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Research Articles

Constituents of Cannabis sativa

XV: Botanical and Chemical Profile of Indian Variants

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Abstract

Wild growing *Cannabis* from different altitudes and locations was collected in northern India. Plant material from each collection was analyzed quantitatively for ten cannabinoids by GC. The average total cannabinoid content of plants growing above 2,000 m was 1.33%, whereas below 2,000 m the average total cannabinoid content was 2.74%. Morphological characteristics were recorded. Data obtained from wild growing Indian *Cannabis* (twenty variants) are compared with data obtained from the same variants grown in Mississippi, USA.

Introduction

Cannabis sativa L. is not indigenous to India but was introduced there as a fiber-yielding plant. After being cultivated for many thousands of years, *Cannabis* spread like a weed on the foothills of the Himalayas. It was found growing from Kashmir in the west to Assam in the east, ranging in altitude from plains to three thousand meter levels. With different types of climate and soils *Cannabis* changed significantly, not only in morphological but also in biochemical aspects. Instead of being chiefly fiber producers, many Indian variants became famous for their hallucinatory and drug purposes [1].

In a study on the altitudinal variation in leaf epidermal patterns of Indian *Cannabis*, SHARMA [2] concluded that leaves of plants growing at low elevations (about 250 m), when compared to ones growing at higher elevations (about 2,000 m), are smaller, dull green in color, and have higher stomatal frequency. He also noted that plants growing at lower elevations have longer and more numerous trichomes. In another study KRISHNAMUNTY and KAUSHAL [3] reported a semi-quantitative analysis of wild grown Indian *Cannabis*. In their

study samples collected from eight different regions of northern India showed a wide percentage range for both cannabidiol (CBD) and (–)-*trans*-Δ^9-tetrahydrocannabinol (Δ^9-THC). However, the relationship between cannabinoid content, botanical feature (often used to determine species) and the altitude at which plants are grown remains uncertain. This prompted us to study the profile of Indian variants grown at different altitudes.

Experimental

In autumn of 1976 samples of wild Indian *Cannabis* were collected from twenty different altitudes in northern India: see Table I. Herbarium specimens were prepared and examined for morphological characteristics. Samples of plant material from these specimens were prepared for chromatographic analysis according to the method reported by TURNER et al. [4], using a Beckman GC-72-5 gas chromatograph equipped with a 2% OV-17 column at 210°. Separation of (–)-Δ^9-*trans*-tetrahydrocannabivarin (THCV) from cannabicyclol (CBL) and CBD from cannabichromene (CBC) was achieved using a 6% OV-1 column at 180° (5). A duplicate cannabinoid analysis was performed.

Seeds of six of these Indian variants were planted in the Research Institute of Pharmaceutical Sciences Medicinal Plant Garden in the spring of 1978. Analytical samples for cannabinoid analysis were taken from the growing plants at 14 and 25 weeks of age. Herbarium specimens were collected from mature plants.

Results and Discussion[1]

According to the "Report of the Indian Hemp Drugs Commission, 1893–1894" [1], it was concluded that *Cannabis* was introduced to India through Kashmir. The report also indicated that there are no botanical characteristics to separate Indian Hemp from European *Cannabis sativa* L.: the only differences recognized in the plant by this report are between wild and cultivated, male and female plants. Most of the wild growing Indian variants were found in an open area with plenty of sunlight, dry and windy climate conditions and in sandy-rocky soils. The plants were dwarf with a rough and branching stem. The leaves were brownish green in color and poorly developed with only three leaflets in most of the cases. Leaf epidermal patterns found were much in agreement with the study of SHARMA [2].

Based on the results of our observations of Indian variants grown at different altitudes and locations in India (Table I) and recent literature [6, 7, 8], we concluded that these plants are indeed *Cannabis sativa* L. A general description of these plants follows:

Plant – annual, dioecious, erect, highly branched, dwarf (0.5–1 m) hardly reaching 1.5 m in height except when growing on refuse or cultivated.

Stem – woody, sturdy, cortex very thin, fiber brittle instead of flexible, pale green with red stripes occasionally, covered with cystolithic trichomes.

Leaves – alternate (lower may be opposite), palmate (1 to 7 leaflets) mostly 3.

Leaflets – coarsely serrate, lanceolate with acute apex central leaflets (4.5 to 15 cm) mostly 7–10 cm, dusty and dull green or shiny and dark green, upper surface covered with brownish glandular trichomes, lower surface

[1] For an extensive reivew on the taxonomy of *Cannabis* see reference no. 6.

Table I

Observation of Indian Variants Grown at Different Altitudes[1].

Location (Elevation)	Habitat and Appearance[2]	Sex[3]	Number of Leafles[4]	Central[4] Leaflet (cm)	Sepals[4] (cm)	Bract[4] (cm)	Achene[4] (cm)
Nai-abadi (245 m)	Sandy soils; plant size extremely small	F	3–5	1.1× 6.5	–	0.50	0.20×0.30
		M	3–5	1.1× 7.0	0.40	–	–
Hoshiarpur (260 m)	Sandy-rocky soils; growing wild, branching	F	3	1.0× 1.0	–	0.60	0.20×0.30
		M	3–5	0.8× 6.5	0.30	–	–
Bahadurpur (275 m)	Short plant; branching	F	3–5	0.6× 4.5	–	0.50	immature
		M	3	0.4× 4.5	0.25	–	–
Tatapani (610 m)	Sandy-rocky soils; near a river open area; tough stems	F	3	0.5× 6.5	–	0.40	0.15×0.25
		M	3	0.4× 5.5	0.40	–	–
Ambota (760 m)	Along roadside; some terraces nearby	F	3–5	0.7× 7.0	–	0.50	0.20×0.30
		M[5]	–	–	0.30	–	–
Mandi (1070 m)	Rocky soils; northeast facing slope; fruits abundant	F	3–5	0.7× 5.5	–	0.50	0.25×0.30
		M[5]	–	–	0.30	–	–
Solon (1370 m)	Dry, loamysoil; south facing slope; fruits abundant	F	3	1.1× 8.0	–	0.70	0.30×0.40
Gwaldam (1980 m)	Along hillside; south facing slope	F	3–5	0.8× 7.0	–	0.50	0.20×0.30
Durgapur (1980 m)	Along a road; small plant	F	3–5	1.0× 7.0	–	0.60	0.20×0.25
Durgapur (1980 m)	Growing on refuse; large plant	F	3–5	1.6×14.0	–	0.60	0.25×0.35
		M	3–5	1.1×12.0	0.30	–	–
Kanlog (1980 m)	Along a slope; forested area nearby	F	3–5	1.2×15.0	–	0.60	0.20×0.25
Khalini (2040 m)	Direct sunlight along secondary roads	F	3–5	1.8× 7.0	–	0.70	0.20×0.30
		M	1–5	0.9× 8.0	0.30	–	–
Summerseat (2070 m)	South facing slope, growing in partial shade; seeds immature	F	3–5	1.1× 8.0	–	0.50	0.25×0.35
		M	3–5	1.5×12.0	0.30	–	–
Khalwava (2100 m)	Sout facing slope; along terraces; young plant; only 18 cm tall	Y	5–7	1.0× 5.5	–	–	–
Simla (2130 m)	South facing slope	F	3–5	0.8× 9.0	–	0.75	0.20×0.30
		M	3–5	0.7×10.0	0.30	–	–
Mashobra (2290 m)	Along hillside; growing on refuse; excellent growth	F	3–5	1.3×10.0	–	0.70	0.25×0.40
Manali (2290 m)	Wasteland; wild open area; crisp climate; gentle slope; strong smell	F	3–4	1.2× 8.5	–	0.50	0.20×0.30
		M	1–3	0.9× 8.0	0.40	–	–

Location (Elevation)	Habitat and Appearance[2]	Sex[3]	Number of Leafles[4]	Central[4] Leaflet (cm)	Sepals[4] (cm)	Bract[4] (cm)	Achene[4] (cm)
Bakhalti (2380 m)	Rocky soils; along a ridge; direct sunlight	F	3–5	0.7×11.0	–	0.70	0.30×0.40
		M	3–5	2.0×13.0	0.35	–	–
Narkanda (2740 m)	Rocky soil; south-facing slope; burnt area in the nearby wood	F	3–5	0.4× 4.0	–	0.40	0.20×0.25
		M	3–5	0.8× 8.0	0.30	–	–
Narkanda (2770 m)	Dry windy area, growing on refuse; excellent growth; extremely strong smell	F	3–5	1.4×10.0	–	0.60	0.25×0.40
		M[5]	3–5	–	0.40	–	–

[1] *Cannabis* herbarium specimens were collected in India in autumn 1976 and stored in the Herbarium, School of Pharmacy, RIPS, University of Mississippi, University, MS 38677.
[2] Observations made by Dr. SHARMA in India in 1976.
[3] F = Female, M = male, Y = Young.
[4] Measurements on original specimen made in Mississippi; average of twenty measures.
[5] Measurement based on flower specimen.

Table II
Observation of Indian Variants Grown in Mississippi[1].

Seed Source (Elevation)	Germination Rate	Sex	Height (m)	Number of Leaflets	Central Leaflet (cm)	Sepal (cm)	Bract (cm)	Achene (cm)
Bahadurpur (275 m)	5 %	F	1.8	5–7	0.6× 8.0	–	0.6	.0.2×0.3
Tatapani[2] (610 m)	5 %	F	1.9	(5)7	0.5× 5.5	–	0.6	0.2×0.3
Gwaldam (1980 m)	80 %	F	2.0	5–7	0.7×11	–	0.7	0.3×0.4
		M	2.0	5–9	0.6× 8	0.30	–	–
Khalini (2040 m)	5 %	F	1.5	5–7	0.6× 7.0	–	0.6	0.2×0.3
Khalwava (2100 m)	20 %	F	2.4	5–9	0.5× 7.5	–	0.6	0.2×0.3
		M	2.3	5	0.7× 8.0	0.35	–	–
Simla (2130 m)	10 %	M	2.5	(5)7	1.0×12.5	0.40	–	–

[1] Seeds were collected in India in autum 1976 and planted on April 25, 1978 at the University of Mississippi. *Cannabis* herbarium specimens were prepared on August 1, 1978 and stored in the Herbarium, School of Pharmacy, RIPS, University of Mississippi, University, MS 38677.
[2] Opposite leaves have been found on the plants originated from Tatapani.

covered with dense unicellular trichomes.

Male inflorescence – panicled, many flowered, sepals 0.25–0.4 cm, pollen pale yellow.

Female inflorescence – racemose, compact, green bracts, brownish in mature, length 4 to 8 cm but mostly 5–6 cm.

Fruit – achene, grayish, dark mottled, tiny, 1.5–3.0 mm in diameter, 2.5–4.0 mm in length.

Although there were distinct morphological differences between different herbarium collections of the Indian grown *Cannabis*, when seeds from these locations were grown under the same conditions, the resulting plants were morphologically similar.

Recently EMBODEN [9], based on the size and color of the achene, classified *Cannabis* into three species of which one embraces three varieties: *C. sativa*, *C. ruderalis*, *C. indica* var. *indica*, *C. indica* var. *afghanica*, and *C. indica* var. *kafiristanica*. SMALL and CRONQUIST [6] concluded that *Cannabis* consists of a single highly variable species, namely *C. sativa*. Based on the Δ^9-THC concentration as well as the size and morphological characters of the achenes, they recognized two subspecies (subsp. *sativa* and subsp. *indica*) and four varieties (*sativa*, *spontanea*, *indica*, and *kafristanica*). We found that the size of achene of Indian variants, although smaller than those of variants cultivated for fiber or seed oil, varied from 1.5–3.0 mm in width and 2.5–4.0 mm in length. Mississippi grown Indian variants, harvested at age 25 weeks, had large size achenes (3.5×5.0 mm). In an examination of our collection of seeds from sixty different countries, we found that all seeds have almost the same color range, namely grayish white to dark gray with black mottling. Thus, we do not feel, based on the size and color of achenes or even the phyllotaxy, that one can discuss several species within the genus *Cannabis*. EMBODEN [9] also indicated that *C. sativa* usually has opposite leaves, whereas *C. indica* generally has alternate leaves. Studying our extensive *Cannabis* herbarium, probably the world's largest, and observing *Cannabis* growing from over 200 seed collections, we have found variants which predominantly have alternate leaves and others which have mainly opposite leaves. However, one can find alternate and opposite leaves in almost all variants.

The cannabinoid analyses of Indian variants grown at different altitudes are shown in Table III. Although each variant contains the same major cannabinoids, the percentage of each cannabinoid varies greatly; some are higher in Δ^9-THC, others higher in CBD. The lowest total cannabinoid content was found in young plants collected at Khalwava (elevation 2,100 m) which were sexually immature. Two collections were made at Durgapur (elevation 1,980 m), but their cannabinoid analyses showed great variation. The one collected on a refuse (containing large amounts of nutrients) had large plants which showed high content of CBD (1.35 %) but extremely low content of Δ^9-THC (0.08 %). On the other hand, plants collected just a few yards away from the refuse and in a more wild state, showed a low content of CBD (0.03 %) and a tremendously high content of Δ^9-THC (4.43 %).

The average cannabinoid content of Indian variants collected below 2,000 m (11 samples) was 2.74 %, while the average of those collected above 2,000 m (9 samples) was 1.33 %. This result was in accordance with the results by SHARMA which showed that the density of trichome was greater on the epidermis of plants grown at lower elevations than at upper elevations [2]. Generally, in warm, dry and windy areas, *Canna-*

Table III
Cannabinoid Analysis of Indian Variants Grown at Different Altitudes[1].

Location	Altitude (m)	CBDV	Δ^9-THCV	CBL	CBD	CBC	CBGM	Δ^8-THC	Δ^9-THC	CBG	CBN	Total[2]	W[3]	S[3]
Nai-abadi	245	0.11	0.05	t[4]	1.50	0.09	0.02	–	0.49	0.02	0.04	2.32	F	II, IV
Hoshiarpur	260	0.42	0.16	0.01	1.86	0.14	0.02	0.01	0.43	0.02	0.04	3.11	F	II, IV
Bahadurpur	275	0.18	0.16	0.04	0.32	0.34	–	0.02	1.14	0.07	0.04	2.31	D	I, IV
Tatapani	610	0.03	0.27	0.05	0.01	0.20	0.02	0.03	0.80	0.04	0.16	1.61	D	I, IV
Ambota	760	0.09	0.79	0.03	0.06	0.24	t	–	1.45	0.05	0.07	2.78	D	I
Mandi	1070	0.04	0.17	0.01	0.07	0.40	–	t	1.20	0.03	0.08	2.00	D	I
Solon	1370	0.07	0.05	t	0.08	0.18	0.03	t	2.36	0.09	0.14	3.00	D	I, IV
Gwaldam	1980	0.03	0.26	0.01	0.04	0.06	–	–	2.89	0.15	0.17	3.61	D	I
Durgapur	1980	0.03	0.05	0.01	0.03	0.32	0.02	–	4.43	0.27	0.24	5.40	D	I, IV
Durgapur	1980	0.03	0.02	t	1.35	0.05	0.02	t	0.08	t	t	1.55	F	III, IV
Kanlog	1980	0.19	0.02	t	2.11	0.03	–	–	0.12	0.01	t	2.48	F	III
Khalini	2040	0.08	0.13	t	0.24	0.04	–	t	0.35	0.01	0.03	0.88	D	I
Summerseat	2070	0.02	0.02	t	0.80	0.12	0.01	t	0.88	0.10	0.05	2.00	F	II, IV
Khalwava	2100	0.23	0.10	t	0.06	0.04	–	t	0.06	t	t	0.85	D	III
Simla	2130	0.04	0.09	0.02	1.13	0.08	0.04	0.01	0.72	0.06	0.05	2.24	F	II, IV
Mashobra	2290	0.02	0.01	0.01	1.03	–	0.02	0.03	0.19	0.01	0.03	1.35	F	III, IV
Manali	2290	0.05	0.21	t	0.11	0.03	0.03	t	0.66	0.03	0.04	1.16	D	I IV
Bakhalti	2380	t	0.02	0.01	0.71	0.08	–	t	0.30	t	0.02	1.14	F	II
Narkanda	2740	0.09	t	t	0.46	0.02	0.07	0.02	0.16	0.02	0.02	0.86	F	III, IV
Narkanda	2770	0.05	0.05	0.01	0.14	0.04	0.03	t	0.88	0.16	0.05	1.51	D	I, IV

[1] Plant materials were collected in India in autumn of 1976 (see Table I). The cannabinoid content was based on the percentage by dry weight.

[2] Total cannabinoids. Average for plants collected below 2,000 m was 2.74% and above 2,000 m was 1.33%.

[3] Classification according to WALLER [12] and SMALL and BECKSTEAD [13]; F = Fiber, D = Drug [12], I, II, III, IV designates phenotypes [13].

[4] trace = less than 0.009%.

bis develops more glandular trichomes on the surface and hence produces more cannabinoids. In previous studies, we have found that the cannabinoid content of *Cannabis* is affected not only by environmental and genetic factors, but also by time of day, age of plants and plant part sampled [10].

In a weekly cannabinoid analysis of a Mississippi grown Indian variant [11] both male and female plants varied in the content of each cannabinoid so much that they could be defined as drug type or as fiber type according to Waller's classification [12]. As shown by the data in Tabes III and IV, Indian *Cannabis* variants grown in India or Mississippi may be drug type or fiber type according to Waller's classification method. This also holds true for Small's classification based on phenotypes [13]. In this case, several variants could be classified into one or more phenotypes.

Indian variants also showed significant amounts of cannabidivarin (CBDV) and Δ^9-THCV. The presence of these propyl homologs in a sample of Indian *Cannabis* grown in Mississippi has been reported [14]. In a study of propyl homologs in 51 variants of known geographical origin, TURNER et al. [15] found that only plants originating in Afghanistan, India, Lebanon, Pakistan, and Sudan contained significant amounts of both CBDV and Δ^9-THCV. *Cannabis* originating in Nigeria and South Africa contained high amounts of Δ^9-THCV, but only traces of CBDV.

Seeds of six Indian variants collected at different altitudes were planted in the *Cannabis* garden, University of Mississippi. The resulting plants showed much uniformity (Table II). The height of plants at 14 weeks of age ranged from 1.5 m to 2.5 m (wild grown average 1.0 m) and the number of leaflets ranged from 5 to 9 with 7 in most cases (wild grown leaflets usually 3). However, the size of reproductive portions of all variants, whether grown in India or Mississippi, were in the same range.

The cannabinoid analysis of Indian variants grown in Mississippi (Table IV) showed less variation of total cannabinoid content than the corresponding wild grown plants. Detectable amounts of cannabigerol monomethylether (CBGM) were present in both Mississippi grown and native Indian variants. This, according to SMALL and BECKSTEAD [13], classifies these variants as phenotype IV.

From this present broad study of Indian *Cannabis* grown both in India and Mississippi, USA, we conclude that *Cannabis* growing in India is, indeed, *Cannabis sativa* L. However, it is impossible to classify all Indian *Cannabis*, whether grown in India or Mississippi, exclusively as fiber or drug type since approximately half of the variants are drug and half are fiber (see Table III). It is also impossible to classify each variant as a single phenotype using SMALL and BECKSTEAD's [13] method. Phenotype classification, as well as drug and fiber classifications, will vary according to age, sex, time of harvest, plant part harvested, etc.

Similar studies are underway on several variants of Russian and Mexican *Cannabis*.

Table IV
Cannabinoid Analysis of Indian Variants Grown in Mississippi[1].

Seed Source Altitude	SEX	CBDV	Δ^9-THCV	CBL	CBD	CBC	CBGM	Δ^8-THC	Δ^9-THC	CBG	CBN	Total[2]	W[3]	S[3]
Bahadurpur (275 m)	F	0.18	0.10	0.01	0.65	0.03	–	0.04	0.26	–	t	1.27	F	III
Tatapani (610 m)	F	t	0.14	t	t	0.11	t	t	0.95	0.02	t	1.22	D	I
Gwaldam (1980 m)	F	0.03	0.11	0.01	t	0.07	0.01	0.02	0.61	–	0.02	0.88	D	I, IV
Khalini (2040 m)	F	0.08	0.10	t	0.41	0.08	0.05	0.05	0.60	t	t	1.37	D	I, IV
Khalwava (2100 m)	F	0.03	0.36	0.02	t	0.12	0.02	0.02	0.82	0.02	–	1.41	D	I, IV
	M	0.64	0.11	t	0.82	0.05	0.03	0.07	0.24	0.02	–	1.98	F	III, IV
Simla (2130 m)	M	0.01	t	t	0.27	0.08	0.06	0.03	0.74	0.03	t	1.22	D	I, IV
Khalini[4] (2040 m)	F	0.12	0.18	t	0.81	0.15	0.02	0.13	1.06	0.04	0.02	2.53	D	II, IV
Simla[4] (2130 m)	X	t	t	t	0.67	0.10	0.05	0.03	0.56	0.09	0.03	1.53	F	II, IV

[1] Seeds were collected in India in autumn 1976 and planted on April 25, 1978 at the University of Mississippi. Leaves from F (female), M (male) and X (mixed) plants were collected on August 1, 1978 for cannabinoid analysis. The cannabinoid content on the table was based on the percentage of the dried plant material.

[3] Classification according to WALLER [12] and SMALL and BECKSTEAD [13]; F = Fiber, D = Drug [12], I, II, III, IV designates phenotypes [13].

[2] Total cannabinoids.

[4] Collected and analyzed on October 30, 1978.

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