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The Effect of Feeding With Pumpkin Seed Membrane on Survival, Development and Longevity in Model Organism

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ABSTRACT
Most wastes generated during food production in the world are released to nature. However, the target and non-target species take these wastes into their organisms through nutrition although their bioavailability is unknown. In this study, the aim was to determine the bioavailability of wasted pumpkin seed membrane (PSM) for species in nature through its effects on the *Drosophila* obesity model along with the increased fat intake. For this purpose, either fat (20%) and PSM (0-2 g) were added to the *Drosophila melanogaster* diet. Survival-development, sex ratio, and adult lifespan were evaluated. As a result, the survival rate decreased and development time increased in insects depending on the amount of PSM added to the food. In the study, fed with PSM and fat intake negatively affected the insect in terms of survival-development and lifespan.

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Model Organizmanın Kabak Çekirdeği Membranı ile Beslemenin Yaşama, Gelişme ve Ömür Uzunluğuna Etkisi

ÖZET
Dünyada gıda üretimi esnasında oluşan atıkların neredeyse birçoğu doğaya bırakılmaktadır. Çevre ve canlılar açısından kullanılabilirliği bilinmeyen ürünlerin beslenme yoluyla organizmaya alınması hedef ve hedef olmayan canlılar açısından önemlidir. Çalışmada hem atık olarak kabak çekirdeği mebranının (KÇM) çevre ve doğada yaşayan türler açısından kullanılabilirliği, hem de artan yağ alımı ile obezite modeli olarak kullanılan *Drosophila*'da etkinin belirlenerek çıkarımlar yapılması amaçlanmıştır. Bu amaçla *Drosophila melanogaster* diyetine yağ (%20) ve KÇM (0-2 g) eklenmiştir. Böcekte yaşama-gelişme, eşey oranı ve ergin ömür uzunluğu değerlendirilmiştir. Sonuç olarak besine ilave edilen KÇM miktarına bağlı olarak böceklerde yaşama oranı azalırken, gelişim süresi artmıştır. Çalışmada beslenmeyle KÇM ve yağ alınması böceği yaşama-gelişim ve ömür uzunluğu açısından olumsuz etkilemiştir.

Araştırma Makalesi

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Anahtar Kelimeler
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INTRODUCTION
Worldwide, pumpkin is cultivated for food (vegetable-nuts) industry, cosmetic industry, animal nutrition, and medical use (oil industry) (Tuncer and Yanmaz, 2011). When examining international cuisine, pumpkin has a wide range of uses in Turkish and regional cuisines. This crop is used extensively, especially in the kitchens of the regions as ingredient from soup to dessert (Cesur and Avcıkurt, 2019; Güleç and Durlu Özkaya, 2019; Kahraman et al., 2019). In Turkey, it is observed that the pumpkin cultivation is growing for nut pumpkins production. In particular, the reasons for this increase of cultivation in recent years are low production costs and longer seed storage time (Altınok et al., 2019). Pumpkin seeds (*Cucurbita pepo* L.) are known to be nutritious and beneficial for health because of being rich in protein, fiber, vitamins, and minerals such as iron, zinc, calcium, magnesium, and copper (Syed et al., 2019; Valdez-Arjona and Ramírez-Mella, 2019; Alshehry, 2020). Pumpkin seeds and its oil are used in herbal medicine for the treatment of different diseases. Some studies show their positive effects on diseases such as diabetes, high cholesterol, liver functions, gallbladder, prostate

gland, depression, and gastrointestinal parasites (Syed et al., 2019; Valdez-Arjona and Ramírez-Mella, 2019; Alshehry, 2020; Majid et al., 2020). The wastes generated in food production from the agricultural origin are often used as animal feed or disposed (Konca, 2014; Yanmaz, 2014). Pumpkin seed is surrounded by a soft shell and a natural membrane. It is known that this natural membrane has the potential to pollute the environment after the seeds are roasted (Çankaya and Özcan, 2017; Syed et al., 2019; Alshehry, 2020). Nuts with a high rate of waste formation are pumpkin seeds, pistachios, hazelnuts, almonds, carobs, peanuts, and sunflower seeds (Gültepe and Bayram, 2019; Valdez-Arjona and Ramírez-Mella, 2019; Gültekin Özgüven et al., 2020). As the nuts industry wastes contain some nutrients required for animal, the objective of this study aimed to evaluate the use of PSM. The PSM is surrounded by a thin membrane-coating separated from the core during roasting. It cannot be used in human and animal nutrition in contrast to the other plant seed wastes. According to recent studies, these membrane structures are powdered with a special application and found to be available as natural additives in the clothing and paper industries. This substance has high water retention properties, which makes PSM usable (Çankaya and Özcan, 2017). Most bioactive phytochemicals are found in the outer shell of the seed (Liu et al., 2014). Thus, in this study, it was aimed to test the bioavailability of pumpkin seed membrane (PSM) in an animal model. In nutritional research, the fruit fly *Drosophila melanogaster* Meigen is used as an important model organism. This fly is fed by a daily standard diet that includes glaze or potatoes, yeast, sugar sucrose, fungicide, and agar. The number of replicates has no ethical concern, its easy supply and cultivating in laboratory conditions make it the preferred model. To perform studies on diseases such as obesity and diabetes, which are the result of poor nutrition today, necessary research can be done by adding the desired products (high-fat diet, high sugar diet, etc.) to the standard diet (Lüersen et al., 2019; Poças et al., 2020). This study also evaluated the effect of feeding with PSM on the model organism's development and lifespan. Besides, the use of PSM in the laboratory was tested by using its water retention capacity. As recycling has become an important issue, this study also aimed to determine whether the PSM can be disposed of in the soil.

MATERIAL and METHOD

In this study, commercial (Pınar Nuts Products, Nevşehir type, pointed pumpkin) PSM produced in 2018-2019 was used by adding to the standard insect diet (SD) without freezing and after sterilization (30-40^{o}C) and removal of rough parts. The flies used in the experiments were the White type (W^{1118}) strain of *D. melanogaster* (Drosophilidae). This stock had been maintained for five years in the Laboratory. The flies were kept at a constant temperature of 25 ± 2°C on an SD composed of potato flour, sucrose, dried yeast, agar, nipagin, and vitamin C (Güneş, 2016). The flies were kept in 12 hours at the light and 12 hours at darkness except during the transfers onto a fresh medium (usually once weekly). The humidity of the experimental chamber was 70-60%.

Experimental Design

In this study, for a High-fat diet (HFD), palmitic acid was dissolved within Tween-80 (1%) and added 20% to the hot SD (Sun et al., 2010). HFD groups were added to experiments because of the increased fat intake in non-target organisms such as humans. Similar to the previous study (Güneş and Biçer Bayram, 2019), the upper and lower limits of PSM use were determined. The six different amounts of PSM (0.05, 1 and 2 g) were added to 100 ml of SD. All of the experiments were carried out under conditions where the stock culture of insects was grown. From the culture, the females and males (5 virgin/7 male; N: 100/each of trials) with the same age were used for experiments, they mated and the eggs were collected after six hours. The culture vials containing only the SD were used as negative control; high fat containing diets were used as a positive control (PC). Firstly, newly 1st stage larvae (72 hour, N: 100) were grown up to the adult stage by inoculating on the experimental pattern. The larvae of the insect were grown up to the adult stage with the PSM in different concentrations and the developmental stages were followed daily. After pupation, pupae were removed, offsprings were counted every day, and sexes were determined by Stereomicroscope (SZ61, Olympus, C3X33, Japan). Secondly, the same age mated adults were raised in the vials including SD, PSM, and PC. The effects of PSM on longevity were studied separately in 25 females and 25 males, which were grown in the experimental nutrients. Foods were replaced with fresh ones twice a week, and dead-alive adults were recorded. The experiment was conducted until the last fly died. The experiments were repeated 4 times in thermal cabins (Çolak et al., 2014).

Statistical analyses

One-way Variance Analysis (ANOVA; SPSS version 18.0, Inc., Chicago, IL, USA) and LSD tests were used to determine the importance of the difference between averages. Survival rates and longevity were analyzed by the Chi-square test. The longevity values of the experimental groups were subjected to Duncan's one-way range test (P<0.05).

RESULTS

The development, sex ratio, and longevity data are presented in the table below (Table 1; Figure 1). As data were evaluated in pupae fed by PSM, the highest survival ratio was obtained in pupae fed with 0.05 g PSM (99.5%). It was observed that increasing the rate of PSM (1 and 2 g) reduced the rate of larval survival or even reduced the lethal concentration below 50, and this decrease cannot be prevented by adding PSM with the HFD added to the feed. In larval developmental times; although only PSM additions were statistically very similar compared to control, it was found that the development of the insects fed with the HFD group increased 2-fold, and with the addition of PSM, the developmental time extended 3 times of the normal developmental time (approximately 12days) (Table 1). The survival rate in the pupal period was found to be 100% in 0.05 g PSM, 70% at 1 g and 20% at 2 g. These rates changed with HFD and the rate of pupae decreased from 70% in 1 g PSM to 50% with the addition of HFD to the feed. In terms of the developmental time of being pup; contrary to what was observed in the larval phase, the addition of PSM extended the pupal development period and even reached 3-3.5 times the duration with the addition of HFD and PSM to the feed (Table 1). While the rate of adult life in the group fed with 0.05 g PSM was around 99%, its increased amount in the feeding (2 g) was found to reduce the survival ratio by 4.5 times. With the addition of HFD to the feed, the survival rates of adults remained the same when 2 g PSM was added. When adult development times were evaluated, they were extended 3-4 days with PSM addition, 5 days with HFD addition, and approximately 11 days in the adult when both were added (Table 1). Although the change in sex ratio was not statistically different between PSM and the other groups, the proportion of male individuals in HFD-fed insects was greater than the proportion of females (Güneş and Biçer Bayram, 2019).

Çizelge 1. Kabak çekirdeği membranı (KÇM) ile oluşturulan besin gruplarının D. melanogaster yaşama, gelişim ve cinsiyet oranına etkisi

Table 1. Effect of nutritional groups formed with pumpkin seed membrane (PSM) on D. melanogaster survival, development time and sex ratio

(g/L)	Survival to third instar (%) (M*± S.E)	Time to third instar (days) (M*±S.E)	Survival to pupal stage (%) (M*±S.E)	Time to pupal stage (days) (M*±S.E)	Survival to adult stage (%) (M*± S.E)	Time to adult stage (days) (M*± S.E)	Sex ratio (%) Female/Male (M*± S.E)
Control	98.8 ± 0.1^{a}	3.81 ± 0.2^{ab}	90.8 ± 0.1^{a}	4.80 ± 0.2^{a}	80.4 ± 0.2^{b}	7.35 ± 0.1^{a}	55/54±0.5^{d}
HFD	40.3 ± 1.7^{cd}	6.57 ± 2.2^{c}	40.0 ± 0.8^{c}	9.14 ± 0.5^{c}	40.0 ± 2.3^{c}	12.75 ± 0.2^{c}	26/74±0.4^{a}
PSM 0.05g/L	99.5 ± 0.1^{a}	4.4 ± 0.2^{b}	99.5 ± 0.1^{a}	7.8 ± 0.2^{b}	99.5 ± 0.2^{a}	10.8 ± 0.1^{b}	50/50±0.5^{c}
PSM 1 g/L	70.0 ± 0.1^{b}	4.7 ± 0.2^{b}	70.0 ± 0.1^{b}	8.4 ± 0.2^{bc}	70.0 ± 0.2^{b}	10.7 ± 0.1^{b}	43/57±0.5^{b}
PSM 2 g/L	20.0 ± 0.1^{d}	3.0 ± 0.2^{a}	20.0 ± 0.1^{d}	8.0 ± 0.2^{b}	20.0 ± 0.2^{d}	11.5 ± 0.1^{c}	50/50±0.5^{c}
PSM 0.05g/L+HFD	71.3 ± 1.8^{b}	7.8 ± 0.2^{c}	70.5 ± 2.1^{b}	11.0 ± 0.2^{d}	70.3 ± 2.1^{b}	14.3 ± 0.4^{d}	50/50±2.1^{c}
PSM 1 g/L + HFD	51.3 ± 1.8^{c}	10.3 ± 0.2^{d}	50.5 ± 2.1^{c}	13.1 ± 0.4^{e}	50.3 ± 2.1^{c}	16.3 ± 0.4^{e}	50/50±1.73^{c}
PSM 2 g/L + HFD	21.0 ± 1.8^{d}	11.8 ± 0.2^{e}	20.5 ± 2.1^{d}	15.3 ± 0.4^{f}	20.0 ± 2.1^{d}	18.3 ± 0.4^{f}	50/50±1.29^{c}

*Average of four replicates, 100 larvae used for each repeat, ^{a,b,c,d,e,f} Values within a row with different superscripts differ significantly at P<0.05 (χ2 test, LSD Test), Control: Standard insect diet, M: Mean, HFD: Hihg-fat diet (20%), PSM: Pumpkin seed membrane, S.E (Standart Error)

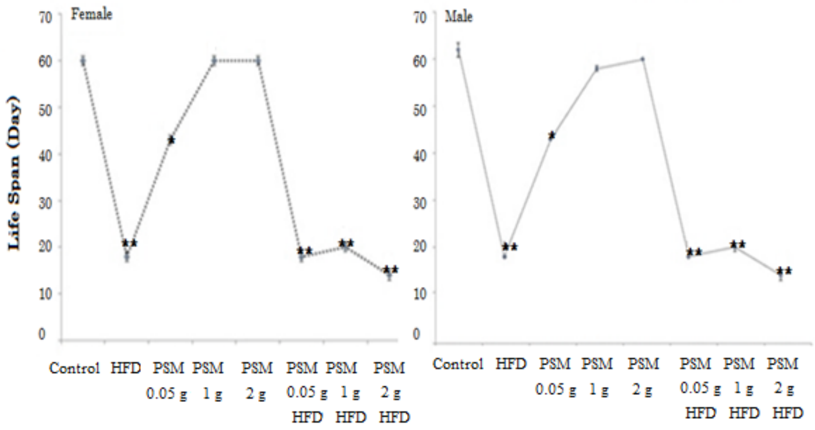
Çizelge 2. Kabak çekirdeği membranı (KÇM) ile oluşturulan beslenme gruplarının böceğin ömür uzunluğuna etkisi

Table 2. The effect of feeding groups formed with pumpkin seed membrane (PSM) on the lifespan of the insect

(g/L)	Female Max. Life Span (N:100)	Female life span (days) (M. ± S.E)	Male Max. Life span (N:100)	Male life span (days) (M. ± S.E)
Control	62.5	60.0 ± 1.2^{a}	65.0	62.0 ± 1.5^{a}
HFD	21.5	18.0 ± 1.6^{b}	18.5	18.0 ± 0.3^{b}
PSM 0.05g/L	43.0	43.0 ± 0.1^{c}	43.0	43.0 ± 0.1^{c}
PSM 1 g/L	62.5	60.0 ± 1.2^{a}	59.5	58.0 ± 0.6^{a}
PSM 2 g/L	60.5	60.0 ± 0.3^{a}	60.0	60.0 ± 0.1^{a}
PSM 0.05g/L + HFD	18.0	18.3 ± 0.1^{b}	18.0	18.3 ± 0.1^{b}
PSM 1 g/L + HFD	39.0	20.0 ± 1.0^{b}	36.0	20.0 ± 0.8^{b}
PSM 2 g/L + HFD	18.3	18.0 ± 1.0^{b}	18.3	18.0 ± 1.0^{b}

^{a,b,c,d,e,f} Values within a row with different superscripts differ significantly at P<0.05 (Duncan test), Max=Maximum, Control: Standard insect diet, M: Mean, HFD: Hihg-fat diet (20%), PSM: Pumpkin seed membrane, S.E (Standart Error)

As for females, while the control group is statistically similar and had near-average longevity compared to groups fed with 1 and 2 g PSM, HFD was found to negatively affect the length of life and reduced it approximately 2-3 times. In the PSM and HFD group, it was determined that there were individuals between 18-20 days old and that the length of life decreased by 3% compared to other groups without changes in male and female ratio (Table 2). If the longevity of male individuals was evaluated, similar results occurred in females, but the maximum longevity of males fed with fat and 1 g PSM was 3 days shorter than females (Table 2; Figure 1).



Şekil 1. Kadınların ve erkeklerin ortalama ömür uzunluğu (gün); * P <0.05, ** P <0.01,
*Figure1. The average life span of females and males (days); *P<0.05, **P<0.01,*

DISCUSSION

Under normal circumstances, the rate of survival of *Drosophila melanogaster* can be as low as 98% in larvae and up to 80% in the development of the adult stage. The duration of development is about 7-8 days in total from larvae to adults (Güneş et al., 2017). In the study, it was determined that 2 g PSM prevented the development of the insects, decreased the rate of survival with the addition of HFD, and delayed the developmental period by 3-3.5 times in 18 days. This experiment was designed in line with the increased prevalence of obesity and fat intake in humans. Studies with *Drosophila* species showed that *Cucurbita pepo* L. led to 79% survival (Goñi et al., 1998). Nutrients and their ingredients can reduce or increase the rate of survival (Güneş, 2016). Palmitic acid, especially used in HFD, is known to negatively affect the development of life (Güneş et al., 2019). Feeding with a low amount of PSM increased the survival rate by approximately 5% compared to control group, and addition of PSM to the HFD caused a 14% decrease in the survival rate of adult insects. In the developmental period of the same individuals fed with 0.05 g PSM, the duration of adultness increased by approximately 3 days compared to control, while the duration of development increased by 6 days to14 days in those fed with fat-added diet.

In the study, there was no significant difference in sex ratios except for those fed HFD although nutrition affects sex ratio (Güneş et al., 2019). Research on nutrition and photoperiods supports the results of the present study (Koç and Gülel, 2006). However, while some nutrients increase the female ratio (Güneş et al., 2019), sometimes an increase in the male ratio can be observed (Arıca et al., 2017). Since sex ratio determines the population density, amount, and variety of nutrients affect the survival, reproduction, and egg-laying of the species, thereby directly influence population size (Koç and Gülel, 2006).

The addition of HFD to the diet causes the larvae to become a gel and remain small, which decreases the percentage of survival, but the nutrients taken during the larval period are known to affect survival, adult size, and rate of development (Chippindale et al., 1997). In insects, environmental factors such as stress, temperature, climate, light, nutrition, humidity as well as genetic factors such as mutation or inter-species diversity affect life longevity and aging (Carey et al., 2002; Çakır and Sarıkaya, 2004; Uysal et al., 2015). Population density affects body size and maturity, while maturity affects the length of life in individuals (Benli and Türkoğlu, 2017). Therefore, in this study, life-longevity experimental groups were designed where only the nutrition varied among groups. Under normal circumstances, the life length of female and male individuals can vary between 60-80 days (Uysal and Semerdöken, 2011; Uysal et al., 2015). In this study, it was determined that females lived an average of 62 days and males lived about 65 days, and these lengths were not affected by the addition of PSM to the diet. However, with HFD added to the diet, it was determined that the life span was shorter 47 days in individuals, the addition of PSM did not affect the longevity (Table 1). Although it is known that under normal circumstances males live longer than females (Lints et al., 1983; Good and Tatar; 2001; Koç and Gülel, 2006), in this study females live a day or two more when the average days of life were compared between males and females. Similar nutritional experiments with different herbal extracts (Çolak et al., 2014) support in this study results of female and male life longevity in the study. Factors such as dietary content, egg formation, and early maturation are thought to be the factors that reduce the length of life of females (Prowse and Partridge, 1997; Good and Tatar, 2001; Arking et al., 2002; Novoseltsev et al., 2003). In this study, there was a negative correlation between PSM+HFD and lifetime in female and male individuals (R=0.110 for males and R=0.180 for females).

CONCLUSION

Vegetable wastes are either re-used as animal feed or disposed, with or without processing, directly or indirectly to nature. In this study, the effects of PSM, which is known to cause discomfort in the lungs of animals when taken through inhalation, were investigated to determine whether swallowing/eating would cause similar negative outcomes. Only the addition of PSM at a low amount (0.05 and 1 g) to the diet was determined not to adversely affect the development of the insects and their longevity. However, the intake of PSM together with HFD prevented the formation of eggs, reducing the population density and survival-development. PSM, which can hold water, is considered not to be available in the laboratory due to usage limitation at low amounts (g/L) and a high amount of toxic properties. Detailed studies are needed to evaluate its safe use as animal feed. With this approach presented in this study, the basis for advancing studies is thought to be established.

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Researchers' Contribution Rate Statement Summary

The authors declare that they have contributed equally to the article.

Conflict of Interest Statement

There is no conflict of interest between the article authors.

KAYNAKLAR

Alshehry GA 2020. Preparation and nutritional properties of cookies from the partial replacement of wheat flour using pumpkin seeds powder. World 9: 48-56.

Altınok H, Çolak EŞ, Altınok MA 2019. Kayseri ili çerezlik kabak meyvelerinde çürüme: risk faktörleri ve öneriler. Ziraat Fakültesi Derg 14: 166-176.

Arıca ŞÇ, Demirci S, Özyılmaz A, et al. 2017. The effects of some macroalgae on viability of *Drosophila melanogaster*. Su Ürünleri Derg 34: 451-454.

Arking R, Buck S, Novoseltev V, et al. 2002. Genomic plasticity, energy allocations, and the extended longevity phenotypes of *Drosophila*. Ageing Research Reviews 1: 209-228.

Benli D, Türkoğlu Ş 2017. The effect of some food preservatives on percentage of survival and longevity in *Drosophila melanogaster*. Cumhuriyet Science Journal 38: 461-472.

Carey J, Liedo P, Harshman L, et al. 2002. Life history response of mediterranean fruit flies to dietary restriction. Aging Cell 1: 140-148.

Cesur R, Avcıkurt C 2019. Gastronomi turizmi kapsamında doğu karadeniz yerel yiyeceklerinin tanınma ve deneyimlenme durumları üzerine bir araştırma. IVth International Gastronomy Tourism Studies Congress 19-21 September (Ugtak), pp.698-705, Nevşehir.

Chippindale A, Alipaz J, Chen H, et al. 1997. Experimental evolution of accelerated development in *Drosophila*. 1. developmental speed and larval survival. Evolution 51: 1536-1551.

Çakır Ş, Sarıkaya R 2004. Bazı organik fosforlu insektisitlerin *Drosophila melanogaster*'in yaşama yüzdesi üzerine etkisi. Gazi Eğitim Fakültesi Derg24: 71-80.

Çankaya N, Özcan M 2017. A new functional additive obtained by using jet pulse fılters: pumpkin seed membrane. Tabkon pp.343.

Çolak DA, Ayar A, Uysal H 2014. The effects of *Punica granatum* L ethanol extract including the antioxidant flavonoids on *Drosophila melanogaster* lifespan. Journal of Applied Biological Sciences 8: 06-09.

Goñı B, Martinez M, Valenta V et al. 1998. Preliminary data on the *Drosophila* species (Diptera, Drosophilidae) from Uruguay. Revista Brasileira de Entomologia 42: 131-140.

Good T, Tatar M 2001. Age-specific mortality and reproduction respond to adult dietary restriction in *Drosophila melanogaster*. Journal of Insect Physiology 47: 1467- 1473.

Güleç H, Durlu-Özkaya F 2019. Gastronomi turizminde Bursa. IVth International Gastronomy Tourism Studies Congress 19-21 September 2019. pp.329-336, Nevşehir.

Gültepe E, Bayram İ 2019. Bitkisel ve endüstriyel atıkların ruminant beslemede etkin kullanımının hayvan verimi üzerine etkileri. Turkiye Klinikleri Animal Nutrition and Nutritional Diseases-Special Topics 5: 14-21.

Güneş E 2016. Besinler ve beslenme çalışmalarında *Drosophila*. KSÜ Doğa Bilimleri Dergisi 19: 236-243.

Güneş E, Biçer Bayram Ş 2019. Kabak çekirdeği zarının in vivo kullanımı. Ganud International Conference On Gastronomy, Nutrition And

Dietetics pp.303-310. Gaziantep.

Güneş E, Biçer Bayram Ş, Erçetin H 2019. Palm yağının in vivo kullanımı. Ulusal Çevre Bilimleri Araştırma Dergisi 2: 61-68.

Güneş E, Erdal MO, Gemi L 2017. The effect of nanofiber on the biological traits of *Drosophila melanogaster*. Sakarya Üniversitesi Fen Bilimleri Enstitüsü Derg 21: 1608-1612.

Kahraman Ü, Etyemez S, Gençoğlu U 2019. Hatay mutfağında tatlı ve reçeller. IV^th^ International Gastronomy Tourism Studies Congress 19-21 September (Ugtak). pp.512-520, Nevşehir.

Koç Y, Gülel A 2006. Fotoperiyot ve besin çeşidinin *Drosophila melanogaster* Meigen, 1830 (Diptera: Drosophilidae) un gelişim süresi, ömür uzunluğu, verim ve eşey oranına etkisi. Anadolu Tarım Bilimleri Derg 21: 204-212.

Konca Y 2014. Çekirdek Kabağı Artıklarından Silaj Yapma İmkanları. Çerezlik Kabak Çalıştayı pp.88-98.

Lints FA, Bourgois M, Delalieux A, et al. 1983. Does the female life span exceed that of the male: a study in *Drosophila melanogaster*. Gerontology 29: 336-352.

Liu T, Han Yu X, Gao E, et al. 2014. Hepatoprotective effect of active constituents ısolated from mung beans (*Phaseolus radiatus*) in an alcohol induced liver injury mouse model. Journal of Food Biochemistry 38: 453-459.

Luersen K, Röder T, Rimbach G 2019. *Drosophila melanogaster* in nutrition research the importance of standardizing experimental diets. Genes Nutrition 14: 1-5.

Majıd AK, Ahmed Z, Khan R 2020. Effect of pumpkin seed oil on cholesterol fractions and systolic/diastolic blood pressure. Food Science and Technology 40: 1-10

Novoseltsev V, Novoseltseva J, Boyko S et al. 2003. What fecundity patterns indicate about aging and longevity: Insights from *Drosophila* studies. Journals of Gerontology Series A-Biological Sciences and Medical Sciences 58: 484-494.

Özgüven M, Beyde B, Özçelik B 2020. Atıkların değerlendirmesi: fındık (*Corylus avellana* L.) ve antep fıstığı (*Pistacia vera* L.) iç zarlarından elde edilen fenolikçe zengin ekstraktlara lipozomal taşıma sistemlerinin uygulanabilirliği. Avrupa Bilim ve Teknoloji Derg 19: 241-246.

Poças GM, Crosbie AE, Mirth CK 2020. When does diet matter? the roles of larval and adult nutrition in regulating adult size traits in *Drosophila melanogaster*. Journal of Insect Physiology 104051.

Prowse N, Partridge L 1997. The effects of reproduction on longevity and fertility in male *Drosophila melanogaster*. Journal of Insect Physiology 43: 501-512.

Sun X, Seebergerb J, Alberico T et al. 2010. Açai palm fruit (*Euterpe oleracea Mart.*) pulp improves survival of flies on a high fat diet. Experimental gerontology 45: 243-251.

Syed QA, Akram M, Shukat R 2019. Nutritional and therapeutic importance of the pumpkin Seeds. Seed 21: 15798-15803.

Tuncer B, Yanmaz R 2011. Hibrid çerezlik kabak (*Cucurbita pepo* L.) tohumlarında yağ asitleri ve e vitamini içeriğinin belirlenmesi. Türkiye 6. Ulusal Bahçe Bitkileri Kongresi, 383-386.

Uysal H, Semerdöken S 2011. Sentetik Gıda Boyalarının *Drosophila melanogaster*'in Oregon R Soyunda Larval Toksisite ve Ergin Ömür Uzunluğu Üzerine Etkilerinin Belirlenmesi. Kafkas Üniversitesi Fen Bilimleri Enstitüsü Dergisi 4: 71-87.

Uysal H, Unver S, Kizilet H 2015. The effects of neonicotinoids on the longevity of the male and female populations of *Drosophila melanogaster*. Ekoloji Dergisi 24: 57-63.

Valdez-arjona LP, Ramírez-Mella M 2019. Pumpkin waste as livestock feed: ımpact on nutrition and animal health and on quality of meat, milk, and egg. Animals 9: 769.

Yanmaz R 2014. Türkiye'nin çekirdek potansiyeli. Çerezlik Kabak Çalıştayı Bildirileri pp.1-12.