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ORIGINAL RESEARCH ARTICLE

Some biological aspects of honey bee colonies in relation to the age of beeswax combs

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A study was carried out to investigate the influence of differently aged wax combs (foundation as zero, 1, 2, 3 and 4 – 6 years old) on some biological aspects that affect the productivity of honey bee colonies. Twenty-five package colonies were equally divided and situated on each of the tested combs during the spring of two successive years (2018 and 2019). The obtained results revealed that worker brood areas, worker population, worker life span, weights of newly emerged workers and drones, and honey yield significantly increased with fresh combs. However, drone brood areas increased with old combs, and wax combs age had no effect on worker survivorship. It could be concluded that the wax combs aged from zero (foundation) to three years old (light color combs) are more preferable in the performance and productivity of honey bee colonies than the older (dark color combs) ones.

Keywords: wax comb age; honey bee colony; worker and drone brood; worker life span; honey production; weight of emerged workers and drones

Introduction

Western honey bee colonies build a set of wax combs inside their protected nests in tree cavities, hollows, or various human-designed hives. These combs play an important role in the inner homeostasis of the honey bee colonies. Bees use them as nurseries for rearing immature stages (eggs, larvae and pupae) as well as for food storage (Seeley, 1985). Smell, which increases with frequent use within the colony, supports the communication cues and the nest-mate recognition either between individuals of the same nest or for intra-colonial members (Breed & Stiller, 1992, 1998; Free, 1987; Namdar et al., 2007;Yang et al., 2010).

In wildlife, colony growth requires the construction of new wax combs that are quickly filled with eggs laid by the queen, so the workers gradually turn to storing food in the old ones (Free & Williams, 2009; Winston, 1987). But Berry and Delaplane (2001), mentioned that in spite of the survivorship of brood being higher in old combs, colonies which established on new combs produced more brood and larger bees than those originated on the old ones.

The construction of new combs requires a large amount of honey to be consumed then secreted as wax. Seeley (1985) and Nogueira-Couto and Couto (2006) pointed out that the secretion of one kg of wax requires a quantity of sugar equivalent to 6–7 kg of honey. This reflects negatively on colony productivity (Alber, 1974; Asadi Dizaji et al., 2007; Pratt, 2004; Taha et al., 2010).

Wax combs are light yellow in color at the beginning of construction yet gradually turn dark and brittle with frequent use in rearing brood and food storage (Free & Williams, 2009; Hepburn, 1998; Winston, 1987). The dark color of wax combs is a result of the accumulation of cocoon layers, ecdysis skins of larvae and pupae (Coggshall & Morse, 1984), and storage of pollen and propolis (Free & Williams, 2009), in addition to many unlimited pollutants that get absorbed inside the wax as time progresses (Hepburn & Kurstjens, 1988; Tulloch, 1980). The most common pollutants are spores of fungi and bacteria (Baily & Ball, 1991; Gilliam, 1985; Koenig et al., 1986; Nelson & Gochnauer, 1982), as well as chemical compounds (Morse, 1986; Smith & Wilcox, 1990;Wu et al., 2011).

The continued reuse of wax comb for brood rearing increases its weight (Zovaro, 2007) and causes a significant decrease in cell diameters and volumes (Abdellatif, 1965; Hepburn & Kurstjens, 1988). This change reflects negatively on colony performance through the disturbance of egg laying by the queen (Koeniger, 1970), as well as the significant decrease in the weight of the newly emerged workers which was also investigated (Abdellatif, 1965; Asadi Dizaji et al., 2007; Berry & Delaplane, 2001; Buchner, 1953).

Old combs are those used in brood rearing and storing pollen and honey for one or more years, so, these

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combs should have light, dark and black color according to Berry and Delaplane (2001) and Asadi Dizaji et al. (2007). On the other hand, Arnaut de Toledo et al. (2015) considered combs of light color that those ranged from recently built white combs to those of light brown color.

The main objective of this study is to evaluate the impact of combs aged from zero to six years old on some biological features and performance of honey, bee colonies under sub-tropical conditions.

Materials and methods

The present study was undertaken in the apiary of Agricultural Experimental Station, Faculty of Agriculture, Cairo University at Giza governorate during the spring season (March to June) of two successive years (2018 and 2019).

Preparing of experimental colonies

In each year, 25 packages of first hybrid Carniolan bees (1.5 kg each) were brought from Damietta governorate (North Egypt). These packages were packed from colonies previously treated with formic acid (5 times at 5 days intervals) and oxytetracycline powder at the end of both Autumn and Winter. Each package bees was headed by one of sister open mated queens aged 5–6 months. These queens were reared during the preceding August from the same breeding stock (pure Carniolan queen), the same comb of young brood and the same patch of grafting. Many of these newly emerged virgin queens were introduced into nuclei (each nucleus contained two brood combs and one comb of honey) and left for mating in the same producing region. Randomly, each package colony was situated directly onto five of known age wax combs in 10-frame Langstroth hive on 10 March 2018 and on 15 March 2019. A sample of workers was collected from each colony to determine the mean worker weight. So, the starting worker population of each package colony was calculated by dividing the weight of each package bees on the mean weight per bee.

The tested ages of wax combs and colonies arrangement

The experimental colonies were divided into five blocks, with five colonies each. Colonies of each block contained one type of the following wax comb ages: foundation, one, two, three and from four to six years old combs. Except for foundation, all the tested wax combs were annually coded and previously used in rearing brood. The blocks were arranged at 2 m from each other and 1 m apart for colonies within each block. The entrances of all colonies were faced toward the south direction.

Parameters of study

The quantity of produced worker and drone brood, the body weight of newly emerged workers and drones, and worker populations of the tested colonies were measured three times at 21 day intervals from 31 March to 12 May in 2018, and from 5 April to 17 May in 2019.

1. Measurement of worker and drone brood

The areas of brood, (eggs, larvae and sealed brood) were measured to the nearest one cm^2 on both sides of each tested comb with a Plexiglas grid. These brood areas were converted into number of cells assuming that 1 cm^2 contained 4.1 cells for foundation, 1 and 2 years old combs (light combs) and contained 4 cells for the rest type of combs (dark combs), (Berry & Delaplane, 2001; Hassan, 1995).

2. Determination of colony population and honey production

Before sunset on the day of measurement, each hive with bees was weighed, then reweighing after brushed out the bees in another box. On the base of the mean worker weight for the sample taken from each colony, the total worker population (WP) of each colony was calculated (Woyke, 1984). The last worker population in 2019 was determined on 25 May, after the main nectar flow from Egyptian clover (*Trifolium alexandrinum* L.) was extracted on 20 May. The combs of honey of each colony within each comb age treatment were individually weighed before and after honey extraction. Therefore, the amount of honey harvested per colony of each comb age treatment calculated from the difference in comb weight before and after extraction (Avni et al., 2009; Taha & Al-Kahtani, 2013)

3. Determination of life span and survival percentage of workers

Mean length of worker life span in each colony was calculated by dividing total bee-days (number of workers multiplied by 42) by the number of bees that emerged in the previous days (combined 42, WB2, and 21, WB1, earlier brood counts) (Woyke, 1984). Worker survival percentage was determined in each tested colony by dividing worker population (WP) into the total brood cells (TBC) that were counted 42 days earlier (WB1 + WB2) according to Woyke (1984).

4. Weight of newly emerged workers and drones

The weight of newly emerged workers and drones was determined by collecting the individuals from combs incubated on 33 ± 1 C°. for 12 h and weighed by a digital balance of 0.001 gm. (Alfalah et al., 2012)

Table 1. Mean of worker population(no.) and brood areas (cm^2) after two months of establishing colonies on wax combs of different ages during active season of two successive years (2018 & 2019) All figures in thousands; WB = worker brood; WP = worker population; - = not take in consideration in the 2 years.

Year Date	Foundation		1 Year		2 Years		3 Years		4-6 Years		Mean/Year	
	WB	WP	WB	WP	WB	WP	WB	WP	WB	WP	WB	WP
2018												
Mar. 31	2.16	–	2.8	–	2.88	–	2.01	–	1.5	–	2.271	–
Apr. 21	3	13.42	3.25	15.13	3.21	15.65	2.62	11.66	2.35	7.984	2.886	12.769
May 12	4.1	21.19	4.58	25.218	4.4	23.39	4.16	18.18	3.02	13.282	4.051	20.252
Mean	**3.089**	**17.305**	**3.545**	**20.174**	**3.495**	**19.52**	**2.929**	**14.92**	**2.289**	**10.633**	**3.069**	**16.51**
±SD	0.155	0.433	0.115	0.507	0.134	0.341	0.056	0.201	0.084	0.165	0.509	3.879
	b	**B**	**a**	**A**	**a**	**A**	**b**	**C**	**c**	**D**	**b***	**B****
2019												
Apr. 5	1.95	–	2.53	–	2.64	–	2.3	–	1.76	–	2.235	–
Apr. 26	3.57	13.714	3.63	15.524	3.8	17.11	2.83	13.21	2.6	8.45	3.286	13.602
May 17	4.87	22.714	5.54	26.91	5.26	25.236	4	19	3.25	13.508	4.584	21.474
Mean	**3.463**	**18.214**	**3.899**	**21.217**	**3.9**	**21.173**	**3.042**	**16.105**	**2.538**	**10.979**	**3.368**	**17.5376**
±SD	0.135	0.349	0.169	0.518	0.075	0.486	0.175	0.471	0.054	0.271	0.585	4.251
	b	**B**	**a**	**A**	**a**	**A**	**c**	**C**	**d**	**D**	**a***	**A****
Mean/ Comb age	**3.276**	**17.76**	**3.722**	**20.696**	**3.698**	**20.347**	**2.985**	**15.513**	**2.413**	**10.806**	**3.219**	**17.024**
±SD	0.265	0.643	0.251	0.738	0.286	1.169	0.08	0.838	0.176	0.245	0.212	0.726
	b	**B**	**a**	**A**	**a**	**A**	**c**	**C**	**d**	**D**		

a = Means in the same row with the same small letter are not significantly differed according to Duncan's Multiple Range Test at .05 probabilities.
A= Means in the same row with the same capital letter are not significantly differed according to Duncan's Multiple Range test at .05 probabilities.
a*= Means in the same column with the same small letter with star are not significantly differed according to Duncan's Multiple Range test at .05 probabilities; **A****= Means in the same column with the same capital letter with two stars are not significantly differed according to Duncan's Multiple Range test at .05 probabilities.

Statistical analysis

Data was analyzed with a two-way ANOVA with season (2018, 2019) as random blocking factor and wax comb age as fixed effect of interest. The MSTAT program, (version 6.4) was used in this manner and the means of treatments compared by Duncan's Multiple Range Test at 5% (Snedecor & Cochran, 1980).The correlation (*r*) and regression (*b*) coefficients were calculated for the various parameters (as dependent variable, y) based on wax comb ages(as independent variable, x).

Results

Worker and drone brood production

Means of worker brood areas that were produced in honey bee colonies established on wax combs of different ages during spring of two successive years are presented in Table 1. Results of all tested comb ages were significantly higher in the second year than in the first one. However, colonies maintained on one (3722 cm^2) and two (3698 cm^2) year old combs had a significant increase in brood areas than those of the other comb ages. On the other hand, there was a significant increase in reared brood areas of colonies established on foundations (3276 cm^2) than on both 3 (2985 cm^2) and $4-6$ (2413 cm^2) years old combs. A significant difference was, also found between the two later old combs.

Highly significant negative correlation ($r = -0.7520$, $n = 50$, $p < 0.01$) was found between the areas of worker brood (y) and wax comb ages (x). Therefore, based on comb age under similar circumstances, the expected worker brood areas ($\hat{y}$) can obtain by the regression equation $\hat{y} = 3747.1 - 1.0782$ x (standard error, SE, of the regression coefficient, b, = 1.0249).

In contrast, highly significant positive correlation ($r = 0.6433$, $n = 50$, $p < 0.01$) was recorded for the produced areas of drones brood (Table 2). Therefore, the expected drone brood production can be attained by the equation $\hat{y} = 267.7 + 1.1934$ x (SE of b = 1.0700). The percentages of drone brood to worker brood had the same trend and decreased from the oldest to the newest combs, (29.0 ± 4.2%, 15.0 ± 6.8%, 10.7 ± 5.1%, 8.8 ± 5.0% and 9.3 ± 2.9%, respectively).

Colony worker population

There was a great reduction in workers population after the first 21 days from situated colonies on combs of different ages during the two studied seasons, (Table 3). The highest rate of worker loss was recorded for the oldest comb age, (70.7%) followed by comb aged 3 years old (63.6%) and foundation (59.9%). While combs aged one and two years old reported the lowest rate of workers reduction (48.8% and 50.7%, respectively). After this period, the increase in worker populations was highly significant in colonies situated on one and two years old combs than those on the other comb ages during the two years (Figure 1a and b). Foundation ranked the second category followed by 3 years and lastly $4-6$ years old combs. Therefore, the worker population may fall within the following regression equation as $\hat{y} = 21359.9 - 1.1225x$ ($r = -0.8260$, $p < 0.01$, SE of b = 1.0349, $n = 50$) for the 2 years.

Table 2. Means of drone brood areas (cm^2), percentage of worker survival, length of worker life (in days), weight of newly emerged workers and drones (in mg.) and honey production (in Kg.) for colonies established on wax combs of different ages during active seasons of two successive years (2018 & 2019).

Year	Foundation	1 Year	2 Years	3 Years	4-6 Years	Mean/Year
		Areas of drone brood (cm^2)				
2018	223.7	188.1	247.1	296.7	596.9	310.5 **B**
2019	391.9	480.2	557.3	600.2	812.9	568.5 **A**
Mean	**307.8 d**	**334.15 d**	**402.2 c**	**448.45 b**	**704.88 a**	**439.5**
±SD	37.6	65.4	69.4	67.9	48.3	57.7
		% of worker survival				
2018	69.4	72.5	70.3	73.1	72.4	71.5 **A**
2019	72.8	76.4	71.8	72.1	72.6	73.1 **A**
Mean	**71.1 a**	**74.45 a**	**71.05 a**	**72.6 a**	**72.5 a**	**72.3**
±SD	6.0	7.5	5.1	5.8	7.7	
		length of worker life span (in days)				
2018	29.2	30.0	29.7	27.4	24.0	28.1 **A**
2019	27.2	28.7	28.5	28.2	22.3	27.0 **A**
Mean	**28.2 a**	**29.3 a**	**29.1 a**	**27.8 a**	**23.1 b**	**27.5**
±SD	1.4	1.0	0.9	0.6	1.2	1.0
		Worker weight (mg.)				
2018	124.1	120.1	111.6	100.5	94.3	110.1 **A**
2019	122.5	121.2	110.8	100.9	93.5	109.7 **A**
Mean	**123.3 a**	**120.6 a**	**111.2 b**	**100.7 c**	**93.9 d**	**109.9**
±SD	2.4	4.1	4.2	3.3	2.6	
		Drone weight (mg.)				
2018	261.8	260.3	258.3	243.4	197.4	244.3 **A**
2019	264.8	261.5	259.9	243.4	199.8	245.9 **A**
Mean	**263.3 a**	**260.9 a**	**259.1 a**	**243.4 b**	**198.6 c**	**245.1**
±SD	6.4	3.9	4.1	4.8	4.7	
		Honey production (Kg.)				
2018	8.7	10	9.2	6.6	6	8.1 **B**
2019	11.5	9.5	10.7	7.7	5.4	9.0 **A**
Mean	**10.1 a**	**9.8 a**	**10.0 a**	**7.2 b**	**5.7 b**	**8.5**
±SD	1.0	1.2	1.1	1.4	1.3	

Means in the same row or column with the same letter (for each parameter) are not significantly differed according to Duncan's Multiple Range Test at .05 probabilities.

Table 3. Mean numbers and percentages of lost workers after 21 days of situated colonies on different ages wax combs during active seasons of two successive years (2018 and 2019).

worker population	Foundation	1 year	2 years	3 years	4-6 years	mean/year
2018						
Starting population	15.62	15.55	15.63	15.63	15.61	
Population after 21 days	6.372	7.866	7.712	5.154	4.016	
No. lost workers after 21 days	9.251	7.682	7.916	10.475	11.591	
Lost % after 21 days	**59.2**	**49.4**	**50.6**	**67.0**	**74.3**	**60.1**
±SD	3.7	4.5	3.0	8.3	3.7	**A**
2019						
Starting population	15.74	15.48	15.53	15.52	15.73	
Population after 21 days	6.218	8.044	7.638	7.88	5.18	
No. lost workers after 21 days	9.526	7.437	7.885	9.337	10.399	
Lost % after 21 days	**60.5**	**48.0**	**50.8**	**60.2**	**67.1**	**56.7**
±SD	3.6	4.6	5.9	7.7	4.5	**A**
% of mean loss / year	**59.9 b**	**48.7 c**	**50.7 c**	**63.6 b**	**70.7 a**	58.4
±SD	3.5	4.4	4.4	6.4	5.8	

Means in the same row or column with the same letter are not significantly differed according to Duncan's Multiple Range Test at .05 probability.

Weight of emerged workers and drones

Results in Table 2 indicate that old combs have a negative influence on the emerged worker and drone weights. Workers produced from colonies established on foundation (123.3 mg) and 1-year-old combs (120.6 mg) recorded significant weight increase compared to other tested comb ages. The lightest worker weight produced comes from the oldest combs, (93.9 mg). Therefore, predicted change in worker weights according to change in comb age follows the equation: $\hat{y} = 124.6 - 1.0612\ x$ ($r = -0.9587$, $p < 0.01$, SE of $b = 1.0153$, $n = 50$).

Also, insignificant differences between weights of drones produced from colonies established on foundation (263.3 mg), 1 (260.9 mg) and 2 (259.1 mg) year old combs, (Table 2). The lightest drone weight resulted

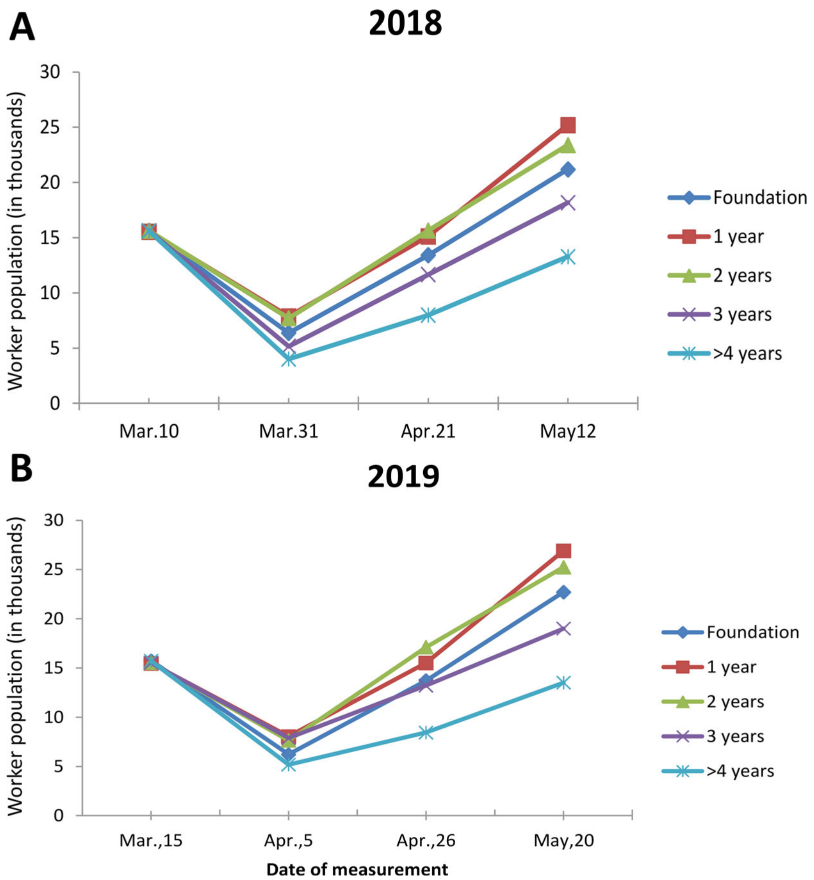

Figure 1. Mean of worker populations (in thousands) after 2 months of establishing colonies on wax combs of different ages during active season of 2018 (a) and 2019 (b).

from the oldest combs (198.6 mg). The change in drone weight can be predicted by applying the equation: $\hat{y} = 266.6–1.0266\ x$, ($r = -0.4832$, $p < 0.01$, SE of $b = 1.0130$, $n = 50$).

Survivorship and life span of workers

The worker survivorship percentage of different ages tested combs do not differ significantly during the two seasons and ranged from 71.1% to 74 5%, (Table 2).

The shortest life span was for workers of the oldest combs (23.1 days) while it ranged from 27.8 – 29.3 days for the rest without significant differences between them (Table 2).The regression equation for the expected worker life span was $\hat{y} = 30.0 - 1.0428\ x$, ($r = -0.6244$, $p < 0.01$, SE of $b = 1.0167$, $n = 50$) for the 2 years.

Honey production

There were significant differences in honey production between the two years. Honey produced from colonies that had wax combs of foundation (10.1 kg.), one (9.8 kg.) and two (10.0 kg.) years old were significantly higher than those produced from three (7.2 kg) and four- six (5.7 kg.) years old (Table 2). The regression equation of the expected honey yield (y) was $\hat{y} = 10.64 - 1.1269x$, ($r = -0.6785$, $p < 0.01$, SE of $b = 1.0442$, $n = 50$) for the 2 years.

Discussion

Concerning the age of new and old combs, our results are not completely in agreement with those reported by Berry and Delaplane (2001) and Asadi Dizaji et al. (2007). They classified new and old combs according to their use in brood rearing. They considered new combs those newly drawn white combs which were not previously used in brood rearing, while the old ones are those colored from light to black and included from one year old to unknown age. However, this study accurately classifies by age and revealed that combs aged from zero to three years old had a color ranged from whitish to light brown and were considered as effective light combs. While combs aged four or more years old, had a color ranged from brown to black and were considered not efficient dark combs.

Worker and drone brood production

It is clear from the present results that new combs promote an increase in worker brood production but hinder the production of drone brood. A reverse effect resulted from old combs as reported by Szabo (1983) and Colter (1994). This may be due to the new combs being more suitable for laying eggs by a queen, as indicated by Koeniger (1970) who showed that the queen uses its forelegs for discrimination between the diameters for worker and drone brood cells. The fresh combs had, also, a wide area of regular and large size worker brood cells, so they are enhancing worker brood rearing (Abdellatif,1965; Berry & Delaplane, 2001; Free & Williams,2009; Yang et al., 2010).

On the other hand, the continued reuse of wax comb in brood rearing and food storage increase the accumulation of wax, propolis, and debris from ecdysis of brood within the comb cells which makes them smaller and combs become heavy and dark as noticed in wax combs aged more than 3 years old (Zovaro, 2007). Besides, the old combs have a high proportion of irregular comb cells resulting from the transformation of worker cells to drone cells (Free, 1967), as well as the increase in built drone brood cells caused a significant decrease in the reared amounts of worker brood against drone brood (Johansson & Johansson, 1971; Nazzi, 2016; Schmickl & Crailsheim, 2002, 2004; Seeley, 2002).

Population, survivorship and life span of workers

The high rate of worker reduction after the first 21 days of starting the experiment, especially in the foundation and the oldest comb treatments could be attributed to two different explanations. The first is concerned with foundation treatment since it needs more labor of colony workers to build combs from foundation sheets (Jaycox & Guynn, 1974). Besides, the presence of different amounts of brood that filled quickly any completed cells by the active queen (Eckert et al., 1994). This may negatively affect the worker life span by sapping the physiological energy of them causing a high rate of workers mortality during that period of the experiment (Eyer et al., 2017; Rueppell et al., 2009; Smedal et al., 2009; Visscher & Dukas, 1997). However, the negative effects of foundation on workers stop after the comb has been built (Jaycox & Guynn, 1974).

The second explanation is that the highest reduction in the oldest wax comb population may be attributed to exposing workers to numerous foreign toxic contaminants sequestered in these combs (Smith & Wilcox, 1990). These contaminants may alter the signature phenotype and nest-mate recognition cues which are continuously causing an increase in drifting of returning foragers to their colonies (Berry & Delaplane, 2001; Breed et al., 1988a, 1988b; D'ettorre et al., 2006).

The present results indicate that colonies established on both foundations, 1, 2 and 3 year old combs enabled to rear significant amounts of worker brood during April and May compared to those on the oldest wax combs. Therefore, a high rate of healthy new emerged workers in the new (light) wax combs resulted in an increase in worker populations by 64.4%, 91.5%, 88.3% and 43.6% for the mentioned treatments in comparison to 4 – 6 years old combs, respectively. These results strongly agree with those reported by Wille and Cerig (1976), Woyke (1984), Taha and Al-Kahtani (2013) and Abd Al-Fattah et al. (2016).

The length of worker life span is correlated with its weight at emergence (Maurizio & Hodges, 1950; Winston, 1980) and the ratio of larvae to adults in the colony (Abd Al-Fattah et al., 2010; Sakagami & Fukuda, 1968). Therefore, the old combs (very dark and black colour) may produce weak workers with light weights (Berry & Delaplane, 2001), short wings, proboscis and hind legs (Alfalah et al., 2012). This negatively reflects on their flight activity and nursing efficiency (Free & Spencer-Booth, 1959; Neukirch, 1982) and explains the increase in worker populations in colonies of new, (white and light colour) combs. So, replacing the wax combs aged more than three years old with fresh ones can be recommended to worldwide beekeepers to increase colony health and productivity. This conclusion is in harmony with the results of Gilliam (1985), Koenig et al. (1986), Morse (1986), Message and Goncalves (1995), Piccirillo and De Jong (2003, 2004) and Wu et al. (2011).

Weight of newly emerged workers and drones

The reduction of worker's weight as a result of repeated use of the comb may be attributed to a continuous decrease of cell size due to the accumulation of cocoon's layers and fecal debris that are deposited by the brood development during successive generations. The weight of emerged workers reduced by 19% and 8% after 68 and 70 generations of reared brood in combs as reported by Buchner (1953) and Abdellatif (1965), respectively. The oldest combs in the present study produced about 85 generations of brood, which caused a great reduction in worker weight by 31.3% compared to combs built from foundation. In addition, brood reared in old comb cells are more exposed to injuries during the development resulting in exhausted workers (Gilliam,1985; Koenig et al., 1986; Message & Goncalves, 1995; Piccirillo & De Jong, 2003, 2004).

However, our study finding aligns with Hassan (1995) and Schlüns et al. (2003) when both adult longevity of drones, their semen volume, and quality are considered. The honey bee drones are responsible for producing semen and transmitting it to the virgin queen during the mating flight. The sperm numbers in *A. mellifera* L. drones depend on body size, which mostly depends on the quantity of food provided to larvae (Abd Al-Fattah et al., 2016; Czekońska et al., 2015; Schmickl & Crailsheim, 2004) and the size of the cells that drones are reared in (Alfalah et al., 2012; Free, 1957). Therefore, drones with light weights that resulted from old combs could negatively have an effect on their longevity and quantity of semen produced. Hassan (1995) found a positive correlation between drone pupa weight and both adult longevity and its semen volume.

Honey production

Honey production is one of the most important targets in beekeeping worldwide (Szabo & Lefkovitch, 1989). It is relative to population size (Harbo, 1986). Therefore, the present results are consistent with previous findings of many researchers: Farrar (1937); Szabo (1982); Woyke (1984); Jevtic et al. (2009) and Taha and Al-Kahtani (2013) concluded that honey yield is governed by the interaction of three primary factors including average daily brood production, length of worker life and individual productivity of a worker.

It is noteworthy that, colonies of the oldest combs contained the largest areas of reared drone brood and subsequently adult drones which may negatively reflect on honey production. Our results confirm those found by Seeley (2002) who studied the effect of drone comb on a honey bee colony's production of honey for three successive years and reported that colonies with a natural amount of drone comb do indeed produce less honey than those with little or no drone comb. He explains that, in annual overall, a colony sacrifices some 7–12 kg of honey to rear its drones.

Conclusions

In this study, there was a significant increase in worker brood, colony worker population, life span of workers, weights of emerged workers and drones, and honey yield, as well as a hindering of brood and adults of drones in newer wax combs compared to old ones. Therefore, the continuous replacement of wax combs aged more than three years old (dark and black color) by new ones (light color) will improve colony productivity. In addition, it will help in protecting worldwide honey bee colonies from unlimited numbers of living and nonliving contaminants which may contribute to avoiding colony collapse disorder.

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