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PROPOLIS IMPACT ON THE HONEY BEE LIFE SPAN, VARROA MITE INFESTATION AND POPULATION GROWTH OF THE COLONY

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ABSTRACT

Effect of providing the hives with additional propolis in nine colonies of *Apis mellifera* in domesticated hives was investigated. Significantly more sealed brood were found in colonies contained empty combs previously sprayed with propolis extract (320 inch^2/colony) and colonies reared in painted hives with propolis (286 inch^2/colony) than those reared in untreated colonies (196 inch^2/colony) during early summer in June. While in September only bees reared in colonies contained sprayed combs with propolis extract had significantly more sealed brood (143 inch^2/colony) than those reared in untreated colonies (99 inch^2/colony). Biggest number of fallen varroa mites (60 mites before and 170 mites after acaricide application) was recorded in untreated colonies, before and after application of the acaricide. Smallest number of fallen varroa mites was (6 mites before and 31 mites after acaricide application) recorded in colonies reared in painted hives with propolis. Adult workers developed as larvae in colonies contained empty combs previously sprayed with propolis extract and also in colonies reared in painted hives with propolis lived longer than those developed as larvae in untreated colonies in both laboratory and field experiments. The proportion of living workers developed as larvae and reared as adults inside their colonies in the field in October from the three treatments, painted, sprayed colonies and untreated colonies (control) after four weeks were 20%, 27% and 15% respectively, these workers continued alive as winter bees.

KEYWORDS: Bee longevity; propolis; population growth; brood; varroa mite

1. INTRODUCTION

There is a huge decline in honey bee colonies in various regions of the planet, most likely due to various factors including pathogens, parasite; using pesticides via pest management, and also insufficient nutrition, all together affect individual health and survival of the colony. It is necessary to know the factors that influencing individual stressors and the interaction among these factors in order to find suitable solution for the maintaining and developing healthy colonies (Simone-Finstrom *et al.*, 2017).

Bees are social insects; they have special behavioral defenses to aid bees to protect their colonies from intruders. An example of these behaviors is the foraging worker bees to collect sticky material secreted from plant buds and using these material in the nest construction particularly as interior layer inside their hives, called a propolis envelope. Propolis characterized by many features, it can suppress the growth of pathogens (bacteria, fungi and some viruses) and can be obtained from bee hives for using in human medication. However, the advantages of propolis to benefit honey bees colonies has been poorly studied (Simone-Finstrom *et al.*, 2010).

It is known that honey bees forage searching for fundamental material (nectar and pollen) in addition to water; the most important element for maintenance of the colony. Honey bees forage for plant resins, not for nutrition, but for nest construction. Plants are characterized by guarding the young leaf buds or other parts from diseases, and herbivore by secreting sticky exudate which is called Resin (Langenheim, 2003). Due to the health benefits of antimicrobial resins, they are collected and kept by animals including bees, in order to improve the immune system of the bee population (Simone-Finstrom and Spivak, 2010; Borba *et al.*, 2015).

The colony of honey bees nests in hollow tree cavity, while preparing the new nest by removing the loose, decayed wood from walls and depositing propolis in the cracks to make it hard and regular (Seeley and Morse 1976).

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Beekeepers, avoid dealing with colonies that gather large amounts of propolis because it is the sticky materials makes opening and managing hives in standard beekeeping equipment more difficult (Fearnley, 2001). It is important to know that the colony of honey bees do not build a propolis envelope in standard hives because the inner walls of the boxes are hard and smooth, therefore they do not stimulate collection and deposition of propolis. Instead of the envelope, bees deposit propolis only in cracks and large opening particularly in fall, in manmade hive bodies and not as a continuous envelope as they do within a tree cavity (Simone-Finstrom and Spivak, 2010).

Studies have explained that the presence of a propolis envelope enshrouding the nest area is a basic component of honey bee colony health (Simone-Finstrom *et al.*, 2009; Borba *et al.*, 2015; Borba *et al.*, 2017). Function of propolis envelope is an antimicrobial, or (disinfectant) layer around the nest, and thus as an external layer of the colony immune defense. Resin collection depends on the colony demand as well as on the genetic of the bee population, but how and what can stimulate bees to collect more propolis is not understood (Simone-Finstrom *et al.*, 2017)

components with various modes of action that are presence in natural products might give effective solution to varroa mite problem. One of such natural products is propolis (bee glue), a combination of different compounds collected by honeybees from plants, mixed with wax and used in the construction and protection of the beehive (Bankova *et al.*, 2016).

Function of propolis against the ectoparasitic mite (*Varroa destructor)* Anderson and Trueman, (2000) has been investigated and showed narcotic and lethal effects. Rate of mortality and Length of narcosis depended on the extraction procedure, contact time and concentration of propolis. Some authors proposed that some flavonoid components of propolis have insecticidal or inhibition of insect larval development effects (Garedew *et al.*, 2002). Varroa mites (*V. destructor)* are very sensitive to propolis. The varroacidal action of propolis appear to be paradoxical, since propolis and *V. destructor* mites are normally found together in the beehive, and the mites walk on thin propolis layers throughout the hive (Garedew *et.*, 2003). laboratory experiments showed that the direct exposure to ethanolic propolis extracts cause high number of mite mortality (Drescher *et al.*, 2017). Effects of low concentrations of propolis may reduce Varroa mite's mobility and results in less tolerant mites against environmental changes (Garedew *et.*,2003). Borba *et al.*(2015), does not found any differences in the mite infestation between colonies with and without a propolis envelope after two years of study. Regarding differences in the colony-level in chemical composition of the collected propolis. Popova *et al.*(2014), confirmed that propolis inside colonies maintained very low mite infestation without using acaracide were distinct in their chemical composition compared to propolis inside colonies with high mite infestation. Scientific evidence suggested that some components of are able to influence longevity of the workers (Castella *et al.*,2008). Although propolis has been used as a natural and traditional material for human medications since ancient times (Simone-Finstrom and Spivak, 2010), advantages of propolis for honey bee colony health were not appreciated until the last ten years. Understanding honey bees natural behavioral defense helps beekeeping community to improve beekeeping practices to enhance their natural behaviors and defenses. The process of keeping of the *Apis mellifera* species by humans using managed hives has overlapped with a very important natural defense mechanism of the honey bee colony, the construction of a propolis envelope. Simone-Finstrom (2010) and Borba (2015) confirm that the propolis envelope serves as an external antimicrobial layer around the colony, providing basic benefits to adult bees immunity.

Aims of the study

In scientific literature there is a continuing discussion concerning the benefits of propolis for human health, but the rule of propolis to bee health and maintenance of the colony is poorly understood.

In the light of all of this information, this study aimed to investigate effect of providing bee colonies in domesticated hives with additional amounts of propolis using two different methods, considering population growth of the colony and the lifespan of the individual bees in addition to the level of varroa mite infestation the most important indicators to the colony health.

2. MATERIALS AND METHODS

3. All experiments were conducted in the apiary of college of agriculture; university of Duhok;

Summel; Duhok province, from the last week of April to the end of October 2018.

3.1. Preparation of Experimental Colonies:

Nine colonies of honeybee (*Apis mellifera* L.) each with 5 to 6 combs were used, the tested colonies headed with young active queens. All these colonies provided with screen bottom boards, and were randomly divided into three treatments (each of three colonies); the first treatment was untreated (used as control). The other treatments were continuously provided with additional amounts of propolis during the six months of the experiment, but these two treatments were received the additional amounts of propolis in two different ways. The experimental colonies were organized as the following:

1. The first treatment; control treatment these colonies were naturally reared (consist of three replicates).

2. The second treatment; treatment of painting colonies with solvent propolis monthly, (consist of three replicates). The interior walls of the hives were painted with a thin layer of solvent propolis by using painting brush; this solvent propolis was prepared in the laboratory; 200 gm. of freshly collected propolis was added to 200ml of warm water inside water bath. After drying or evaporation of the water from propolis these hives were transferred to apiary again (Simone-Finstrom, Borba, *et al.*, 2017).

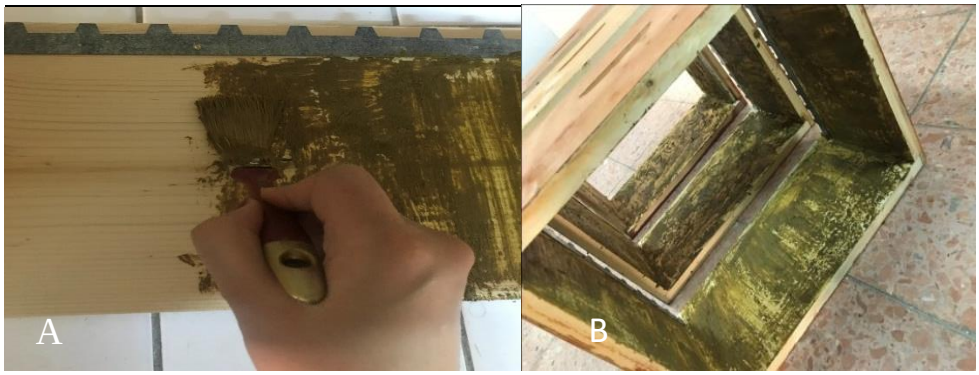


Fig. (1): Steps of painting interior walls of the hives with solvent propolis.

Third treatment; spraying empty combs with propolis extract, (consist of three replicates). Colonies of this treatment were treated differently, the empty combs inside the brood chamber of these colonies were exposed to spray of propolis extract; this extract prepared using a simple method of extraction; 200g of propolis with 200ml of alcohol. Because of propolis is too sticky to be broken, parts of the freshly collected propolis were kept in a freezer for few hours then immediately broken into small pieces in order to increase the contact surface between propolis and alcohol, both materials mixed together and the mixture left for about two days then applied to the colonies. This process was monthly performed (Fao.org, 2017).

Fig. (2): Spraying empty combs with propolis extract.

To investigate the effect of each treatment on the colony health and population growth, all tested colonies were weekly inspected in order to obtain the total area of sealed brood in addition to the number of fallen varroa mites on the surface of the screen bottom boards; the average of each month was calculated. Moreover, the life span of individual bees was calculated in both laboratory and in the field.

3.2. Life span of individual bees

This experiment was performed in both laboratory (inside small cages) and in the field (inside the hives). Newly emerged workers were placed in small wooden cages with one glass side; all cages were provided with a source of water and a small piece of comb containing honey. Dead workers were counted and removed from the cages in three days interval (Yang *et al.*, 2017), figure(3).

Fig. (3): Wooden cages containing newly emerged workers inside the incubator.

Two combs containing sealed brood with emerging workers were transferred into an incubator at 34ºC and 30 to 50 newly emerged workers for the laboratory experiment or 70 to 100 newly emerged workers for the field experiment were collected from each colony (according to the number of newly emerged workers available on each experimental comb).

Each colony was treated in the same way to obtain a large number of bees of similar age (day 0). Then these newly emerged workers were labeled with different numbers and colors. The labeled newly emerged workers were returned back to their native colonies at the same day. The presence of these workers inside the colonies were recorded in three days intervals, figure (4).

Fig. (4): Labeled newly emerged workers with different colors and numbers.

2.3. Varroa mite infestation level

Fallen varroa mites on the bottom boards were counted in three days intervals. All colonies treated with Organic natural strips for varroa as a detection method. And the numbers of fallen varroa mites before and after treatment were counted.

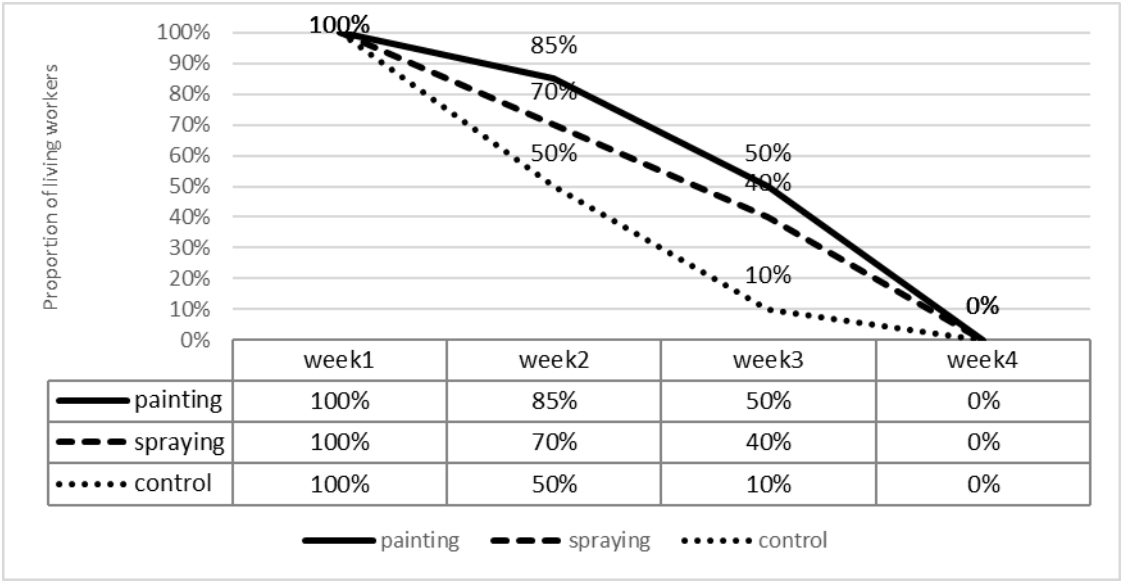
4. STATISTICAL ALALYSIS

Statistical analyses and graphing were presented using the GraphPad Prism Version 8 software. This analysis was performed using one way ANOVA followed by Dunnett's multiple comparisons test to determine any significant difference ($p < 0.05$) among the means of painting the interior walls with propolis solvent treatment and spraying empty combs with propolis extract treatment when compared to the control group and p values are shown. Data on the graph are shown as standard deviation and mean of triplicated samples. Significant differences were considered if the p value was < 0.05 and data are presented as mean ± SD of triplicated samples.

5. RESULTS

5.1. Lifespan of individual bees

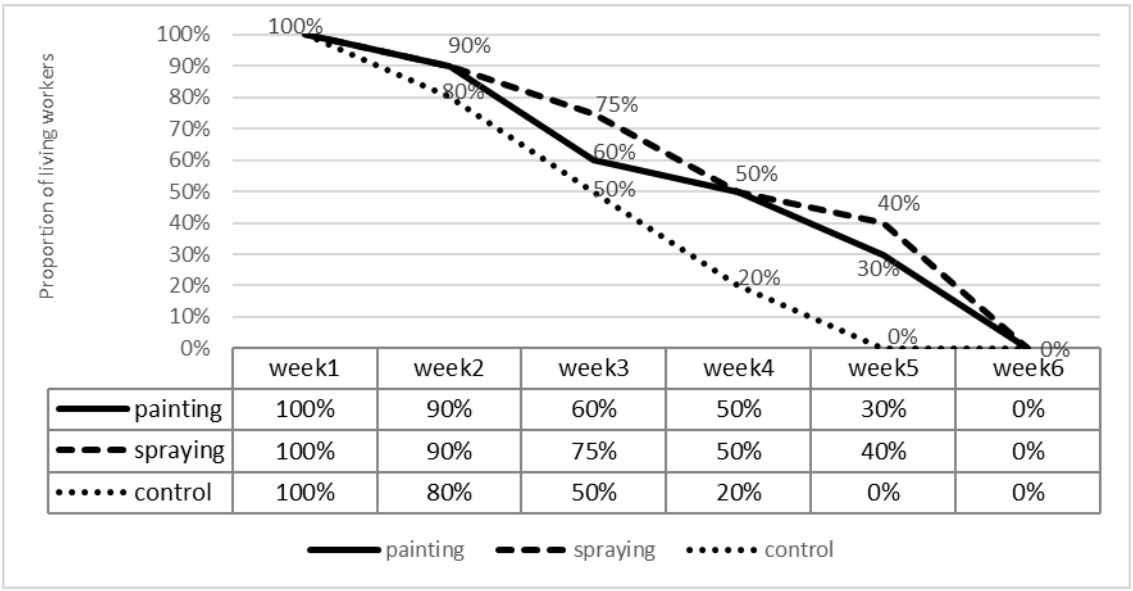
Proportion of living workers developed as larvae in untreated colonies and reared as adults inside small cages in the laboratory in May after two weeks was 50%, then 10% after three weeks. While 50% of workers developed as larvae in painted colonies and colonies contained sprayed combs with propolis extract continued to the end of the third week, but all bees were died at the end of week four, figure (5).



	week1	week2	week3	week4
painting	100%	85%	50%	0%
spraying	100%	70%	40%	0%
control	100%	50%	10%	0%

Fig. (5): Proportion of living workers developed as larvae in colonies treated with additional propolis compared to those developed in untreated colonies and reared as adults in the laboratory in May.

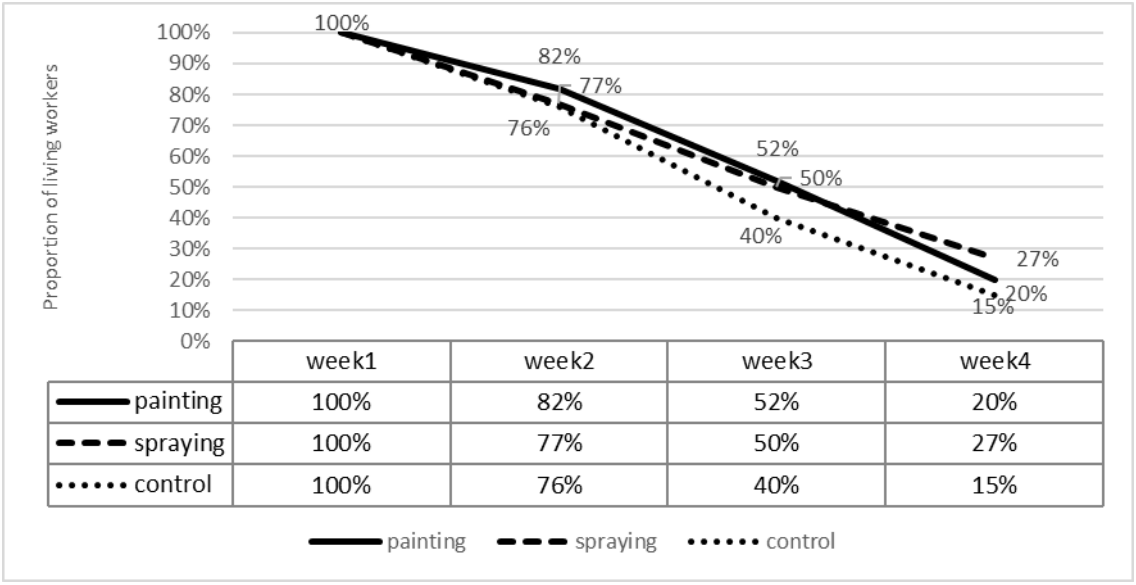
Proportion of living workers inside the cages from the three treatments, untreated (control), painted and sprayed colonies after three weeks were 50%, 60% and75% respectively, figure (6).



	week1	week2	week3	week4	week5	week6
painting	100%	90%	60%	50%	30%	0%
spraying	100%	90%	75%	50%	40%	0%
control	100%	80%	50%	20%	0%	0%

Fig. (6): Proportion of living workers developed as larvae in colonies treated with additional propolis compared to those developed in untreated colonies and reared as adults in the laboratory in September.

Figure (7) shows that proportion of living workers developed as larvae and reared as adults inside their colonies in the field in October from the three treatments, untreated (control), painted and sprayed colonies after four weeks were 15%, 20% and 27% respectively, these workers continued alive as winter bees.



	week1	week2	week3	week4
painting	100%	82%	52%	20%
spraying	100%	77%	50%	27%
control	100%	76%	40%	15%

Fig. (7): Proportion of living workers developed as larvae and reared as adults in colonies treated with additional propolis compared to those developed in untreated colonies in the field in October.

4.2. Population growth of the colony

Analyses of variance (ANOVA) showed significant differences at ($p < 0.05$) between untreated colonies and colonies treated with additional propolis. Bees reared in colonies contained empty combs previously sprayed with propolis extract had significantly more sealed brood (320 inch^2/colony) than those reared in untreated colonies (196 inch^2/colony) during early summer in June. Colonies reared in painted hives with solvent propolis also had significantly more sealed brood (286 inch^2/colony) than those reared in untreated colonies during early summer in June, figure (8); table (1).

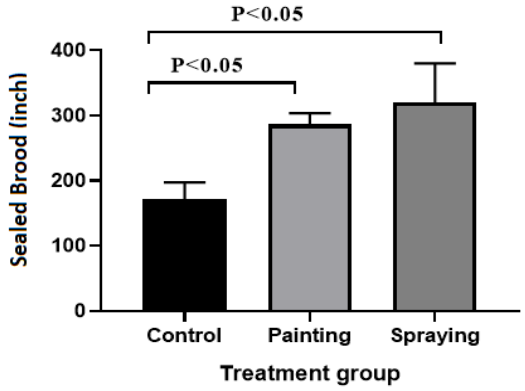


Fig. (8) Number of closed brood in three groups of colonies, untreated colonies(control)compared to colonies provided with additional propolis in May.

While in September only bees reared in colonies contained sprayed combs with propolis extract had significantly more sealed brood (143 inch^2/colony) than those reared in untreated colonies (99 inch^2/colony), significant differences were not found between colonies reared in painted hives with solvent propolis and untreated colonies but bees reared in painted hives had slightly more sealed brood (103 inch^2/colony) than those reared in untreated colonies, figure (9); table (1).

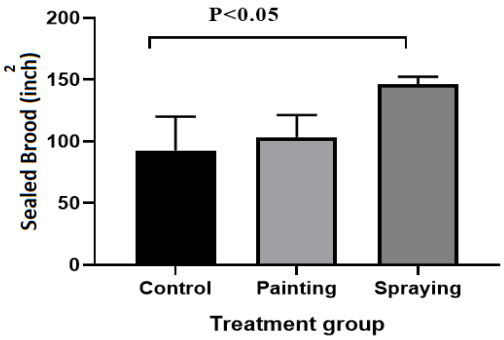


Fig.9. Number of closed brood in three groups of colonies, untreated colonies (control) compared to colonies provided with additional propolis in September.

4.3. Varroa mite infestation level

Numbers of fallen varroa mites on the bottom boards during September in untreated colonies were (60 mites before and 170 mites after acaricide application) higher than colonies of both treatments in which in painted colonies were 6 mites before and 31 mites after acaricide application, while in spraying treatment were 20 mites before and 27 mites after acaricide application. This trend was appeared before application of the acaricide and after application. Biggest number of fallen varroa mites was recorded in untreated colonies in both cases, before and after application of the acaricide. Smallest numbers of fallen varroa mites were recorded in colonies reared in painted hives, figure (10); table (1).

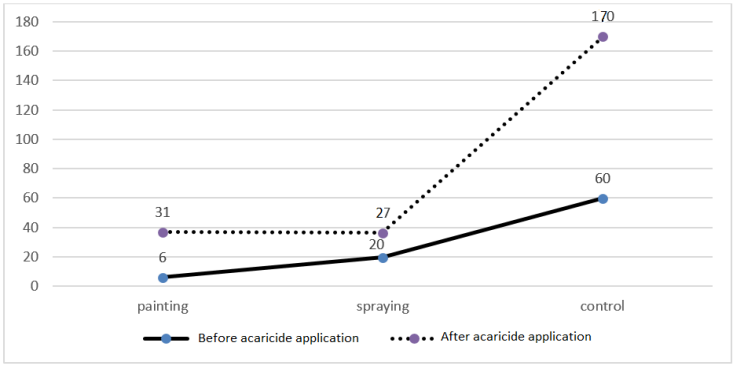


Fig. (10):Number of fallen varroa mites on the bottom boards during September in untreated colonies compared to those in colonies provided with additional propolis.

Table (1): Number of sealed brood in three treatment of colonies, in June and September, and Number of fallen varroa mites on the bottom boards during September in untreated colonies (control) compared to colonies provided with additional propolis.

Treatments	Sealed brood inch2		Number of fallen varroa mite per colony	
	June	September	Before acaricide application	After acaricide application
control	196	99	60	170
Painting	286	103	6	31
spraying	320	143	20	27

5. DISCUSSION

Honeybee worker's lifespan is an important factor to be considered in beekeeping. There is a considerable relationship between foraging behavior and mortality. Our results concerning the prolonged life span of workers developed in treated colonies with additional propolis are consistent with recent studies had been performed in other countries, they explained that propolis, as a natural complex material contain large amount of antioxidants. The flexibility of honey bees life span is evident. European honey bee workers live in the summer approximately 6 weeks and in the winter up to 6 months. This indicates that environmental factors affect worker longevity (Seeley, 1996). Confirm that the production of reactive oxygen species (ROS) via cellular metabolism directly affect in the aging process.

Manrique and Soares (2002) have reported that honey production and propolis production are positively correlated. It is also known that greater food availability positively affects the longevity of bees (Graham, 1997).

To increase the production of honey bee products, it is important to know the factors that influence yield. It is known that colony productivity is highly affected by its health. Brood viability is also affected by colony size, which in turn affects the capacity of the bees to maintain optimal temperature and humidity conditions in the brood nest (Garófalo, 1977).Worker longevity is also affected by environmental conditions, presence of nectar and pollen, brood area, the adult bee population and infestation by Varroa (Malone et al., 1995). Propolis, as a natural mixture full of antioxidants, is certainly understudied in regard to its potential effects against oxidative stress. Oxidative stress is natural in the progression of aging in honey bee (Vance *et al.*, 2009).

More brood area was found in colonies treated with additional propolis. It is worth mentioning that combs treated with spraying of propolis extract were more acceptable for the queen to lay eggs that was most

likely caused the presence of more brood area in the colonies contained these combs. Studies have demonstrated that increased brood viability and longer lifespan are affected by the amount of propolis inside the hive.

The biggest number of fallen varroa mites found in treated colonies with additional propolis are not in agreement with the results of, Borba *et al.*, (2015) who were not found differences in Varroa mite infestation between colonies provided with this envelope and those without propolis envelope over two years of study. However, many recent studies demonstrated identical results that were found in our study. From German propolis Ethanolic extracts caused 100% mortality due to contact with 10% propolis extract (Garedew *et al.*, 2002). Moreover, mite exposure to extracts at concentrations as low as 0.5% caused narcotic effects leading to lower heat production and metabolic rates (Garedew *et al.*, 2002; Garedew et al., 2003). Sub lethal effects of low concentrations of propolis may reduce Varroa mite's mobility, as well as make them less capable of dealing with environmental stressors e.g., high temperatures (Garedewa *et al.*, 2003).

Drescher *et al.*,(2017) concluded through the study of raw propolis, that the survival of varroa mites in the laboratory was not affected after exposing to the propolis. Concerning the differences in chemical composition of propolis in the colony-level Popova *et al.*, (2014) confirmed that propolis from colonies with very low mite infestation levels without using acaricides were differed in its chemical composition compared to propolis from colonies contained high level of mites. Therefore, impacts of more natural applications of propolis against Varroa mites should be explored

6. CONCLUSION

According to our investigations it seems that honey bee foragers not only search for food and water, but they are searching for the most valuable material to maintain the individual health and colony productivity. They perform this task through the collection of sticky materials from plant buds and combine them with some secretions from their bodies in order to protect their brood and the whole colony from pathogens, pesticides, parasites and other intruders. Thereby it is necessary for beekeepers community to understand the rule of propolis in addition to the food and the rich forage before establishing their apiaries. As a contribution from scientific researchers we can advise beekeeping community in our area to start selecting for propolis collection instead of selecting against because of its sticky nature. It is also possible to provide the hives with additional amounts of propolis in order to maintain the colony health and productivity with minimum parasites and maximum lifespan for individual adult bees.

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پوختە

هاتە دیارکرن کو کارتێکرنا پێ دانا شانێت مێشا هنگڤینی (*Apis mellifera*) ب ڕێژەیەکا زێدە یا پێنداڤێ. ڕێژا کرما زێدەتر بو د وان شانا دا ئەوین پەروازێن وان هاتینە ڕەشاندن ب گیراوێ پێنداڤێ (320 inch^2/colony) و هەر وەسا ل وان شانا ئەوێن دیوارێن وانا هاتینە بویاغکرن ب پێنداڤێ (286 inch^2/colony) ژ وان شانا ئەوێن بشێوەیەکێ سروشتی هاتینە خودان کرن (196 inch^2/colony) ل دەسپێکا وەرزێ هاڤینێ هەیڤا خزیرانێ. بەلێ ل هەیڤا ئەیلونێ دا بتنێ ئەوان شانێت پەروازێن وان هاتینە ڕەشاندن ب گیراوێ پێنداڤێ ڕێژەیەکا زێدەترهەبو(143 inch^2/colony) ژ کرمان ژ وان شانا ئەوێن دیوارێن وانا هاتینە بویاغکرن ب پێنداڤێ (103 inch^2/colony)وئەوێن سروشتی هاتینە خودان کرن(99 inch^2/colony).

ڕێژا پتر ژ ڤاروایێن کەتی لسەر بوتوم بوردی هاتنە تومارکرن د وان شانێت سروشتی هاتینە خودان کرن(60 ڤاروا بەری و170 ڤاروا پشتی بکارئینانا قرکەری), و کێمترین ڕێژا ڤاروایێن کەتی لسەر بوتوم بوردی هاتنە تومارکرن ل شانێت دیوارێن وانا هاتینە بویاغکرن ب پێنداڤێ (6 ڤاروا بەری و31 ڤاروا پشتی بکارئینانا قرکەری).

شولکەرێن مێشا هنگڤینی ئەوێن وەك کرم مەزن بوین دناڤ شانێت پەروازێن وان هاتینە ڕەشاندن بگیراوێ پێنداڤێ و هەروەسا دوان شانا دا ئەوێن دیوارێن وانا هاتینە بویاغکرن ب پێنداڤێ تەمەنێ وانا درێژ تر بوژ ئەوان مێشا ئەوێن بشێوەیەکێ سروشتی هاتینە خودان کرن, د هەردوو تاقیکرنادا یا تاقیگەهێ ویا مێشگەهێ. و ڕێژا شولکەرێن مێشا هنگڤینی ئەوێن وەك کرم و ئەدالت مەزن بوین ل مێشگەهێ ل هەیڤا ئەیلونێ ود هەرسێ تریتمێنتادا پشتی چار حەفتیا ببو 20%,27% و 15%و ببەردەوامی, وئەو مێشێت ماینە ساخ دئ دبەردەوام بن بو زڤستانێ.

الخلاصة

تم التحقق من تأثير تزويد خلايا النحل (*Apis mellifera*) بكميات اضافية من العكبر. الحضنة المقفلة كانت معنويا اكثر في الخلايا التي احتوت على اطارات مرشوشة بمستخلص العكبر (320 inch^2/colony)وايضا في الخلايا التي تم طلاء جدرانها بالعكبر (286 inch^2/colony) من التي ربيت طبيعا بدون اي اضافات (196 inch^2/colony) في بداية الصيف شهرحزيران. ولكن في شهر ايلول فقط الخلايا التي احتوت على اطارات مرشوشة بمستخلص العكبر كانت لديها نسبة اكبر من الحضنة المقفلة (143 inch^2/colony) من الخلايا المطلية جدرانها الداخلية بالعكبر (103 inch^2/colony) و الخلايا التي ربيت طبيعيا(99 inch^2/colony).

اكبر عدد من الفاروا المتساقطة على الالواح السفلية تم تسجيلها في الخلايا التي ربيت طبيعيا (60 حلم قبل و170حلم بعد تطبيق المبيد) ,و اقل عدد من الفاروا المتساقطة على الالواح السفلية تم تسجيلها في الخلايا التي تم طلاء جدرانها بالعكبر(6 حلم قبل و31حلم بعد تطبيق المبيد).

الشغلات التي قضت طورها اليرقي في الخلايا التي احتوت على اطارات مرشوشة بمستخلص العكبر و ايضا الخلايا المطلية جدرانها بالعكبر كانت اطول عمرا من الشغلات التي ربيت طبيعيا في كلتا الحالتين في المختبر و في الحقل. نسبة الشغالات التي قظت طورها اليرقي و كبالغة في الحقل في شهر ايلول في المعاملات الثلاثة بعد اربعة اسابيع كانت 20%,27% و15% و على التوالي, و هذه الشغالات اكمت حياتها للشتاء.