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Fatty acid composition of Achene oils from five Moroccan climatic cultivars of *Cannabis*

By **A. Merzouki** and **J. Molero Mesa**

Departamento de Biología Vegetal, Facultad de Farmacia, Campus de Cartuja. 18071 Granada. España

RESUMEN

Composición en ácidos grasos del aceite de los aquenios de cinco variedades climáticas marroquíes de *Cannabis*.

Se estudia la composición en ácidos grasos del aceite de los aquenios de cinco variedades climáticas del cáñamo (*Cannabis sativa* L) cultivadas en el norte de Marruecos. Predomina el ácido linoleico (40 a 45%) seguido por el linolénico (12 a 17%) y el oleico (7 a 10%). Las diferencias en la composición de los ácidos grasos del aceite se atribuyen a factores ambientales.

PALABRAS-CLAVE: Acido graso (composición) – Aquenio – Cannabis – Marruecos.

SUMMARY

Fatty acid composition of Achene oils from five Moroccan climatic cultivars of *Cannabis*.

The fatty acid composition of achene oil from five *Cannabis* climatic cultivars cultivated in the nort of Morrocco is determined. Linoleic acid predominated (40 to 45%), followed by linolenic (12 to 17%) and oleic (7 to 10%) acids. Differences in the fatty acid composition of oils are attributed to environmental factors.

KEY-WORDS: Achene – Cannabis – Fatty acid (composition) – Morocco.

1. INTRODUCTION

Hemp (*Cannabis sativa* L.) an herbaceous annual plant is of economic importance as a drug, a fibre and oil seed plant. It's considered as one of the oldest of cultivated plants and appears to have originated in northern China (Hui Lin Li, 1974), where it grew in the wild state still around 5200 to 6200 years ago. Actually hemp spread throughout most of the temperature and tropic regions of the world.

Both genetic and environmental conditions influenced the biosynthesis process in hemp plant, essentially cannabinoids composition. Warm, dry and windy conditions were believed to induce a higher density of resin glands where the biosynthesis of cannabinoids takes place (Crombie, 1977; Valle et al., 1978; Braut-Boucher, 1980; Mahlberg and Hemphill, 1983). Murari et al., (1983), estimated higher contents of cannabinoids, in the same varieties, when hemp grown in a continental climate than in a maritime climate.

One of many compounds secondary metabolites produced by hemp is oil, resulting from the plant lipid synthesis and achenes are the primary storage tissue for accumulating oil. Achenes of *Cannabis* contain around 30-35% oil, which is brownish-green in colour and smells of the seed (Vaughan, 1970). The oil possesses marked drying properties, and it's used in the manufacture of paint, soaps, varnishes, printing inks and an edible oil. Many countries cultivate hemp as an oil-seed plant, like Manchuria, Chile, Turkey, China, Formosa, Hungary, Czechoslovakia and Francia, the main world cultivator is the ancient USSR. The average annual production of seed being in the region was 250.000 tons (Altschul cited in Vaughan, 1970).

In previous studies (Merzouki and Molero Mesa, 1995; Merzouki et al., 1996a and b) about *Cannabis* chimiotaxonomy (seed morphology and cannabinoids composition), we have concluded that *Cannabis* cultivated in the Rif belongs to the drug phenotype and the genus was monotype (*Cannabis sativa L.).*

The Rif, space of *Cannabis* cultivation in Morocco, is characterized by a high ecological variety where bioclimates vary from arid to perhumid (Ben Abid, 1982).

The purpose of this study was to determine oil concentrations and differences in the fatty acid composition of the oil in achene from the five «*Cannabis* climatic varieties» cultivated in the north of Morocco.

2. MATERIAL AND METHODS

When ripe, achenes of *Cannabis* were collected from different localities of the Rif (North of Morocco),

1-Ketama, 2-Jebha, 3-Bouhmed, 4-Chaouen and 5-Bad Taza. In each locality, 5 *Cannabis* populations are selected. From achenes of each population, one sample was prepared.

Achene samples were milled and the oil was extracted n-hexane in a Soxhlet apparatus. The extract was dried over anhydrous sodium sulphate and the solvent removed under reduced pressure in a rotary film evaporator. Fatty acid composition of solvent-extracted oils from achenes was determined using transesterification of the triacylglycerols with a solution 0,5N KOH in methanol (Sonanini and Weber in Lawi-Berger, 1982) and the methyl esters analysed by gas chromatography uing a glass column (20 m long and 2.25 mm ID) coated with SP-2330. The detector was a flame ionization detector. A fatty acid standard containing the fatty acids myristic (C:14), palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), arachidic (C20), behenic (C22:0), lignoceric (C24:0). Fatty acid peaks were identified by comparing the fatty acid methyl ester peaks and retention times of standards with sample peaks. Standard solution of margaric acid methyl ester was used for quantification. Seed oil and fatty acid concentrations were determined from one sample per population (Five samples per locality). Means and standard deviation of means are presented to give some ideas of the variability of oil and fatty acids within each variety. Variance analyse (ANOVA) was used to compare oil and fatty acid concentrations between varieties. Correlation analyses were used to examine the relationships among fatty acid composition and ecological and geographical parameters [T° Max. (°C), T° min. (°C), precipitation (mm), altitude (m), latitude and longitude].

3. RESULTS AND DISCUSSION

Oil concentrations of 5 «climatic *Cannabis* varieties» from the Rif are summarized in Table I.

Table I
Means and standard deviation (SD) of seed oil concentration (%) from five Rifian *Cannabis* varieties

Variétés	N	Mean	S.D.
Kétama	5	33.84	1.29
Jebha	5	33.72	1.62
Bouhmed	5	30.96	1.29
Chaouen	5	34.60	1.28
Bad Taza	5	32.48	0.91

N = number of samples

Values varied from 30.96% to 34.60%. The highest mean oil concentration was observed in *Cannabis* seeds from Chaouen and the lowest mean was observed in Bouhmed locality.

Difference analysis between mean oil concentrations of each locality (variance analysis) was consigned in Table II, and reveal that seed oil concentration from Bouhmed locality vary significantly with seed oil concentrations of Kétama, Jebha, Chaouen localities at P=0.01, and vary significantly at P=0.05 with this from Bab Taza.

Table II
Significant difference at 95% and 99% confidence for mean between seed oil concentrations from five Rifian *Cannabis* localities

	Ketama	Jebha	Bouhmed	Chaouen	BabTaza
Ketama	1				
Jebha	NS	1			
Bouhmed	**	**	1		
Chaouen	NS	NS	**	1	
Bab Taza	NS	NS	*	**	1

* Significant difference at P=0.05
** Significant difference at P=0.01
NS Non significant difference

The fatty acid composition from *Cannabis* achenes is summarized in Table III and Fig. 1. The major fatty acids observed were C18:1, C18:2 and C18:3 in each variety. The lowest one is C24 which varied from 0.31 in Ketama to 0.76 µMol/g oil in Chaouen. Results of variance analysis were summarized in Table IV. Difference analysis of each fatty acid between localities reveal that fatty acid concentrations from Bouhmed achene variety presents a clear difference with the other varieties. The myristic, palmitic, oleic, linoleic, behenic and lignoceric acids from Bouhmed achene variety are a highly significant difference to the others varieties. Achene oil from Bab Taza location are characterized by 3 fatty acids, oleic (C18:1), linoleic (C18:2) and arachidic (C20). These fatty acids are significantly different to thus from others localities.

Seiler (1986) concluded that environmental factors, especially temperature, during the period of achene development and maturation affect both the concentration and composition of oil in maturing sunflower achenes. Therefore, differences in fatty acid composition found in *Cannabis* achene oils can be attributed to environmental factors.

Results of Rifian *Cannabis* varieties show that the achene oils have similar fatty acid composition than the varieties analysed by Lawi-Berger (1982), who has compared fibre and drug varieties. In such study, the author reveal that comparison of fatty acid

Table III

Fatty acid concentrations (μMol/1g achene oil) and ecological, and geographical factors of 5 Cannabis climatic varieties from Morocco

	Localities				
	Ketama	**Jebha**	**Bouhmed**	**Chaouen**	**Bab Taza**
	Mean (Standard deviation)				
Alt. (m)	1338-1510	95-270	55-150	342-400	765-880
P (mm)	1504 (114.58)	195.6 (52.86)	289.2 (99.37)	993.20 (75.15)	1044 (135.11)
Tmax°C	31.3 (1. 51)	32.06 (3.29)	29.5 (0.58)	30.80 (2.07)	30.8 (1.28)
Tmin°C	–0.02 (1.21)	4.14 (0.73)	7.26 (0.74)	3.66 (1.07)	3.3 (0.67)
Lat.	34°48'-34°56'	35°09'-35°11'	34°25'-32°27'	35°11'- 35°50'	35°09'-35°15'
Long.	4°33'-4°42'	4°32'-4°38'	4°25'-4°55'	5°09'-5°11'	5°07'-5°14'
C14	10.64 (1.43)	9.46 (0.94)	12.80 (0.78)	9.92 (0.88)	9.04 (0.53)
C16	175.72 (7.25)	192.04 (6.05)	167.42 (12.60)	157.12 (11.92)	229.7 (17.27)
C18	65.56 (2.50)	58.70 (2.53)	53.90 (7.53)	62.88 (3.23)	54.64 (3.31)
C18: 1	313.54 (7.36)	320.26 (12.94)	264.14 (8.47)	358.36 (7.40)	343.26 (11.19)
C18: 2	1427.94 (19.25)	1424. 14 (57.55)	1227.34 (24.58)	1343.76 (25.04)	1625.64 (18.40)
C18: 3	624.74 (11.54)	548.28 (3.96)	530.62 (67.46)	431.62 (7.45)	540.48 (17.30)
C20	40.58 (3.60)	56.36 (3.65)	33.90 (2.83)	36.16 (2.23)	80.80 (6.28)
C22	4.68 (0.37)	5.24 (0.67)	3.18 (0.78)	4.48 (0.67)	5.96 (1.33)
C24	0.50 (0.07)	0.66 (0.15)	0.31 (0.11)	0.52 (0.14)	0.76 (0.11)

Alt. = Altitude
P = Precipitation

Table IV

Difference analysis of means of fatty acid concentrations between all localities

	Kétama	**Jebha**	**Bouhmed**	**Chaouen**	**Bab Taza**
Kétama	—				
Jebha	1^{ns} 2^{**} 3^{**} 4^{ns} 5^{ns} 6^{**} 7^{**} 8^{ns} 9^{ns}	–			
Bouhmed	1^{**} 2^{*} 3^{**} 4^{**} 5^{**} 6^{*} 7^{*} 8^{*} 9^{*}	1^{**} 2^{**} 3^{ns} 4^{**} 5^{**} 6^{ns} 7^{**} 8^{**} 9^{**}	–		
Chaouen	1^{**} 2^{**} 3^{ns} 4^{**} 5^{**} 6^{**} 7^{*} 8^{ns} 9^{ns}	1^{ns} 2^{**} 3^{*} 4^{**} 5^{*} 6^{**} 7^{**} 8^{ns} 9^{ns}	1^{**} 2^{**} 3^{*} 4^{**} 5^{**} 6^{**} 7^{ns} 8^{*} 9^{*}	–	
Bab Taza	1^{*} 2^{*} 3^{**} 4^{**} 5^{**} 6^{**} 7^{**} 8^{ns} 9^{**}	1^{ns} 2^{ns} 3^{ns} 4^{**} 5^{**} 6^{ns} 7^{**} 8^{ns} 9^{ns}	1^{**} 2^{**} 3^{ns} 4^{*} 5^{**} 6^{ns} 7^{**} 8^{**} 9^{**}	1^{**} 2^{**} 3^{**} 4^{*} 5^{**} 6^{**} 7^{*} 8^{ns} 9^{*}	–

1- C14, 2-C16, 3-C18, 4-C18:1, 5-C18:2, 6-C18:3, 7-C20, 8-C22, 9-C24
* Significant difference at 95%
** Significant difference at 99%
ns:Non significant difference

concentrations don't permit to distinguish fibre or drug *Cannabis* variety.

The variability observed in fatty acids of the five *Cannabis* varieties cultivated in the Rif region was undoubtedly caused by differences in environment at different locations.

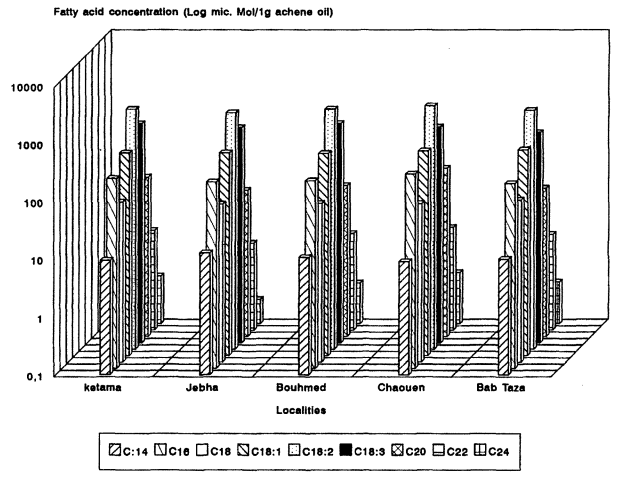

Figure 1

Fatty acid concentrations of 5 *Cannabis* climatic varieties from Morocco

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