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Cannabinoids Bee Pollen: A Presence of Phytochemical Cannabidiol (CBD) in *Cannabis sativa* L. (Hemp) on The Characteristics of *Apis mellifera* L. Raising Protocol in Samoeng, Chiang Mai, Thailand

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Abstract:

Honey bees (Apis mellifera L.) are internationally kept by the beekeeping industry due to they are relatively gentle and calm easy to obtain, excellent foragers, moderate tendency to swarm, and good, compact brood pattern resulting in a strong workforce for a high productivity. While Cannabidiol (CBD) are unique phytochemical compounds encounter only in Cannabis sativa (hemp) plants. This experimental research aimed to investigate a presence of Cannabidiol (CBD) via a prototype of bee-raising protocol foraging in hemp cultivar to collect hemp pollens. Theoretically, in-hived stored hemp pollen shall hold phytochemicals as being abundance in hemp plants, thru biological (biotic) extraction process. In the field experiment, seed production hemp plot in Samoeng, Chiang Mai was fully covered by mosquito net to confine honey bees during male flowering. The extraction of CBD of in-hived stored pollen samples and it intensity was conducted by GC-MS technique. A repeated measures ANOVA model was conducted for statistical analysis. The result was presences of phytochemical compounds, Cannabidiol (CBD) in hemp plants to in-hived honey bee pollen as bee raising produce, significantly (P-value < 0.0009). This first found experiment result would open up opportunities in developing Thailand agroindustry.

Keywords: *Apis mellifera, Cannabis sativa L. (Hemp), Cannabidiol (CBD), GC-MS, Repeated Measures ANOVA*

I. INTRODUCTION

Honey bees (*Apis mellifera* L.) are widely kept by the beekeeping industry since then due to they are relatively gentle and calm easy to obtain, moderate to high cleaning behaviour, white capping in common, excellent foragers, moderate tendency to swarm, and good, compact brood pattern resulting in a strong workforce for collecting a good amount of nectar and pollen [1], [2]. Their characteristics considerably generate the good yields for honey bee produces [3]. Cannabidiol (CBD) is a non-psychoactive compound. It has been well recognizing as one of potent Cannabinoids, a unique phytochemical constituent encounters only in *Cannabis sativa* (hemp) plants. CBD has been used in pharmaceutical manufacturing in western medicines i.e. a pure concentrate of CBD used to treat severe forms of Epilepsy, as well as in the herbal supplements industry [4]. Its unique characteristics and therapeutically bioactive potency would be vital in medicinal, healthcare purposes if one could manage to get this powerful phytochemical compound to honey bee produces in a symbiosis process. Recent studies have documented the importance of hemp pollen in supporting a diverse community of honey bees during periods of floral resource scarcity [5], [6]. Mass flowering crops such hemp cultivar can support pollinator populations foraging [7], [8], but the incorporation of novel hemp pollens into the diets

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of general bees has yet been shown to have detrimental effects on larval development [9]. The enthusiasms are to investigate and identify cannabidiol (CBD) in *Cannabis sativa* plant being transferred by means of honey bee raising protocol into in-hive stored pollens via 'cannabinoids biological/ biotic extraction' process as the main objective of this experimental research.

II. Methods

A. Field experiment method

The total of 9 hives of raising honey bees (*Apis mellifera* L.) were moved to hemp plot where hemps were planted for seed and fibre production in Baan Khong Khark Luang, Samoeng, Chiang Mai, Thailand (Latitude: 18°53′45.75″ North and Longitude: 98°42′23.31″ East) where elevation of 720 metre above mean sea level, average temperature of 18.8-29.6 degree Celsius, average air humidity of 53.6-95 percent (at mean of 74.3%) and rainfall average of 1,075.5 mm. and photoperiod of 11-13 hours daily. 6 Honey beehives were confined within mosquito net (mesh 16 threads per square inch) covering the hemp plot to ensure collected data of only hemp pollen from beehives and also protect bees from other predators during experimental period (Figure 1). The other 3 beehives were set up outside the mosquito net area as controlled experimental units. The in-hive-stored Pollens, during the male flowering peak, were collected from brood frames into prepared and sterilized vials on every other days (on September 14, 16 and 18 between 13.00 and 16.00 hours) in volumes of approximately 10 grams from each hive for sufficient laboratory investigation.

Figure 1 Experimental *Cannabis sativa* L. cultivar and foraging honey bees

B. Laboratory

Instruments: Gas Chromatograph/Mass Spectrometry Detector (GC/MSD) for Cannabidiol (CBD) as being incumbent technique used in Thailand [10]-[12]

Gas chromatography: Agilent technologies made in China Model 6890 N, Oven 100 °C hold 1 min, 10 °C/min to 300°C hold 9.0 min., Post time 5 min. at 330 °C, Total run time 30 min., Helium carrier gas flow 1.0 mL/min, Column DB 5MS Agilent technologies made in USA 0.25 mm x 30m x 0.25 micron of film thickness, Inlet split 20:1 volume of injection 1 uL., Inlet temperature 280 °C, Auxiliary temperature 280 °C; **Mass spectrometer detector**: Agilent technologies made in USA Model 5973 inert, Scan mode 40 – 500 m/z, MS Quadrupole temperature 150 °C, MS Source temperature 230 °C; **Database Agilent technologies USA**: Wiley version 9; Basic instrument: Ultrasonic bath: BRANSON 3510 USA, Vortex mixer: Genie 2 USA, Water bath: Memmert WNE21 Germany, Freezer -20°C: Sanyo Japan; **Reagent**: Hexane (HPLC Grade) Labsan Ireland, Cannabinoids standard CBD: RESTEX (34014) USA.

III. Results

Laboratory results

Table 1 Cannabidiol (CBD) intensity/ abundances being detected from in-hive stored pollen samples.

		Measure no. by each collection			
Experimental Unit no.1 (Treatment # 1)	Hive #1	**CM62/10544-001**	**T1H1-1**	**T1H1-2**	**T1H1-3**
		CBD (RT 18.03 min.)	2,348 23.48%	2,335 23.35%	1,954 19.54%
	Hive #2	**CM62/10544-002**	**T1H2-1**	**T1H2-2**	**T1H2-3**
		CBD (RT 18.03 min.)	2,703 27.03%	2,600 26.00%	2,428 24.28%
	Hive #3	**CM62/10544-003**	**T1H3-1**	**T1H3-2**	**T1H3-3**
		CBD (RT 18.03 min.)	2,426 24.26%	2,445 24.45%	2,728 27.28%

Remark: CBD unit is mg/kg

Table 1 Cannabidiol (CBD) intensity/ abundances being detected from in-hive stored pollen samples (Cont.)

		Measure no. by each collection			
Experimental Unit no.2 (Treatment # 2)	Hive #1	**CM62/10544-001**	**T2H1-1**	**T2H1-2**	**T2H1-3**
		CBD (RT 18.03 min.)	9,418 94.18%	10,903 109.03%	10,152 101.52%
	Hive #2	**CM62/10544-002**	**T2H2-1**	**T2H2-2**	**T2H2-3**
		CBD (RT 18.03 min.)	10,209 102.09%	9,470 94.70%	8,021 80.21%
	Hive #3	**CM62/10544-003**	**T2H3-1**	**T2H3-2**	**T2H3-3**
		CBD (RT 18.03 min.)	7,252 72.52%	7,663 76.63%	8,482 84.82%

Remark: CBD unit is mg/kg

Table 1 Cannabidiol (CBD) intensity/ abundances being detected from in-hive stored pollen samples (Cont.)

Measure no. by each collection

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Experimental Unit no.3 (Treatment # 3)	Hive #1	**CM62/10544-001**	**T3H1-1**	**T3H1-2**	**T3H1-3**
		CBD	6,060	6,824	7,288
		(RT 18.03 min.)	60.60%	68.24%	72.88%
	Hive #2	**CM62/10544-002**	**T3H2-1**	**T3H2-2**	**T3H2-3**
		CBD	8,786	5,768	6,005
		(RT 18.03 min.)	87.86%	57.68%	60.05%
	Hive #3	**CM62/10544-003**	**T3H3-1**	**T3H3-2**	**T3H3-3**
		CBD	9,180	9,166	7,777
		(RT 18.03 min.)	91.80%	91.66%	77.77%
Limit of Quantification-LOQ			1.00	1.00	1.00
Limit of Detection-LOD			0.50	0.50	0.50

Remark: CBD unit, LOQ, and LOD are mg/kg

Statistical analysis

Using the Repeated measures ANOVA model by paralleling means across measure variables that were based on repeated observations. As seen comparison and analysis below.

Table 2 Detected Cannabidiol (CBD) abundance (mg/kg) classified by the experimental units

Experimental Unit (Treatment#)	Sampling Unit (Beehive#)	Measure 1	Measure 2	Measure 3
1	1	2,348	2,335	1,954
	2	2,703	2,600	2,428
	3	2,426	2,445	2,728
2	1	9418	10,903	10,152
	2	10,209	9,470	8,021
	3	7,252	7,663	8,482
3	1	6,060	6,824	7,288
	2	8,786	5,768	6,005
	3	9,180	9,166	7,777

Table 3 Analysis of Variance of detected Cannabidiol (CBD) abundance/ intensity

Sources of Variation	Sum of Squares	Degrees of Freedom
Experimental Unit	214,219,297.556	2
Discrepancy	16,104,120.444	6
Sum of between Groups	230,323,418.00	8
Time of Measure	722,644.222	2
Time of Measure * Exp. Unit	1,262,698.889	4
Discrepancy	10,276,331.556	12
Sum of Within Group	12,261,674.67	18
Sum Total	**242,585,092.67**	**26**

Table 3 Analysis of Variance of detected Cannabidiol (CBD) abundance/ intensity (Cont.)

Sources of Variation	Root Means Square (RMF)	F	P-value
Experimental Unit	107,109,648.778	39.906	<0.0009
Discrepancy	2,684,020.074		
Sum of between Groups			
Time of Measure	361,322.111	0.422	0.665
Time of Measure * Exp. Unit	315,674.722	0.369	0.826
Discrepancy	856,360.963		

From the test, it found that

Where $F_1 = 39.906$ It could be concluded that each experimental unit contains different means of detected CBD P-value < 0.0009. When a pair test is given, the findings were that none of means differences of experimental unit 2 and 3 is found with greater means than experimental unit 1.

Where $F_2 = 0.422$ It could be concluded that none of different means is found in each measure of detected CBD P-value = 0.665.

Where $F_3 = 0.369$ It could be concluded that none of different interaction within measure is found with experimental unit P-value = 0.826.

The finding could be interpreted that among 3 different treatments, as above statistical result shown; among the group of treatments, each treatment has different means of detected CBD which is significant (P-value < 0.0009). While none of different means within each group shown no statistically significant difference (by each measure and within each treatment P-value > 0.05). Therefore, all repeated measure data within a group of treatments are the same means of detected CBD. When comparing average means among all treatments; detected CBD abundance of treatment 2 (with diluted syrup in-hive fed and detained bees within netted hemp cultivar) and treatment 3 (without feeding, with free foraging bees in opened hemp cultivar) are greater than treatment 1 (with diluted honey in-hive fed and detained bees within netted hemp cultivar). This is noticeable that under the same protocol of bee raising in order to manage honey bee colony at most readiness stage for foraging C*annabis sativa* L. (hemp); the artificial feeding with carbohydrate source (nectar) might be influential in CBD intensity containing in bee produce yields. However, the valuable finding in this experiment has been clearly possible that targeted cannabinoids (CBD) is transferred to bee produces via a biological/ biotic extraction process according to the research objective.

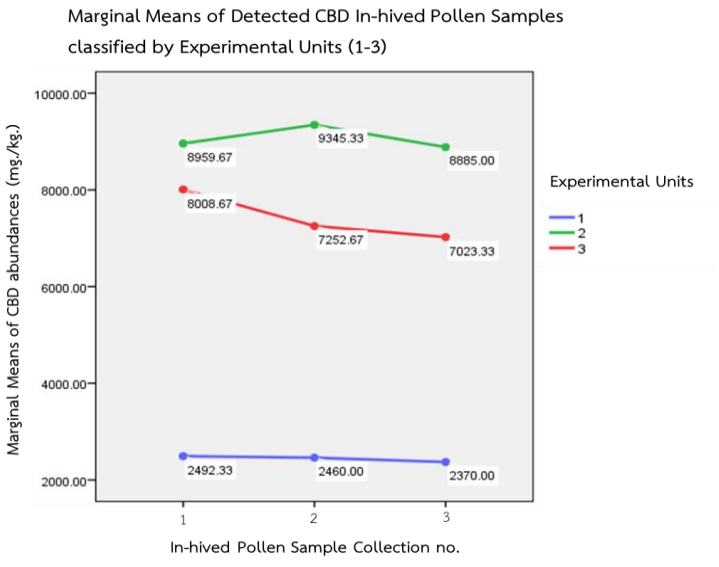

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Figure 2 Marginal Means of detected Cannabidiol (CBD) illustrated by experimental units

CONCLUSION

The presence of CBD in honey bee pollen will sparking a possibility in managing bee at hemp cultivar. This worthy information would be ultimately useful in terms of business development in the future, especially for CBD which is high in Cannabis sativa L. (hemp), namely CBD infused honey thru the biological/ biotic extraction. This would be tailor-made on demand for health food industry. Obviously, under the same protocol of bee raising in order to manage honey bee colony at most readiness stage for foraging Cannabis sativa L. (hemp); the artificial feeding with carbohydrate source (nectar) was probably influential in CBD intensity reflecting to bee produce yields. The theory on phytochemicals transfer from plants, especially prolific flowers to bee. This was also considered as a biological (biotic) extraction in natural rather than chemicals used by laboratory or industry. Since this first found experiment on how to manage bees to verify the natural extraction of cannabinoids, which are rich in Cannabis sativa L. (industrial hemp) depends upon their strains and landraces. Since cannabis sativa L. (hemp) plants produce large amounts of pollen that are attractive to bees, thus to manage bees was a crucial experiment. With some techniques deployed in order to calm bees for their utmost efficiency while induce stress to hemp plants expected to optimize level of outputs both from bees and plants. During the time of seasonal dearth of natural/ crop foods availability in apiaries, commercially grow hemps should be one of alternatives to develop model of Thailand beekeeping industry which have been struggling with climate changes vastly reflect to commercial crop plants and cultivation practices along with inefficiently controllable uses of pesticides and herbicides. This experiment could be an inspiration for further exploration of studies on negative impacts on bee reproductive system. Presently, according to techniques have been adopting for extraction either for medical, recreation or other purposes for food supplements generally using the biosynthetic extraction methods. While a biological (biotic) extraction for natural produces could be adaptably used in other sectors i.e. nutritional supplements in Thai Traditional and complementary medicine and also for green agroindustry purposes.

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