

## Effect of wax comb age on Honey bee activity (*Apis mellifer meda*)

<sup>1</sup>Alan. Krim , <sup>2</sup>Rukhosh. Rashed 

<sup>1</sup>University of Sulaimani/ College of Agricultural Engineering Science/ Department of Biotechnology and Crop Sciences /Sulaymaniyah/Iraq, alan.karim@univsul.edu.iq

<sup>2</sup>University of Sulaimani/ College of Agricultural Engineering Science/ Department of Horticulture/Sulaymaniyah/Iraq, rukhosh.rashed@univsul.edu.iq

### Abstract

This study was conducted on *Apis mellifera meda* hybrid from April to the end of October 2024 including three seasons; spring, summer, and autumn, in the Sulaimani-Kurdistan region. The effect of wax comb age on brood rearing, the activity of workers in honey and pollen storage, and the size of workers' bodies. Used combs with Foundation as (new comb), and combs aged more than 3 years old as an (old comb). The highest average of sealed and unsealed brood areas was 107.363, 62.99 inch<sup>2</sup> in the spring season, in the colonies which improved with new combs, the highest average of the honey storage area was 73.706 inch<sup>2</sup> in the summer season, the highest average of the pollen storage area was 56.376 inch<sup>2</sup> in the spring season in the colonies which improved with new combs. The highest average of the worker's body length and width was 12.205, 4.348 mm, in the colonies enhanced with new combs. Thus, the results show that regularly replacing aged combs with new ones is useful to improve the activity and productivity of honey bee colonies.

**Key Words:** Combs age, Brood area, Body size, Management of Combs, Beeswax.

### I. Introduction

Honey bees are social insects that play an initial role in nature by pollinating wild and cultivated plants, they live in colonies that contain several cast (Queen, Workers, Drones) with broods, approximately 15,000 to 60,000 individuals (Southwick and Heldmaier, 1987, Breeze et al., 2011). Honey bees have several activities inside the colony such as honey and pollen storing, brood rearing, and regulating internal temperature to the proper range (Southwick and Heldmaier, 1987). Also, they have external activities including foraging by workers and flight activity of the queen and drones. Well-known that changes in ecological abiotic factors such as temperature and relative humidity affect those activities (Abou-Shaara et al., 2017). Before the introduction of domestication, the honey bee, *Apis mellifera* L., lived in the wild and constructed nests in protected locations such as caves or hollowed-out trunks of trees. The first thing that was done in the nest was to construct a wax comb, which is considered an essential component of the honey bee colony (Pratt, 1998). The dimensions of these protected areas might influence the quantity and size of combs, hence indicating the length of honeybee colonies (Seeley, 2014). Honey bees in nature are responsible for managing the affairs of the colony and benefit, ideally, from the wax comb that is made in nature. However, following the bee's domestication and human involvement in colony management, several detrimental practices were unintentionally altered, such as using wax combs for bee production across multiple generations (Alfalah et al., 2012). It has been observed that as time passes, the comb's cell components narrow, turn dark brown, and gain weight (Pratt, 1998). In domesticated honeybees, comb management is a prevalent practice among beekeepers, as it is essential for optimizing honey output. Framed combs are inserted or substituted to enhance the vitality of honeybee colonies and



their output, as well as to cultivate many generations of honeybees (Shawer et al., 2020). Furthermore, as the age of the comb increases, the diameters of the cells decrease due to the accumulation of silks and faecal materials generated by the developing larvae. With each new generation of honeybees, the mass ratio of silk fibers to wax substance rises and the wax cells get smaller with time (Al-Kahtani and Taha, 2021).. Additionally, reduced cell size in honey-storing combs should lead to diminished honey storage capacity. The utilization of the new wax comb has been shown to positively impact brood expansion and enhance the colony's honey production (Dizaji et al., 2008). However, because a comb comprises roughly 1200 g of wax and the energy or resources needed to produce a comb can equal 7.5 kg of honey, many beekeepers are reluctant to replace old wax combs regularly, believing this method is not cost-effective (Seeley, 2014). Our interactions with numerous beekeepers revealed that they often do not replace the old wax comb, continuing to use it beyond its expiration, under the belief that this practice is economically beneficial. They lack awareness of the effects associated with that. This research was conducted to assess the potential benefits or damages that may arise from the prolonged use of wax combs in a colony.

## II. Materials and Methods

The study was performed from May to October of 2024 within the apiary of Kani Bardina in Sulaymaniyah City-Iraqi Kurdistan (35°38'21"N,45°24'25"E), to evaluate the effect of different wax comb ages on sealed and unsealed brood areas, pollen and honey areas, and body size of worker honey bees in different ages. The subspecies of *Apis Mellifera* L. was used: *Apis mellifera meda* hybrid. Data was collected at locations from 9 colonies, each of which had five combs with different wax ages (just framed as control, foundation as new comb, and more than 3 years old as old combs). Colonies exhibited comparable strength, comprising approximately 8000 individuals.

The selected colonies were divided into three groups, each group included three replicates (colonies), the first group (control) was supplied by just frame, the second improved by foundation combs, and the last was supplied by more than 3-year-old combs.

### The Total Area of Broods, Stored Pollen and Honey

The total area of brood, pollen, and honey was measured across three seasons in 2024: spring from May 1 to May 15, summer from July 1 to July 15, and autumn from September 1 to September 15, utilizing the standard Langstroth frame.(Targany, 2008, Shamdin, 2003). The standard Langstroth frame utilizes a specialized foundation crafted from

silk, designed into squares measuring 1 inch. The brood, pollen, and honey areas were assessed following the application of the standard Langstroth frame on each comb. As shown in figure (1).

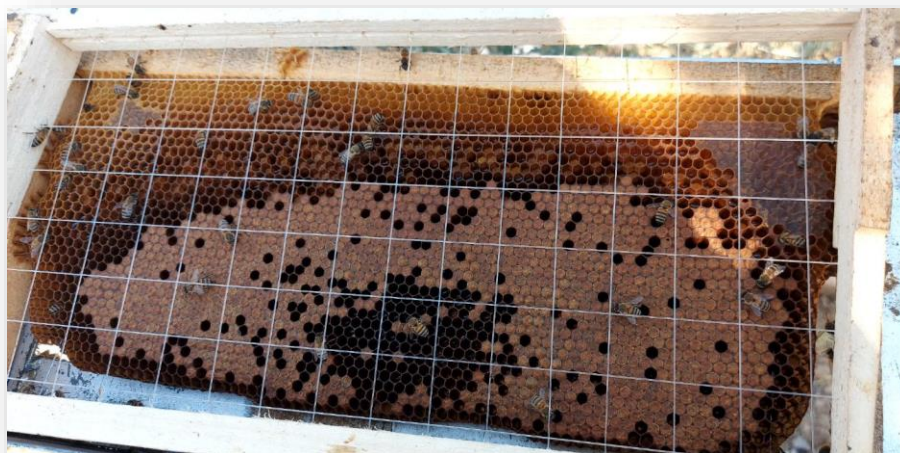


Figure 1: The measurements frame divided by inch<sup>2</sup>

#### Worker's labelling is based on the following steps:

Installation of the workers' excluders on either side of the sealed brood comb during the transition from pupa to adult stage. The excluder was repaired after the young workers were shielded with hexagons on both sides, followed by their removal using a bee brush. The captured frames were subsequently returned to their original positions within the experimental hives (Rashed, 2021).

Newly emerging workers were identified in a closed comb using 3x3 mm sieve space excluders. The head capsule's average width was 3.7249 mm, whereas the third and fourth abdominal tergum was 4.4145 mm. (Rashed, 2021). As shown in figure (2).



Figure 2: The stained newly emerged workers beneath the excluder before release.

### Preparation of specimens for study

#### Sampling

Samples were collected from experimental colonies that included a significant number of labelled workers with established ages. Fifteen young workers of specified ages were collected from each hive using forceps. Live specimens were positioned in plastic containers that contained a piece of cotton saturated with glacial acetic acid. (Pantin, 1946). The specimens were taken to the laboratory to be studied for the desired qualities.

#### Worker body size measurements

The body dimensions of worker honey bees were assessed using an electronic caliper.

#### Statistical analysis

The XLSTAT tool (version 2019 .2.2) was used to statistically analyze the results using a factorial RCBD design with triple replicates. To test for differences in comb ages, one-way analysis of variance (ANOVA) was used, and Duncan's multiple range test was used to ascertain the correlation coefficient (r) and mean differences at  $P=0.05$ .

## III. Result and Discussion

The average of sealed and unsealed brood areas is significantly higher ( $p<0.05$ ) in the new comb than in the old. While the averages in spring were (107.363,62.990), and (77.327,34.253) inch<sup>2</sup>, (Table 1) (Figure 3), in summer were (60.807,46.737), (38.850,24.173) inch<sup>2</sup> (Table 2) (Figure 4), and in autumn were (28.797,21.927), and (16.663,15.450) inch<sup>2</sup> (Table 3) (Figure 5), for new combs(foundation), old combs (more than 3-year-old). Those results agree with the study performed by Berry and Delaplane (2001) The total area of sealed brood was significantly higher in fresh combs than in old comb colonies. Also, another study observed that there are significantly higher rates in brood areas that reared with the colony supplied by the foundation compared to colonies that improved with combs that were more than 3 years old. (Abd Al-Fattah et al., 2021).

In addition, the colonies with new combs were more active in honey and pollen storage in spring (55.667,56.337) inch<sup>2</sup>, summer (73.707,45.410) inch<sup>2</sup>, and autumn (52.193,12.187) inch<sup>2</sup>, compared to old combs colonies



(29.513,30.433), (41.217,28.760) inch<sup>2</sup>, and (35.480,4.191) inch<sup>2</sup> under significant level of ( $p < 0.05$ ). (Figure 3,4,5). This result agrees with Taha et al. (2021) They observed in their study that the bees were more active in storing honey and pollen in colonies that improved with combs at 1 to 3 years old than the colonies that improved with combs at more than 3 years old.

The body length and width of workers were significantly increased in fresh comb colonies compared to old comb colonies (12.205,4.408) mm, and (11.672,4.243) mm, under a significant level of ( $p < 0.05$ ), (Table 4) (Figure 6). This result agreed with Berry and Delaplane (2001) and Abdellatif (1965) Have emphasized that honeybees have smaller bodies due to space constraints and relative food reduction when cell diameter or volume is reduced. Also Al-Kahtani (2018) Show that the body size of worker bees is significantly affected by the comb's age, which the workers came from naturally constructed combs bigger than those that came from old comb colonies.

Table 1: Spring, sealed and unsealed brood, and honey and pollen area, in relation to comb age.

Wax comb age	Sealed brood	Unsealed brood	Honey	Pollen
Foundation	107.363 <sup>a</sup>	62.99 <sup>a</sup>	55.666 <sup>a</sup>	56.376 <sup>a</sup>
Control	85.633 <sup>ab</sup>	53.22 <sup>b</sup>	32.623 <sup>b</sup>	30.823 <sup>b</sup>
Old comb	77.326 <sup>b</sup>	34.25 <sup>c</sup>	29.513 <sup>b</sup>	30.433 <sup>b</sup>

The means with the same letter are not significantly different.

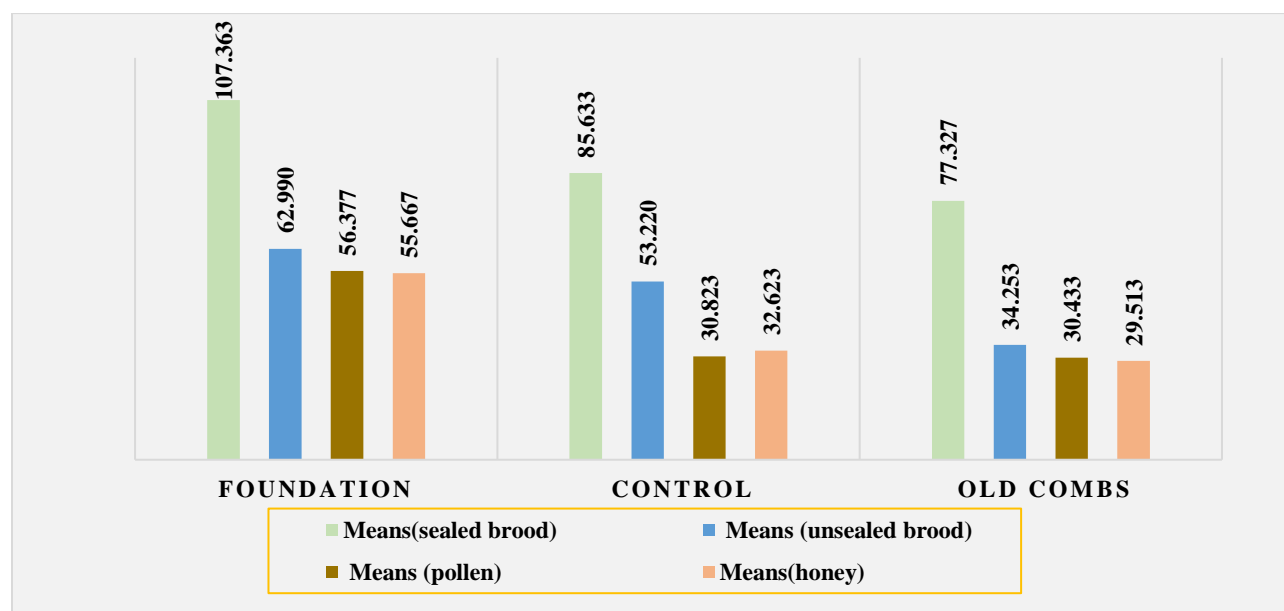


Figure 3: spring, sealed and unsealed brood, honey and pollen storage area, in relation to comb age.

Table 2: Summer, sealed and unsealed brood, and honey and pollen storage areas, in relation to comb age.

Wax comb age	Sealed brood	Unsealed brood	Honey	Pollen
Foundation	60.806 <sup>a</sup>	46.736 <sup>a</sup>	73.706 <sup>a</sup>	45.4 <sup>a</sup>
Control	47.993 <sup>b</sup>	31.096 <sup>b</sup>	41.216 <sup>b</sup>	29.833 <sup>b</sup>
Old comb	38.85 <sup>c</sup>	24.173 <sup>b</sup>	33.33 <sup>b</sup>	28.76 <sup>b</sup>

The means with the same letter are not significantly different.

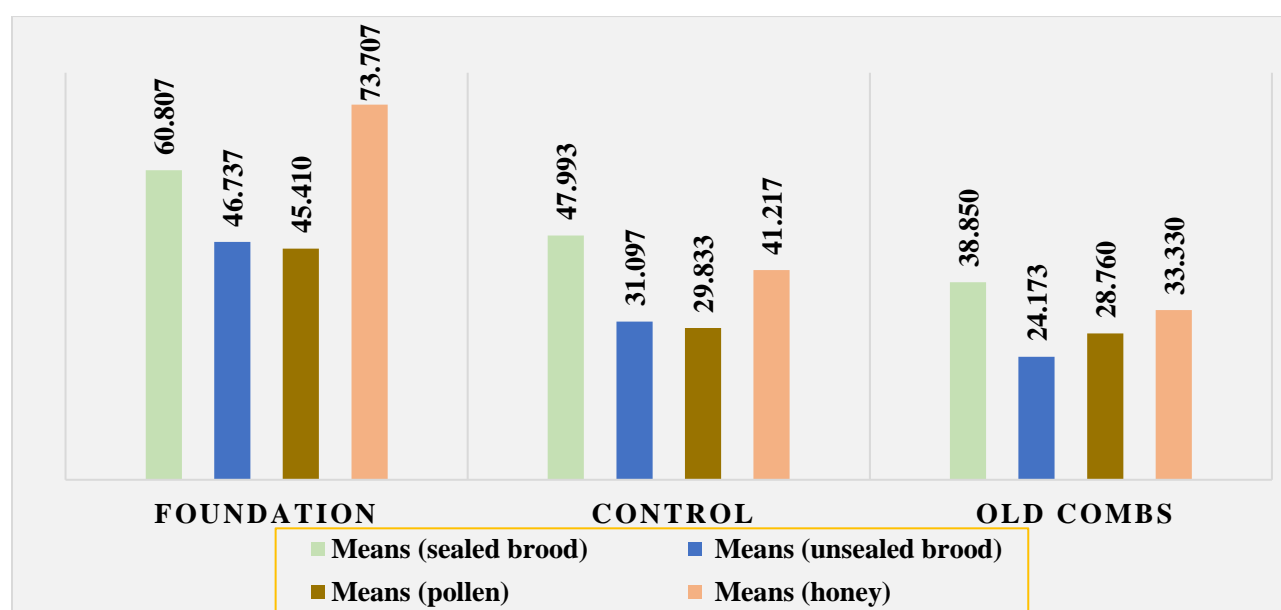


Figure 4: Summer, sealed and unsealed brood, and honey and pollen storage areas, in relation to comb age.

Table 3: Autumn, sealed and unsealed brood, and honey and pollen storage areas, in relation to comb age.

Wax comb age	Sealed brood	Unsealed brood	Honey	Pollen
Foundation	28.796 <sup>a</sup>	21.926 <sup>a</sup>	52.193 <sup>a</sup>	12.186 <sup>a</sup>
Control	16.663 <sup>b</sup>	15.45 <sup>b</sup>	35.48 <sup>b</sup>	5.083 <sup>b</sup>
Old comb	14.223 <sup>b</sup>	11.363 <sup>c</sup>	31.12 <sup>b</sup>	4.196 <sup>b</sup>

The means with the same letter are not significantly different.



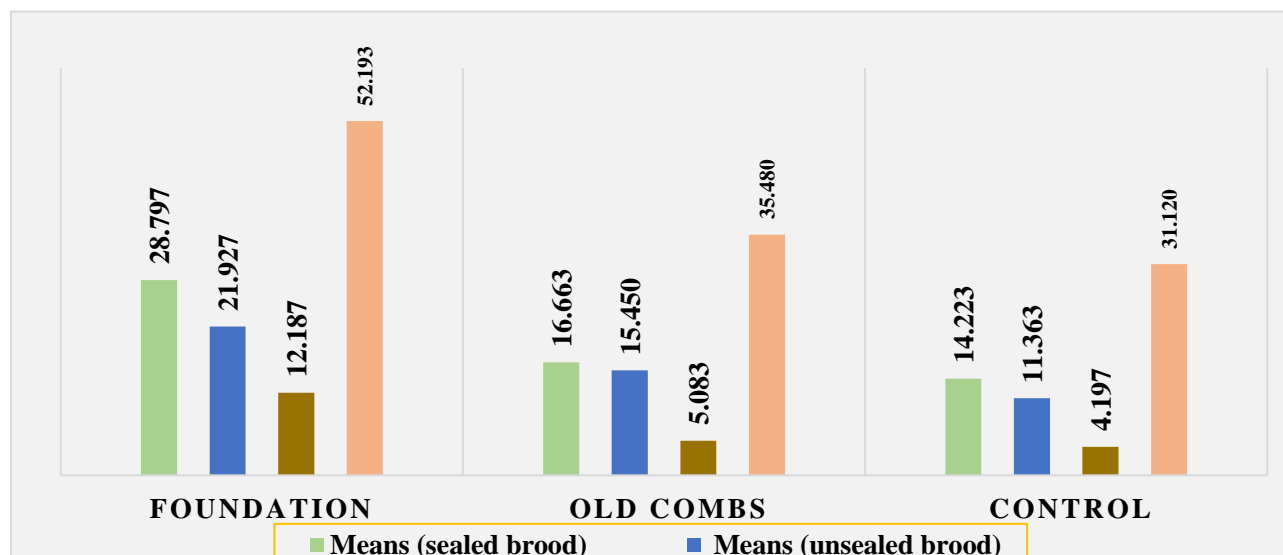


Figure 5: Autumn, Sealed and unsealed brood, and honey and pollen storage areas, in relation to comb ages.

Table 4: The body length and width of workers, in relation to comb age.

Wax comb age	Body length	Body width
Foundation	12.205 <sup>a</sup>	4.348 <sup>a</sup>
Control	12.135 <sup>a</sup>	4.322 <sup>a</sup>
Old comb	11.672 <sup>b</sup>	4.247 <sup>a</sup>

The means with the same letter are not significantly different

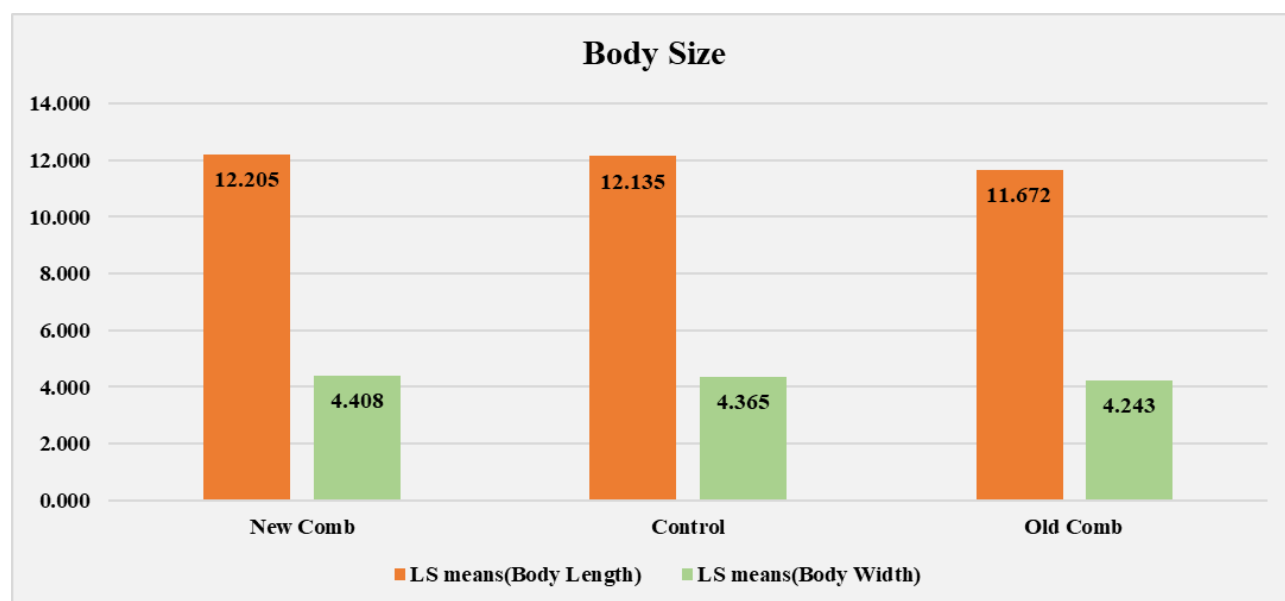


Figure 6: The length and width of the worker's body, in relation to comb ages.

#### IV. Conclusion

In conclusion we can say that significantly increase in worker brood and workers' activity in collecting honey and pollen according to rearing bees on new combs compared to rearing on more than 3-year-old combs. In addition, the comb ages hurt the body size of workers, while those come from old combs that are smaller than those of new combs. Thus, colony productivity will increase if wax combs older than three years (dark and black) are regularly replaced with fresh ones (light colour or foundation).



## V. Reference

- Abd Al-Fattah, M. A. A.-W., Yehia Ibrahim, Y. and Ibrahim Haggag, M. (2021) 'Some biological aspects of honey bee colonies in relation to the age of beeswax combs', *Journal of Apicultural Research*, 60(3), pp. 405-413.
- Abdellatif, M. (1965) 'Comb cell size and its effect on the body weight of the worker bee', *Apis mellifera*, pp. 86-87.
- Abou-Shaara, H., Owayss, A., Ibrahim, Y. and Basuny, N. (2017) 'A review of impacts of temperature and relative humidity on various activities of honey bees', *Insectes sociaux*, 64, pp. 455-463.
- Al-Kahtani, S. N. (2018) 'Morphometric characteristics of Carniolan honeybee workers in relation to age of comb', *Sci. J. King Faisal Univ*, 19, pp. 47-54.
- Al-Kahtani, S. N. and Taha, E.-K. A. (2021) 'Effect of comb age on cell measurements and worker body size', *PLoS One*, 16(12), pp. e0260865.
- Alfalah, H. A., Shaibi, T., Tawfiq, M. M. and Mogrby, A. (2012) 'The effects of wax comb age on some morphometrics characteristics of honey bee (*Apis mellifera* L.) workers', *Persian Gulf Crop Prot*, 1, pp. 18-23.
- Berry, J. A. and Delaplane, K. S. (2001) 'Effects of comb age on honey bee colony growth and brood survivorship', *Journal of Apicultural Research*, 40(1), pp. 3-8.
- Breeze, T. D., Bailey, A. P., Balcombe, K. G. and Potts, S. G. (2011) 'Pollination services in the UK: How important are honeybees?', *Agriculture, Ecosystems & Environment*, 142(3-4), pp. 137-143.
- Dizaji, A., Alishah, H., Shaddel, A. and Sis, N. (2008) 'Effects of comb wax age on the brood and honey product performance in honey bee'.
- Pantin, C. F. A. (1946) 'Notes on microscopical technique for zoologists', (*No Title*).
- Pratt, S. C. (1998) 'Condition-dependent timing of comb construction by honeybee colonies: how do workers know when to start building?', *Animal behaviour*, 56(3), pp. 603-610.
- Rashed, R. J. (2021) 'The Morphometrical study of some honeybee (*Apis mellifera* L.)(Hymenoptera: Apidae) races in Sulaimani-Iraqi Kurdistan Region'.
- Seeley, T. D. (2014) *Honeybee ecology: a study of adaptation in social life*. Princeton University Press.
- Shamdin, Z. (2003) *Effect of supplemental proteins and vitamins on the development of specific tissues with special concern to their fine structures in relation to the activity of honey bee workers Apis mellifera L.(Hymenoptera: Apidae)*. M. Sc. thesis. College of Agriculture, Dohuk University.
- Shawer, M. B., Elnabawy, E. M., Mousa, K. M., Gaber, S. and Ueno, T. (2020) 'Impact of different comb age on morphological and biological characteristics of honeybee workers (*Apis mellifera* L.)'.
- Southwick, E. E. and Heldmaier, G. (1987) 'Temperature control in honey bee colonies', *Bioscience*, 37(6), pp. 395-399.
- Taha, E.-K. A., Rakha, O. M., Elnabawy, E.-S. M., Hassan, M. M. and Shawer, D. M. (2021) 'Comb age significantly influences the productivity of the honeybee (*Apis mellifera*) colony', *Journal of King Saud University-Science*, 33(4), pp. 101436.
- Targany, Y. (2008) *Effect of rich proteins diet on the activities of honey bee colonies Apis mellifera L.(Hymenopter: Apidae)*. M. Sc. Thesis. College of Agriculture, Univ of Salahaddin, Erbil.