

ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

EFFECTS OF APILARNIL AND QUEEN BEE LARVAE ON LARVAL MORTALITY AND LONGEVITY IN *DROSOPHILA MELANOGASTER*

Apilarnil ve Kraliçe Arı Larvasının *Drosophila melanogaster*'in Larval Mortalitesi ve Uzun Ömürlülüğü Üzerine Etkileri

Aybüke Afra BABACAN^{1*}, Arif AYAR²

^{1*}Health Services Department, Sabuncuoğlu Şerefeddin Vocational School of Health Services, Amasya University, Amasya, TÜRKİYE, Corresponding Author / Yazışma yazarı E- mail: afra.babacan@amasya.edu.tr, ORCID No: 0000-0003-4333-4732.

²Medical Laboratory Techniques Department, Sabuncuoğlu Şerefeddin Vocational School of Health Services, Amasya University, Amasya, TÜRKİYE, E-mail: arif.ayar@amasya.edu.tr, ORCID: 0000-0003-0473-4653

Received / Geliş: 01.06.2024

Accepted / Kabul:03.10.2024

DOI: 10.31467/uluaricilik.1494204

ABSTRACT

The basic mechanisms of ageing and longevity are not yet fully understood. More studies are needed to correlate this situation with functional foods and food supplements that have been used frequently recently. Bee products are in the first place in the use of natural products and food supplements. It has been reported that drone larvae (apilarnil) and queen bee larvae, which have become popular in recent years, support the protection of health due to their high nutritional value, but no studies have been conducted on their life-extending efficacy. For this purpose, the efficacy of these two bee products on life span and mortality was investigated in our study. Apilarnil and queen bee larvae lyophilisates added to *Drosophila melanogaster* medium at different concentrations (0.5; 1.0; 2.5 and 5.0 mg/ml medium) were studied separately in male and female populations for treatment and control groups. As a result, apilarnil at 5 mg/ml concentration showed the best effect in terms of larval mortality compared to the control group, while the most effective group in terms of mean life span was determined as queen bee larvae with 83.1±3.53 days. In general, both bee products increased the life span of flies in parallel with the increase in concentration in both female and male populations. These results were statistically significant at p<0.05 level compared to the control group. In our study, it was concluded that apilarnil and queen bee larvae lyophilisates can be used in terms of life-length increasing activity, but the underlying mechanisms should be elucidated by detailed studies.

Keywords: *Drosophila melanogaster*, Apilarnil, Queen bee larvae, Larval mortality, Longevity

ÖZ

Yaşlanma ve uzun ömürlülüğün temel mekanizmaları henüz tam olarak anlaşılamamıştır. Bu durumun son zamanlarda sıkça kullanılan işlevsel gıdalar ve gıda takviyeleri ile ilişkilendirilebilmesi için daha çok çalışmaya ihtiyaç duyulmaktadır. Arı ürünleri ise, doğal ürünler ve gıda takviyeleri için kullanımda ilk sıralardadır. Son yıllarda popüler hale gelen erkek arı larvası (apilarnil) ve kraliçe (ana arı) arı larvasının da yüksek besin değeri nedeniyle sağlığın korunmasına destek olduğu bildirilmiş ancak ömür uzatıcı etkinliği üzerine çalışmalar yapılmamıştır. Bu amaçla çalışmamızda bu iki arı ürününün ömür uzunluğu ve mortalite üzerine etkinliği araştırılmıştır. *Drosophila melanogaster* besiyerine farklı konsantrasyonlarda (0.5; 1.0; 2.5 ve 5.0 mg/ml besiyeri) eklenen apilarnil ve kraliçe arı larvası liyofilizatları, tedavi ve kontrol grupları için erkek ve dişi popülasyonlarda ayrı ayrı çalışılmıştır. Sonuçta kontrol grubuna göre larval mortalite açısından en iyi etkiyi 5 mg/ml konsantrasyondaki

ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

apilarnil gösterirken, ortalama ömür uzunluğu bakımından ise en etkili grup 83.1 ± 3.53 gün ile kraliçe arı larvası olarak belirlenmiştir. Genel olarak her iki arı ürünü de sineklerin yaşam sürelerini hem dişi hem de erkek popülasyonlarında konsantrasyon artışına paralel olarak artırmıştır. Bu sonuçlar kontrol grubuna göre $p<0.05$ düzeyinde istatistiksel olarak anlamlı bulunmuştur. Çalışmamız ile apilarnil ve kraliçe arı larvası liyofilizatlarının ömür uzunluğunu artırıcı etkinliği açısından kullanılabileceği ancak altta yatan mekanizmaların ayrıntılı çalışmalarla aydınlatılması gerektiği sonucuna ulaşılmıştır.

Anahtar Kelimeler: *Drosophila melanogaster*, Apilarnil, Kraliçe arı larvası, Larval mortalite, Ömür uzunluğu

GENİŞLETİLMİŞ ÖZET

Amaç: Arılar, dünya yaşamında birçok alanda katkısı bulunan canlılardır. Polinasyondan sonra en önemli katkılarında biri, insan sağlığına sunduğu kendi ürettikleri ürünleridir. Erkek arı larvası (apilarnil) ve kraliçe (ana arı) arı larvası apiterapotik ürünler arasında son yıllarda kullanımı popüler hale gelmiş ürünlerdir. Yüksek besin değeri taşımaları nedeniyle beslenmeye destek olmakta ve bu yüzden çeşitli formları piyasaya sürülmektedir. Bu ürünler hem yumurta hem de larva yapısı nedeniyle yüksek biyolojik aktivite göstermektedir. Bu sebeple tam gıda olarak tanımlanmaktadır. Kraliçe arı larvası ve apilarnilin hem gıda olarak tüketilmesi hem de apiterapide kullanımı için içerik analizlerinin yapılması, kullanım dozlarının belirlenmesi, canlılarda yararlı ya da toksik etkinliklerinin ortaya çıkarılması oldukça önemlidir. Bu çalışmanın amacı da apilarnil ve kraliçe arı larvasının toksisite ya da beslenme araştırmalarında sıkça kullanılan model organizma olan *Drosophila melanogaster* (meyve sineği) üzerinde larval mortalite ve ömür uzunluğunun değerlendirilerek literatürdeki eksiklikleri gidermektir.

Gereç ve Yöntem: Deneysel çalışmamızda gıda takviyesi olarak kullanılan liyofilize apilarnil ve kraliçe arı larvaları Amasya şehrinde faaliyet gösteren bir apiterapi ürünleri işletmesinden temin edildi. Çalışmamızda kullandığımız *D. melanogaster*, Amasya Üniversitesi Fen-Edebiyat Fakültesi Biyolojik Araştırma Laboratuvarı'nda yıllardır çoğaltılarak saklanmaktadır. Standart *D. melanogaster* besiyerine farklı konsantrasyonlarda (0.5; 1.0; 2.5 ve 5.0 mg/ml) eklenen apilarnil ve kraliçe arı larvası ekstraktları, uygulama ve kontrol grupları için erkek ve dişi popülasyonlarda ayrı ayrı çalışılmıştır. Öncelikle larval mortalite çalışması yapılmış, daha sonra uygun bulunan konsantrasyonlarda ömür uzunluğu deneyleri yapılmıştır. Her deney seti üç kez tekrar edilmiş ve

elde edilen bulguların ortalamaları alınarak istatistiksel değerlendirmeler yapılmıştır.

Bulgular ve tartışma: Her iki cinsiyetteki kraliçe arı larvası uygulama gruplarında tüm konsantrasyonlar karşılaştırıldığında, en uzun maksimum yaşam süresi 5 mg/ml tedavi grubunda ve erkeklerde (92 ± 3.17) gözlemlendi; en düşük maksimum yaşam süresi kontrol grubunda (66 ± 0.41) gözlemlendi. Bu sonuçlara göre apilarnil ortamındaki 2.5 mg/ml konsantrasyonu kullandığımız apilarnil ve kraliçe arı larvalarının larval mortalite ve yaşam süreleri üzerindeki etkileri açısından her iki cinsiyet grubunda da etkili olmuştur. Ortalama yaşam süresi açısından gözlenen bu fark her iki cinsiyet grubunda da $p<0.05$ düzeyinde istatistiksel olarak anlamlıydı.

Sonuç: Çalışmadan elde edilen bulguların değerlendirilmesi ile bireylerin yaşadığı olumsuzluklar ve kullanılan kimyasal ilaçlar ile yapılan tedavi protokollerinin yarattığı güçlü yan etkiler düşünüldüğünde, antioksidan ve antikanserojen etkisi olan ve sağlığı destekleyen gıda takviyelerinin geliştirilmesi önem arz etmektedir. Zengin besin içeriğine sahip olan apilarnilin ve kraliçe arı larvasının *D. melanogaster* ömrü üzerindeki etkinliğinin literatüre önemli katkı sağlayacağı düşünülmektedir.

INTRODUCTION

Many reasons such as the increase in the elderly population in the world, the decrease in the rate of physical activity, the prevalence of harmful habits such as smoking and alcohol, and the change in lifestyles are increasing the rate of global diseases (Onur et al. 2018). These effects can also bring some difficulties in people's quality of life, daily functions and treatment adherence (Duran 2011). Increasing diseases with artificial living conditions, especially drug resistance and side effects of chemical drugs in the body have led individuals to seek alternative solutions (Sorucu 2019). In different

ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

countries of the world, with the need for food that develops due to population growth the solution to the problem in the context of the search for a food source and the search for a food source can be an alternative food for people to utilize the resources. For this purpose, insects and insect products are used as a protein source in many countries of the world. It is consumed as a food product that is a source of food (Hakan et al. 2021).

In order to eliminate nutritional deficiencies, imbalances and protein deficiencies, research is carried out on various functional food sources. Insects have started to be consumed as food in order to use food sources effectively and honeybees are also included in this consumption (Isidorov et al. 2016). As a result of the research, it has been reported that drone bee larvae (apilarnil) and queen (queen) larvae support health protection due to their high nutritional value. The name Apilarnil was created by Romanian scientist Nicolae Iliesiu, from the Latin name for bees 'api' (*Apis mellifera*), 'lar' from larvae and initials 'nil' (Erdem and Ozkok 2018). Apilarnil is a drone larvae in the 3–7-day larval stage before they pupate. It shows high biological activity due to both egg and larval structure. Apilarnil is defined as "whole food" because it contains all essential amino acids, which are the basic building blocks of our body (Topal et al. 2018).

Queen bee larva is another popular bee larva recently. It is obtained by collecting 3-day-old queen bee larvae from the thimble, which is naturally present in the queen bee cell during the production of royal jelly before the milk harvest (Margaoan et al. 2017). It is estimated that the biggest difference between apilarnil and queen bee larvae is due to the total protein content of the queen bee, as it is fed with pure royal jelly (Keskiner 2021). The chemical composition of apilarnil and queen bee larva homogenate was investigated by GC-MS (Gas Chromatography-Mass Spectrometry). The contents of apilarnil homogenate were 73.75% water, 9.47% total protein, 8.38% lipid, 0.38% fructose and 3.55% glucose. The contents of queen bee larva homogenate were 75.17% water, 12.03% total protein, 10.30% lipid, 1.25% fructose, 2.10% glucose and 0.08% sucrose (Isidorov et al. 2016).

It is appropriate to collect apilarnil and queen bee larvae in April-May when the best quality food form is preserved. The best process applied to preserve the nutritional values is lyophilization and thus fresh

apilarnil and queen bee larvae lyophilized can be safely stored at -15 °C for 1 year (Bruneau 2015).

In the study by Isidorov et al., the biological properties of apilarnil were investigated and some pharmacological chemical substances have been found to show activity. In their studies, apilarnils with the queen bees in queen larvae. In terms of sugar content, glucose was more dominant in apilarnil larvae, while in queen larvae trehalose was predominant. Amino acid content and essential amino acids of apilarnil homogenates amount was lower than that of queen homogenates. In this study, the chemical composition of apilarnil was determined by GC-MS (Gas Chromatography-Mass Spectrometry) (Isidorov et al. 2016). Studies have revealed that apilarnil, a bee product, has the potential to shed light on scientific studies.

The recent initiation of studies on apilarnil, the determination of its chemical content and the reduction of its effects on animal subjects on tissue and cellular basis and the results obtained have become promising (Dong et al. 2018, Hakan et al. 2021, Hamamci et al. 2020, Isidorov et al. 2016). Apilarnil and queen bee larvae are powerful energy providers that stimulate oxidative processes. The accumulation of oxidative damage has been shown to play an important role in some advanced-age diseases and the aging process. Aging and longevity have been the subject of curiosity of people for years. For this reason, it has never lost its topicality in the scientific world. This biological process is quite complex and complicated. Model organisms are materials used to understand and analyze biological processes. The most basic life process character to describe the aging process is lifespan (Coskun 2023).

Longevity is affected by all factors that reduce viability. From this point of view, the effects of apilarnil and queen bee larvae, which have high biological activity, on the life span of adult individuals of *Drosophila melanogaster* were tried to be determined. Basic metabolic molecular pathways are well conserved and approximately 75% of known human disease genes have sequences of interest in *D. melanogaster*.

D. melanogaster has been recognized as a valuable model and has gained interest in nutritional intervention studies. The effects of food on larval mortality and survival were evaluated to investigate food-related pathophysiological mechanisms, including inflammation and stress response.

ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

MATERIAL AND METHODS

Material

Lyophilized apilarnil and queen bee larvae used as food supplements in our experimental study were obtained from an apitherapy products business operating in our region. *D. melanogaster*, which we used in our studies, has been hybridized for years in the Biological Research Laboratory of the Faculty of Science and Letters of Amasya University. The short

life cycle (9-10 days), high number of offspring, low rearing conditions and easy observation of possible variations make *D. melanogaster* an ideal experimental organism. In our experimental study, Oregon (R) (wild type) strain of *D. melanogaster* with normal round, red eyes and no mutant characters was used to determine lifespan (Figure 1). The environment of *D. melanogaster* is at 40% - 60% RH, 25 ± 1 °C and under permanent dark conditions.



Figure 1. Images of female and male adult individuals and their developmental periods used in the study

Methods

Lyophilized apilarnil and queen bee larvae were dissolved in distilled water to prepare a 100 ml stock solution at 5mg/ml. 1.5 g of *D. melanogaster* ready-made medium (Instant *D. melanogaster* Medium, purchased from Carolina Biological Supply Company) and apilarnil or queen bee larvae dissolved in 5 ml distilled water were added to 50 ml falcon tubes. In the larval mortality assay, sufficient numbers of male and female individuals of stock *D. melanogaster* were transferred to fresh medium for larval mortality or survival rate tests and kept in an incubator at 25°C and 40-60% relative humidity, humidity for 25 days. The third stage larvae obtained after three days (72 ± 4 h) were transferred to a medium containing different concentrations of apilarnil and queen bee larvae (0.5-1-2.5 and 5 mg/ml medium). Distilled water was used for the control group. For each experimental group, 100 larvae were used. Test tubes were sterilised. The mouths of the tubes were closed with cotton plugs and placed inside the tubes and the larvae were allowed to mature. During this process, all experimental groups were checked daily and

counted for 7 days after the first adult fly was seen. Counts were recorded twice daily, separating males and females. All experiments were repeated 3 times.

In our study, 3rd instar larvae *D. melanogaster* larvae were used to determine larval mortality, and *D. melanogaster* individuals were used to determine lifespan (Figure 1). Males and females of the Oregon R strain were crossbred in culture bottles to create preliminary stocks. Individuals reaching the 3rd larval stage were separated under tap water, and 3rd stage larvae collected with the help of fine-mesh sieves were weighed at the determined concentrations (0.5-1-2.5 and 5 mg/ml) and containing apilarnil or queen bee larvae dissolved in 5 ml of water. *D. melanogaster* was transferred to glass bottles containing ready-made medium. 100 larvae were placed in each bottle and expected to develop into adult flies.

The study was carried out in triplicate. The results obtained were averaged.

The effects of apilarnil and queen bee larvae on lifespan were studied separately in the male and female sexes of *D. melanogaster*. For this purpose,

ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

preliminary stocks were created by crosses in culture bottles containing fresh nutrient medium to obtain same-aged individuals. Unmated female and male flies of the same age (1-3 days old) emerging from the pupa were separated and 100 adult flies were collected for each concentration. Collected adult flies were fed for 10 days in media containing apilarnil or queen bee larvae at different concentrations (0.5-1.0-2.5 and 5 mg/ml). At the end of 10 days, the adult flies, which were taken to the medium-free medium, were transferred to the new medium every three days, while counting was continued and the numbers were noted. All culture bottles were kept in suitable temperature cabinets (25 ± 1 °C). During the experiment, the foods were refreshed every three days. In all control and treatment groups, counting and practice were continued until the last individual died.

Statistical analysis: The analysis of the data we obtained as a result of our study was made with the SPSS version 27.0 (Statistical Package for the Social Sciences) program. For this purpose, the "One-way Analysis of Variance" (One-way ANOVA) method was used. The Duncan test was evaluated at a probability level of 0.05 for data from longevity studies ($p < 0.05$). Larval mortality graphs and survival curves of adult individuals were drawn using the Microsoft Windows Office Excel program.

RESULTS

In our experimental study, it was observed that apilarnil and queen bee larvae increased larval mortality and mean lifespan in both female and male individuals in all treatment groups (0.5; 1.0; 2.5 and 5 mg/ml) compared to the control. From the results obtained from the larval mortality studies, it was determined that the highest larval mortality rate was observed in the 1 mg/ml queen bee larvae treatment group (40%) and 0.5 mg/ml queen bee larvae treatment group (20%) (Table 1). The best survival was observed in the 5 mg/ml apilarnil treatment group (95%) and the 5 mg/ml queen bee larvae treatment group (94%) (Table 1).

In the second stage of our study, larvae were collected with a new experimental setup and substance applications were made at determined doses from larva to adulthood. 100 male and female adult individuals obtained from these larvae were fed on standard media and their mortality rates were monitored throughout their lifespan. All studies were repeated 3 times and the averages were taken. Then, the importance controls of the differences between the means obtained as a result of pairwise comparisons of the study groups and control groups were also determined (Table 1-3, Figure 2-4).

Table 1. Survival and mortality rates of larvae chronically fed with different concentrations of Apilarnil (APL) and Queen Bee Larvae (QBL)

Experiment Sets	N	Mortality Rate (%) \pm S.E.	Survival Rate (%) \pm S.E.
Control	100	5 \pm 0.04 ^a	95 \pm 1.12 ^a
0.5 mg/ml APL	100	14 \pm 1.05 ^b	86 \pm 1.04 ^b
1 mg/ml APL	100	18 \pm 1.04 ^b	82 \pm 2.04 ^b
2.5 mg/ml APL	100	7 \pm 0.04 ^a	93 \pm 1.14 ^a
5 mg/ml APL	100	5 \pm 0.08 ^a	95 \pm 1.10 ^a
0.5 mg/ml QBL	100	20 \pm 1.11 ^b	80 \pm 0.94 ^b
1 mg/ml QBL	100	40 \pm 1.14 ^c	60 \pm 1.01 ^c
2.5 mg/ml QBL	100	11 \pm 0.94 ^{ab}	89 \pm 1.04 ^{ab}
5 mg/ml QBL	100	6 \pm 0.02 ^a	94 \pm 1.94 ^a

S.E.: Standard Error, n: number of larvae, QBL: Queen Bee Larvae, APL: Apilarnil, ^{a-d}Values belonging to experimental groups with different letters in the same column are significant at $p < 0.05$ level.

When the maximum lifespan lengths were analyzed, the longest mean lifespan in apilarnil treatment groups was determined as 103 \pm 1.98 and 75 \pm 1.04 days in the 5 mg/ml treatment group in males and

the lowest maximum lifespan in 0.5 mg/ml treatment group in males, respectively (Table 2 and Figure 2).

When the maximum lifespan lengths were analyzed, the longest mean lifespan in apilarnil treatment

ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

groups was determined as 110 ± 1.49 and 75 ± 1.09 days in 5 mg/ml treatment group in females and the lowest maximum lifespan in 1 mg/ml treatment group in females, respectively (Table 2 and Figure 3).

When all concentrations were compared in apilarnil treatment groups in both sexes, the longest maximum life span was observed in 5 mg/ml treatment group and females (110 ± 1.49); the lowest maximum life span was observed in 0.5 mg/ml treatment group and males (75 ± 1.04) (Tables 2).

Table 2. Lifespan data obtained from larvae treated with Apilarnil (APL)

Experiment Sets	Sex	N	Maximum Lifespan (Days) \pm S.E.	Average Lifespan (Days) \pm S.E.
Control	Female	100	66 ± 1.04^a	65.3 ± 1.06^a
	Male	100	66 ± 1.04^a	63.4 ± 1.01^a
0.5 mg/ml APL	Female	100	$78 \pm 1.18^*$	$75.8 \pm 1.28^*$
	Male	100	75 ± 1.04^b	74.7 ± 1.07^b
1 mg/ml APL	Female	100	75 ± 1.12^b	72.6 ± 1.29^{ab}
	Male	100	95 ± 2.18^c	70.7 ± 1.84^a
2.5 mg/ml APL	Female	100	101 ± 2.64^d	79.7 ± 1.96^{bc}
	Male	100	98 ± 2.15^c	78.6 ± 1.78^{bc}
5 mg/ml APL	Female	100	110 ± 3.49^d	72.8 ± 1.24^{ab}
	Male	100	103 ± 2.98^d	70.8 ± 1.88^a

S.E.: Standard Error, N: Number of larvae, APL: Apilarnil, ^{a-d} Values belonging to experimental groups with different letters in the same column are significant at $p < 0.05$ level.

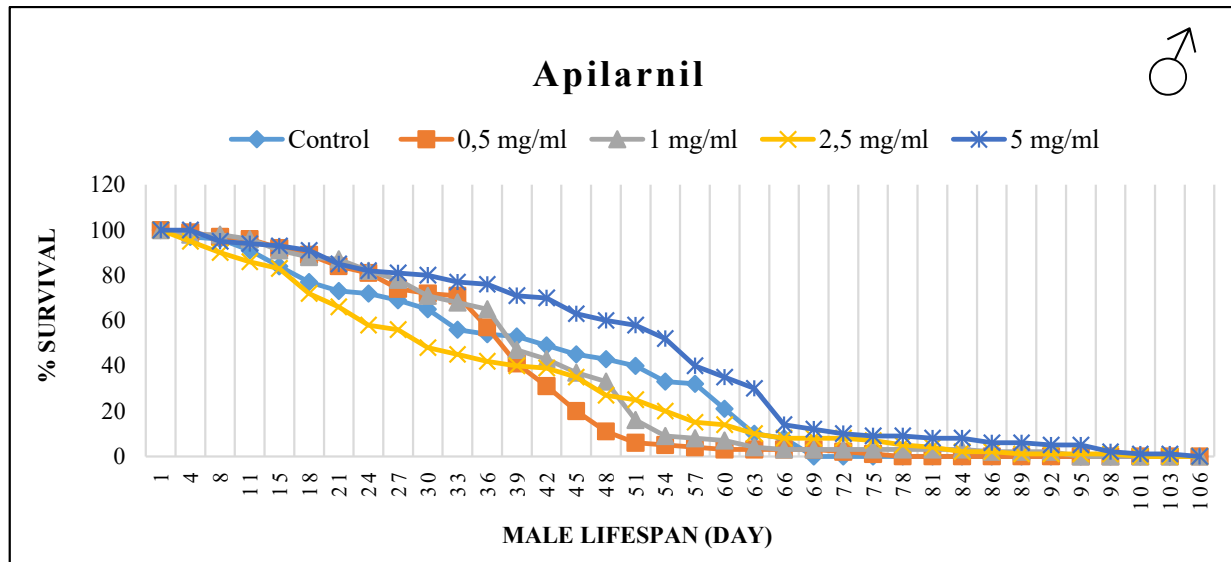


Figure 2. Survival curves of male *D. melanogaster* individuals living on apilarnil medium at different concentrations during their adult lives

When the maximum life span lengths were analyzed, the longest average life span was determined as 92 ± 1.075 and 75 ± 1.052 days in the 5 mg/ml treatment group in males and the lowest maximum

life span was determined as 92 ± 1.075 and 75 ± 1.052 days in the 1 mg/ml treatment group in males, respectively (Table 3 and Figure3).

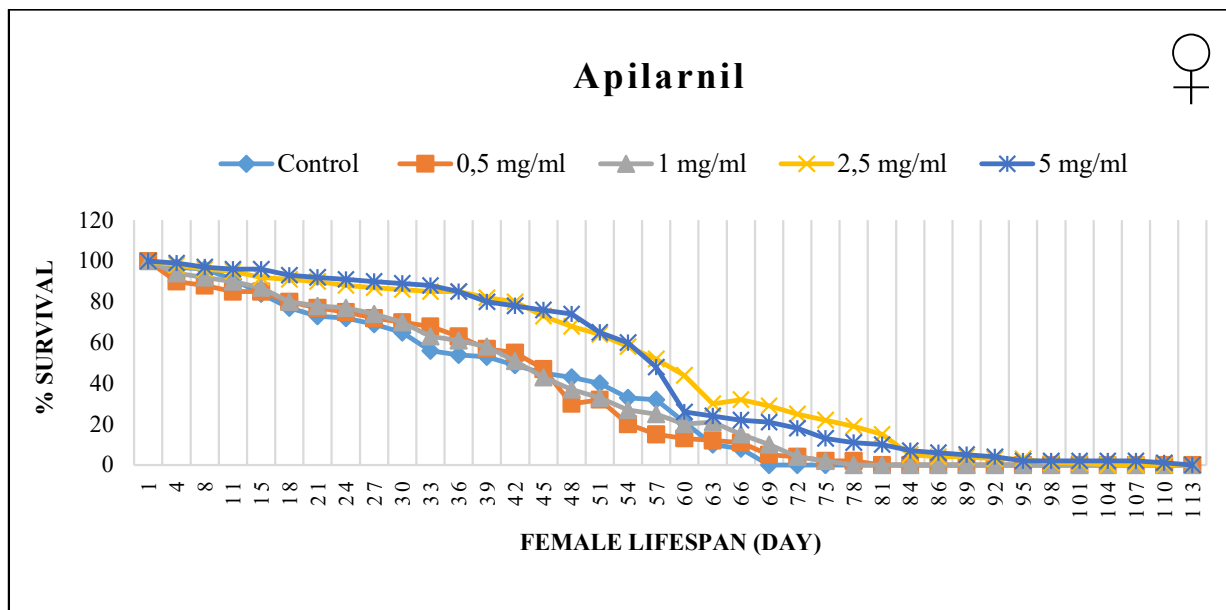


Figure 3. Survival curves of female *D. melanogaster* individuals living on apilarnil medium at different concentrations during their adult lives

Table 3. Lifespan data obtained from larvae treated with queen bee larvae (QBL)

Experiment Sets	Sex	N	Maximum Lifespan (Days) ± S.E.	Average Lifespan (Days) ± S.E.
Control	Female	100	66±0.41 ^a	62.4±1.54 ^b
	Male	100	66±0.41 ^a	60.1±1.18 ^b
0.5 mg/ml QBL	Female	100	72±1.48 ^b	70.8±1.68 ^b
	Male	100	78±2.07 ^c	75.7±1.87 ^c
1 mg/ml QBL	Female	100	70±1.02 ^b	66.6±2.20 ^c
	Male	100	75±2.05 ^c	68.7±2.52 ^c
2.5 mg/ml QBL	Female	100	75±2.09 ^c	71.2±3.22 ^d
	Male	100	89±3.09 ^d	80.7±3.08 ^d
5 mg/ml QBL	Female	100	72±1.09 ^a	68.8±3.39 ^d
	Male	100	92±3.17 ^d	83.1±3.53 ^d

S.E.: Standard Error, N: Number of larvae, QBL: Queen Bee Larvae, ^{a-d} Values belonging to experimental groups with different letters in the same column are significant at p<0.05 level.

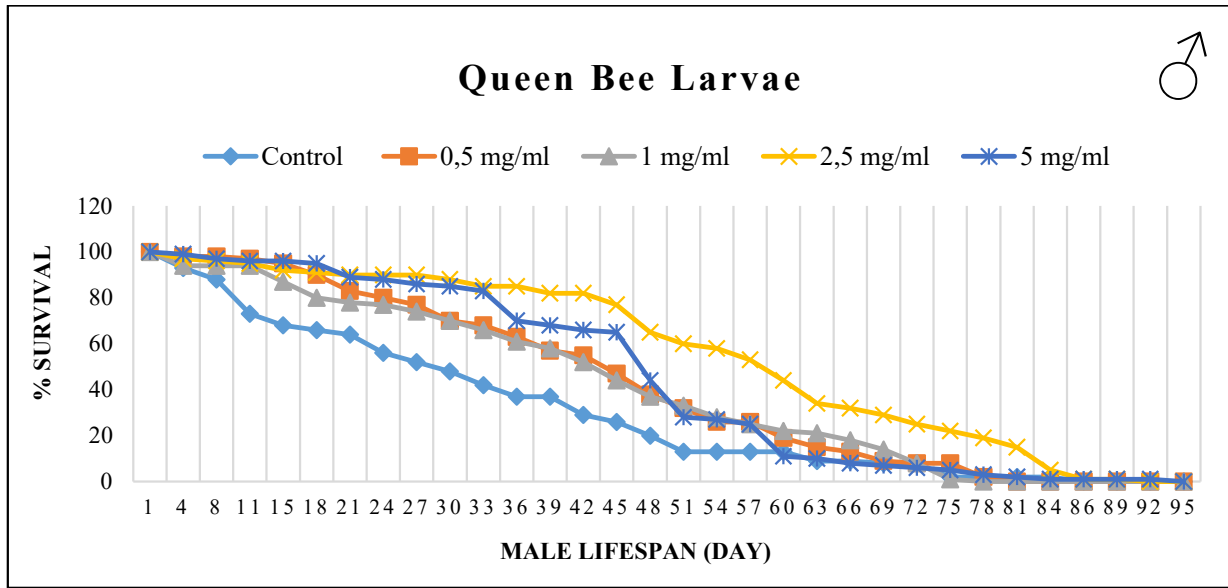


Figure 3. Survival curves of male *D. melanogaster* individuals living in different concentrations of queen bee larvae medium during their adult live

When the maximum life spans were examined in females, the longest maximum life span and mean life span in the queen bee larvae treatment groups were determined as 75 ± 2.09 and 70 ± 1.02 days in

the 2.5 mg/ml treatment group, respectively, while the lowest were determined in the control group, respectively (Table 3 and Figure 4).

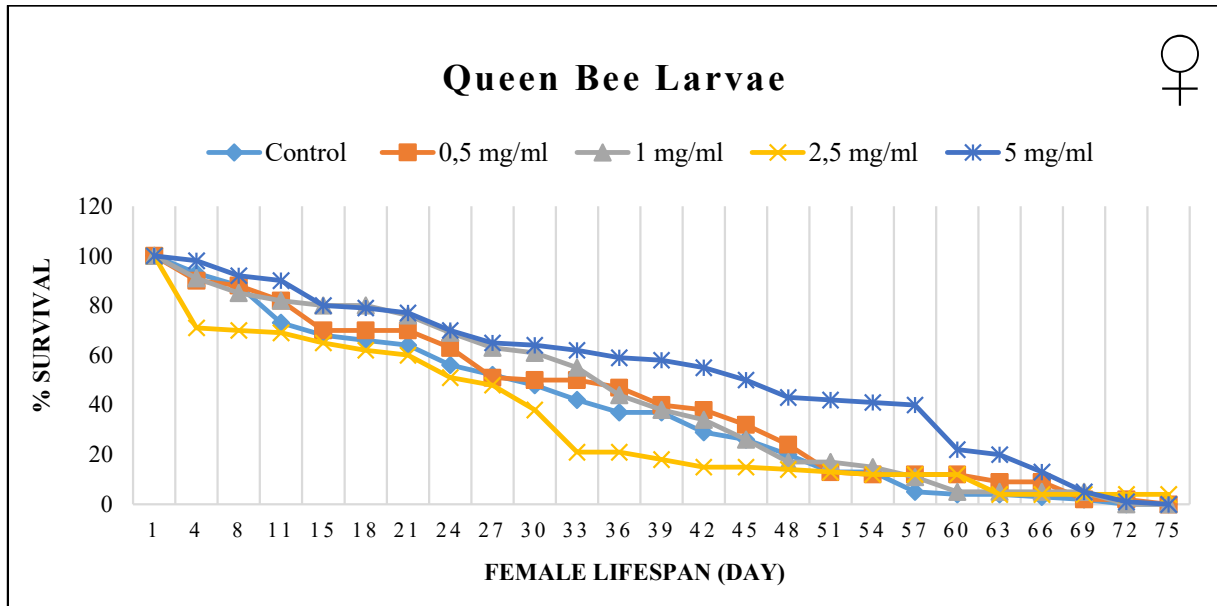


Figure 4. Survival curves of female *D. melanogaster* individuals living in different concentrations of queen bee larvae medium during their adult live

ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

When all concentrations were compared in queen bee larvae treatment groups of both sexes, the longest maximum lifespan was observed in the 5 mg/ml treatment group and males (92 ± 3.17); the lowest maximum lifespan was observed in the control group males and females (66 ± 0.41) (Tables 2 and 3). However, the apilarnil and queen larvae treatment group showed higher lifespan prolonging activity than the distilled water control group in both sexes in both treatment groups.

According to these results, 2.5 mg/ml concentration in apilarnil medium was effective in both sex groups in terms of the effects on apilarnil and queen bee larvae larval mortality and lifespan, which we used in our experiments; 5 mg/ml concentration was effective in queen bee larvae medium. This difference observed in terms of mean lifespan was statistically significant at $p<0.05$ level in both sex groups.

DISCUSSION

Apilarnil and queen bee larvae are powerful energy providers that stimulate oxidative processes. Many bee products within the scope of apitherapy products have been examined and shown to have antioxidant properties due to their powerful energy-providing effects that stimulate oxidative processes. It was determined that the most popular bee products of recent times, apilarnil, which we used in our experiments, and the queen bee larva, adult *D. melanogaster*, reduced mortality.

Today, various functional resources are used due to problems such as the increase in diseases, malnutrition, unbalanced nutrition and insufficient protein intake due to various reasons. Insects have recently been consumed as a food source. Honey bees are also evaluated in this category, and bee products have many positive effects on human health. Apilarnil and queen bee larvae are some of them. It has an important place in medical use with its high nutritional value. In our study, it was observed that the support of apilarnil and queen bee larvae applied to the 3rd stage larvae had a positive effect on the death of adult *Drosophila melanogaster*, thus increasing the lifespan. We think that this positive effect is due to its antioxidant properties. When the literature is examined, there is no study investigating the effects of apilarnil and queen bee larvae on *Drosophila melanogaster*. It has mostly been studied on other organisms, while

studies on *Drosophila melanogaster* are other bee products such as honey, propolis, royal jelly and bee venom. Apilarnil has a positive effect on problems such as loss of appetite, hypoproteinemia, premature aging, depression in the elderly, genital diseases, hormone and vitamin deficiency. One of the important reasons why the queen bee resists pathogens is that she is fed with pure royal jelly for life.

Apilarnil has a positive effect on problems such as loss of appetite, hypoproteinemia, premature aging, depression in the elderly, genital diseases, hormone and vitamin deficiency due to various reasons. One of the important reasons why the queen bee resists pathogens is that she is fed with pure royal jelly for life (Yang et al 2017).

As a result of the study of Yucel et al., apilarnil (drone bee larvae); testosterone levels of 14.80 ± 0.05 ng/g, progesterone levels of 14.40 ± 0.05 ng/g were found to be at high levels, at the same time conjugated linoleic acid was determined as a fatty acid marker with a level of 52.62%. According to the results, it was understood that apilarnil has an important place in terms of bioproperty (Yucel et al. 2019). In a study by Hamamcı et al., apilarnil reduced the decrease in SOD and CAT levels in the brain with sepsis. At the same time, apilarnil decreased the increase in MDA, XOD and testis-1 levels in the septic brain, and as the dose of apilarnil increased, the number of degenerated neurons due to sepsis decreased. Apilarnil reduced the high levels of proinflammatory cytokines (IL-6, TNF- α , IL-1 β) induced by sepsis. As a result, apilarnil prevented sepsis-related apoptosis in the brain (Hamamcı et al. 2020). In the 2020 study, it was observed that apilarnil from bee products had a positive effect on oxidative stress, proinflammatory cytokine production and increased apoptosis caused by LPS application. As the doses of Apilarnil increased, the TLR4 / HMGB-1 / NF-kB signaling pathway was inhibited and liver injury decreased. In line with these results, it was thought that apilarnil, which gained importance in terms of biological content, could be an alternative treatment for sepsis (Doganyigit et al. 2020). Another study was conducted on Wistar rats.

As a result of the study, it was observed that apilarnil is a powerful energy source that stimulates oxidative processes with an intense catabolic effect (Kogalniceanu et al. 2010). The most important

ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

markers of antioxidant activity are amino acid sequences and molecular weight.

Dong et al. found a relationship between the hydrophobic amino acid, which is abundant in the content of queen bee larvae, and its high antioxidant activity (Dong et al. 2018). In this study, low (2.5 g/broiler) and high (7.5 g/broiler) doses of apilarnil were administered to male and female broilers between 28 and 55 days. As a result of the study, it was observed that blood sugar and cholesterol levels were lower in the group administered high-dose apilarnil, along with a decrease in fear and stress in animals (Altan et al 2013). In another research paper, it was seen that apilarnil applied to wild boars had a positive effect on sexual dysfunction.

It has been observed to increase the fertility rate by 76.4% in pigs (Bolatovna et al. 2015). Former beekeepers living in Romania consumed queen bee larvae in their entirety in order to prevent disease transmission when they got seasonal flu (Strant 2016). In this paper by Cruz et al., the effect of Brazilian *Pampa biome* honey on adult *Drosophila melanogaster* was investigated. Honey is protected against wing posture error and molecular changes related to mitochondrial pathways induced by hypoxia/reoxygenation. An upright wing posture was observed in some of the flies after reoxygenation, this acquired trait was also associated with death (Cruz et al. 2018).

In a study by Ayikobua et al. on the treatment of parkinsonism in *Drosophila melanogaster*, the effects of propolis and levodopa were compared. Propolis alone had a positive effect on motor activity, antioxidants and life span in *Drosophila melanogaster* compared to PINK1 flies. In combination with levodopa, propolis improved physiological parameters better at lower concentrations in Parkinsonism *Drosophila melanogaster* and showed a positive effect on the side effects of levodopa (Ayikobua et al. 2020).

The contents of this article, *Drosophila melanogaster* was fed with Perga called bee bread and it was observed that this product showed positive effects on vital parameters. While the effect on mortality rates of *Drosophila melanogaster* 3rd instar larvae was 94% in the control group, it was 98% in larvae fed with Perga (Fidan et al. 2020).

Many experimental studies on bee products have been conducted in the literature. The experimental

study of apilarnil and queen bee larvae has only recently begun to enter the literature. As seen in our study, apilarnil and queen bee larvae on *Drosophila melanogaster* showed a positive effect on longevity with their important biocaracteristics and contents. Apitherapy, which is seen as a supporter of medicine, works on many bee products. In this context, apilarnil and queen bee larvae are thought to have an important place in this field. In this way, the use of natural products whose contents have been analysed and biocaracteristics have been determined should be widespread. Since this study is the first study of apilarnil and queen bee larvae, which are the most popular bee products of recent times, on *Drosophila melanogaster*, it is thought that it will make a long-lasting contribution to the literature.

Conclusion: In this study, apilarnil (drone bee larvae) and black widow bee larvae, the most popular bee products of recent times in apitherapy, were used. Their effects on mortality and longevity of Oregon R wild larvae of *D. melanogaster* were examined. For this purpose, the results of different concentrations of selected apitherapy products were compared with the results of the control group. As a result of our study, an increase in the number of maturing larvae was observed for both bee products compared to the control group. It was also found to prolong the lifespan of *D. melanogaster*. Although the basic mechanisms of aging and longevity are not yet fully understood, it is argued that this process can be delayed. Today, studies on humans on this subject are not enough. It is thought that apilarnil, which has a rich nutritional content, and its effectiveness on the lifespan of queen bee larvae will make a significant contribution to the literature.

Author Contributions: AAB and AA conceived and planned the experiments. AAB and AA carried out the experiments. AAB and AA contributed to sample preparation. AAB and AA contributed to the interpretation of the results. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Conflict of Interest: The authors declared that there is no conflict of interest.

Data Availability Statement: The data supporting this study's findings are available from the corresponding author upon reasonable request.

ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

Ethical Statement: This study does not present any ethical concerns. There is no need to obtain an ethics committee for the this article.

Financial support: No financial support was received for this research.

REFERENCES

- Altan Ö, Yücel B, Açıkgöz Z, Şeremet Ç, Kösoğlu M, Turgan N, Özgönül AM. Apilarnil reduces fear and advances sexual development in male broilers but has no effect on growth. *British Poultry Science*. 2013; 54(3): 355-361. doi.org/10.1080/00071668.2013.791382
- Ayikobua ET, Kasolo J, Kasozi KI, Eze ED, Safiriyu A, Ninsiima HI, Kiyimba K, Namulema J, Jjesero E, Ssempijja F, Semuyaba I, Mwandah DC, Kimanje KR, Kalange M, Okpanachi AO, Nansunga M. Synergistic action of propolis with levodopa in the management of Parkinsonism in *Drosophila melanogaster*. *J Complement Integr Med*. 2020; 17(3). doi: 10.1515/jcim-2019-0136
- Bolatovna KS, Rustenov A, Eleuqalieva N, Omirzak T, Eleuqalieva N, Akhanov. Improving reproductive qualities of pigs using the drone brood homogenate. *Biol Med*. 2015; 7(2): 091-15.
- Bruneau E. First steps for good beekeeping practices-guide for apitherapy products. *Apitherapy Symposium Book Of Abstracts*, page:40, 2015.
- Coşkun, R. Bitkisel aspir yağının oksidatif stres ve ömür uzunluğu üzerine etkilerinin araştırılması. Master thesis. Amasya University, Türkiye, 2023.
- Cruz LC, Ecker A, Rodrigues NR, Martins IK, Posser T, Maciel FE, Franco JL. Honey protects against wings posture error and molecular changes related to mitochondrial pathways induced by hypoxia/reoxygenation in adult *Drosophila melanogaster*. *Chemico-Biological Interactions*. 2018; 291, 245-252. doi.org/10.1016/j.cbi.2018.06.033
- Doğanyığıt Z, Okan A, Kaymak E, Pandır D, Silici S. Sıçanlarda lipopolisakkarit kaynaklı karaciğer hasarına karşı apilarnil koruyucu etkilerinin TLR 4/HMGB-1/NF-κB yolu ile araştırılması. *Biyotıp ve Farmakoterapi*. 2020; 125:109967.
- Dong D, Dong M, Liu K, Lu Y, Y B., Antioxidant activity of queen bee larvae processed by enzymatic hydrolysis. *Journal of Food Processing and Preservation*. 2018; 42(2):13461.
- Duran ET. Kanser tedavisinin yan etkilerine yönelik alternatif uygulamalar. *SDÜ Tıp Fakültesi Dergisi*. 2011; 18(2): 72-77.
- Erdem B, Özkök A. Can food supplement produced from apilarnil be an alternative to testosterone replacement therapy. *Hacettepe Journal of Biology and Chemistry*. 2018; 45(4): 635-638. DOI: 10.15671/HJBC.2018.207.
- Fidan M, Ayar A, Konar V. Effects of perga (bee bread) on metamorphosis, mortality and fecundity in *Drosophila melanogaster*. *Sabuncuoğlu Serefeddin Health Sciences*. 2020; 2(2): 1-15.
- Hakan İ, İlkaya M, Ebubekir İ. Apilarnilin kimyasal içeriği, biyoaktif özelliği ve insan sağlığı açısından bazı hastalıkların tıbbi tedavisinde destekleyici potansiyeli. *Uluslararası Gıda Tarım ve Hayvan Bilimleri Dergisi*. 2021; 1(1): 1-7.
- Hamamci M, Doganyigit Z, Silici S, Okan A, Kaymak E, Yılmaz S, Inan LE. Apilarnil: a novel neuroprotective candidate. *Acta Neurologica Taiwanica*. 2020; 29(2): 33-45.
- Isidorov VA, Bakier S, Stocki M. GC-MS investigation of the chemical composition of honeybee drone and queen larvae homogenate. *Journal of Apicultural Science*. 2016; 60 (1): 111-120.
- Keskiner AA. Gıda takviyesi olarak kullanılan bazı apiterapik ürünlerin çeşitli kanser hücre hatlarında etkinliğinin belirlenmesi. Master thesis. Amasya University, Türkiye, 2021.
- Kogalniceanu S, Lancrajan I, Ardelean G. Changes of the glucidic metabolism determined by the physical effort of the treatment with the aslavital and apilarnil. *J Med Aradian*. 2010; 3:33-41.
- Mărgăoan R, Mărgăritas LA, Dezmirean DS, Bobiş O, Bonta V, Cătană C, Margın MG. Comparative study on quality parameters of royal jelly, apilarnil and queen bee larvae triturate. *Bulletin of The University of Agricultural Sciences and Veterinary Medicine*

ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

- Cluj-Napoca Animal Science and Biotechnologies. 2017; 74(1). DOI:10.15835/buasvmcn-asb: 12622
- Onur E, Nalbantsoy A, Kışla D. İmmünoterapi ve propolisin kanser immünoterapisinde kullanım potansiyeli. Food and Health. 2018; 4(4): 231-246. DOI: 10.3153/FH18023
- Sorucu A. Arı ürünleri ve apiterapi. Veteriner Farmakoloji ve Toksikoloji Derneği Bülteni. 2019; 10(1): 1-15.
- Strant M. Personal experiences in apitherapy. Cluj Napoca. 2016.
- Topal E, Strant M, Yücel B, Kösoğlu M, Margaoan R, Dayıoğlu M. Ana ve erkek arı larvalarının biyokimyasal özellikleri ve apiterapötik kullanımı. Hayvansal Üretim. 2018; 59(2): 77-82. doi.org/10.29185/hayuretim.455478
- Yang W, Y Tian, M Han, X Miao. Longevity extension of worker honey bees (*Apis mellifera* L.) by royal jelly: optimal dose and active ingredient. Peerj. 2017; https://doi.org/10.7717/peerj.3118
- Yücel B, Şahin H, Yıldız O, Kolaylı S. Apilarnilin biyoaktif bileşenleri ve etki mekanizması. Hayvansal Üretim. 2019; 60 (2), 125-130. https://doi.org/10.29185/hayuretim.591007