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# EFFECT OF FEEDING WITH FROZEN AND DRIED POLLEN ON THE DEVELOPMENT OF BEE COLONIES

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***Abstract***

*This study aimed to examine the impact of feeding with frozen and dried pollen on the development of bee colonies (Apis mellifera L.). The study monitored changes in the strength of the bee colonies (SBC), the amount of sealed worker bee brood (SWBB), and food reserves (honey and pollen) before and after feeding during the Autumn and Spring periods. The experiment was conducted with one control group (CG) and two experimental groups - two bee colonies fed with frozen pollen (GFFP) and two bee colonies fed with dried pollen (GFDP). The results showed that during the Spring period, the SBC fed with frozen pollen was 36.52% higher than the SBC of the CG. For the same period, a higher value of the amount of pollen and the amount of SWBB was also observed in the GFFP compared to its value in the CG. In the Autumn period, the amount of brood in GFDP was 51.20% more than in the CG. In the GFFP, a greater amount of brood was also found compared to the CG, with the difference being 48.30%.*

***Key words**: Apis mellifera L., development of bee colonies, feeding, frozen and dried pollen.*

## INTRODUCTION

The development of bee colonies throughout the year is a dynamic process associated with adaptation to constantly changing environmental factors (Cebotari & Buzu, 2023). The number and functions of the separate individuals, as well as the relationships between them, are constantly changing and in this way, the maximum utilization of the available honey-bearing vegetation is reached.
According to Nedyalkov et al. (1991), the year-round life of the colony is divided into two periods - a period of active work and a period of relative rest, associated with the seasons spring - summer and autumn - winter, respectively. The period of active work begins with the laying of the first eggs by the queen bee in January - February. Bee activity increases when they begin to feed the first larvae in the hive. With the increase in the proportion of flowering vegetation in the spring, the brood in the bee colonies gradually increases and reaches its amaximum in May-June, when in one day the queen bee lays 1500-2000 eggs. At the beginning of the main pasture, the colony reaches the highest point in its development during the year. Collecting pollen in autumn is very important for replenishing the reserves in the bees' fat body. This, in turn, ensures proper wintering and early brood rearing in the spring (Bizhev et al., 2003).
In periods when brood is being raised in the bee nests, the "nurse" bees need a large amount of protein, which they obtain from the pollen entering the hive. It is carried by the "forager" bees. They use the secretion of their salivary glands to shape the collected pollen from plants into pollen grains, which they place in baskets made of hairs on the third pair of legs (Garbuzov & Ratnieks, 2014). When the "forager" bees arrive at the hive, they place the pollen grains in the comb cells, compacting them, replenishing the cells with honey and finally sealing them with wax caps (Nicolson et al., 2018).
After the pollen cells in the bee comb are sealed, lactic acid fermentation takes place with the participation of strains of lactic acid bacteria (Nepi et al., 2018). Thanks to the fermentation, acidity increases and thus prevents unwanted processes in the pollen, and also improves its nutritional value. After these changes in the pollen, it becomes "bee bread", which is the main source of protein for bees. The availability of pollen in bee colonies is particularly important in the production of royal jelly and the rearing of larvae and young worker bees (Abou-Shaara, 2014).
The development of modern agriculture has largely led to a change in the natural habitats of

pollinators. This also limits the diversity of floral resources for honey bees (Biesmeijer et al., 2006). Furthermore, seeding large areas with agricultural crops of the same species reduces the diversity of flowering plant species within the flight radius of bees around hives (Grundel et al., 2010; Rands & Whitney, 2010). Mass flowering monocultures provide temporary availability of nectar and pollen for bees (Todd et al., 2016). For these reasons, the loss of biodiversity of floral resources can lead to suboptimal bee nutrition, which in turn leads to reduced bee immunity and poor health (Dolezal et al., 2019; De Grandi-Hoffman et al., 2010).

The natural foods for bees (nectar, manna, honey, pollen, royal jelly) contain all the nutrients necessary for the normal development of bee individuals. Regardless, in beekeeping practice, supplemental feeding of bee colonies is practiced, aiming to compensate for the lack of food coming into the bee colonies from nature. Supplemental feeding of bee colonies is also relevant in connection with global warming and climate change. The presence of long periods of drought and lack of nectar and pollen in nature leads to stress in the development of the colonies and to their death.

Regarding the proper breeding and development of bee colonies, we consider it reasonable to study the effect of supplemental feeding of bees with dried and freshly collected frozen pollen.

## MATERIALS AND METHODS

The survey was conducted in the fall of 2022 and the spring of 2023 at the Educational Experimental Base of the Beekeeping section at the Faculty of Agriculture of Trakia University - Stara Zagora. Feeding of bee colonies of the local honey bee (*Apis mellifera* L.) settled in Dadant-Blatt 10-frame hives was carried out. The colonies were previously equalized by the age of the queen bees, strength, amount of brood, and food reserves. Two experimental and one control groups were formed with two bee colonies each, as follows:

- **1$^{st}$ Experimental group (GFDP)** – six feedings at seven days' intervals with 160 g polyfloral bee honey mixed with 40 g of dried pollen;

- **2$^{nd}$ Experimental group (GFFP)** – six feedings at seven days' intervals with 160 g polyfloral bee honey mixed with 40 g of frozen fresh pollen;

- **Control group (CG)** – six feedings at seven days' intervals with 200 g polyfloral bee honey;

The development of the bee colonies from the experimental and control groups was monitored in terms of the following indicators - strength, amount of sealed worker brood and food reserves (honey and pollen) in the autumn and spring periods, before and after feeding. Classic methods established in beekeeping were used to determine the indicated parameters.

**1. Determining the strength of bee colonies (SBC)**

To determine the strength of the bee colonies, it is taken into account that in the Dadant-Blatt hive, a densely occupied inter-frame space accommodates about 0.250 kg of bees. The inter-frame spaces occupied with bees are counted and their number is multiplied by 0.250 kg. Since the weight of one worker bee is approximately 100 mg, it is assumed that there are 10,000 bees in 1 kg.

**2. Determining the amount of sealed worker brood (SWBB)**

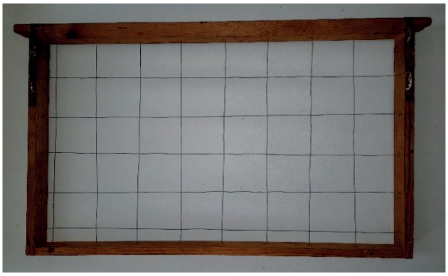
When determining the amount of sealed worker brood, a measuring frame with 5/5 cm squares was used, which was placed on the brood comb (Figure 1). One square of the measuring frame has an area of 25 $cm^2$. One square centimeter accommodates 4 worker cells, and 25 $cm^2$ – 100 cells (Nenchev & Zhelyazkova, 2010).

From the specified amount of brood, the bees will hatch in a period of 12 days, because the pupa stage (sealed brood) lasts 12 days. For this reason, the brood measurement is carried out over 12-day periods.

**3. Determining the amount of sealed honey and pollen in the combs**

- amount of honey – only the sealed honey is determined

To determine the area of sealed honey, a measuring frame with 5/5 cm squares is placed over the measured honeycomb (Figure 2). The area of one square in the measuring frame is 25 $cm^2$.



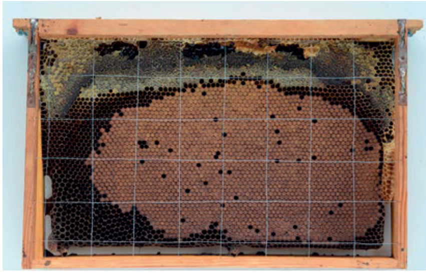


Figure 1. Determining the area of sealed brood with a measuring frame (own source)

Figure 2. Determining the area of sealed honey with a measuring frame (own source)

When determining the amount of honey in kilograms, it is assumed that there is 0.350 kg of honey on an area of 1 $dm^2$ (100 $cm^2$) of the comb filled on both sides, 0.175 kg on one side, respectively (Nenchev & Zhelyazkova, 2010). After counting the filled squares, the area of the sealed honey is determined by the following formula (Lazarov & Dineva, 2022):

$S_{hf} = \text{n}.25, \text{cm}^2$

where $n$ is the number of squares filled with honey.

The total amount of honey on one side of the comb is calculated by the following formula:

$$Q_{hf} = \frac{S_{hf}.0{,}175}{100}, kg$$

where: Qhf – amount of honey, kg;
Shf – area of the sealed honey, $cm^2$

- amount of pollen – determined with a measuring frame with 5/5 cm squares. Within one square of the measuring frame, there are 25 $cm^2$. The measuring frame is placed on the pollen combs counting the squares filled with pollen.

Statistical software IBM SPSS Statistics 26.0 (NY, USA) was used for data processing. Univariate ANOVA analysis was used to study the effect of supplemental feeding of bee colonies with frozen and dried pollen on the development of bee colonies in the autumn and spring periods. Significant differences between the groups at $p < 0.05$ were calculated by the Post Hoc procedure with the Scheffe test. Levene's test was applied to verify the data variations.

## RESULTS AND DISCUSSIONS

### 1. Bee colony strength

Figure 3 shows the results from the Univariate ANOVA analysis regarding the bee colony strength for the periods after spring and autumn supplemental feeding with dried and frozen fresh pollen.

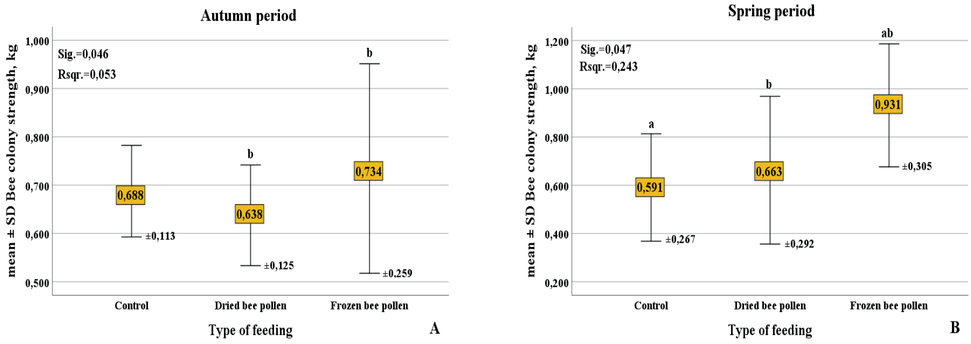



Figure 3. Strength of bee colonies, kg

In the autumn period after feeding the bee colonies, close average values of the strength of the bee colonies were found in the experimental and control groups: GFDP - 0.638±0.113 kg, GFFP - 0.734±0.125 kg and CG - 0.688±0.113 kg, respectively. A significant difference was reported between the two experimental groups (Figure 3A).

A significant difference in the strength of bee colonies fed with frozen pollen and the CG was observed during the spring period (Figure 3B). The strength of bee colonies from this group was 0.931±0.305 kg, and for colonies from the CG, it was 0.591±0.267 kg. The indicated difference is 36.52%, which is indicative of the effect of feeding with frozen pollen. Probably, with this way of storing the pollen, its qualities are preserved to the greatest extent compared to drying it. De Grandi-Hoffman et al. (2016) found that feeding bee colonies with floral pollen before wintering and in early spring positively influences their wintering ability and supports the development of bee colonies during the spring period. The authors establish that bees fed with floral pollen were stronger, healthier, and more active. In colonies fed with dried pollen during this period of the year, the difference compared to the control group is insignificant - 11%. Similar to the autumn period, a statistically significant difference between the two experimental groups fed with frozen and dried pollen was recorded in the spring. The coefficients of determination $R^2$ (0.053; 0.243) for both periods show that from 5.3% to 24.5% of the variations in the SBC are due to the type of feeding. This again confirms the significance of the type of pollen used for feeding to the strength of bee colonies.

## 2. Amount of honey

Figure 4 presents the results from the Univariate ANOVA analysis regarding the presence of sealed honey in bee nests for the period after spring and autumn feeding with dried and frozen fresh pollen.

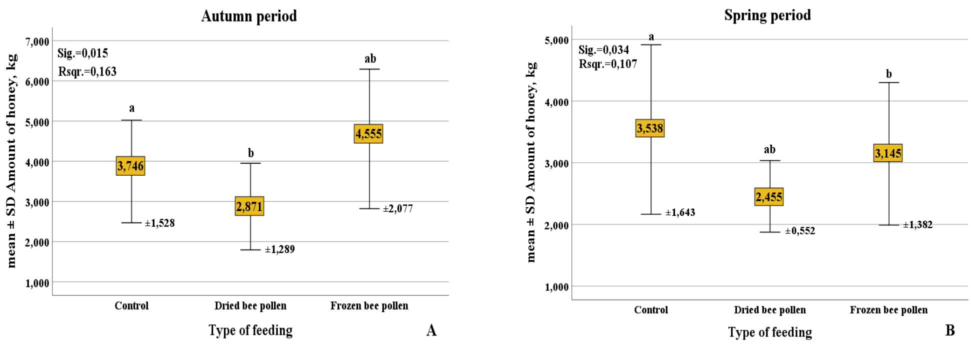



Figure 4. Amount of honey in bee colonies, kg

In the autumn period, it was found that in the GFFP, the average value of the amount of honey was 4.555±2.077 kg, which is 17.76% higher compared to the value of this parameter in the colonies from the CG. A probable reason for this is that during this period the strength of colonies (number of bees) from this group was higher (GFFP - 0.734±0.125 kg) and CG (0.688±0.113 kg), respectively (Fig. 3A). The analysis of the results for the autumn period illustrated on Figure 4A shows that the average value of the amount of honey of the GFDP (2.871±1.289 kg) was lower than that of the CG (3.746±1.528 kg). It can be assumed that this is also due to the lesser strength of the colonies from the experimental group fed with dried pollen compared to the strength of the families from the CG (Figure 3A). A significant difference was found regarding the amount of honey in bee nests between the two experimental groups.

After the spring feeding, statistically significant differences were recorded between the average values of the amounts of honey obtained from the CG (3.538±1.643 kg) and the GFDP, as well as between the two experimental groups - GFDP (2.455±0.552 kg) and GFFP (3.145 ±1.382 kg). The coefficients of determination $R^2$ (0.107 and 0.163) for the two periods show that from 10.7% to 16.3% of the variations in the amounts of honey produced by the bee colonies are due to the type of their supplemental feeding, which reflects on their strength.

### 3. Surface area bee pollen

Figure 5 presents the results of the Univariate ANOVA analysis regarding the area of bee pollen in the combs stored by the bee colonies for the periods after the spring and autumn feeding with dried and frozen fresh pollen.

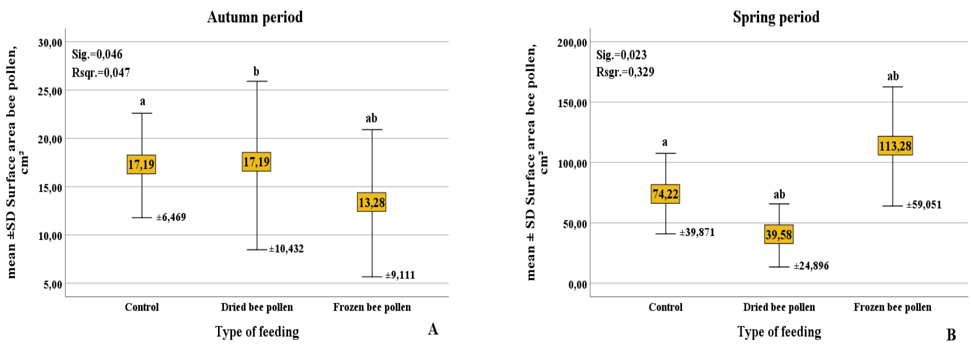



Figure 5. Bee pollen area, $cm^2$

When analyzing the results for pollen content in bee colonies after feeding, it was found that in the autumn period, the value of this parameter for the GFDP was the same as for the CG (17.19 $cm^2$), (Figure 5A). The value of the indicator in the GFFP pollen was even lower (13.28 $cm^2$). A possible reason for this is that bee colonies do not store the extra pollen supplied by the experiment.

During the spring period (Figure 5B), significantly larger areas of pollen in combs were observed in the GFFP than in the CG, 113.28±59.051 $cm^2$ and 74.22±39.58 $cm^2$, respectively. This difference is meaningful. It is possible that the bees mix and process the pollen that is additionally provided to them in the experiment with that which they bring into the hives from nature and store it in the combs. The presence of pollen in the bee colonies during this period is particularly important in the production of royal jelly and the rearing of the larvae and young worker bees (Abou-Shaara, 2014). Significant differences were found in the pollen content of bee colonies fed with dried and fresh frozen pollen, both in autumn and in spring. The coefficients of determination $R^2$ (0.047 and 0.329) for the two periods show that from 4.7% to 32.9% of the variations in pollen content of the combs are due to the type of feeding of the bee colonies.

### 4. Surface area capped brood

Figure 6 presents the results of Univariate ANOVA analysis on the area of sealed worker brood for the periods following spring and autumn feeding with dried and frozen fresh pollen.

Regarding the influence of supplemental feeding of the bee families and brood keeping in the autumn period, it was observed that in the colonies receiving dried pollen, the amount of brood was 390.63±337.781 cm$^2$, which is 51.20% more than in the CG (190.630±252.446 cm$^2$), (Figure 6). This is a significant difference, and bearing in mind that for the successful wintering of bee colonies, it is necessary to provide them with a large number of worker bees to hatch in the autumn, it is not to be neglected at all. Some authors establish a relationship between the nutritional value of pollen and the development, reproduction, and productivity of bee colonies (Radev et al., 2014). In the GFFP, a greater amount of brood was also found compared to the CG, the difference being 48.30% (Figure 6A). These results show the beneficial effect of supplemental feeding of bee colonies during the autumn period, when in nature the availability of pollen is limited. A similar statement is shared by other authors (Bizhev et al., 2003).

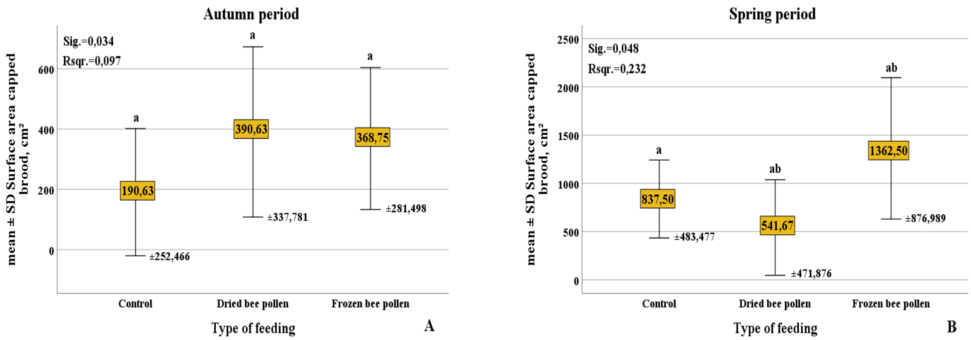



Figure 6. Area of capped worker brood, cm$^2$

Seasonal and climatic changes throughout the year lead to a significant decrease in resources from nectar-producing plants in nature (Şahin et al., 2015). When natural nectar-producing vegetation is insufficient, the egg-laying rate of the queen bee decreases, which in turn leads to a reduction in the quantity of brood and the number of bees in the bee colony.

Figure 6B shows that during the spring period feeding colonies with dried pollen does not lead to an increase in the amount of brood. In this group, its amount is less than in the CG. Analysing the results for this indicator in the GFFP during the spring period, it can be seen that the amount of brood is 38.58% higher than its level in the CG, and this difference is statistically significant (Figure 6B).

The coefficients of determination $R^2$ (0.097 and 0.232) for the two periods show that from 9.7% to 23.2% of the variations in the amount of brood are due to the type of feeding of the bee colonies.

## CONCLUSIONS

During the spring period, the strength of bee colonies from the group fed with frozen pollen was 0.931±0.305 kg, and for colonies from the control group, it was 0.591±0.267 kg. The indicated difference is 36.52%, which is indicative of the effect of feeding with frozen pollen during this period of the year. In the colonies fed with dried pollen during the same period of the year, the difference compared to the control group is insignificant - 11%.

During the spring period, significantly larger areas of pollen in the combs were observed in the group fed with frozen pollen than in the control group, 113.28±59.051 cm$^2$ and 74.22±39.58 cm$^2$, respectively. This difference is significant, and it is likely that the bees mix and process the pollen that is additionally provided to them in the experiment with the one they bring into the hives from nature and store it in the combs.

During the autumn period, it was observed that in the colonies receiving dried pollen, the amount of brood was 390.63±337.781 $cm^2$, which is 51.20% more than in the control group (190.630±252.446 $cm^2$). In the group fed with frozen pollen for this period, a greater amount of brood was also found compared to the control group, the difference being 48.30%. The results for this indicator show that the amount of brood in the group fed with frozen pollen in the spring period is 38.58% higher than its value in the control group.

For all studied parameters, significant differences were found between the two experimental groups, both in the autumn and in the spring period, which confirms the authors' thesis that the type of pollen used for supplemental feeding has a significant impact on the development of bee colonies.

## REFERENCES


Abou-Shaara, H. F. (2014). The Foraging Behaviour of Honey Bees, *Apis Mellifera*. *Veterinarni Medicina*. 59, 1–10.

Biesmeijer, J. C., Roberts, S. P. M., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., Schaffers, A. P., Potts, S. G., Kleukers, R., Thomas, C. D., Settele, J., & Kunin, W. E. (2006). Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science*, 313, 351–354.

Bizhev, B., Simidchiev, T., Mitev, B., Venov, B. & Nedyalkov, S. (2003). Practical beekeeping, *PH - Christian,* Sofia. [in Bulgarian]

Cebotari, V., & Buzu, I. (2023). Climatic changes of atmospheric precipitation and the vital activity of bees. *Scientific Papers. Series D. Animal Science, LXVI* (2), 30-43.

De Grandi-Hoffman, G., Chen, Y., Huang, E. & Huang, M. H. (2010). The effect of diet on protein concentration, hypopharyngeal gland development and virus load in worker honey bees (*Apis mellifera* L.). *Journal of Insect Physiology*, 56, 1184–1191.

De Grandi-Hoffman, G., Chen, Y., Rivera, R., Carroll, M., Chambers, M., Hidalgo, G. & de Jong E. W.(2016). Honey bee colonies provided with natural forage have lower pathogen loads and higher overwinter survival than those fed protein supplements. *Apidologie*, 47, 186–196.

Dolezal, A. G., Carrillo-Tripp, J., Judd, T. M., Allen Miller, W., Bonning, B. C. & Toth, A. L. (2019). Interacting stressors matter: Diet quality and virus infection in honeybee health. *Royal Society Open Science*, 6, 181803.

Garbuzov, M. & Ratnieks, F. L. W. (2014). Quantifying Variation among Garden Plants in Attractiveness to Bees and Other Flower-Visiting Insects. *Functional Ecology*, 28, 364–374.

Grundel, R., Jean, R. P., Frohnapple, K. J., Glowacki, G. A., Scott, P. E. & Pavlovic, N. B. (2010). Floral and nesting resources, habitat structure, and fire influence bee distribution across an open-forest gradient. *Ecological Applications,* 20, 1678–1692.

Lazarov S. & Dineva G. (2022). Determining the amount of capped honey in honeycombs with AutoCAD program. *Bulgarian Journal of Animal Husbandry*, 59(1), 23-31.

Nedyalkov, S., Lazarov, A., Mitev, B., Radoev, L., Bizhev, B., Petkov, V., Ivanov, T. & Nenchev, P. (1991), Bee encyclopedia, *Zemizdat*, Shumen. [in Bulgarian]

Nenchev, P & Zhelyazkova, I. (2010). Beekeeping. *Academic Publishing House*, Trakia University, Stara Zagora, Bulgaria, 138-147. [in Bulgarian]

Nepi, M., Grasso, D. A. & Mancuso, S. (2018). Nectar in Plant–Insect Mutualistic Relationships: From Food Reward to Partner Manipulation. *Frontiers in Plant Science,* 9, 1063.

Nicolson, S. W., Da Silva Das Neves, S., Human, H. & Pirk, C. W. W. (2018). Digestibility and Nutritional Value of Fresh and Stored Pollen for Honey Bees (*Apis mellifera scutellata*). *Journal of Insect Physiology,* 107, 302–308.

Radev, Z., Liolios, V., Tananaki, C. & Thrasyvoulou, A. (2014). The impact of the nutritive value of pollen on the development, reproduction and productivity of honey bee (*Apis Mellifera* L.) Bulg. J. Agric. Sci., 20, 685–689.

Rands, S. A. & Whitney, H. M. (2010). Effects of pollinator density-dependent preferences on field margin visitations in the midst of agricultural monocultures: A modelling approach. *Ecological Modelling,* 221, 1310–1316.

Şahin, M., Topal, E. & Özsoy N. (2015). Altunoğlu E. İklim Değişikliğinin Meyvecilik ve Arıcılık Üzerine Etkileri. J. Anatol. Nat. Sci., 6, 147–154.

Todd, K. J., Gardiner, M. M. & Lindquist, E. D. (2016). Mass flowering crops as a conservation resource for wild pollinators (*Hymenoptera: Apoidea*). *Journal of the Kansas Entomological Society,* 89, 158–167.