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Impact of Certain Egyptian Wax Foundation Types and Their Chemical Composition on Biological Activities of Honey Bees

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Abstract The quality of certain beeswax foundation sheets locally marketed that named by Etman, Mady and Nasr and natural beeswax secretion by bees was determined. Quality was evaluated which based on the effect of beeswax foundation sheets on the biological activities of honey bee colonies. Also, the chemical analysis of these natural beeswax or foundation sheets was detected by Gas Chromatography (GC) and Mass Spectrometry (MS).Those parameters were carried out all through unique blooming seasons of citrus, clover and black cumin in three governorates of Egypt. In April season the studies showed significant differences in the average area of comb built (drawn) by honey bee colonies after one month in Qalubia region. Also, at the same region a significant area of wax cells built were used for worker brood in Mady beeswax foundation followed by Etman and Nasr beeswax foundation. Mady wax foundation had a significant area of honey stored at Qalubia region. Tested colonies at Beni Sauef built a highly area of wax cells (243.3 inch^2) of Etman wax foundation followed by Assiut (203.3 inch^2) and Qalubia region (133.6 inch^2). In June period Beni Sauef recorded a significant value of natural secreted wax cells (373.33 inch^2) followed by of Assiut (350 inch^2) and Qalubia (221.66 inch^2) after one month of test technique. It is noticed that the natural secreted wax cells were used for worker brood rearing at Qalubia region. Results indicated that during June period bee workers in the tested colonies used natural secreted wax cells for honey stored and drone brood rearing at Beni Sauef and Assiut regions.

Keywords: honey bees, beeswax foundation, chemical composition, biological activities

Introduction

Honey bee workers produce the beeswax that is the wax gland secretion of bee workers. Wax secretion is the highest activity in bees from 9 to 15 days old according to the needs of the bee colony (Skowronek, 1973). Through beekeeping processes the quality of recycled beeswax in honey bee colonies is important to preserve the natural properties of apiarian products and to avoid the rejection of foundation beeswax sheets by the bees. Some physicochemical

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parameters are detecting the beeswax adulterations, but it is not useful to identify unequivocally the added product (Tulloch and Hoffman, 1972; Tulloch, 1980). According to determine these parameters, adulteration percentages close to 5–10% can be detected if products such as paraffin, tallow or stearic acid are mixed with beeswax (Bernal *et al.*, 2005).

The components of beeswax are belonging to the chemical groups of esters (67%), hydrocarbons (14%), fatty acids (12%) and alcohols (1%) (Tulloch, 1980). The linear saturated hydrocarbons (n –alkanes) are the most numerous group of hydrocarbons in beeswax (Streibl *et al.*, 1966). In the beekeeping industry the beeswax is used to produce the comb foundation (Waś *et al.,* 2014a). The amount of even numbered *n*-alkanes (C22- C32) is significantly higher in darker coloured beeswax than light coloured beeswax. This may be returned to the accumulation of cuticular residues found in the darker coloured comb cells. Cuticular residues are known to contain C23-C32 odd and even numbered *n*-alkanes (Namdar et al., 2007 and Waś et al., 2014b). This work aimed to investigate the qualitative and quantitative analysis of beeswax hydrocarbons either wax foundation or naturally secreted by honey bee workers.

Materials and methods

This proposal was carried out through different blooming seasons of citrus, clover and black cumin during the period from 15 March to 31 July 2016. The experiments were conducted in three regions of Egypt, Qualubia, Beni Sueif and Assiut governorates. Thirty six experimental honey bee colonies headed by carniolan hybrid queens were used for this proposal. These colonies were nearly similar in strength, stored food and the number of combs covered with bees. Three types of local marketed beeswax foundation sheets that has commercial name Etman, Mady and Nasr from Egypt were used for this study. Twelve colonies were used for each governorate, nine colonies provided with one beeswax foundation sheet for each colony (three colonies/ wax type). The rest three colonies were used for natural beeswax secretion and provided them by empty wood comb with wire metal for each colony.

Either the beeswax foundation sheet or empty wood combs were inserted inside all tested colonies between honey and brood combs place. Wax cells building by bee workers in beeswax foundation sheets and that natural secreted in empty wood combs were measured in square inches. The measurements were done at 1,2,3,7 and 30 days after treatment.

At the end of experiment area of sealed worker brood, drone brood and stored honey (inch²) were measured according to Fresnay (1962) in wax cells building in beeswax foundation and that have been secreted naturally.

Chemical analysis of beeswax Beeswax samples

Samples of the beeswax foundation originated from the local market of Egypt belonging to the different commercial names Etman, Mady and Nasr were cooperating in the study. Samples of beeswax naturally secreted were obtained from the natural beeswax inside the beehive.

Analysis of beeswax hydrocarbons

Screening of beeswax hydrocarbons with the Gas Chromatography (GC) and Mass Spectrometry (MS) was detected according to the technique by Waś et al., 2014a. Instrument used as follows:

GC/MSD 5977A, Agilent, USA.

Column used: Agilent, HB 5ms -60 ℃-325 ℃ (350 ℃): 30 m x 250 µm x0.25 µm.

Oven program 50 ℃ for 1 min then 10 ℃/min to 320 ℃ for 25 min.

Carrier gas helium (purity: 99.9999) flow was kept constant at 1 ml /min.

Pulsed split less injection, injection pulse pressure 24.95 psi until 1 min.

Inlet: Multimode inlet (MMI), Liner Agilent 5190- 2293: 900 µL.

Inlet Temperature: 280 ℃.

Auxiliary temperature: 330 ℃.

Statistical analysis

All the different data obtained were subjected to analysis of variance (ANOVA) using the SPSS (Statistical Package for the Social Sciences) computer program. The means were compared using Duncan's Multiple Range Test.

Results

Impact of different locally foundation beeswax sheet on the ability of bee workers to build comb cells in two regions Qualubia and Beni Sauef in April was illustrated in Table 1. Whereas, the results showed significant differences in the average area of comb built (drawn) by honey bee colonies after one month in Qalubia region. Average area of comb built by bees reached to 237.6 inch² in Mady beeswax foundation followed by Etman (188.6 inch²) and Nasr (180.6 inch²). At Beni Sauef region no significant differences were found especially after one month of experiment. Regarding to the t-value honey

bee workers at Qalubia governorate produced a significant value of comb constriction in Mady and Nasr beeswax foundation after one month of insert the foundation sheet in the colonies. At Qualubia region significant area of wax cells built were used for worker brood in Mady beeswax foundation (154.0 inch^2) followed by Etman and Nasr beeswax foundation (141.0 & 129.3 inch^2). No significant differences were obtained in sealed worker brood area in Beni Sauef region. Sealed drone brood area recorded insignificant values between the tested types of wax foundations in either Qalubia or Beni Sauef regions. The wax cells built contained significant worker brood area at Qalubia than Beni Sauef region in all tested wax foundations According to the area of stored honey Mady wax foundation had a significant area of honey at Qalubia region in vice versa no significant differences were recorded at Beni Sauef region (Table 1). While wax foundation at Qalubia region resulted a significant value of honey stored than Beni Sauef region.

Results obtained from the experiments during June period in Table 2 indicated that colonies provided with Mady wax foundation sheets built a highly mean area of wax cells after one month. Whereas, colonies at Qalubia and Assiut region recorded 173.3 & 206 inch^{2}of wax cells built, respectively. While, colonies in Beni Sauef region built more wax cells when provided with Etman wax foundation sheets (243.3 inch^2) than that of Mady and Nasr wax foundations. Results also showed that there were significant differences between the experimental regions in wax cells built especially with Etman wax foundation during one month of treatment.

Tested colonies at Beni Sauef built a highly area of wax cells (243.3 inch^2) followed by Assiut (203.3 inch^2) and Qalubia region (133.6 inch^2). Regarding to the area of the wax cells which was built in the different tested wax foundations. The majority of these cells was used for worker brood rearing especially at Beni Sauef region (Table 2). While, wax cells built for worker drone were highly number in Etman wax foundation (1.0 3 inch^2) at Qalubia region but they were more in Mady wax foundation at Assiut (10.3 inch^2) and Beni Sauef (2.3 inch^2) regions. A highly mean area of Nasr wax foundations was used for honey storage at Assiut (40 inch^2) and Beni Sauef (10 inch^2) regions. In Qalubia region Mady wax foundations were the best type for honey stored (95 inch^2).

Table 1. Effect of different locally foundation eeswaxheet on comb building and biological activities of honey bee colonies during April 2016

Governo-rate	Type of wax foundation	Area of comb built (inch²)					Sealed worker brood area (inch²)	Sealed drone brood area (inch²)	Honey area (inch²)
		24 h	48 h	72 h	7 days	Month			
Qalubia	Etman	0.0	28.0a	83.0a	100.0	188.6a	141.0 a	1.0	45.0 a
	Mady	0.0	20.0b	91.0a	100.0	237.6b	154.0 b	1.0	75.6 b
	Nasr	0.0	20.6b	72.6b	95.0	180.6a	129.3 c	0.6	45.3 a
	F-value	0.0	10.2**	13.03**	3.0 ^{NS}	66.4**	42.3**	0.1 ^{NS}	54.0**
Beni	Etman	61.0	113.6a	116.0	127.6	174.3	112.3	1.0	1.3
Sauef	Mady	48.6	97.0b	116.0	122.0	162.0	110.6	1.0	1.3
	Nasr	50.3	106.0ab	116.0	121.6	163.0	112.0	1.3	2.3
	F-value	3.1 ^{NS}	9.4**	0.0 ^{NS}	1.5 ^{NS}	1.1 ^{NS}	0.2 ^{NS}	0.5 ^{NS}	0.1 ^{NS}
t- value	Etman	--	--	--	--	1.21 ^{NS}	12.68**	0.0 ^{NS}	13.73**
	Mady	--	--	--	--	42.89**	21.37**	0.0 ^{NS}	49.86**
	Nasr	--	--	--	--	3.64*	5.20**	0.7 ^{NS}	13.44**

F- value for comparison between wax foundation types in each region. *: Significant; ** : Highly significant; NS: Non-significant. Means in a column with dissimilar letters differ significantly at 0.05 level of probability. t-value for comparison between regions in each wax foundation types

Table 2. Effect of different locally foundation beeswax sheet on comb building and biological activities of honey bee colonies during June 2016.

Governorate	Type of wax foundation	Wax secretion (inch²)					Sealed worker brood area (inch²)	Sealed drone brood area (inch²)	Honey area (inch²)
		24 h	48 h	72 h	7 days	Month			
Qalubia	Etman	12.0 a	20.0 b	80.0 b	100.0 a	133.6 a A	90.0 a B	1.0	90.0a A
	Mady	0.0 b	35.0 a	100.0 a	108.3 a	173.3 a	95.0 a B	0.6	95.0a A
	Nasr	0.0 b	18.0 b	68.6 b	100.0 a	155.0 a	116.6 b B	0.3	36.6b A
	F -value	432.0**	86.3**	9.5*	0.5 ^{NS}	1.0 ^{NS}	5.6*	0.3 ^{NS}	51.3**
Beni Sauef	Etman	119.3	190.0	233.0 a	237.0	243.3 B	222.0 a A	2.3	9.3 B
	Mady	111.6	193.3	208.0 b	234.6	231.6	210.3 b A	3.0	9.3 C
	Nasr	97.0	200.3	216.6 b	237.3	236.6	215.3 b A	1.3	10.0 B
	F- value	0.8 ^{NS}	0.1 ^{NS}	7.3*	0.6 ^{NS}	1.7 ^{NS}	15.6**	0.5 ^{NS}	0.3 ^{NS}
Assiut	Etman	6.6	12.6	32.6 a	170.0 ab	203.3 C	80.0 B	3.0 a	26.6 B
	Mady	2.3	7.3	27.3 a	176.6 a	206.6	73.3 B	10.3 b	33.3 B
	Nasr	0.0	12.0	58.0 b	103.3 b	188.3	113.3 B	0.3 a	40.0 A
	F -value	1.3 ^{NS}	0.5 ^{NS}	8.7*	4.3 ^{NS}	0.2 ^{NS}	0.7 ^{NS}	3.5 *	0.5 ^{NS}
F –value ˣ (Etman)		--	--	--	--	25.62**	19.28**	0.90 ^{NS}	29.52**
F –value ˣ (Mady)		--	--	--	--	0.81 ^{NS}	15.72**	3.24 ^{NS}	66.66**
F –value ˣ (Nassar)		--	--	--	--	4.28 ^{NS}	81.03**	0.77 ^{NS}	24.49**

NS: Non-significant, Means in a column with dissimilar letters (a,b and c) differ significantly 05 level of probability between types of wax foundation for each region.

F –value ˣ : for comparison between regions in each wax foundation types by figures; A,B and C at 0.05 level of probability.

Natural secreted wax cells built by honey bee workers in colonies were determined in the experimental regions and illustrated in Table 3. In April data showed that after one month of insert the empty wood comb there were significant differences in the area of natural secreted wax cells built between colonies at Qalubia (234.33 inch²) and Beni Sauef (223.6 inch²) regions. While, in June period Beni Sauef recorded a significant value of natural secreted wax cells (373.33 inch²) followed by Assiut (350 inch²) and Qalubia (221.66 inch²) after one month of experiment procedure.

It is noticed that the natural secreted wax cells were used for worker brood rearing at Qalubia region either in April (96.66 inch²) or June (101.6 inch²). In contrast most of the natural secreted wax cells were used for drone brood rearing (6.0 inch²) and stored honey (200 inch²) at Beni Sauef region. Results also indicated that during June period bee workers in the tested colonies used natural secreted wax cells for honey stored (356.66 & 321.66 inch²) and drone brood rearing (10.66 & 10.0 inch²) at Beni Sauef and Assiut regions, respectively.

Table 3. Beeswax naturally secreted by honey bee workers in different regions of Egypt.

Governorate	**Wax secretion (inch²)**					**Sealed worker brood area (inch²)**	**Sealed drone brood area (inch²)**	**Honey area (inch²)**
	24 h	48 h	72 h	7 days	Month			
April								
Qalubia	8.00	24.33	54.00	71.33	243.33	96.66	7	116.66
Beni Sauef	71.33	103.00	162.66	222.00	223.66	0.00	6.0	200
t- value	5.52 **	11.89 **	7.12**	6.41 **	4.37*	8.04**	1.22 NS	8.98**
June								
Qalubia	3.33 a	16.00 a	54.66 a	89.66 a	221.66 a	101.6 a	4.33 b	116.66 a
Beni Sauef	97.66 b	198.66 b	288.33 b	333.33 b	373.33 b	0.00 b	10.66 a	356.66 b
Assiut	80.00 c	180.00 c	265.00 b	306.66 b	350.00 b	11.66 b	10.0 a	321.66 c
F- value	107.94**	389.64**	106.05**	133.63 **	46.47**	66.86**	23.35**	177.97**

t-value for comparison between regions in April for natural beeswax secretion. F- value for comparison between regions in June for natural beeswax secretion. *: Significant; ** : Highly significant; NS: Non-significant. Means in a column with dissimilar letters differ significantly at 0.05 level of probability.

Results indicated that samples analyses of foundation beeswax sheet and naturally secreted bees wax has a long chain n-alkanes contained from 20 to 36 carbon atoms in their molecule (Table 4). Foundation beeswax sheet produced by Mady Company had a higher content of the total alkanes, whereas the n-alkanes appeared from C20H42 to C36H74 in comparison with the other tested samples which contained n-alkanes from C22H46 to C36H74. The hydrocarbon compounds Eicosene and Heneicosane were found only in wax foundations from Mady Company. Although, the other resulted compounds in Table 4 beginning from Docosane to Hexatriacontane were found in either Etman & Nasr foundation beeswax sheets or wax naturally secreted by bee workers.

Table 4. Contents of n-alkanes (g/100g) in different beeswax foundations and in beeswax naturally secreted by bee workers.

No.	Retention time (min)				Compound	Formula
	Etman	Mady	Nasr	Natural secretion		
1	--	18.582	--	--	Eicosane	C20H42
2	--	19.524	--	--	Heneicosane	C21H44
3	20.418	20.425	20.421	20.4	Docosane	C22H46
4	21.286	21.293	21.287	21.29	Tricosane	C23H72
5	22.116	22.127	22.116	22.108	Tetracosane	C24H50
6	22.93	22.936	22.924	22.917	Pentacosane	C25H52
7	23.692	23.713	23.699	23.693	Hexacosane	C26H54
8	24.468	24.47	24.461	24.455	Heptacosane	C27H56
9	25.164	25.181	25.177	25.164	Octacosane	C28H58
10	25.873	25.878	25.88	25.867	Nonacosane	C29H60
11	26.53	26.53	26.543	26.53	Triacontane	C30H62
12	27.186	27.189	27.192	27.179	Hentriacontane	C31H64
13	27.802	27.803	27.809	27.796	Dotriacontane	C32H66
14	28.425	28.426	28.432	28.419	Tetratriacontane	C34H70
15	29.088	29.093	29.101	29.082	Hexatriacontane	C36H74

Discussion

Wax foundation sheet managements is an important part of the beekeeping practice and produce comb wax cells by the bee workers. In colonies combs used for brood rearing (worker and drone brood), stored honey and pollen grains. Our results showed that the bee ability for cells building on wax foundation sheets differed from wax type to another and the region of experimental procedure. These findings may be regarding to the honey bee hybrids breeding in each region. Whereas, most of bee hybrids rearing at Assiut

and Beni Sauef regions is carniolan Egyptian race cross but at Qalubia region is carniolan Italian race cross. Also, the blooming source of nectar crops in April is caraway, black cumin and anise plants at Assiut and Beni Sauef regions. While at Qalubia region is citrus crop. In June the main source of nectar is clover crops that were grown around the apiaries in all experimental regions. Our results are in line with those obtained by Bozina (1960) found that Italian bees secreted the greatest amount of bees wax and built combs the fastest. The Russian race was the last in this respect.

The parameters of some physico- chemical values differ from one country to another the parameters. The origin of the beeswax could be related to the environmental and geographical factors which play a significant role in beeswax composition (Serra *et al.*, 1989). In the present study we observed that Mady wax foundation sheets had a higher content of n-alkanes appeared from C20H42 to C36H74 in comparison with the other tested samples which contained n-alkanes from C22H46 to C36H74. Differences in n-alkane content may be caused by added some other external components during industrial operations. The presence of some adulterated to beeswax foundation can affect the values of some physico- chemical parameters (Vit *et al.*, 1992). Foundation beeswax sheets quality considers one of the importances of apiarists and a determinant factor in the honey bee colony development (Bernal *et al.*, 2005). Recycled beeswax quality is of concern to preserve the natural properties of bee hive products and to keep the bees for building wax cells of foundation beeswax sheets.

The results indicated that the dominant group hydrocarbons of either beeswax foundation or natural secreted beeswax are alkanes containing from 20 to 35 carbon atoms. These results are in line with those reported by other authors (Jimenez *et al.*, 2004; Serra Bonvehi and Ornantes Bermejo, 2012; Maia and Nunes, 2013; Waś *et al.*, 2014b). Differences in the quantitative composition (e.g., hydrocarbons) of the beeswax obtained from different species and races were shown by Brand-Garnys and Sprenger (1988), and Aichholz and Lorbeer (2000).

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