บทความวิจัย

การสำรวจกัญชงที่ปลูกในประเทศไทย โดยใช้เทคนิคแก๊สโครมาโทรกราฟี-แมสสเปคโตรเมตรี

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บทคัดย่อ

ในการสำรวจนี้ได้สุ่มสำรวจตัวอย่างกัญชงจำนวน 58 ตัวอย่าง จากแหล่งปลูกหลักในจังหวัดเชียงใหม่และจังหวัดตาก วิเคราะห์ปริมาณสารออกฤทธิ์หลัก ได้แก่ Δ^9-Tetrahydrocannabinol (Δ^9-THC), Cannabidiol (CBD) และ Cannabinol (CBN) ด้วยเทคนิคแก๊สโครมาโทรกราฟี-แมสสเปคโตรเมตรี และแบ่งชนิด *Cannabis* ด้วยลักษณะทางเคมี โดยเทียบตามเกณฑ์ 3 อย่าง ที่ได้เคยมีรายงาน คือ %Δ^9-THC, phenotypic index และอัตราส่วน THC/CBD เมื่อพิจารณาปริมาณสารออกฤทธิ์ต่อจิตประสาท Δ^9-THC พบว่า 91.38 เปอร์เซ็นต์ จัดเป็นพืชเสพติด และ 8.62 เปอร์เซ็นต์ จัดเป็นพืชเส้นใย เมื่อรายงานตามพื้นที่พบว่า ตัวอย่าง 73.33 เปอร์เซ็นต์ จากพื้นที่เชียงใหม่ และ 97.67 เปอร์เซ็นต์ จากพื้นที่ตาก จัดเป็นพืชเสพติด จากการศึกษานี้คณะผู้วิจัยจึงเสนอแนะว่าการจัดจำแนกชนิด *Cannabis* ควรใช้ปริมาณ Δ^9-THC เป็นเกณฑ์หลัก จากนั้นจึงใช้ phenotypic index และอัตราส่วน THC/CBD เป็นเกณฑ์รอง นอกจากนี้ควรพิจารณาปัจจัยต่างๆ ที่อาจมีผลต่อการเพิ่มของปริมาณสารออกฤทธิ์ เช่น เมล็ดพันธุ์ การผสมข้ามของละอองเกสรในแปลงปลูก และปัจจัยด้านสภาพแวดล้อม เพื่อเป็นการป้องกันการแพร่กระจายของการปลูกพืชเสพติด

คำสำคัญ: กัญชง, Δ^9 -Tetrahydrocannabinol, Cannabidiol, Cannabinol

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Survey of *Cannabis sativa* L. "Fiber Type" Cultivated in Thailand Using Gas Chromatography-Mass Spectrometry Data

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ABSTRACT

A total number of 58 *Cannabis sativa* L. "fiber type" plants were randomly sampled from the major cultivation areas in Chiangmai and Tak provinces. The 3 major cannabinoids compounds; Δ^9-tetrahydrocannabinol Δ^9-THC), cannabidiol (CBD), and cannabinol (CBN) were analyzed by gas chromatography-mass spectrometry (GC-MS). Classification of *Cannabis* chemotypes according to 3 published criteria; % Δ^9-THC, the phenotypic index, and the THC/CBD ratio, were brought to consideration and discussed. Regarding the level of psychoactive Δ^9-THC compound, results suggested that 91.38% of the plants were "drug type", and 8.62% were "fiber type". It is also reported that 73.33% of *C. sativa* L. sampled from Chiangmai, and 97.67% of the *C. sativa* L. sampled from Tak were classified as "drug type". From this study, we suggest that chemotype classification should be based on the level of Δ^9-THC compound, and the phenotypic index and THC/CBD ratio should be used as secondary criteria. Factors which may affect the cannabinoid contents in plants, such as seed stocks, cross pollination in the field, and environmental factors, must be taken into consideration to prevent the expansion of drug type cultivation.

Keywords: *Cannabis sativa* L., Δ^9 -Tetrahydrocannabinol, Cannabidiol, Cannabinol

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Introduction

Cannabis sativa L. is a source for oil, food, medicine and fiber [1]. The "fiber type" *Cannabis sativa* L. (industrial hemp, hemp or "kanchong") is a widely cultivated plant throughout the world. In Thailand the cultivation of *C. sativa* L. for fiber is wide spread among the Hmong hill tribes. The annual hemp cultivation starts in the rainy season during May to July. Seeds stocks collected from the mature plants are used in the next cultivation.

The different *Cannabis* types are difficult to distinguish from their morphological characteristics alone, posing a problem for drug control in the field. Chemical analysis of the major cannabinoids content, Δ^9 -tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD), and cannabinol (CBN), has been used to classify the *Cannabis* types [4-7]. According to the EU guideline [4], a maximum limit of 0.3% Δ^9-THC content is allowed for cultivated fiber type plants. It is reported that there will be no psychoactive effect if the Δ^9-THC content is lower than 0.3%. This criterion is accepted in most countries [2-5]. Fetterman *et al.* described the phenotypic index $\frac{\%\ \Delta^9\text{-THC} + \%\ \text{CBN}}{\%\ \text{CBD}}$ to classify the 2 chemotypes of *Cannabis;* fiber and drug types [6]. If the index exceeds 1, the plant is classified as "phenotype I" or "drug type". If the index is lower than 1, the plant is classified as "phenotype II" or "non-drug type". In 1973, Small and Beckstead determined 3 chemotypes of *Cannabis* using the THC/CBD ratio; "chemotype I" or "drug type" plants have a high THC/CBD ratio (>>1.0), "chemotype II" or "intermediate type" plants have an intermediate ratio (close to 1.0), and "chemotype III" or "fiber type" plants have a low THC/CBD ratio (<<1.0) [7].

The variation in cannabinoids content may derive from environmental conditions and the variation in the plants themselves [8]. Cross pollination occurring in the field cultivations resulted in fiber-type hybrids that produced high concentrations of psychoactive components. Thus, re-cultivation using the progeny's seeds would result in the expansion of drug type in the field. Furthermore, West *et al,* (1998) reported variation in cannabinoids content of the fiber type plants in different cultivation areas [9].

Forensic detection and identification of *C. sativa* L. are based on the chemical analysis of cannabinoids which are unique to the plant [7]. Confirmatory test methods performed in forensic laboratories such as TLC, HPLC, GC, and GC-MS can offer both quantitative and qualitative data of the analyzed samples [2, 3, 5]. However, these methods are at different levels of sensitivity. In this study, the cannabinoids *in Cannabis* plants were analyzed by GC-MS technique to evaluate the current situation of hemp cultivation in Thailand. GC-MS is more accurate than TLC and other analytical techniques. Cannabinoids were vaporized and then trapped by the mass detector. The peak area of mass ions was used for quantitative analysis of compound.

Under the Thai Narcotics Act of B.E. 2522 (1979), the fiber type plant is treated as a *"Cannabis sativa"* [10], which prohibits its commercial cultivation. Cultivation of fiber type *Cannabis* is under the control of the Office of the Narcotics Control Board (ONCB) and the Food and Drug Administration (FDA), Thailand. Recently, the Royal Project Foundation of Thailand realized the economical potential and is planning to promote the commercial cultivation of the fiber type *Cannabis* [2, 3]. It is then necessary to survey the current situation of the cultivated fiber type in the field before large scale commercial cultivation is permitted.

Materials and Methods

Plant Material

A total of 58 mature *Cannabis sativa* L. plants were randomly sampled by ONCB officers from 10 major cultivation areas in Chiangmai and Tak provinces. The leaf part (Figure 1) was collected and oven-dried at 40°C.

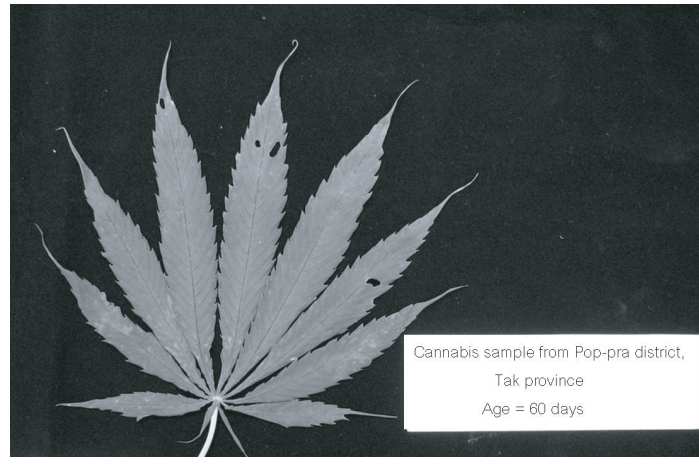

Figure 1 A leaf of *Cannabis sativa* L. from Tak province.

Sample Preparation

Cannabinoids were extracted from 20 mg of pulverized sample macerated in 1 ml of n-hexane overnight. Then, 10 µl of 1000 ppm diphenylamine (internal standard) was added to a 500-µl aliquot of the extract before the solvent was evaporated under N_2 steam and reconstituted in 500 µl of ethyl acetate.

Standard solutions of Δ^9-THC, CBD, and CBN were prepared by adding 10 µl of 1000 ppm diphenylamine (internal standard) to 500-µl aliquots of 500 ppm THC, 530 ppm CBD, and 52 ppm CBN in methanol.

Analysis of cannabinoids by Gas chromatography-mass spectrometry

One microliter of each prepared samples was injected to GC-MS instrument (Agilent, model 5973) installed with a HP-5MS crosslinked 5% phenylmethylsilicone column (30 m × 0.25 mm I.D., film thickness 0.25 µm, Hewlett-Packard). The condition was modified from M. Stefanidou *et al.* [8] as follows: initial oven temperature 100 °C; 0 min; rate 15 °C/min; final temperature 300 °C; final time 8.00 min; injection port temperature 220 °C; interface temperature 300 °C. Split mode with ratio 20: 1. The helium flow-rate was 1 ml/min. The results were analyzed using ChemStation software (Agilent).

The area ratio between the cannabinoids and the internal standard was used for quantitative analysis. The major ions for Δ^9-THC are m/z 299, 314, and 231, for CBD are m/z 231, 174, and 314 and for CBN are m/z 295, 238, and 310 and diphenylamine is m/z 169.

Results

Analysis of the cannabinoid contents in 58 randomly collected *C. sativa* L. plants showed varying concentrations of the cannabinoid contents within the samples. The retention times of Δ^9-THC, CBD, CBN, and diphenylamine were 12.79, 12.29, 13.14, and 7.06 min, respectively (see figure 2). Table 1 showed the content of cannabinoids and chemical classification of each plant in the sample group. The percentage of Δ^9-THC content in the surveyed plants varied between 0.05%-11.90%. There were only 5 plants (Hp03, Hp09, Hp10, Hp16 and Hp59) which the level of Δ^9-THC was lower than 0.3%. Based on the Δ^9-THC content, results suggested that 91.38% of the plants randomly collected from 2 major cultivation areas were “drug type”, and 8.62% were classified as “fiber type”. The percentages of CBD and CBN content varied between 0.01%-1.33%, and 6.6×10^{-6}% - 1.0×10^{-2}%. Classification of the chemotypes according to the different proportion of 3 major cannabinoids showed that the phenotypic index ($\frac{\%\ \Delta^9\text{-THC} + \%\ \text{CBN}}{\%\ \text{CBD}}$) of 56 plants were more than 1, and 2 plants (Hp10 and Hp16) were less than 1, suggesting that 96.55% were “drug type” or chemotype I, and

3.45% of the plants were "fiber type" or chemotype II. When only the Δ^9-THC and CBD contents were taken to account, the THC/CBD ratio of 55 plants were more than 1, one (Hp59) was approximately 1, and 2 plants (Hp10 and Hp16) were less than 1. Hence, 94.83%, 1.72%, and 3.45% of the plants were "drug type" or chemotype I, "intermediate drug type" or chemotype II, and "fiber type" or chemotype III, respectively.

The percentage of drug and fiber types plants in Chiangmai and Tak provinces are shown in table 2. A total number of 15 plants were sampled from Chiangmai province. The Δ^9-THC content in 4 plants (Hp03, Hp09, Hp10, and Hp16) were less than 0.3% and 11 plants were more than 0.3%. The level of Δ^9-THC suggested that 26.67% of the plants were "fiber type" or chemotype II and 73.33% were "drug type" or chemotype I. However, the phenotypic index and THC/CBD ratio showed that 13.33% (2 plants; Hp10 and Hp16) were "fiber type" or chemotype II and 86.67% were "drug type" or chemotype I.

Of the 43 plants sampled from Tak province, the level of Δ^9-THC exceeded 0.3% in 42 plants, only one plant (Hp59) that was lower than 0.3%. The level of Δ^9-THC suggested that 97.67% of the plants were "drug type" and 2.33% were "fiber type". However, all 43 plants had the phenotypic index more than 1, suggesting that all plants in this group were "drug type" or chemotype I. The THC/CBD ratio suggested that 42 plants in this group were of the "drug type" as when considered by the Δ^9-THC content. Only one sample that had the THC/CBD ratio close to 1 (Hp59) which was classified as "intermediate drug type" or chemotype II.

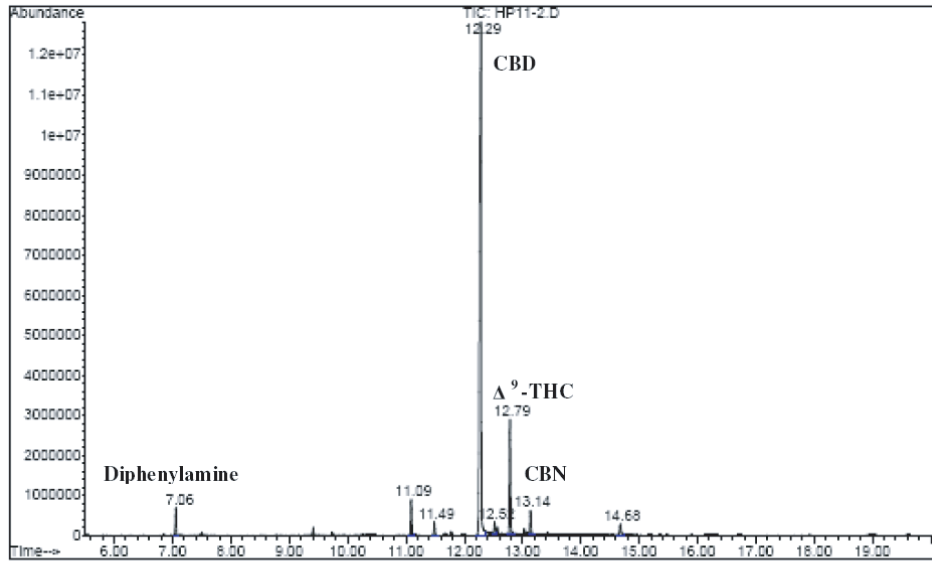

Figure 2 Total ion chromatogram of diphenylamine (internal standard), Δ^9-THC, CBD, and CBN standard solutions. The retention times were 7.06, 12.79, 12.29, and 13.14 min, respectively.

Table 1 The percentage of cannabinoid contents and the phenotypic index of each sample.

Samples ID	Cannabinoids content			Chemical characterization		
	%Δ^9-THC	%CBD	%CBN	Phenotypic idex $\frac{\%\Delta^9\text{-THC}+\%\text{CBN}}{\%\text{ CBD}}$	$\frac{\text{THC}}{\text{CBD}}$ ratio	
					Ratio	Chemotype
Chiangmai province, Queen Sirikit Botanic Garden (QSBG)-1						
Hp03	0.11	0.01	1.5E-04	11.02 >1	11.0 >1	I
Chiangmai province, 1013 m. from sea level, New Hmong Mae-Sa Village Moo. 6, Pong-yang, Mae-rim, Coordinate MA851857.						
Hp09	0.29	0.13	7.6E-03	2.29 >1	2.23 >1	I
Hp10	0.18	0.85	2.9E-03	0.22 <1	0.21 <1	III
Hp11	2.67	1.33	3.1E-02	2.03 >1	2.00 >1	I
Hp12	1.10	0.03	5.3E-02	38.43 >1	36.66 >1	I
Chiangmai province, Queen Sirikit Botanic Garden (QSBG)-2						
Hp16	0.14	0.30	1.3E-03	0.47 <1	0.46 <1	III
Hp18	1.17	0.28	1.8E-02	4.24 >1	4.17 >1	I
Hp19	1.18	0.20	6.3E-03	5.93 >1	5.90 >1	I
Hp21	1.50	0.16	6.6E-06	9.38 >1	9.38 >1	I
Hp24	2.60	0.39	2.9E-02	6.74 >1	6.66 >1	I
Hp25	11.90	0.30	1.0E-02	39.70 >1	39.66 >1	I
Hp27	11.00	0.30	2.0E-02	36.73 >1	36.66 >1	I
Hp29	7.32	0.12	6.4E-02	61.53 >1	61.00 >1	I
Hp32	9.61	0.26	9.1E-02	37.31 >1	36.96 >1	I
Hp34	2.98	0.51	1.5E-02	5.87 >1	5.84 >1	I
Tak province, Hmong Kee-ree-ras Village, Pop-pra.						
Hp35	2.03	0.05	9.4E-03	40.79 >1	40.60 >1	I
Hp36	1.46	0.03	7.1E-03	48.90 >1	48.66 >1	I
Hp37	0.61	0.01	1.5E-03	61.15 >1	61.00 >1	I
Hp38	3.39	0.04	7.3E-03	84.93 >1	84.75 >1	I
Hp39	4.70	0.13	3.4E-02	36.42 >1	36.15 >1	I
Hp40	2.20	0.10	8.4E-03	22.08 >1	22.00 >1	I
Hp41	2.00	0.03	5.9E-03	66.86 >1	66.66 >1	I
Hp42	11.30	0.30	5.6E-02	37.85 >1	37.66 >1	I
Hp43	3.10	0.14	1.4E-02	22.24 >1	22.14 >1	I

Table 1 (cont.) The percentage of cannabinoid contents and the phenotypic index of each sample.

Samples ID	Cannabinoids content			Chemical characterization		
	%Δ^9-THC	%CBD	%CBN	Phenotypic idex $\frac{\%\Delta^9\text{-THC}+\%\text{CBN}}{\%\text{ CBD}}$	$\frac{\text{THC}}{\text{CBD}}$ ratio	
					Ratio	Chemotype
Tak province, Hmong Kee-ree-ras Village, Pop-pra.						
Hp44	0.60	0.02	3.0E-03	30.15 >1	30.0 >1	I
Hp45	3.01	0.10	4.3E-02	30.53 >1	30.1 >1	I
Tak province, New Kee-ree-ras Village (Rom-glao) Moo. 9, Kee-ree-ras, Pop-pra, Coordinate MU810212, 853 m. from sea level.						
Hp46	6.36	0.10	2.7E-03	63.62 >1	63.6 >1	I
Hp47	1.80	0.01	7.6E-04	180.08 >1	180.00 >1	I
Hp48	1.20	0.01	6.0E-04	120.06 >1	120.00 >1	I
Hp49	3.68	0.06	1.8E-03	61.36 >1	61.33 >1	I
Hp50	2.50	0.04	1.4E-03	62.54 >1	62.50 >1	I
Hp51	2.20	0.20	7.5E-04	11.00 >1	11.00 >1	I
Hp52	4.80	0.06	2.0E-03	80.03 >1	80.00 >1	I
Hp53	1.40	0.03	5.0E-04	46.68 >1	46.66 >1	I
Hp54	2.60	0.40	9.0E-04	6.50 >1	6.50 >1	I
Hp55	2.10	0.05	8.0E-04	42.02 >1	42.00 >1	I
Tak province, New Kee-ree-ras village (Rom-glao) Moo. 9, Kee-ree-ras, Pop-pra, Coordinate MU 809201, 842 m. from sea level.						
Hp56	1.30	0.20	9.0E-04	6.51 >1	6.50 >1	I
Hp57	1.70	0.03	9.0E-03	56.96 >1	56.66 >1	I
Tak province, Ruam Thai pattana village 4, Ruam-Thai Pattana, Pop-pra, Coordinate MU 808151, 721m. from sea level.						
Hp58	9.14	0.16	2.5E-03	57.14 >1	57.13 >1	I
Hp59	0.05	0.04	5.0E-05	1.25 >1	1.25 ≈ 1	II
Hp60	3.94	0.51	3.1E-03	7.73 >1	7.72 >1	I

Table 1 (cont.) The percentage of cannabinoid contents and the phenotypic index of each sample.

Samples ID	Cannabinoids content			Chemical characterization		
	%Δ^9-THC	%CBD	%CBN	Phenotypic idex $\frac{\%\Delta^9\text{-THC}+\%\text{CBN}}{\%\text{ CBD}}$	$\frac{\text{THC}}{\text{CBD}}$ ratio	
					Ratio	Chemotype
Tak province, New Kee-ree-ras village (Rom-glao) Moo. 9, Kee-ree-ras, Pop-pra, Coordinate MU 798201, 830 m. from sea level.						
Hp61	8.50	0.16	7.1E-03	53.17 >1	53.13 >1	I
Hp62	2.58	0.06	2.8E-03	43.05 >1	43.00 >1	I
Hp63	8.40	0.12	4.4E-03	70.04 >1	70.00 >1	I
Hp64	9.70	0.30	6.0E-03	32.35 >1	32.33 >1	I
Hp65	6.60	0.11	5.4E-03	60.05 >1	60.00 >1	I
Hp66	10.17	0.21	5.9E-03	48.45 >1	48.42 >1	I
Hp67	3.05	0.09	3.6E-03	33.92 >1	33.88 >1	I
Hp68	2.47	0.10	3.0E-03	24.73 >1	24.70 >1	I
Hp69	2.10	0.35	3.0E-03	6.01 >1	6.00 >1	I
Tak province, New Kee-ree-ras village (Rom-glao) Moo. 9, Kee-ree-ras, Pop-pra, Coordinate MU 814219, 864 m. from sea level.						
Hp70	0.96	0.02	6.5E-04	48.03 >1	48.00 >1	I
Hp71	4.10	0.10	2.3E-03	41.02 >1	41.00 >1	I
Hp72	1.16	0.02	1.2E-03	58.06 >1	58.00 >1	I
Tak province, Ton Ma-muang village Moo. 13, Mae-Tor, Muang, Coordinate MU 990460, 824 m. from sea level.						
Hp73	5.02	0.06	7.3E-03	83.78 >1	83.66 >1	I
Hp74	1.48	0.08	1.5E-03	18.52 >1	18.50 >1	I
Hp75	2.33	0.02	3.4E-03	116.67 >1	116.50 >1	I
Hp76	3.74	0.03	2.9E-03	124.76 >1	124.66 >1	I
Hp77	9.70	0.20	1.0E-02	48.55 >1	48.50 >1	I

Table 2 Percentage of *Cannabis* types in Chiangmai and Tak provinces.

Criteria / Area (No. of plants)	Δ^9-THC		Phenotypic index		THC/CBD ratio		
	Drug (%)	Fiber (%)	Drug (%)	Fiber (%)	Drug (%)	Intermediate (%)	Fiber (%)
Chiangmai province (15 plants)	11(73.33)	4(26.67)	13(86.67)	2(13.33)	13(86.67)	0	2(13.33)
Tak province (43 plants)	42(97.67)	1(2.33)	43(100)	0	42(97.67)	1(2.33)	0
Total (58 plants)	53(91.38)	5(8.62)	56(96.55)	2(3.45)	55(94.83)	1(1.72)	2(3.45)

Discussion

In this study, the GC-MS technique was used for cannabinoid analysis of the *C. sativa* L. samples. The cannabinoids contents in the samples were determined based-on the one point calibration of Δ^9-THC, CBD, and CBN reference standards. Comparing to multi-points calibration curves, the results may not be as accurate for samples which have values much lower and much higher than the calibration value. However, these values were good representatives for quantitative and qualitative analysis of plant samples for the survey of the situation of hemp cultivation in Thailand.

Considering only the Δ^9-THC content, results showed that 91.38% of the *C. sativa* L. plants randomly sampled from the cultivation areas were chemically classified as "drug type", but when the phenotypic index and THC/CBD ratio were considered, 96.55% and 94.83% of the sample group was "drug type". The conflicting classification of the *Cannabis* types was demonstrated in 3 plants; Hp03, Hp09, and Hp59. The reported Δ^9-THC content of less than 0.3% suggested that Hp03, Hp09, and Hp59 were "fiber type" plants. However, this contradiction with the phenotypic index of the 3 plants of "more than 1" indicated that all three samples are "drug type" plants. In addition, the THC/CBD ratio suggested that Hp03 and Hp09 were "drug type" plants, and Hp59 was an "intermediate drug type" as the ratio was "more than 1" and "close to 1", respectively. As a result, percentage of plants that were classified as "fiber type" reduced from 8.62% to 3.45% of the sample group.

To control the cultivation of hemp, it is suggested that classification according to the published criteria should be in the given priority. Firstly, the *Cannabis* type should

be determined by the cut-off point of 0.3% Δ^9-THC because plants with Δ^9-THC content of less than 0.3% is reported to have no psychoactive effect. If further clarification of the *Cannabis* types is required for classification, the phenotypic index and/or the THC/CBD ratio should then be considered. Under this circumstance, the "intermediate drug type" with less than 0.3% Δ^9-THC content would then be accounted as a "fiber type" plant. These would benefit the promotion of commercial cultivation of fiber type hemp under a controlled level.

The variation of cannabinoid contents in the samples may be a result of cross pollination and/or together with environmental effect on plant growth. Hemp was cultivated from seed stock collected from the mature plants grown in the past season. These seeds may occur from cross pollination of different chemotype plants resulting in a mix of hybrid seeds. Moreover, environmental conditions such as climate, sunlight, the quality of soil in the cultivation field, and high altitude can induce *Cannabis* plants to produce high amount of psychoactive components.

Conclusion

We report in this study that 91.38%, of the *C. sativa* L. "fiber type" plants randomly collected from the cultivation areas in Thailand, were classified as "drug type" plants, due to the presence of Δ^9-THC content more than 0.3%. In detail, 73.33% of *C. sativa* L. plants sampled from Chiangmai, and 97.67% of the *C. sativa* L. plants sampled from Tak were classified as "drug type". From the drug control point of view, the Δ^9-THC content is the most important index for the determination of *Cannabis* chemotypes because of the psychotic effect of the Δ^9-THC compound. Other classification of *Cannabis* types based on the amount of cannabinoids can be used as secondary criteria for further verification. It is suggested that the authority must also consider factors which may affect the induction of cannabinoids contents in plants, such as seed stocks, cross pollination in the field, and environmental factors, to prevent the expansion of drug type cultivation.

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References

1. Gregory, L. D. 2003. Global Drug Enforcement: Practical Investigative Techniques. California. CRC Press.
2. Kitpipit, T. 2006. Forensic Detection of Processed Marijuana. Bangkok. Master Thesis of Mahidol University.
3. Watanasiri, S. 2006. Forensic Detection and Discrimination of *Cannabis* Types. Bangkok. Master thesis of Mahidol University.
4. Reglement (CEE). 1989. Reglement No. 1164/89, Relatif Aux Modalites Concernant L'aide Pour Le Lin Textile Et Le Chanvre. 28 April 1989.
5. Division of Narcotics Drugs, United Nation, Vienna. 1987. Recommended Methods for Testing *Cannabis:* Mannual for Use by National Narcotics Laboratories ST/NAR/8.
6. Fetterman, P. S., Keith, E. S., Waller, C. W., Guerrero, O., Doorenbos, N. J. and Quimby, M. W. 1971. Mississippi-Grown *Cannabis sativa* L. Preliminary Observation on Chemical Definition of Phenotype and Variations in Tetrahydrocannabinol Content Versus Age, Sex and Plant Parts. *Journal of Pharmaceutical Sciences* 60: 1246-1249.
7. Hillig, K. W. and Mahlberg, P. G. A. 2004. Chemotaxonomic Analysis of Cannabinoids Variation in *Cannabis* (Cannabaceae). *American Journal of Botany* 91(6): 966-975.
8. Stefanidou, M., Dona, A., Athanaselis, S., Papoutsis, I. and Koutselinis, A. 1998. The Cannabinoids Content of Marihuana Sample Seized in Greece and its Forensic Application. *Forensic Science International* 95: 153-162.
9. West, D. P. 1998. Hemp and Marijuana: Myths and Realities. Wisconsin. North American Industrial Hemp Council.
10. The Narcotics Act of B. E. 1979. Food and Drug Administration, Thailand.

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