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MELLIFERA RESEARCH ARTICLE

Effects of Royal Jelly Supplementation on Growth Plate Zones and Longitudinal Growth in Young Rats

Özgür PİRGON*[1], Müge ATAR[1], Metin ÇİRİŞ[2], Murat SEVER[1]

[1]Süleyman Demirel University, Departments of Pediatric Endocrinology and Diabetes, Isparta, TURKEY

[2]Süleyman Demirel University Pathology Faculty of Medicine, Isparta, TURKEY

** Corresponding author e-mail:ozgurpirgon@gmail.com*

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A B S T R A C T

Royal jelly (RJ) is secreted by the mandibular glands of worker honeybees as an essential food for the queen bee larva. In recent years, families have often used RJ supplementation for their children's growth. We investigated the effects of RJ supplementation on the growth plate of young rats and evaluated the hormone levels such as estradiol, growth hormone (GH) and insulin like growth factor-1 (IGF-1). A total of 30 rats aged 7 days were randomly divided into two groups of 15. For 15 days, 50 mg/kg of RJ was administered once a day by gavage to RJ group. Plasma estradiol, growth hormone (GH) and IGF-I levels were measured. Mean weight and tail length changes were significantly higher in the RJ group than the control group at the end of the study ($p<0.001$ and $p=0.04$). Plasma growth hormone and estradiol levels were significantly increased in the RJ group ($p=0.03$ and $p=0.04$) and the total height of the growth plate was measured significantly higher in RJ group than the control rats ($p<0.001$). In addition, the percentage of estrogen receptor expression on the growth plate was stated as 81.3% in the proliferative zone of RJ group, and as 14.3% in the control group ($p<0.001$). Our data suggested that the administration of RJ caused longitudinal bone growth and also increased estradiol and growth hormone levels, but our findings also provided the evidence of some potential estrogenic effects of RJ on growth plate.

Keywords: Royal Jelly, growth, growth hormone, estrogenic activities, growth plate

Introduction

As growth failure is one of major causes of concern and anxiety to children, adolescents, and parents, there is interest in growth promotion during childhood and adolescence. Royal jelly (RJ) is the most commonly used product of apitherapy and is in frequent use by parents as a growth supplement for children (1–3 g daily) [1,2]. RJ is yogurt-like bee milk secreted by the hypopharyngeal and mandibular glands of

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worker honeybees as an essential food for the queen bee larva. RJ contains proteins, carbohydrates, fats, free amino acids, vitamins, and minerals [3]. It is partially soluble in water and highly acidic (pH 3.4–4.5) with a density of 1.1 g/mL [4]. 10-Hydroxy-2E-decenoic (10-HDA) acid is the most important active ingredients in RJ, and the 10-HDA content can be considered as an index for estimation of quality [5,6] (Fig. 1). Many studies have reported that RJ has some potential estrogenic effects [7,8]. This honey bee-excreted biological fluid possesses estrogen-like activity, yet the compounds mediating its estrogenic effects are largely unknown. Suzuki et al [9] and Narita et al [10] demonstrated a weak estrogenic activity of RJ that it competes with 17-beta estradiol in binding to the human estrogen receptors alfa and beta, although it is much weaker than diethylstilbestrol in terms of binding affinity.

RJ is also used extensively in commercial nutritional supplements, medical products, and cosmetics and as a growth supplementation for children in many countries. Growth of long bones occurs at the growth plate, a thin layer of cartilage that separates the epiphysis from the metaphysis. An improvement in the height is significantly associated with the bone growth in length occurs at the growth plate by endochondral ossification [11]. The growth plate is regulated by a multitude of genetic and hormonal factors, growth factors, environment, and nutrition [12]. The purpose of the present study was to investigate the effects of RJ administration on the growth plate of young rats and to evaluate the hormone levels such as estradiol, growth hormone (GH) and insulin like growth factor-1 (IGF-1).

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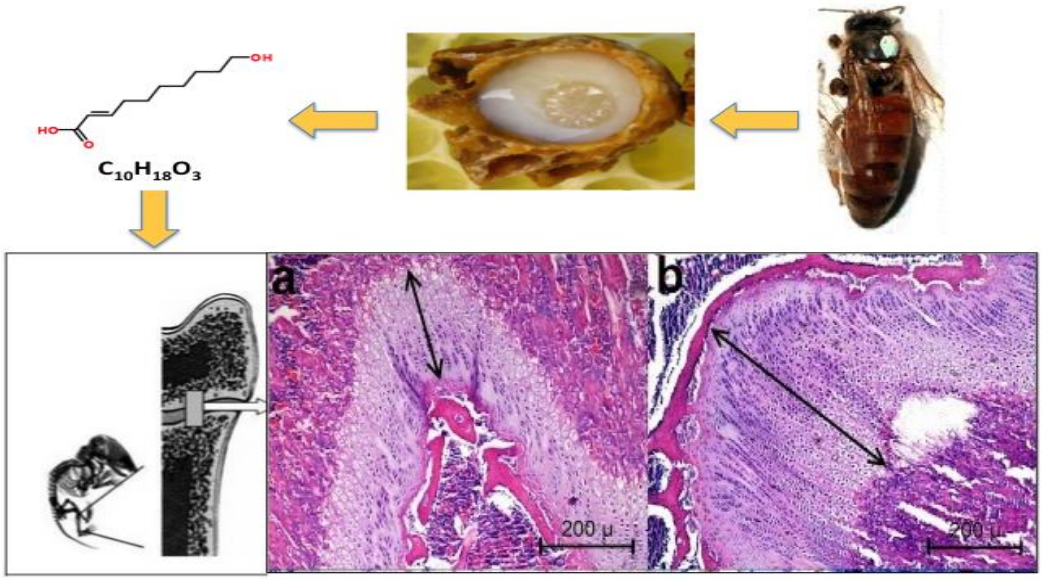

Figure 1. Chemical profile of 10-Hydroxy-2-Decenoic Acid (also called 10-HDA or royal jelly acid). It is a kind of special active substance which exists only in royal jelly in the nature.

Materials and Methods

Animals

A total of 30 Sprague-Dawley rats aged 7 days old were randomly divided into two groups each containing of 15. All the rats were breast-fed and kept in standard laboratory conditions of 22±2°C, humidity 55±5%, and a 12-hour light-dark cycle. The breastfeeding rats were not provided with food pellets during the study period but had free access to drinking water. The RJ group was administered with 50 mg/kg of RJ, once a day, by oral gavage for 15 days. The control group received 1 mL of distilled water by oral gavage once daily for 15 days. On day 0 as baseline, then weekly on Day 8, and Day 15 the animals were weighed using electronic scales accurate to ± 0.001 g (A&DGF600, Japan) and tail length was measured. The RJ was well tolerated by all the animals. There was no death in the observation period. After 15 days, the 3-week-old rats were killed and the growth plates were isolated from the proximal tibiae of each rat, thus giving a total of 30 growth plates for each group. All stages of the experiment were conducted according to the guidelines of the Institutional Ethics Committee of the S.Demirel

University (Approval number: 4062-TU2-14/03), in the line with the European Union guidelines on Animal Care.

Hormone measurements

At the end of the study, blood samples were taken from the trunk of the decapitated rats and collected into heparinized tubes at the moment of sacrifice. Plasma was separated by centrifugation at 1200× g for 15 minutes at 4°C and stored at –20°C until analysis. Plasma GH (mouse/rat rGH E023, Mediagnost, Reutlingen, Germany) and IGF-1 (mouse/rat IGF1 REF E25, Mediagnost, Reutlingen, Germany) levels were measured using ELISA kits. The minimum detection limit, and intra- and inter-assay variability for the IGF-1 ELISA kit were 90 pg/ml, 6.7%, and 6.8%, respectively. Plasma estradiol levels were measured using BioVision rat ELISA Kit (Mountain View, CA). This kit shows no species cross-reactivity. Detection range was 2–50 ng/L. These kits are highly specific and sensitive, and also have a small sample volume requirement, making them ideal for young rats.

Analysis of the growth plate

The growth plate is a highly complex, spatially polarized structure that consists of three layers: the stem cell zone, the proliferative zone and the hypertrophic zone. Histomorphometric measurements and immunohistochemical examinations were performed on the growth plate. Histomorphology of the growth plate was assessed from 5-µm sections of paraffin-embedded tissues following hematoxylin–eosin staining. The height of the growth plate was determined using the complete Olympus BX51 equipment (Olympus Optical Co. Ltd. Tokyo) consisting of a microscope connected to a computer. The images were transferred and analyzed with the Image J software. Overall growth plate height was measured by determining the central region on the long axis of the tibia. Horizontal lines were drawn along the contours of both the epiphysis on the proximal side of the growth plate and the chondro-osseous junction on the distal side of the growth plate. Growth plate zone heights were totaled to give the total growth plate height representing the distance between the primary and secondary centers of ossification. The total height of the growth plate was calculated as the average of 10–20 measurements/growth plate.

Immunohistochemical analysis

Immunohistochemical analysis for Ki-67, Estrogen receptor (ER) and IGF-1 receptor was performed on formalin-fixed, paraffin embedded tissues, using the streptavidin-biotin-peroxidase technique. The sections

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were incubated with precisely diluted mouse monoclonal antibodies against Ki-67 (Rabbit monoclonal [SP6], MA, USA), Estrogen Receptor (Rabbit polyclonal to ER alpha, MA, USA) and IGF-1 receptor (IGF-1, Rabbit polyclonal to IGF-1 Receptor, MA, USA). Positive stained cells of proliferative and hypertrophic chondrocytes per column were counted using Olympus BX51 equipment. The percentages of IGF-1 and ER-positive chondrocytes were assessed in the proliferative and hypertrophic zones of the growth plate. The Ki-67 cell proliferation index was calculated on the basis of the percentage of positive stained nuclei [13]. At least 2000 cells in the growth plate were counted in each group. Positive cells were counted as recommended by Iamaroon et al [14].

Statistical analysis

All data were presented as the means ± standard deviation (SD) for each group. The differences between the groups were evaluated using an unpaired t-test. (SPSS version 17, Chicago, IL). Differences were considered significant at a value of $p<0.05$.

Results and Discussion

The changes of weight and tail length

The changes of weight and tail length of the groups were shown in Table 1. The body weights and tail lengths were similar in both groups at baseline. Following the RJ administration, there was a marked increment in mean weight change in the RJ group compared with the control group (41.4±7.1 g *vs.* 31.5±4.8 g, $p<0.001$). The change of the tail length was significantly higher in the RJ group than in the control group (3.7±0.6 cm *vs.* 3.6 ± 0.3 cm, $p=0.04$) (Fig. 2).

Table 1. Weight and tail length changes in rats supplemented with Royal Jelly (RJ) for 15 days

		Royal Jelly	Control	p
Weight (grams)				
	Baseline	46.5 ± 6.1	48.7 ± 3.4	0.756
	8^{th} day	69.7 ± 9.2	59.9 ± 4.4	0.001
	15^{th} day	87.9 ± 11.9	80.3 ± 6.7	0.04
	Changes (1-15th days)	41.4 ± 7.1	31.5 ± 4.8	<0.001
Tail length (cm)				

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	Baseline	7.8 ± 0.5	7.5 ± 0.3	0.161
	8th day	9.8 ± 0.5	9.2 ± 0.4	0.04
	15th day	11.5 ± 0.5	11.1 ± 0.4	0.06
	Changes (1-15th days)	3.7 ± 0.6	3.6 ± 0.3	0.04

Hormonal measurements at the end of the study

Hormonal measurements at the end of the study were presented in Table 2. Compared with the control group, the GH levels were found to be higher in the RJ group following RJ administration (2.8±0.7 ng/dL *vs.* 1.05±0.6 ng/dL, p=0.03).

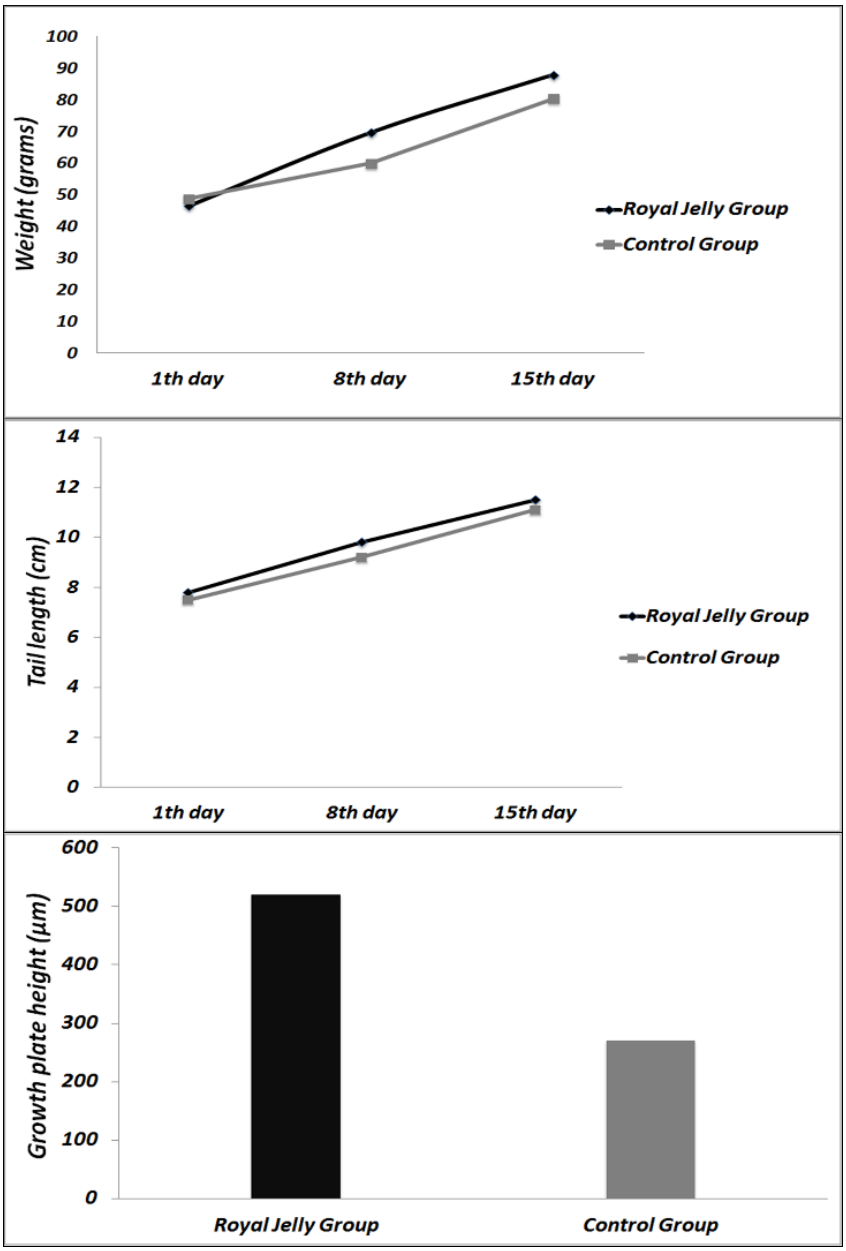

Figure 2. Mean weight, tail length measurements at baseline, 8th and 15th days. At the end of the study, tibial growth plate heights of the groups were shown.

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Table 2. Effects of Royal Jelly administration on hormone levels

	Royal Jelly	Control	p
Estradiol (pg/mL)	708 ± 53	582 ± 85	0.04
Growth hormone (ng/dl)	2.8 ± 0.7	1.05 ± 0.6	0.03
IGF-1 (ng/dL)	404 ± 4	207 ± 27	0.175

The plasma estradiol level was also higher in the RJ group (708±53 pg/mL vs. 582±85 pg/mL, p=0.04) than the controls at the end of the study. However; there was no significant difference among the groups for IGF-I levels (404±4 ng/dL *vs.* 207±27 ng/dL, p=0.175) (Fig. 3).

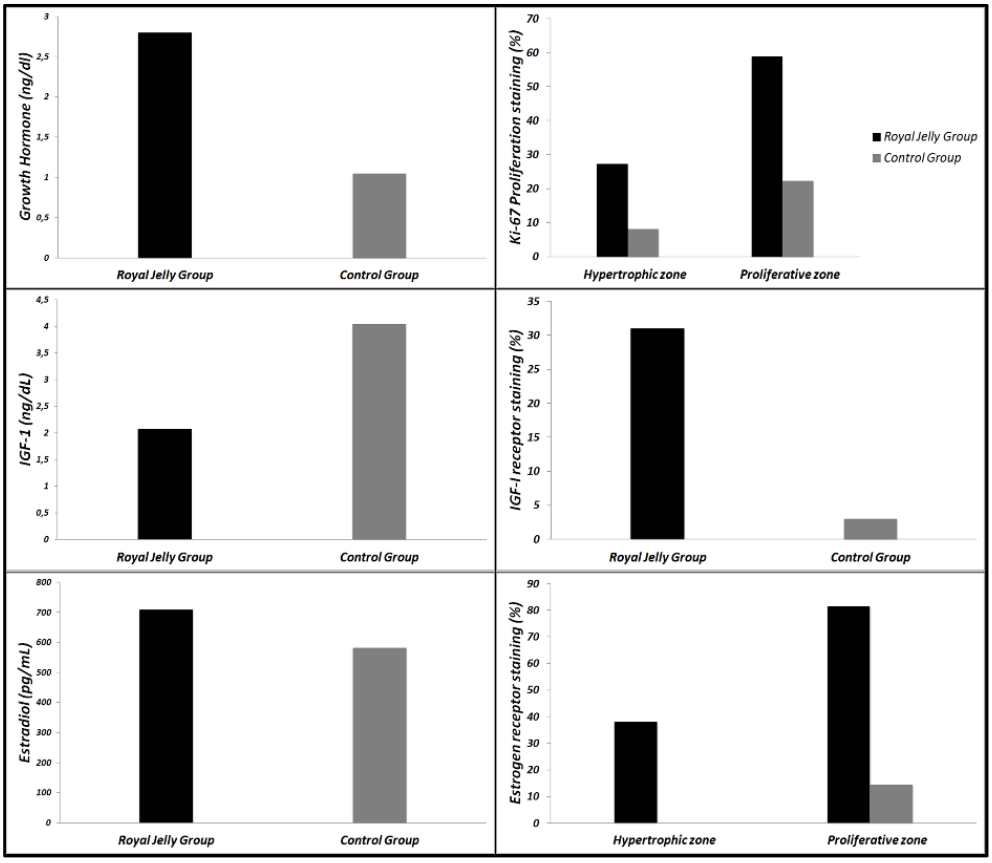

Figure 3. The effects of oral administration of Royal Jelly on hormone levels (Growth hormone, IGF-I and estradiol) and on the growth plate (immunohistochemical stainings; Ki-67 proliferation, IGF-1 receptor and estrogen receptor stainings, results are expressed as % of cell count).

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Comparisons of growth plate measurements

The tibial growth plates at the termination of the experiment on Day 15 are presented in Fig. 4. The total height of the growth plate was measured as 520±10 μm in the RJ group, and as 270±8 μm in the control group ($p<0.001$) at the end of the study (Table 3, Fig. 4a and b). The proportions were determined for the cell density (percentage of cell counts) in the proliferative and hypertrophic zones of the growth plate. Increased estrogen receptor expressions were determined in the proliferative zones of the RJ group compared to the control group (81.3±17.6% *vs.* 14.3±16.3%, $p<0.001$). The percentage of the estrogen receptor expressions in the RJ group were also significantly increased in the hypertrophic zones ($p<0.001$) (Fig. 4g and h). The IGF-1 receptor expression staining was determined as higher expression percentages in the growth plates of the RJ group than in the control group (31±14.7% *vs.* 3±5%, $p<0.001$) (Fig. 4e and f). We found that Ki-67 staining was highly expressed in the hypertrophic zones of the RJ group (27.3±7.9% *vs.* 8±4.6%, $p<0.001$) compared to the control group and there was a significant difference in the proliferative zones among the groups (59±14.9% *vs.* 22.3±17.8%, $p<0.001$) (Fig. 4c and d). In recent years, families have often used RJ supplementation for their children's growth. The present report investigated the possible effects of oral RJ administration on the longitudinal growth and the growth plate of the young rats. We demonstrated in this study that RJ might have some benefits in weight gain and the longitudinal growth, which is an effect linked to increased plasma GH and estradiol levels. Many systemic hormones regulate the longitudinal bone growth including GH, IGF-1, insulin, thyroid hormone, glucocorticoids and sex steroids [15]. In this study, we found higher plasma GH and estradiol levels in the blood samples of the RJ administered group compared to the control group at the end of the study. A study by Narita et al [10] showed that oral administration of RJ to normal female mice caused an increase in bone content and up regulation of gene expression of type I procollagen. The difference of plasma IGF-1 levels was not significant among the groups. It has been argued that IGF-1 locally produced in the growth plate is of greater importance in the regulation of growth plate cartilage than systemic levels of IGF-1 [16]. We detected that the IGF-1 receptors were expressed

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significantly higher percentage staining in the growth plate of the RJ rats than the control rats although no significant difference in IGF-1 levels was found among the groups. These data support the view that is a more local action of IGF-1 which directly targets the growth plate chondrocytes. Ki-67 is a human nuclear protein the expression of which is strictly associated with cell proliferation and which is widely used in routine pathology as a "proliferation marker" to measure the growth fraction of cells [17]. The expression of the Ki-67 positive cells (the Ki-67 labeling index) is closely associated with cell proliferation [17, 18]. Although the rate of new cell production in the proliferative zone is an important factor in bone formation, the hypertrophic zone plays a key role as well [19]. We showed increased Ki-67 expressions particularly in the proliferative zone of the growth plate of the RJ group compared to the control group growth plates (Figs. 4c and d). We found also highly expressed IGF-1 and estrogen receptors locally in the growth plate chondrocytes following RJ administration. These data suggest that the content of RJ (most probably the estrogen compounds of RJ) can promote the growth stimulation in young rats. Estrogens are important endocrine regulators of skeletal growth and maintenance in both females and males [20, 21].

In present study, RJ was seen to increase the longitudinal growth, but it also had estrogenic effects on the growth plate zones in growing rats. We detected significantly higher estradiol and GH levels in blood samples and positive estrogen receptor expressions were demonstrated on growth plate in the RJ administered rats compared to the control group (Figs. 4g and h). Both of the estrogen receptors alfa and beta are expressed by bone and growth plate cartilage of humans and other species [22, 23]. Estrogens are crucial regulators of the GH/IGF-1 axis [24, 25] and therefore, some of the effects of estrogens on skeletal growth may be indirect via the modulation of the GH/IGF-1 axis. In the current study, a significant increase was determined in the total growth plate height of the rats after 15 days of RJ supplementation, probably through increased estrogenic activity. These findings therefore strongly support the hypothesis that RJ has an estrogenic effect on growth plate chondrocytes and thereby could affect linear bone growth in children administered with orally RJ.

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Table 3. Effects of Royal Jelly administration on growth plate height and growth plate immunohistological staining in growing rats at the end of the study.

	Royal Jelly	Control	p
Growth plate total height (µm)	520 ± 10	270 ± 8	<0.001
Estrogen receptor staining (%)			
Hypertrophic zone	38 ± 20	0 ± 0	<0.001
Proliferative zone	81.3 ± 17.6	14.3 ± 16.3	<0.001
Ki-67 receptor staining (%)			
Hypertrophic zone	27.3 ± 7.9	8 ± 11.6	<0.001
Proliferative zone	59 ± 14.9	22.3 ± 17.8	<0.001
IGF-1 receptor staining (%)	31 ± 14.7	3 ± 5	<0.001

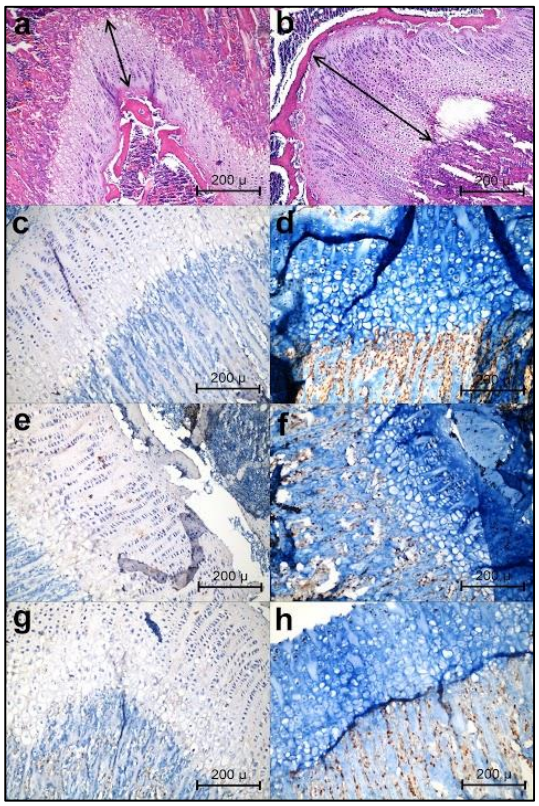

Figure 4. Total growth plate and receptor expressions of the growth plates of RJ group (b, d, f and h) and the control group (a, c, e and g). Images of growth plates from the proximal tibia at the end of the study. (Black arrows indicate the total growth plate height). A positive reaction for Ki-67 (d), IGF-1 receptors (f) and estrogen receptors (h) was observed as

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Conclusion

This study has some limitations which have to be pointed out. The small study group do not allow us to draw any conclusion about the effectiveness of this RJ supplementation on growth. Furthermore, the follow-up was limited. Larger series with long-term follow-up are needed to confirm the effectiveness of the RJ on human studies.
In conclusion, exposure of young rats to RJ by orally caused increased estradiol and growth hormone levels and also longitudinal bone growth, but our findings also provided the evidence of some potential estrogenic effects of RJ on growth plate. However, increased estrogenic activity is also important for the cessation of growth by inducing growth plate closure and this effect of RJ may diminish the final height potential. It can be said that despite the common usage of RJ by parents for children, there still seems to be much to study and learn about the effects of RJ on children.

Arı Sütünün Genç Ratların Büyümesine ve Büyüme Plağı Zonları Üzerine Etkisi

Öz: Kraliçe arıların ana besin öğesini oluşturan Arı sütü, işçi arıların mandibular bezlerinden salgılanmaktadır. Son yıllarda, ailelerin çocuklarının büyümesine yardımcı olmak için Arısütü'nü uygulamasında artış olduğu gözlenmektedir. Bu çalışmada arısütü verilen genç sıçanlarda büyüme plağı zonlarına olan etkisi ve hormonal etkilerini (östrodiol, büyüme hormone ve insulin benzeri büyüme faktörü-1) araştırdık. 30 adet 7 günlük sıçan randomize olarak iki gruba ayrıldı. Bir gruba annesütünün yanısıra 15 gün süresince gavaj yolu ile günde 50 mg/kg Arı sütü uygulandı. Çalışmanın sonunda RJ verilen grubun göre ortalama ağırlığı kontrol grubuna daha fazla ve ortalama kuyruk uzunlukları daha uzundu ($p<0.001$; $p=0.04$). Plazma büyüme hormonu ve östradiol seviyesi RJ g rubunda daha yüksek olarak sonuçlandı ($p=0.03$; $p=0.04$). Büyüme plağının uzunluğu RJ grubunda control grubuna göre daha uzun olduğu tespit edildi. Ayrıca büyüme plağının proliferative zonunda östrojen reseptör ekspresyonu RJ grubunda control grubuna göre daha fazla olduğu gözlendi ($p<0.001$). Çalışma sonunda büyüme plağı zonları, östrodiol, büyüme hormonu ve insulin benzeri büyüme faktörü-1 ölçüldü. Bu çalışma sonucunda Arı sütü'nün büyüme plağı zonlarında ve serumda östrodiol ile büyüme hormonunun artışı olduğu ancak büyüme plağının uzamasının Arısütünün potansiyel östrojenik etkisine bağlı olacağı belirtildi.

Anahtar Kelimeler: Arı sütü, büyüme, büyüme hormonu, östrojenik aktiviteler, büyüme plağı

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MELLIFERA RESEARCH ARTICLE

Chemical Content and Bioactive Properties of Drone Larvae (Apilarnil)

Sibel SİLİCİ*

Erciyes University, Agriculture Faculty, Department of Agricultural Biotechnology, Kayseri, TURKEY

** Corresponding author e-mail:sibelsilici@gmail.com*

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A B S T R A C T

In this study, nutrients, amino acid content and bioactive properties of drone larvae (apilarnil) were determined. According to HPLC-UV analysis, 16 amino acids were determined in apilarnil. Only tryptophan was not detected from the essential amino acids. The highest amino acid amount was lysine amino acid (7198 mg /100g) and the lowest amino acid was methionine (500 mg/100g). According to the results of the research, moisture; ash; protein carbohydrate and lipid contents of apilarnil were found to be 4.43, 4.07, 48.75, 21.62 and 21.13 g/100 g apilarnil respectively. To determine the bioactive properties of apilarnil, the Folin-Ciocalteu method for phenolic content, phosphomolybdenum method for antioxidant activity and DPPH method for antiradical activity were used. The total phenolic content of apilarnil was 834.05 (mg GAE/100 g) and the antioxidant activity was 90.91 mg AAE/g. Antiradical activity inhibition level of drone larva (apilarnil) was found to be 81.61%. As a result, the beneficial biological activity of apilarnil may be due to its antioxidant activity.

Keywords: Apilarnil, drone larvae, phenolic, antioxidant, antiradical, amino acid

Introduction

Drone larvae (apilarnil) is one of the honey bee products that are not known much in our country. Apilarnil is a bee product obtained by lyophilizing after the collection of drone larvae at the age of 3-7 days from drone cels. It has a homogeneous, milky, yellowish gray color and a bitter taste. After the larval cells are closed, the nutrient composition of the larva changes during the pupal phase. Therefore, it is appropriate to collect apilarnil in the larval stage where the highest quality nutritional form is preserved.Since the larvae will die during harvesting, the larvae should be consumed or processed quickly because the protein in its structure may be disrupted [1].

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Studies on drone larvae mainly focused on the reproductive functions of farm animals such as broilers, pigs and rams [2-6]. However, there are also studies on the chemical content of drone larvae [7-11]. Apilarnil contains approximately 25-35% dry matter, 9-12% protein, 6-10% carbohydrate, 5-8% lipid, 2% ash and 3% unidentified substance [12,13]. The protein content of apilarnil is higher than other nutrients. The variety and richness of the pollen sources on which honey bees feeds affect the vitamin content of apilarnil. Vitamins (A, B1, B6, choline etc.) and minerals (Ca, P, Na, Zn, Mn, Fe, Cu and K) contained in larvae increase the quality of drone [14]. Apilarnil has a high level of antioxidant properties. This feature is due to the rich polyphenols in the structure [15]. In addition, apilarnil has been found to be rich in male sex hormones, especially testosterone, and has an androgenic effect that enhances male sex features [16,17]. Furthermore, apilarnil has been shown to be a natural anabolic agent in male individuals because it increases body muscle weight [2]. Apilarnil, a powerful source that stimulates oxidative processes that produce energy due to its strong catabolic effect in the body. It prevents glycogen loss in muscles In order to achieve the desired performance [1]. Determination of the chemical content of apillarnil, which has been found to have beneficial biological activities, is necessary to explain these activities. Therefore, in this study, the nutrients, amino acid content and bioactive properties determined of drone larvae (Apilarnil) produced in Turkey.

Materials and Methods

The drone larvae samples (n=6) used in the study were obtained from hives in Erciyes University Agricultural Application and Research Center in May 2018 and collected at the age of 7 days. Collected samples were frozen at -20 °C. It was then dried by lyophilization and pulverized.

Nutrient content

Determination of ash, crude fat and crude protein in apilarnil samples was carried out using standard analytical procedures, Association of Official Analytical Chemists [18], 920.153, 991.36, and 960.52 respectively. Moisture content was determined using a vacuum oven at 60 °C and weighing until a constant weight. The results were expressed in grams per 100 g

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of fresh weight. The ash content was determined gravimetrically following incineration in an oven at 550°C and weighing until constant weight. Nitrogen determination was performed using micro-Kjeldahl method. Then a conversion factor of 6.25 was used for converting percentage of nitrogen in the sample into percentages of protein. Energy content was determined by at-water method. All analyses were carried out in triplicate [18].

Amino acid content

The analyzes were carried out using High Performance Liquid chromatograph (Perkin-Elmer Corp. Norwalk, USA), equipped with a UV detector. The amino acid content of the apilarnil was determined by the method of Jensen et al. [19]. Briefly, 0.2 g of sample was weighed into 15 mL of flask and 8 ml of 6.0 N HCl was added, sealed well and hydrolysed at 110 ^{o}C for 24 h. After this procedure, 1 mL with drawn and 45% vacuum dried. The sample was redissolved with stirring with 5 mL of 0.02 N HCl and centrifuged at 5,000 rpm. For derivatization, 20 µL of the aminoacid standard solution was placed in vial and dried in a vacuum oven at 65 °C for 2 hours. 30 µL of methanol-water-TEA (2:2:1) were then added and dried at 65 °C for 10 minutes. Then, 30 µL of derivatizing reagent methanol-water-TEA (7:1:1) (v/v) was added, vortexed for 30 h and allowed to stand at room temperature for 20 min. The resulting solution was vacuum dried at 65 °C for 15 min. Diluent containing 5% acetonitrile was added, vortexed for 15 h. The sample was then injected into HPLC.

Total phenolic content

The powdered samples were weighed to 0.5 g in the weighing vessel. It was dissolved with 5 mL of purified water. 0.1 mL of the prepared solution was taken and diluted with 5 mL of purified water and vortexed. 0.5 mL of Folin-ciocalteu reagent was added and mixed well for 3 minutes by vortexing. 1 mL of a solution of 35 g of Na_2CO_3 in 1 L of water prepared in a separate beaker was added to the mixture. Incubate for 1 hour at room temperature and in the dark. After incubation, spectrophotometer was measured at 725 nm.

Antioxidant activity

0.5 g of the powdered samples were weighed in the weighing vessel. It was dissolved with 5 mL of purified water. Sulfuric acid was added and the final volume was completed to 200 mL. 0.4 mL of samples were taken. 4 mL of prepared reagent solution was added. Mix well with vortex. Incubated in a hot water bath at 95oC for 1 hour 30 minutes. As a result of

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the incubation process, the tubes were taken into a container filled with tap water and kept waiting for 5 minutes and cooled. Spectrophotometer was measured at 695 nm wavelength.

Antiradical Activity

The powdered samples were weighed to 0.5 g in the weighing vessel. It was dissolved with 5 mL of purified water. It was vortexed and mixed well and 0.3 mL of DPPH (0.1 mM) was added. 2.4 mL of 99% ethanol was added and vortexed. The mixture was stirred for 30 min. It was kept at room temperature and in the dark. The spectrophotometer was measured at 517 nm.

Statistical analysis

All chemical assays were carried out in triplicate and the data were expressed as means ± standard deviations (SD).

Results and Discussion

The nutrient content of drone larvae is given in Table 1. As shown in Table 1, energy value was 472 kcal/100g; moisture; ash; protein carbohydrate and lipid contents were 21.13 g / 100g. 4.43 g/100g, 4.07 g/100g, 48.75 g/100g (NX6.25), 21.62 g/100g, respectively.

Table 1. Nutrient content of drone laerva (Apilarnil) (n=6)

Nurition element	Mean±SD
Energy	472± 2.3kcal/100g
Moisture	4.43±0.5 g/100g
Ash	4.07±0.8 g/100 g
Protein	48.75±4.2 g/100 g (NX6.25)
Carbohydrate	21.62±1.2 g/100g
Lipit (acid hyrdolysis)	21.13±1.3 g/100g

The amino acid profile of the drone larva is given in Table 2. The amino acids alanine, aspartic acid, methionine, glutamic acid, phenylalanine, lysine, histidine, tyrosine, glycine, valine, leucine, isoleucine, threonine, serine, proline and arginine were determined in apilarnil as shown in the table. Leucine, isoleucine, valine, lysine,

methionine, phenylalanine and threonine amino acids were identified as essential amino acids and only tryptophan could not be detected from essential amino acids. The highest amount of amino acid lysine was 7198 mg/100g, while the lowest amount of amino acid methionine was 500 mg/100g. The amount of other amino acids of alanine, aspatic acid, glutamic acid, phenylalanine histidine, tyrosine, glycine, valine, leucine, isoleucine, threonine, serine, proline and arginine were found 1826 mg/100g, 3571 mg/100g, 5625 mg/100g, 1844 mg/100g, 990 mg/100g, 2021 mg/100g, 1663 mg/100g, 2269 mg/100g, 3258 mg/100g, 2016 mg/100g, 1303 mg/100g, 1610 mg/100g, 3918 mg/100g, and 3005 mg/100g, respectively.

Table 2. Amino acid content of apilarnil (UFLC-UV)

Amino acid	mg/100g	Amino acid	mg/100g
L-Alanine (Ala)	1826±2.8	Glycine (Gly)	1663±2.3
L-Aspartic acid (Asp)	3571±4.3	L-Valine (Val)	2269±2.4
L-Methionine (Met)	500±4.5	L-Leucine (Leu)	3258±1.6
L-Glutamic acid (Glu)	5625±2.3	L-Isoleucine (Ileu)	2016±2.4
L-Phenylalanine (Phe)	1844±3.4	L-Threonine (Thr)	1303±2.8
L-Lysine (Lys)	7198±2.8	L-Serine (Ser)	1610±1.4
L-Histidine (His)	990±1.5	L-Proline (Pro)	3918±2.6
L-Tyrosine (Tyr)	2021±1.6	L-Arginine (Arg)	3005±4.7

Total phenolic content and antioxidant and antiradical activity of apilarnil were 834 mg GAE/100 g, 90.91 mg AAE/g and 81.61 %, respectively.

Table 3. The bioactivity of apilarnil

Bioactivity parameters	Mean ±SD
Total phenolic content (mg GAE/100 g)	834.05± 4,07
Antioxidant activity (mg AAE/g)	90.91± 8,16
Antiradical activity(% İnhibition)	81.61± 0,14

Honey is the most well-known honey bee products. However, besides honey, there are other honey bee products that are nutritious and supportive to treatment. Pollen, royal jelly and propolis have become one of the most researched bee products in recent years. However, products such as bee bread (perga) and drone larvae (apilarnil) are unknown products with little study. Apitherapy; is a form of use of bees and bee products as a protective and complementary application method in the treatment of some diseases. However, chemical and biological properties must be known for successful application of these products.

Hu and Li have determined the weight, moisture, protein, fat, ash and amino acid content of worker bee larvae and pupae at the age of 7-20 days [20]. In their research, they analyzed the vitamin and mineral contents of larva and pupa. They found that the moisture content of pupae and larvae was 81.69-73.48%, protein content of them was 42.36-48.47%, fat level was 15.75-20.63% and ash content was 9.99-18.47%. They were detected 17 amino acids in dry matter, especially glutamate, aspartate, lysine and leucine content was found to be higher and ranged between 26.35-44.72%. In addition, vitamin C and vitamin D, such as high content of vitamins have been identified [20]. Compared to this study, the fat and protein content of apilarnil was higher and the ash content was lower in our study. In another study, Barnutiu et al. studied fresh apilarnil obtained from Transylvanian.The water content of apilarnil changed between 69.70-76.44% and an average of 72.06%. They reported that the ash content was less than 1%, and only 3 samples were above 1%. They found the total lipid content of the samples to be between 1.29-4.51% and 3.8% on average. They reported that the total protein content varied between 4.55-9.95% [8]. Since we used lyophilized drone larvae in our study, nutrient contents were found to be higher than this study results.

Margaoan et al. compared queen and drone larvae in terms of quality parameters.. They identified carbohydrates by HPLC-IR and stated that seven carbohydrate compounds were identified n queen and drone larvae, predominantly glucose, fructose and sucrose. They determined lipid profile by Soxhlet method, total protein content by Kjeldahl method and free amino acids by LC-MS analysis. They identified a total of 31 amino acids in apilarnil and reported that nine of them were essential to humans. They found the moisture content of the bee larva to be 73.25 ± 0.02 and the protein content to 9.47 ± 0.13%. In addition, the total amount of essential amino acid in apilarnil 655.86 reported that the total amount of amino acids is 1830.07 [21]. In another study, the amino acid content of larvae of worker, queen and drone collected from different regions were determined [22]. Samples were obtained from four different regions of Russia and analyzed in five independent studies. As a result

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of this study, amino acid contents of bee larvae were found as 37.57 - 40.57% in drone larvae; 35.06 - 38.42% in queen bee larva and 35.61 - 35.71% in worker bee larva. Essential amino acid contents in drone larvae were 15.45 - 16.28%, 18.92 - 19.01% for queen larvae, 15.95-16.96% for worker larvae, respectively, and the total amino acid contents were between 39.73-42.77%, 51.25-54.79% and 44.74-47.70%, respectively [22].

In a comparative study on bioactivity of bee products; the antioxidant activities of multifloral honey, bee pollen, royal jelly and propolis were 58.89, 42.37, 59.02 and 267.37 mgAAE/g, respectively, while their antiradical activities (% inhibition) were reported as 28.44, 89.66.5.72 and 96.14, respectively [23]. When we compare the results of this research with our research, antioxidant and antiradical activities of apilarnil were found to be higher than other bee products lower than propolis.

Conclusion

In this study, it was determined that apilarnil produced in our country is an important food source.In addition, it was found that apilarnil shows antioxidant and antiradical activity. The antioxidant activity detected in apilarnil can be held responsible for the biological properties determined in scientific studies. However, the determination of the chemical composition that is the source of these effects is important to determine the possible beneficial biological effects. Therefore, there is a need for further research on the determination of the detailed chemical composition and biological activity of drone larvae.

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Erkek Arı Larvalarının (Apilarnil) Kimyasal İçeriği ve Biyoaktif özellikleri

Öz: Bu çalışmada, erkek arı larvasının (apilarnil) besin elementleri, aminoasit içeriği ve biyoaktif özellikleri belirlenmiştir. HPLC-UV ile yapılan analiz sonuçlarına göre apilarnil'de 16 amino asit belirlenmiştir. Esansiyel amino asitlerden yalnızca triptofan tespit edilememiştir. En yüksek amino asit miktarı 7198 mg/100g olan lizin amino asidi iken en düşük metionin amino asidi olup 500 mg/100g olarak belirlenmiştir. Elde edilen sonuçlara göre apilarnilin nem, kül, protein, karbonhidrat ve lipit içeriği sırasıyla 4.43, 4.07, 48.75, 21.62 ve 21.13 g/100 g dır. Apilarnilin biyoaktif özelliklerini belirlemede fenolik madde içeriği için Folin-Ciocalteu metodu, antioksidan

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aktivite için fosfomolibden metodu ve antiradikal aktivite için DPPH metodu kullanılmıştır. Apilarnilin toplam fenolik madde içeriği 834,05 (mg GAE/100 g) iken antioksidan aktivitesi 90,91 mg AAE/g bulunmuştur. Erkek arı larvasının (apilarnil) antiradikal aktivite inhibisyon düzeyi % 81.61 olarak tespit edilmiştir. Sonuç olarak, apilarnilin faydalı biyolojik aktivitesi antioksidan aktivitesinden kaynaklanıyor olabilir.

Anahtar Kelimeler: Apilarnil, arı larvası, fenolik, antioksidan, antiradikal, aminoasit

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MELLIFERA REVIEW ARTICLE

The Effects of Environmental Problems on Honey Bees in view of Sustainable Life

Ekin VAROL*, Banu YÜCEL

Ege University, Faculty of Agriculture, Department of Animal Science, Bornova-İzmir,TURKEY

** Corresponding author e-mail:ekin.varol@gmail.com*

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A B S T R A C T

The balance and the continuity of nature is decreasing day by day due to environmental problems experienced in parallel with social, economic and industrial development. Today, this increase has reached a point that threatens the magnificent life circle that has emerged as a result of thousands of years of accumulation of nature. The balance of nature is deteriorating as a result of excessive and unconscious consumption of natural resources and increasing world population. The importance of honey bees in the ecosystem is indisputable. Honey bees help pollinate plants, increasing yields and making great contributions to the agricultural ecosystem. However, environmental problems such as pesticides, chemical that used in agriculture, chemicals that use to fight against honey bee pests, predators and diseases, environmental pollution, competitive relations between honey bees, radiation, climate change, global warming, geomagnetic disturbance and Colony Collapse Disorder threatens the life of honey bees. Therefore, it is necessary to better understand environmental problems and their effects on honeybees and to specify precautions on the subject quickly.

Keywords: Honey bee, environmental problems, Pesticides, CCD, global warming

Introduction

Humankind use to take advantage of nature since its existence. However, with the opportunities of developing technology, human started to use nature limitless and consume the nature damagingly. At the beginning, these environmental damages neglected because of nature's self-perpetuating property. Besides, it is considered that nature will remove the pollution by its natural cycle. With the qualitative and quantitative increase of environmental damage, it went beyond the nature's self-perpetuating property and environmental degradation started rapidly

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[1]. There is an ecological balance and a multifaceted interaction between the main elements that make up the environment; air, water and soil. Therefore, contamination of any of the air, water and soil elements affects others negatively. In other words, contamination is not limited only in the area where it is used.

Pollinators as bees have a big influence in ecological interaction. Including honey bees, the most important function of bees, is to provide pollination of various wild and cultivated plants. Many animal species that use plants as food and nest, benefits from honey bees indirectly [2-3].

In recent years negative impacts of environmental problems threatens the life of many animal species, especially honey bees. Pesticides that used in agriculture, chemicals that use to fight against honey bee pests and diseases, environmental pollution, competitive relations between honey bees, radiation, geomagnetic field storms, climate change and global warming, Colony Collapse Disorder (CCD or Maria Celeste Syndrome) that cause remains still unknown, can cause rapid and severe honey bee losses, are the most important environmental problems for honey bees.

Synthetic Chemicals (Pesticides, Herbicides, Insecticides, Fertilizers)

Continuous agricultural protection with hundreds of pests and diseases that causes product losses on cultivated plants, becomes an unavoidable necessity to provide food requirements of rapidly increase in World population. Chemical fight become important years ago with the usage of DDT at plant production and much more types and quantities of insecticides started to use at agricultural areas.

Honeybees that are critically important for pollination and negatively affected by pesticides.

Honey bees that directly contacted with insecticide from plant, could not be able to fly and get back to hive, or even bees are able to reach hive, they die in front of the hive. Many bee poisonings occur by the usage of insecticides on blooming period of the plants. Insecticide particles stick on to pollens those forager bees carries on their hind legs.

Bee poisoning of the insecticides occur in three ways as ingestion, contact and inhalation during collecting nectar and pollen. Piles of dead bees in front of the hive are the most known sign of bee poisoning. Another sign is the decreasing

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population of forager bees [4]. Many pesticides cause increase of aggression on bees. Some symptoms like lethargy, paralysis, abnormal behavior, vomiting nectar, tongue sticking out can be seen on bees that exposed to chlorinated carbon and organophosphate insecticides. Stable bees in front of hive can be observed. Dead young bees can be seen in front of the hive by the chemical poisoning with chemicals like arsenic, methyl parathion [5].

Larvae deaths occur because of the insufficient number of nurse bees that cares nursed cells. In some cases, all bees in the hive can die. Queen bee could be negatively affected by the slow-release agrochemicals, nitrit & nitrate deposits from fertilizers and heavy metals (like arsenic, lead etc.) that bring to the hive by nectar and pollen. Also abnormal behaviors and decreased egg production could be seen on queen bee. Many weak colonies could not survive winter and die.

Pests

As in all animal species, many pests, predators and diseases threaten bee life. Environmental pollution, global warming that caused by waste products and gases, intensive migratory beekeeping and improper practices on beekeeping applications causes the rapid spread of honey bee pests and diseases. Today, *Varroa destructor* is the most harmful pest of honeybees. Varroa is a serious external parasite that causes the decreasing number of adult bees, growth deficiency of young bees, irregular brood pattern, disease-causing pathogen growth, adult bees to leave the hive, low productivity of honey and colony losses in an advanced stage [6].

Loss of Natural Habitat

Loss of natural habitats has major effects on bee colonies. Main reasons of nature destruction are monoculture planting, loss of biodiversity, excessive pasture feeding, irrigation and land clearing. Extinction of the natural flora could cause losses of bee colonies [7]. Bees needs extensive, continuous, interconnected and convenient flora. Small scaled habitats decrease the spreading ability of bees, number of nest areas and available food sources.

Environmental Pollution

Honey bees collect nectar and pollens from plants to feeding. Therefore, they have a continuous interact with environment. While making these activities, they bring some of the chemical substance and waste from environment to hive together with the pollen they collected. In the nature, waste and toxic substances (generally industrial

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gases, gas from the vehicles, pesticides and insecticides) absorbed and stored by the plants. As mentioned before, toxic substances in environment poisons honey bees.

The large part of air pollution causes by anthropological factors (urbanization, industrialization, energy generation, mobile sources and other pollutants). One of the most important results of the air pollution is heavy metal pollution [8].

Heavy metal pollution in the atmosphere caused by chimney and exhaust gases has brought with it various negative effects for people, animals and plants. Not only the vegetative organs of plants but also the generative organs of plants are affected by these negative conditions. One of them is the male reproductive cell; pollen. Various heavy metal cations such as cadmium, cobalt, copper, zinc, lead, nickel and mercury are known to adversely affect direct pollen and indirectly the honey bees that feed on them.

Since bees collect pollen from different kinds of flowers, heavy metals, which are found in large quantities within the plant, cause increased concentration of toxic heavy metals in the body of bees and the honey produced from the nectars of these plants.

Chemical wastes that produced by industrial plants and urban areas; causes the acidification of the soil, the pollution of existing and potential underground and aboveground water resources, the reduction of biological activities and the negative impact of the soil structure.

These substances cause plants to not make adequate use of nutrients in the soil and reduce plant growth, reducing productivity, and toxic levels of certain micronutrients in grown crops. All these negative conditions make it difficult for honeybees to find clean sources of pollen and nectar.

Competitive Relations

Another important problem for honey bees is due to foreign honey bee breeds, ecotypes and hybrids brought from foreign countries in order to increase honey production. Imported bee genotypes restrict the habitats of indigenous races and lead to the gradual extinction of our indigenous gene resources.

Imported queen bee genotypes can compete with native bee races and ecotypes in terms of the use of plant resources and place of home, prevent the pollination of plants in the natural flora, transport parasites and pathogens, and mate with native ecotypes to they can cause genetic divergency [9].

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Global warming

Global climate changes could affect the behavior of honey bees. There is not much information about how bees will react physiologically or adapt to changes in the environment as a result of global warming. Global warming often affects bees indirectly by changes environmental conditions.

The most important effect of global warming on nature is the changes in climates. Climate changes will also change the characteristics of the environment in which includes all living creatures and these changes will indirectly affect the behavior of bee societies that lives in that ecological environment.

As a result of global warming, changes will occur in the vegetation of the ecological system. Due to the interactive relationship with honey bees and ecology, global warming will create pressure on bee colonies [10].

Climate changes due to global warming have several effects on plants. In addition to the expansion of arid and semi-arid areas, increases in the duration and severity of the summer drought will accelerate the desertification process and many plant species will dry up and disappear. Thus, many plant species will change their flowering periods. Honey bees will effect negatively. In addition, changes in air temperature and humidity may also affect biological and behavioral properties of honey bees. Competition between wild bees and honey bees will increase as seasonal changes and the spread of wild bee breeds will be affected. The spread of wild bees to new areas and excessive population surge will cause competition with natural pollinators in the use of herbal resources [11].

Since honey bees are very affected by seasonal conditions and sudden temperature changes, an increase in bee losses can be expected. Due to global warming, winters are "hot" and may not have enough "cooling" that plants need in winter. This can lead to plants not producing enough pollen and nectar in the spring. In the absence of sufficient nectar and pollen in the environment, honey bees colonies are on their way to leave the hive, even if there is honey in their hives, in order to ensure the continuity of future generation. This shows how honey bees are sensitive "bio-indicators" [12]. In other words, during the nectar flow period, honey bees can determine the extent to which nectar and pollen sources are sufficient in

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the environment and can plan their lives [13].

Colony Collapse Disorder (CCD)

Although the cause of colony collapse disorder (CCD), which has been extremely busy with the media in recent months and has caused nervousness among beekeepers, is still unclear, many of the triggering possibilities are being considered. These possibilities include external parasites, adult and infant diseases, known/unknown pathogens, inadequate feeding of adult bees, GMO farming, lack of genetic diversity, stress elements in adult bees, drug residues in honey and wax, new chemicals used in agricultural combat, radiation emitted from base stations [14]. Scientists that investigate colony collapse disorder, found high levels of bacteria, viruses and fungi in the honey stomach of collected bees that cannot return to the hive. Studies have shown that severe infection breaks down the immune system in honey bees due to the high toxic effect. It is stated that the stress that occurs in bees due to frequent displacement of hives may cause bees to become sensitive to diseases and become susceptible to other diseases and parasites.

The most typical characteristic of CCD is that adult bees in healthy-looking hives disappear overnight without any signs of death. Adult bees that leave behind the queen bee and young bees in the hive, do not return to the hive and the number of adult bees in the hive is gradually decreasing [15]. However, those who know bee behavior will understand that this is an extremely unusual event. These hives are weakened because of malnutrition due to the decrease in the number of adult bees and disappear even if they have queen bees. Therefore, such a large scale of the loss of honey bees, makes it difficult to examine CCD. Besides, there is not any clue that the hive carries disease [16].

Although the exact cause has not been understood till today, scientists are expressed their opinions about the measures to be taken in the apiary under suspicion of CCD.

In the apiaries that seen CCD, it is not recommended to combine bees in lost hives with strong hives. If CCD is based on an infection-related factor, it may also cause loss of healthy colonies. It is also suspected that CCD could have a connection with insidious progressive bee diseases such as Nosema. Because the fungal spores seen in honey stomachs of bees performed with CCD suspicion increases the suspicion that Nosema may have caused this [17]. Especially in colonies with Nosema spores,

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the susceptibility to other disease-causing pathogens is also very high.

If Varroa is extremely high in the colony, the use of strong chemicals (oxalic acid, fluvalinate, coumaphos, amitraz, apistan) that have a negative effect on malpighi tubes of bees, should be avoided. After the use of these chemicals, problems are increasing such as rapid discharge of hives and fertilization in the queen bee.

Neonicotinoids applied to the stated plants are the systemic insecticides that used in the control of absorbent insects that harm plants. This group of insecticides has been widely used recently and has a negative effect on honey bees, even at very low doses. Young bees that fed with nectar and pollen brought to the hive are more affected, disrupt brood feeding, and abandoned larvae frames in CCD hives attract attention.

On the other hand, adult field bees, having memory loss by the effect of neonicotinoid group insecticides and lose their ability to navigate. Their immune systems are weakening and they are becoming more susceptible to diseases [18]. The effect can also occur months later. Imidacloprid, thiamethoxam and clothianidine from this neonicotinoid group are strictly prohibited except for intra-greenhouse use in the EU and Turkey [19]. Studies show that systemic insecticides such as imidacloprid cause behavioral disorders in bees, that bees lose their ability to navigate, lose their memory and have difficulty returning to hives. There are also studies that show that radiation waves emitted from increasing base stations adversely affect the ability of bees to navigate.

It is certain that, bee losses are seen all over the world for various reasons. Besides all these, CCD is less harmful in domestic ecotypes than imported bees. Also, these bees have a strong immune system because they have higher adaptability to ecology in the region.

The importance of honey bees in our life is an undeniable fact. Increase of negative effects on environmental conditions destroy gradually of honey bees life also future of the world. Ensuring the sustainability of honey bees life and agriculture systems we have to get precautions for minimizing environmental problems.

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Conclusion

The role and place of bees in the ecosystem are quite large and vital. Unfortunately, due to the degradation of nature and the depletion of natural resources, honey bees are under serious threat. That is why serious precautions should be taken about the factors that threaten the life of bees leading to the loss of nature. Pesticides and insecticides used in agricultural production directly cause bee poisoning and bee deaths. Instead of synthetic chemicals, natural and bee-friendly ways to fight should be investigated and used. Attention should be taken for against pests that threaten the life of honey bees. It is very important that honey bees are accessed to clean water, nectar and pollen sources, so attention should be taken against any pollution that harms nature and bee. Competitive relations between bees should be observed and problems that may arise due to the reunion of indigenous races with imported races should be taken into account. Global warming will affect honeybees as it affects the entire ecosystem and natural order. Therefore, due to climate changes related to global warming, problems that affect bees should be studied.

CCD is a problem that threatens all bees and the cause is still unknown. The typical characteristic of CCD is that adult bees in healthy-looking hives disappear overnight without any signs of death. Adult bees that leave behind the queen bee and young bees in the hive, do not return to the hive and the number of adult bees in the hive is gradually decreasing. Many researchers report that CCD, which causes such major problems, is not a single cause, but a disease that arises as a result of a number of causes, including the use of neonicotinoids. Research and studies on CCD, which has such a negative effect on honey bees, should be increased and developed.

It is very important to make effort to solve these environmental problems which directly threaten the life of honey bees who are of such importance in the ecosystem and solutions should be brought to these problems.

Sürdürülebilir Yaşam Açısından Çevresel Sorunların Balarıları Üzerine Etkileri

Öz: Sosyal ve ekonomik kalkınmaya paralel olarak yaşanan çevre sorunları nedeniyle doğanın dengesi ve sürekliliği her geçen gün bozulmaktadır. Bugün, bu artış doğanın binlerce yıllık birikiminin bir sonucu olarak ortaya çıkan muhteşem yaşam döngüsünü tehdit etmektedir. Doğal

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kaynakların aşırı ve bilinçsiz tüketimi ve artan dünya nüfusunun bir sonucu olarak doğanın dengesi bozulmaktadır. Bal arılarının ekosistemdeki önemi tartışılmazdır. Bal arıları bitkilerin tozlaşmasına yardımcı olur, verimi arttırır ve tarımsal ekosisteme önemli katkılarda bulunurlar. Ancak, pestisitler, tarımda kullanılan kimyasallar, bal arısı zararlıları ve hastalıklarla mücadelede kullanılan kimyasallar, çevre kirliliği, bal arıları arasındaki rekabet ilişkileri, radyasyon, iklim değişikliği, küresel ısınma ve koloni çökme sendromu (CCD) bal arılarının yaşamını tehdit etmektedir. Bu nedenle, çevre sorunlarını ve bunların bal arıları üzerindeki etkilerini daha iyi anlamak ve konuyla ilgili önlemleri hızlı bir şekilde belirlemek gerekmektedir.
Anahtar Kelimeler: Bal arısı, çevre sorunları, Pestisitler, CCD, küresel ısınma

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MELLIFERA RESEARCH ARTICLE

Dynamics of the population *Varroa destructor* at the Level of Local Bee Colonies *Apis mellifera intermissa* in the North Central of Algeria

Assia HABBI-CHERIFI *[1], Noureddine ADJLANE [2], Ferroudja MEDJDOUB-BENSAAD[3], Nizar HADDAD [4]

[1]Faculty of Biological Sciences and Agronomic Sciences, Mouloud Mammeri University, Tizi-Ouzou, ALGERIA.

[2] Department of Agronomy ,M'Hamed Bougara University, Boumerdès, ALGERIA.

[3]Animal Biology and Physiology Laboratory, ENS Kouba, Algiers, ALGERIA

[4]National Agricultural Research Center - NARC, Amman, JORDAN

** Corresponding author e-mail:assia_cherifi@yahoo.fr*

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A B S T R A C T

The population dynamics of the *Varroa destructor* mite in local bee colonies *Apis mellifera intermissa* was studied for about two years (March 2016 to December 2017) in the Tizi-Ouzou region in northern Algeria with a Mediterranean climate. Observations were made monthly on colonies treated against *varroa* mites and have not undergone any acaricide treatment. The development of *varroa* populations was monitored taking into account natural mortality, the rate of adult bees and brood infestation rate. Our results show that the *Varroa* population is very important during the first year of study at untreated colonies divided into two apiaries to register a slight decrease during the second year. In contrast, treated colonies at site 1 have a high level of infestation despite being treated with Bayvarol® in October 2015. Our results suggest that the level of *Varroa* infestation in the colonies varies according to the climatic (seasonal) and internal conditions of each colony.

Keywords: *Apis mellifera intermissa*, *Varroa destructor*, dynamics

Introduction

Varroasis is considered one of the most common and dangerous diseases. It is caused by the parasitic mite *Varroa destructor* which parasitizes both brood and adult bees, thus causing considerable losses for beekeeping in the world and particularly in Algeria [3]. Indeed, trade (sale of queens and swarms) and

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transhumance have resulted in the distribution of the parasite spread from Asia to Europe and America [1]. In Algeria, this parasitosis appeared in 1982 and since several acaricidal molecules have been used but Varroais observed in the colonies all the time.

In order to develop a better strategy for controlling this parasite, study the host-parasite relationship study must have priority. In Algeria, little work is done on the dynamics of the *Varroadestructor* [2,3] For this purpose, we have been interested in this study in the development of *Varroa* populations in colonies already treated against this parasite and in colonies that have not been treated following the seasonal evolution of this parasite in the brood, on the ground adult bee as well as natural mortality.

Materials and Methods

Presentation of the study environment

Our study was conducted at 28 colonies spread over two sites, each with two apiaries contain 7 hives in each. of 7 hives each:

- Site 01 is located in the region of Sid Ali Bounab at an altitude of 400m contains Apiary A whose hives are not treated against the Varroa parasite for more than three years and Apiary A 'whose hives are treated in the month of October 2015.

- Site 02 is in the village Azib Ahmed at an altitude of 200m; it contains the Apiary B whose hives have not been treated for more than three years and the Apiary B' whose beehives are treated in October 2015. The study took place during the period from March 2016 to November 2017.

The study stations are characterized by

- a mild spring with average temperatures ranging between 12 ° C and 19 ° C in 2016 and 14 ° C and 21 ° C in 2017;

- a hot summer with average temperatures ranging from 24 ° C to 27 ° C in 2016 and from 26 ° C to 29 ° C in 2017;

- Quite significant rainfall in the month of March of the year 2016 with 185mm and very low in the month of March 2017 with 29mm.

Three parameters were studied

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-Brood Infestation Rate (TIC): Every 3 weeks, a sample of 25cm2 emerging is opened to determine brood infestation rate. [4]

-Infestation rate of adult bees (TIA): about 100 bees per colony were sampled and poured into a 70º alcohol solution. After the bees are dead, the mixture (alcohol + bees) is agitated well so that the varroa can be detached from its host [5]**;** thereafter we count the varroa found there.

- The natural mortality of Varroa *:* Each hive is equipped with a lange, coated with greasy material, placed on the floor (the plateau) of hives. Each lange is protected by a metal grid preventing bees from accessing it. The count of dead Varroa is done every three weeks throughout the studyperiod.

Results and Discussion

Dynamics of the bee population

The highest number of cells in the operculum brood was recorded in June of the first year in apiaries B and C with a peak of 23785.71 and 20765.14 alveoli respectively. While during the second year, we find that brood development marks a slight regression in all apiaries. However, the colonies of the 4 apiaries marked their peak of evolution in June with the highest average rate of cells recorded at the Rucher B with 18225.14 alveoli.

However, the highest average number of bees is 29542.89 bees recorded during the month of June 2016 at apiary C and there are 23500 bees at apiary B at the end of summer 2017 (Figure 1).

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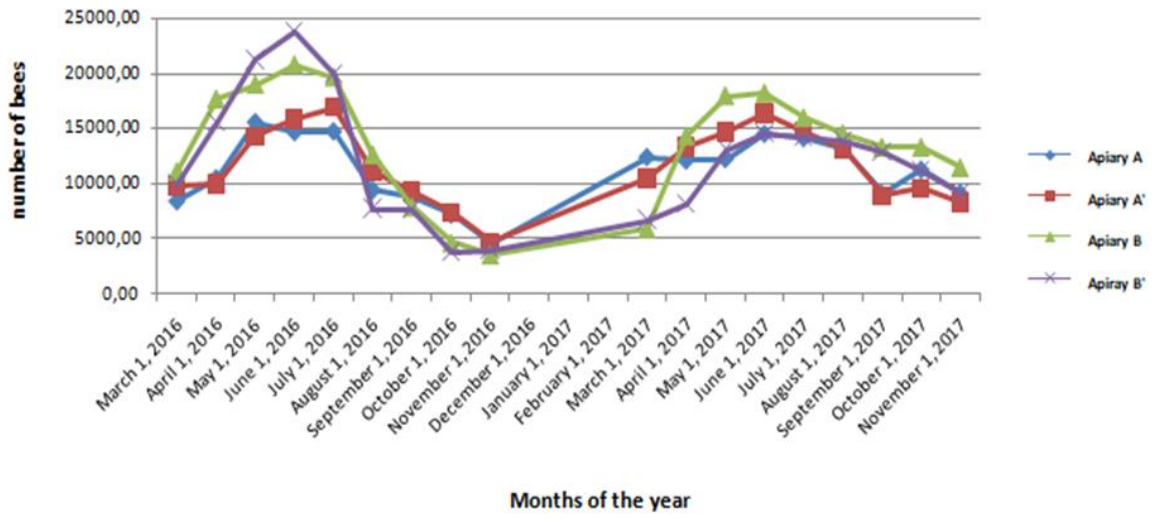

Figure 1. Evaluation of number of cells of the brood capped at the level of the 4 studied apiaries

Dynamics of the varroa population

Brood infestation with *Varroa* shows a very significant difference between the two years of study and between apiaries. However, the highest ICT is recorded in June of 2016 at the B 'Api with an average of 17.43%. However, during the 2017 season, the brood infestation rate is very high in apiary B, with an average of 23.90% registered in July. For apiary A, ICT is very important in June 2016, when an average value of 15.71% was found, while apiary A 'recorded a peak of 9.05% in July of the second year (Figure 2).

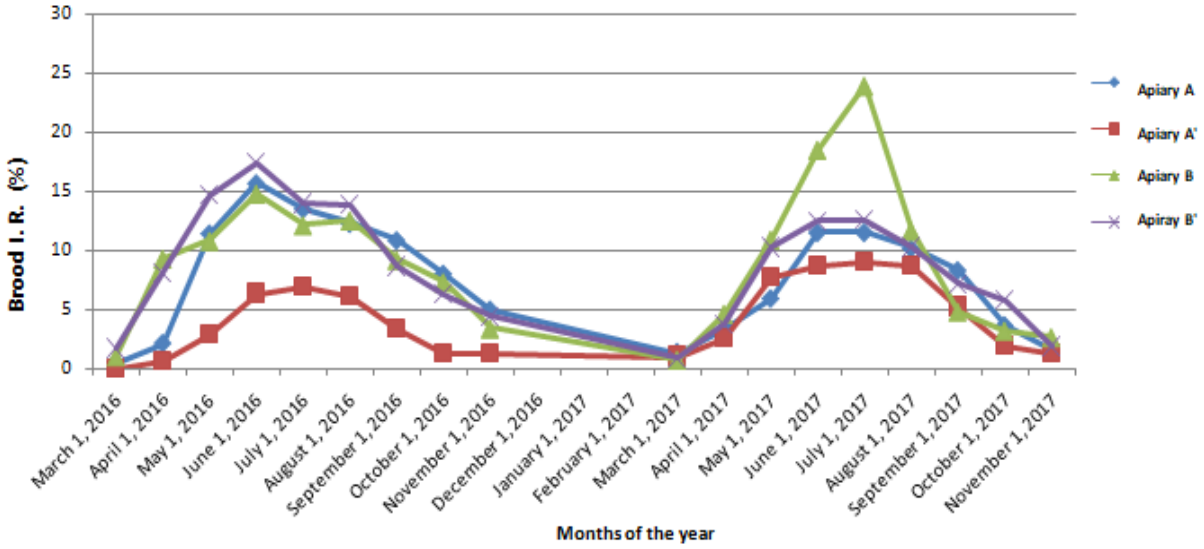

Figure 2. Evaluation of brood infestation rate (TIC) at the four study apiaries

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For the adult bee infestation with *varroa*, we notice that the TIA is very high during the year 2016 in all the apiaries. It also shows that bees from apiaries A and B are the most infested and record an average TIA of 11.81% and 10.95% respectively in November 2016. However, in the second year the TIA will decline in the Apiary B and B 'and A with average rates of 8.72%, 7.83% and 6.29%, recorded in September; while at Api A ', the TIA reached its maximum of 5.28% in November (Figure 3).

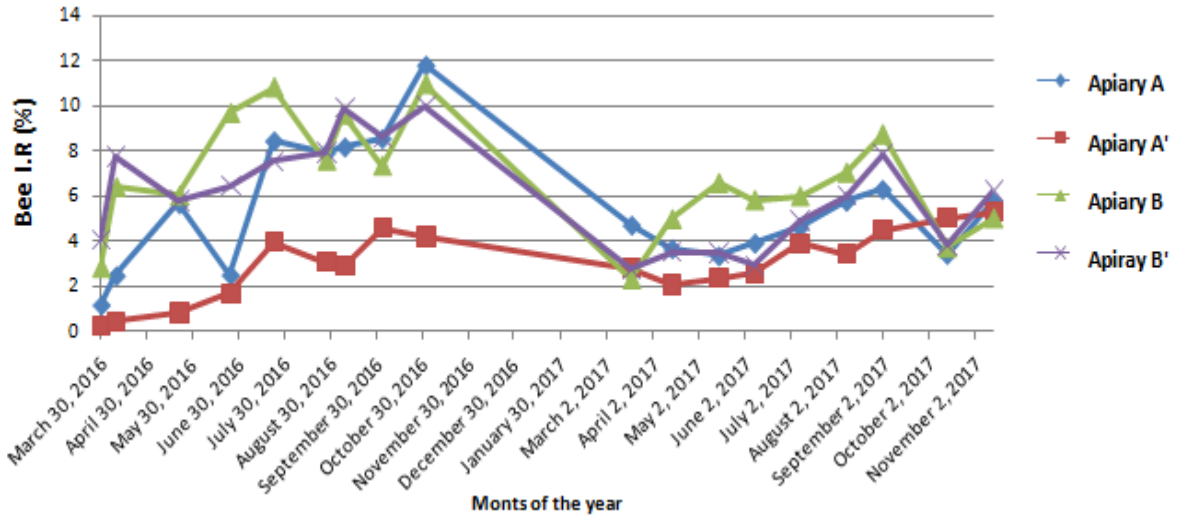

Figure 3. Evaluation of varroa phoretic in the four study apiaries

The comparison of data on changes in natural mortality rates of the *Varroa destructor* during the two years of study shows a gradual increase from March to the September. It also records a highly significant difference between the two years of study and between apiaries. In fact, untreated apiaries A and B record, respectively, an average mortality peak of 711.29 and 890,86 moth in September 2016. In the second year (2017), the mortality rate falls slightly in apiaries A, B and C; while at the level of the treated apiary (A '), the average mortality rates continue to increase to record the maximum of 606 *Varroa* in September (Figure 4).

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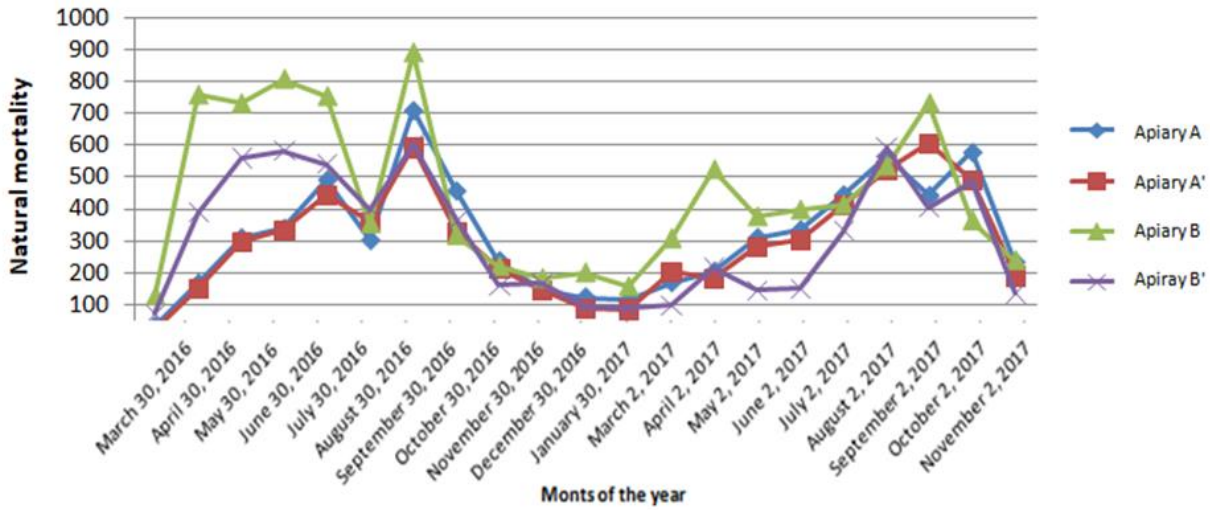

Figure 4. Evaluation of natural mortality in the four study apiaries

In this study, we studied the population dynamics of *varroa* mites in colonies treated against this parasite and colonies that have not been treated. We found that the evolution of the bee population and the *varroa* population differ from one period to another and from one apiary to another.

From the end of March, the queen begins to lay eggs and the colonies resume activity until they reach their peak in June and July in all apiaries. This period coincides with the presence of honey resources (pollen and nectar) food source bees. Then from the end of September, the pollen resources decreases, the queen slowed the laying and the population of bees goes well. The development cycle of the *Apis mellifera intermissa* race is determined by exceptional and seasonal climatic contrasts [6]. Thus, the evolution of the colonies depends on the flowering, the location of the hive and the behavior of the workers as well as the queen [7]. The activity of the colony is also influenced by the climatic conditions [8,9]. Indeed, we found that the colonies studied are more developed during the 2016 season than in 2017. The high temperatures recorded during spring 2017 seem to hinder the activity of the bees. Variable climatic conditions also cause year-to-year differences in colony development [10].

On the other hand, the presence of brood favors the development of the *varroa* population [11]. Indeed, during the first year, the brood is very important which

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favors the entry of the founder into the cells to reproduce. It is also noted that the TIC during the two seasons of our study is at its maxima in June and July. The number of *varroa* in the brood is related to the season and the availability of brood in the hive [12, 13]. Many authors also report that climatic effects on brood production have a significant influence on the growth of brood *varroa* populations [14, 15].

In the Mediterranean climate of California, the growth of parasite populations is not due to a high reproduction rate but to the possibility of *Varroa* breeding continuously in the brood which is present almost all year. [16**]**

However, colonies of untreated apiaries (RA and RB) show significant ICT that differs from one year to another and from one period to another. Indeed, it is higher in June of the first year for the A apiary while at the apiary B, it reaches its maximum in July of the second year. This can be explained by the fact that in a cell we can find more than one individual *Varroa*. A *varroa* female can lay up to seven eggs in the drone cells and six in the worker's alveoli [17]. Nevertheless, during the first year at unprocessed apiaries, the TIA increases each colony is infested at its level according to the factors of tolerance and resistance specific to it [18]. On the other hand, the difference in colony infestation from one year to the next may be due to the fact that workers show much more delousing behavior than other colonies.

The decrease in the *varroa* population during the second year may also be related to high temperatures. reports that experiments have been conducted on the use of heat against *varroa* mites to find the temperature and duration of treatment that can reduce the number of mites without killing bees. He also notes that in some countries, such as Algeria and Morocco, bees manage to cohabit with *varroa* mites. The dynamics of development of the mite is slowed by the temperature of the brood remaining higher than 36 ° C [19].

In Mediterranean climate, a high mortality of *varroa* was recorded; while in the case of semi-arid and arid climates with hot summers, it has been found that high temperatures have a detrimental effect on mites. Climate and phenology are two factors that can control the population dynamics of *Varroa* [2, 20, 21]. However, we note that despite the high infestation rates of the colonies studied, they remain active and rigorous.

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In addition, we have recorded that the fall of mites has continued during the winter, but with low rates, perhaps because the remaining phoretic mites are firmly positioned between the bee segments [22]. We notes that in winter, compared to summer, a smaller number of adult female offspring are recorded, which is due to the high mortality level of male offspring [23]. Nevertheless, in winter, a number of mites continue to leave the colony as they fall with dead bees outside the hive [22].

On the other hand, in the apiaries treated, a reinfestation of the colonies is very remarkable in the apiary B 'which has during the first year preceding the treatment a very high infestation rate compared to the apiary A', at the level of the apiaries treated. of which the development of *varroa* is progressive until reaching the maxima towards the second year. Reinfestation of these two apiaries indicates a decrease in the effectiveness of the Bayvarol® chemical treatment with which they have already been treated.

Conclusion

It appears that the life cycle of *Varroa* is linked to that of its host. It is closely related to the seasonal and internal conditions of each colony. In fact, the population of the parasite increases during the spring period then decreases from September parallel to the decrease in the size of the colony and the amount of the brood of the bee *Apis mellifera intermissa*. In addition, untreated colonies appear to be more resistant to *varroa* mites, since they have not been treated for a long time and do not show signs of weakness or deformations of the wings, which leads us to suppose that our breed is endowed with a certain behavior. considerable hygienic. On the other hand, the weakness of the efficiency of chemical treatment bayvarolis to report, because the already treated colonies are always infested by the *varroa*. Therefore, it is necessary to think about developing an integrated control strategy against varroasis by looking for alternative methods of control that will be economical, do not contaminate the products of the hive and all this to safeguard the honey bee *Apis mellifera.*

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Cezayir'in Merkez Kuzeyindeki *Apis mellifera intermissa* Lokal Arı Kolonilerindeki *Varroa destructor* Populasyon Dinamiği

Öz: *Apis mellifera intermissa* yerel arı kolonilerinde *Varroa destructor* akarlarının populasyon dinamikleri, Akdeniz iklimi olan kuzey Cezayir'deki Tizi-Ouzou bölgesinde yaklaşık iki yıl (Mart 2016 - Aralık 2017) çalışıldı. Varroa akarlarına karşı tedavi edilen kolonilerde aylık olarak gözlemler yapıldı ve herhangi bir akarisit tedavisi uygulanmadı. Varroa populasyonlarının gelişimi, doğal mortalite, yetişkin arı oranı (TIA) ve yavru istilası oranı (TIC) dikkate alınarak izlendi. Elde ettiğimiz sonuçlar, Varroa populasyonunun, ikinci yıl boyunca hafif bir düşüş kaydetmek için iki arı olarak ayrılan işlenmemiş kolonilerde çalışmanın ilk yılında çok önemli olduğunu göstermektedir. Buna karşılık, Ekim 2015'te Bayvarol® ile tedavi edilmesine rağmen RB'de tedavi edilen koloniler yüksek bir istila seviyesine sahiptir. Oysa arı kovanındaki akar miktarı 2017 yılında azami seviyelere ulaşmak için kademeli olarak artmaktadır. koloniler iklimsel (mevsimsel) ve her kolonin iç koşullarına göre değişir.

Anahtar Kelimeler: *Apis mellifera intermissa, Varroa destructor*, dinamik

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MELLIFERA RESEARCH ARTICLE

Bioactive Properties of Blossom and Honeydew Honeys

Sibel SİLİCİ*, Nisa ÜLGEN

Erciyes Üniversitesi, Ziraat Fakültesi, Biyoteknoloji Bölümü, Kayseri, TURKEY

* *Corresponding author e-mail:sibelsilici@gmail.com*

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A B S T R A C T

In this study, it was aimed to determine the bioactive properties of honeydew and blossom honeys produced in Turkey. Botanical origins of honey samples (locust, sunflower, citrus, lavender, coriander, euphorbia, rhododendron, chestnut, carob, thyme, rape, linden, pumpkin, heather, nigella, milk thistle, pine and oak honeys) collected from different geographical regions have been dertemined by pollen analysis. Total phenolic content of honey samples were determined by Folin-Ciocalteu method. The total phenolic content belongs to rape honey with the lowest 70.60 mgGAE/100g and chestnut honey with the highest 212.06 mgGAE/100g. Antioxidant activity of honey samples was determined by phosphomolybdenum method and antiradical activity by DPPH method. The lowest antioxidant activity was found in lavender honey and the highest activity was in citrus honey. The highest antiradical activity was determined in chestnut honey with the lowest antiradical activity in thyme honey; 66.02% and 7.47%, respectively.

Keywords: blossom honey, honeydew honey, phenolic content, antioxidant activity

Introduction

Honey is a sweet and natural food where plant nectar or some insect secretions are collected and processed by honey bee (*Apis mellifera* L.) and stored in the honeycomb cell. According to the source of honey is divided into two classes as honeydew and blossom honeys. Blossom honey is produced by honeybees from the nectar of plants. Honeydew honey is formed by collecting and processing digestive residues of basra (*Marchelina hellenica*) insects fed from the sap of plants [1]. Honeys such as citrus, chestnut, heather and thyme are among the blossom honeys; pine and oak honeys are among the honeydew honeys.

The botanical origin of honey has been determined by pollen analysis, a method that is called melissopalynolgy. The method of pollen analysis, which was elaborated and proposed by the International Commission for Bee Botany

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(ICBB) in 1970, and updated in 1978 [2]. In addition, EU Council Directive (2002) related to honey, it is indicated that the product names may be supplemented by information referring to floral origin, if the product comes mainly or wholly from the indicated source and possesses microscopic, organoleptic and phsico-chemical characteristics of source. The determination of the botanical origin is based on the relative frequencies of nectariferous taxon's pollen types. The frequency classes of pollen grains were given as predominant (>45%), secondary pollen (15-45%), important minör pollen (3-15%) and minör pollen (1-3%). Honey can be defined as unifloral if the "characteristic" pollen exceeds 45%. In addition, it is considered honeydew honey if the ratio HE/PG" exceeds 3. Howeever many pollen types are underrepresented (*Citrus* spp., *Tilia* spp., *Robinia pseudoacacia*) or over-represented (*Eucalyptus* spp., *Castanae sativa*). For instance, to characterize citrus honey as unifloral, *Citrus* spp. pollen must be over 10% while, for chestnut honey, a content of 90% of *Castanea sativa* pollen is required to classidy honey as unifloral [3]. However, in polifloral/multfiloral honeys, no dominant pollen is contained. Therefore, it is generally named according to the geographic region from which multifloral honey is obtained [4-6]. The total amount and composition of phenolic compounds in honey varies depending on the plant species in which the bee collects nectar, the method of collecting the nectar, seasonal and environmental factors, geographical origin and storage conditions. Total phenolic content in honey varies between 5 and 1300 mg/kg. It is strongly believed that the source of phenolic substances in honey is propolis. The flavonoid content, which is an important group of phenolic substances, is approximately 0.5% in pollen, 10% in propolis and about 0.005-0.01% in honey [7].

It is reported that the total phenolic content of honey is related to antioxidant activity Therefore, abundant phenolic compounds are found in dark honeys and such honeys are reported to be more powerful antioxidants than ascorbic acid or vitamin E [8,9]. According to Nagai et al., vitamins B1, B2 and C are degraded in heat treated honeys, and antioxidant activity decreases rapidly as a result of the destruction of peroxidase and catalase enzymes [10].

It is known that dark honeys have higher phenolic content and have higher antioxidant activity than light ones. In a previous study, the phenolic acid and flavonoid contents of blossom and

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honeydew honeys produced in different geographical regions of Turkey were determined and chestnut honey has been reported to contain the highest phenolic content (0.05 mg/g GAE) [9]. In another study, Perez et al. reported that Spain's honeydew honeys have higher antioxidant activity than blossom honeys [11].

Turkey, in terms of honey production and diversity is the country with the best potential in the world. Unfortunately, studies on the biological activity of monofloral honey produced in Turkey is not enough. Therefore, in this study, it was aimed to determine the bioactive properties of 48 different honeydew and blossom honeys produced in Turkey.

Materials and Methods

Honey samples

Honey samples from different provinces of Turkey (Istanbul, Edirne, Antalya, Isparta, Zonguldak, Izmir, Bursa, Mersin, Artvin, Şanlıurfa) were obtained from beekerpers in 2016 (Table 1). In the study,18 different monofloral honeys (locust, sunflower, citrus, lavender, coriander, euphorbia, rhododendron, chestnut, carob, thyme, rape, linden, pumpkin, heather, nigella, milk thistle, pine and oak) were stored in a dark and cool conditions (+4 °C) until analyzed. A total of 48 honey samples were analyzed. All analyzes of the samples were carried out during the year the honeys were produced.

Pollen analysis of honey samples

Pollen analysis of honey samples has been recognized by international beekeeping authorities, Louveaux et al. [2]. Briefly, honey samples were kept in a 45 °C water bath for 10-15 minutes and homogeneity was achieved by mixing. Then 5 g of honey and 10 g of distilled water were mixed in falcon tubes, the mixture was vortexed and centrifuged at 6500 rpm for 20 minutes. Then, water in centrifuged tubes was removed and tubes were left upside down for full drainage. The sediment material was taken from the bottom of the tube and plated on a lam with glycerin gelatin mixture. Glycerin-gelatine mixture and honey were taken with the edge of a sterile needle was transferred to a microscope slide and put on a hotplate set at 40°C. When the gelatine was melted, 18×18 mm cover slips were placed on the samples. Pollen slides were researched with Nickon E 200 microscope and immersion objective (x100) was used for identification of pollens. During microscopic studies all the area, which is 18x18 mm, was checked, 200 pollen was counted for each sample. [12].

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Determination of total phenolic content

Total phenolic content in honey was determined by Folin-Ciocalteu method and read spectrophotometrically [13]. Briefly,1 g of honey sample was made up with 4 mL (1: 4) methanol and vortexed. The prepared solution was filtered through Whatman No. 1 paper. The stock concentrations of the samples were prepared to be 200,000 ppm. Sample incubated at room temperature and dark for 2 hours. The absorbance of the resulting mixtures was read on the spectrometer against the blank at 765 nm wavelength. The spectrophotometer values of the samples were converted according to the formula prepared using the regression coefficient of gallic acid. The total phenolic content of the samples was expressed as mg gallic acid equivalent (GAE/100 g honey) [14].

Determination of antioxidant activity

Antioxidant activities of honey samples were determined according to the phosphomolybdenum method [15]. One g of honey sample was vortexed by adding 9 mL of methanol. The prepared solutions were allowed to stand in a 95 ^{o}C water bath for 90 minutes then cooled in tap water. The absorbance of the samples was read on the spectrophotometer at 695 nm wavelength. Antioxidant activity values of honey samples were expressed as mg ascorbic acid equivalent (AAE/g honey) [14].

Determination of antiradical activity

Free radical scavenging activities of the samples were determined by DPPH (2,2 diphenyl-1-picrylhydrazyl) method by making some modifications in the analysis protocols [16]. One g of honey sample and 4 mL of methanol were vortexed with stirring. 100 µL of this solution was added and 3900 µL of DPPH (1000 µl of 6 × 10^{-5} M DPPH) prepared in methanol was added and the mixture was allowed to stand at room temperature and in the dark for 2 hours. Their absorbance was read on the spectrophotometer at a wavelength of 517 nm.

Statistical Analysis

All chemical assays were carried out in triplicate and the data were expressed as means ± standard deviations (SD).

Results and Discussion

As a result of pollen analysis, it was found that some of the honeys labeled according to beekeeper claims were not monofloral honey. For instance, thyme and carob honeys. Locust, sunflower,linden, lavender, citrus and rhododendron honeys do not contain more than> 45% pollen but they are defined as “unifloral” honey because they show under-represented pollen properties. Other blossom honeys tested were

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identified as “multfiloral”. Pine and oak honey has a honeydew honey feature because the HE/PG value is 3 (Table 1).

Table 1. Botanical and geographical origin, pollen frequency of honey samples

Honey	**n**	**Botanical origin**	**Geographical origin**	**Pollen frequency (%)**
Locust	3	*Robinia pseudoacacia* L.	Muğla	39.70
Sunflower	3	*Helianthus annus* L.	Edirne	38.85
Pine	4	*Pinus spp.*	Muğla	HDE/P>3*
Nigella	2	*Nigella sativa* L..	Antalya	37.38
Heather	2	*Vitex agnus-castus*	Isparta	40.6
Linden	3	*Tilia platyphyllos Scop.*	Zonguldak	41.43
Pumpkin	3	*Cucurbita pepo* L.	Antalya	40.25
Rape	2	*Brassica napus* L.	Diyarbakır	41.77
Carob	3	*Ceratonia siliqua* L.	Antalya	39.15
Thyme	3	*Thymus vulgaris*	Isparta	36.87
Chestnut	4	*Castanea sativa Miller*	Bursa	92.10
Coriander	2	*Coriandrum sativum* L.	Antalya	40.97
Lavender	3	*Lavandula stoechas* L.	Isparta	41.85
Oak	2	*Quercus robur* L.	Zonguldak	HDE/P>3*
Citrus	2	*Citrus spp.*	Mersin	38.92
Rhododendron	3	*Rhododendron* L.	Artvin	35.21
Euphorbia	2	*Euphorbia macroclada Boiss.*	Şanlıurfa	40.72
Milk thistle	2	*Slybum marianum* L.	Diyarbakır	41.45

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Table 2. Total phenolic content, antioxidant and antiradical activity of honeys (Mean±SD)

Honey	Total Phenolic Content (mg GAE/100 g honey)	Antioksidant Activity (mg AAE/g honey)	Antiradical Activity (% inhibition)
Locust	103.45±3.37^{bc*}	83.78±1.71^{g*}	17.39±1.38^{bc}
Sunflower	110.17±4.70^{bc}	98.88±1.44^{h}	41.83±1.90^{de}
Pine	192.30±18.03^{f}	63.42±7.81^{cd}	40.05±22.06^{de}
Nigella	190.13±5.34^{f}	82.40±1.76^{fg}	11.00±0.64^{ab}
Heather	106.47±5.28^{bc}	129.57±11.63^{i}	10.33±0.31^{ab}
Linden	116.90±10.00^{c}	67.61±12.18^{de}	14.19±2.39^{abc}
Pumpkin	75.60±2.51^{a}	45.80±5.56^{ab}	17.47±1.76^{bc}
Rape	70.60±8.01^{a}	70.93±5.40^{def}	46.88±1.96^{e}
Carob	153.40±6.71^{de}	74.41±3.40^{defg}	20.94±1.21^{c}
Thyme	158.25±13.96^{de}	77.07±1.11^{efg}	7.47±1.29^{a}
Chestnut	212.06±12.41^{g}	79.57±2.20^{fg}	66.02±0.97^{f}
Coriander	118.92±6.58^{c}	135.26±1.77^{i}	44.09±0.12^{de}
Lavender	145.12±7.30^{d}	38.30±7.14^{a}	44.83±0.37^{f}
Oak	209.00±32.66^{g}	56.36±21.09^{bc}	41.63±1.29^{de}
Citrus	97.47±2.58^{b}	138.28±2.71^{i}	12.64±1.47^{abc}
Rhododendron	165.35±9.55^{e}	48.70±3.15^{ab}	7.61±1.06^{a}
Euphorbia	153.04±2.83^{de}	84.12±2.03^{g}	11.43±0.78^{ab}
Milk thistle	108.73±3.56^{bc}	103.42±1.99^{h}	43.69±0.28^{de}

Different letters in the same column represent statistically different groups (p <0.05).

As a result of the analysis, a statistically significant difference was found between the total phenolic contents of honey (p <0.05). The total phenolic content of the honeys tested ranged from 70.60-212.06 mg GAE/100 g honey. The total amount of phenolic substances belongs to rapeseed honey with the lowest 70.60 mg GAE/100

g honey and the highest amount of chestnut honey with 212.06 mg GAE/100 g honey. The highest total phenolic content after chestnut honey belongs to oak and pine honey.

The difference between antioxidant activity of honey was found to be statistically significant ($p < 0.05$). Among the honeys tested, the highest antioxidant activity was detected in citrus, coriander and heather honeys, 138.28,135.26 and 129.57 mgAAE/g honey, respectively. The lowest antioxidant activity was found in lavender honey.

The antiradical activity of the honeys analyzed varied between 7.47-66.02%. The highest antiradical activity was found in chestnut honey and the lowest activity was found in thyme honey.

According to the results of this study, labeling honeys according to beekeeper claims can be misleading. Melissopalinological determination of botanical origin of honey is based on the relative frequency of the pollen from the nectar-secreting plant. It is known that this method is time-consuming, requires knowledge and expertise, and involves a laborious counting procedure. In adidition, some difficulties in this method are associated with the need of good experince and knowledge of pollen morphology and the availability of collection of pollen [17]. However, some studies on the chemical composition of honey were performed without pollen analysis, in such cases, botanical origin of honey was based on the claims of local beekepers, when determination of honey origin is performed by considering the predominant flowers surrounding the hive. Even though pollen analysis has some disadvantages or limitations, it is the only way to detect contribution of nectar from other floral origin.

It has been reported in some studies that dark honeys are rich with phenolic compounds and antioxidant activities of honeys with high phenolic content are high. [18,19]. Lachman et al. determined the total phenolic content and antioxidant activity in 40 Czech honeys. The researchers found that the total phenolic content of honey varies between 83.60-242.52 mg GAE/kg [20]. In our study, the total phenolic content of honeys was between 70.59-212.06 mg GAE/100g honey. As a result of the analysis, the highest phenolic content was found in chestnut and honeydew honeys and the lowest in rape honey. In a study by Haroun, it was reported that the total phenolic content of chestnut honey varies between 33.37-77.40 mg GAE/100 g honey [9]. In our study, it was found that the

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phenolic content of the chestnut honey was higher than the values reported by the researcher. Akbulut et al. examined the antioxidant activity and phenolic content of 15 pine honey samples from different regions of Muğla. According to the total phenolic analysis, they found that the polyphenol content of honeys was in the range of 234.9-394.0 mg/100 g [21].

Many researchers reported that there was a significant relationship between antioxidant activity and total polyphenol content of honey. In a study by Silici and Özkök analyzed 66 honey bee products (honey, pollen, royal jelly, propolis) and their mixtures. They determined the total phenolic content of honey samples between 57.59-261.71 mg GAE/100g. Researchers reported that the total phenolic content of honeys examined in citrus honey with the lowest value of 57.59 mg GAE/100 g, and the highest 261.71 mg GAE/100 g for chestnut honey [22]. According to the findings of our study, total phenolic content of citrus honey is higher than (97.47 mg GAE/100 g) their value (57.59 mg GAE/100 g). Although total phenolic content of chestnut honey 212.06 mg GAE/100 g was lower than the value found by Silici and Özkök [22]. In another study, Al et al. was found that, the total amount of phenolic substances were in sunflower 45.00, in linden 38.00 and in multifloral honey 23.00 mg GAE/100 g. In another study, total phenolic content was found to be 110.17 in sunflower and 116.89 GAE/100 g in linden honeys [23]. Therefore, it can be said that the total phenolic content may vary depending on plant origin, climatic conditions and environmental factors from which honeys are taken.

It is known that, the main sources of honey phenolic compounds are plants. Plants biosynthesize a great number of phytochemicals and antioxidants being the major group of bioactive constituents, which might reduce the risk of oxidative damage in living cells [24]. It is shown that honey, depending on the floral source, possesses higher or lower antioxidant or antiradical activity [25,26]. The composition of phytochemicals has an influence on the biological activity of honey; usually, the same compounds have antioxidant activity. Many studies reported that the composition of honey depends on the floral source used to collect nectar; however, seasonal and environmental factors, as well as processing, may also have an effect on the composition of phenolic compounds in honey [26-28].

In honey, phenolic compounds are among the components responsible for antioxidant

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properties [29]. The highest antioxidant activity in the honeys analyzed was found to be citrus 138.28 mg AAE/g and the lowest lavender honey was 38.30 mg AAE/g. Among the honey samples tested, honeys with the highest antioxidant activity were citrus, coriander and heather honey, 138.28, 135.26 and 129.57 mg of AAE/g, respectively. Honeys with the lowest antioxidant activity were lavender 38.30 and pumpkin 45.80 mg AAE/g. Buratti et al. reported that the differences between honey antioxidant activities of different geographical origin of honeys may be due to such as environmental and climatic conditions, ie temperature, humidity, soil structure [30]. Antiradical activity of honey was found to be 7.47% in thyme honey and 66.02% inhibition in chestnut honey (Table 2). There was a statistically significant difference between honeys in terms of total antiradical activities ($p < 0.05$). Akbulut et al. collected pine honeys from Muğla and found antiradical activity of honeys were 35.32%. However, they reported a high correlation between antiradical activity and phenolic content ($r = 0.887$) [31]. They emphasized the importance of pine honey as a good antioxidant source. Our results are similar to the findings of the researchers (40.05% inhibition) in terms of antiradical activity.

Conclusion

It may be concluded that the presence of many factors, which might have an effect on the total phenolic content and antioxidant activity of honey as well as structural variety of such constituents that are biosynthesized by the floral sources of honey.Turkey has a very rich flora makes it possible to produce a large number of different monofloral honey. Pollen and bioactive properties of honey samples obtained in this study were determined and in this sense, contribution was made to the literature. In the following studies, the investigation of other honey types that cannot be included in this study will be complementary to the deficiency in this subject.

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Çiçek ve Salgı Ballarının Biyoaktif Özellikleri

Öz: Bu çalışmada, Türkiye'de üretilen çiçek ve salgı ballarının biyoaktif özelliklerinin belirlenmesi amaçlanmıştır. Farklı coğrafik bölgelerden toplanan bal örneklerinin (akasya, ayçiçek, narenciye, lavanta, kişniş**,** sütleğen, ormangülü, kestane, keçiboynuzu, kekik, kolza, ıhlamur, kabak, hayıt, çörekotu, devedikeni, çam, meşe) polen analizi yapılarak botanik orijinleri tespit edilmiştir. Balların toplam fenolik madde içeriği Folin-Ciocalteu metodu ile belirlenmiştir. Toplam fenolik madde miktarı en düşük 70.60 mg GAE/100 g bal ile kolza balına ve en yüksek 212.06 mg GAE/100 g bal ile kestane balına aittir. Bal örneklerinin antioksidan aktivitesi fosfomolibden metodu, antiradikal aktivitesi ise DPPH metodu ile belirlenmiştir. En düşük antioksidan aktivite lavanta balında en yüksek aktivite ise narenciye balında belirlenmiştir. Analiz edilen ballarda en yüksek antiradikal aktivite% 66.02 ile kestane balında, en düşük antiradikal aktivite %7.47 ile kekik balında belirlenmiştir.

Anahtar Kelimeler: çiçek balı, salgı balı, fenolik içerik, antioksidant aktivite

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