally filtered. Filtrate was discarded and pellets obtained were dried and weighed. The total percentage of alkaloids was estimated as:

Percentage of total alkaloids (%) =

Weight of residue×100/Weight of sample taken

4. Results

4.1 Allocation of Resources (RA)

Allocation of resources to different parts in *Hyoscyamus niger* L. was carried out in different populations (Gulmarg, Pahalgam, Gurez, Qazigund) with varying altitudes. It was observed that the partitioning of resources is not uniform among different natural populations of the plant and also among the different parts of the plants. In the two populations inhabiting comparatively higher altitude (Gulmarg and Pahalgam) much of the resources were allocated towards roots (33.83±5.10 and 28.04±6.33 respectively). However in the

populations inhabiting Gurez and Qazigund resource partitioning showed a shift toward fruits (30.06± 5.23and 32.20± 6.12respectively) and leaves (29.64±6.63 and 31.33±5.12) and least amount of resources were allocated towards flowers among the plant of all the population studied. The percentage of resources allocated towards the flowers varied across populations and it was observed that with increase in altitude the total percentage of resources allocated towards flowers decreased (6.59±1.31 at lowest altitude to 1.32±0.22 at highest altitude) and the same trend was seen in percentage allocation towards leaves, stem and fruits. However, in case of roots, the percentage of resource allocated across populations showed a positive trend with altitudinal gradient and increased from 10.03±4.76 at Qazigund to 33.83±5.10 at Gulmarg.

Table 2 showing the resource allocation of Alkaloids at four different sites

	Population			
	Gulmarg	**Pahalgam**	**Gurez**	**Qazigund**
% R.A Towards Roots	33.83±5.10	28.04±6.33	19.41±4.41	10.03±4.76
% R.A Towards Stem	24.06±3.31	25.90±3.41	27.99±5.51	29.54±9.61
% R.A Towards Leaves	17.08±2.44	19.12±4.17	29.64±6.3	31.33 ±5.12
% R.A Towards Flowers	1.32±0.22	1.69±0.11	4.02±0.04	6.59±1.31
% R.A Towards Fruits	23.68±1.45	25.80±8.53	30.06±5.23	32.20±6.12
Above ground total dry weight (g)	5.50±1.3	6.84±2.6	8.50±1.2	18.51±3.5
Total resource budget per plant (g)	8.40±1.84	9.5±1.2	10.57±1.4	20.83±0.9

4.2 Phytochemical Screening

Phytochemical Screening of crude extracts of *Hyoscyamus niger* L. revealed that petroleum ether, chloroform, methanol and aqueous active fractions of different parts of the plant extracted different phytochemicals depending on their polarity.

In the samples from site I, alkaloids were found to be present in methanolic and aqueous active fractions of leaves and stem, however only methanolic extracts of fruit and root showed positive test for alkaloids. In the samples from site II, methnolic of all the parts (stem, leaves, fruits and roots) and aqueous active fraction of leaves only were found to contain alkaloids. In the samples from site III, methanolic extracts of all the parts (stem, leaves, fruits and roots) contained alkaloids. In the samples from site IV, alkaloids were present only in methanolic extracts of all the parts (stem, leaves, fruits and roots).

Table 3: showing qualitative phytochemical screening of Alkaloids

Body Part		**Stem**				**Leaves**				**Fruit**				**Root**			
Site		**I**	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**
Extract	**Pet. Ether**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Chloroform	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Methnolic	+	+	++	+	+	++	++	+	+	++	+	++	+++	++	++	+
	Aqueous	+	-	-	-	++	+	-	-	-	-	-	-	-	-	-	-

- Absent; + Present

Table 4: showing quantitative phytochemical screening of Alkaloids (mg/g D.W)

Root				**Leaves**				**Fruit**				**Stem**			
I	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**
242.65	**105.2**	**76.58**	**46.71**	**119.72**	**97.12**	**63.17**	**49.92**	**110.84**	**126.08**	**107.19**	**95.2**	**58.52**	**47.19**	**71.44**	**30.12**

5. Discussion

The screening of plant material of the *H. niger* during present study revealed presence of alkaloids in all body parts. The investigation revealed that the plant species constitute a good content of alkaloids which is in conformity with earlier studies taken in this regard [35,36], who also reported presence of alkaloids in the genus *Hyoscyamus*. The amount of alkaloids in mg/g of Dry weight was found to be highest in roots.

Alkaloids were estimated in samples of *H. niger* collected from different altitudes in the Kashmir valley. Spectrophotometric analysis revealed variations in relative content of alkaloids from different altitudinal populations. The study found that the highest content of alkaloids is found in plants collected from Site I (Gulmarg) at an altitude of 2661m asl. The alkaloid content ranged from 30.12mg/g of D.W at site IV (Qazigund; 1679m asl), a low altitude population to 242.84 mg/g of D.W at site I (Gulmarg), a high altitude population. Present investigation revealed alkaloid content increases with increase in altitude, as stress conditions induce polyamine formation which results in nitric oxide biosynthesis that moves freely

through the cells acting as potential chemical elicitor of alkaloid production [37]. The current study is in conformity with previous studies [36] which found that content of total alkaloids in henbane increased with increasing nitrogen levels. Similarly, other studies [38] also corroborate the findings that the concentration and yield of total alkaloid increased by increasing nitrogen rate of application in *H. niger*.

Alkaloid content showed an increase with the increase in the exchangeable calcium content of site. Previous studies [36] have shown that by increasing calcium concentration under hydroponic cultures, the content of alkaloids increased in *H.niger*. It is well established that that cell wall strength and thickness is increased by calcium addition. Calcium is one the most important essential nutrient element in plants, which affects all levels of plant function from metabolism to resource allocation, growth and development.

Since alkaloids are nitrogenous compounds, the availability of nitrogen is expected to play an important role in the biosynthesis and accumulation of alkaloids in plants. As the altitude increases, most of the available nutrients increase because of the reduction in the decomposition due to relatively lower temperature at higher altitudes. The findings are supported by an earlier study [21] which found that with the increase in essential nutrient elements necessary for growth and metabolism cause vigorous vegetation and high phytochemical production in *Datura*. Another study [22] also supported the present findings of increased phytochemical yield with increase in level of fertilizers in medicinal plants. Nitrogen has been found to increase the content of alkaloids in some of the medicinal as well as non-medicinal plants, such as, tobacco, lupines, barley, *Atropa*, *Papaver* [39].

Based on the findings, the study recommends the cultivation of *H. Niger* on large scale for commercial purposes at Gulmarg and adjoining areas in Kashmir valley. The habitat, as per the results obtained is best suited for the commercial cultivation of the plant. Gulmarg is only 50 kilometers from the summer capital Srinagar and is well accessible by a road network. The place is a famous tourist destination and public facilities are better comparatively. Cultivation of H. niger and other medicinal plants can boost the local economy which is reeling under high rate of unemployment. However the present study needs to be corroborated by further studies for the presence of other plant secondary metabolites in the *H. niger*.

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