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POLLEN AND FUNGAL SPORE COMPOSITION VARIATIONS OF HONEYS ACCORDING TO DIFFERENT FEEDING METHODS

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Abstract

Honey samples were collected from two districts of Malatya (Eastern Turkey) Battalgazi and Dogansehir for melissopalynological analysis after the honey season in 2018. The survey was conducted to 3 different feeding groups, Glucose, Sucrose, Bee Feed and a Control group in each study area. The samples were prepared according to Louveaux *et al.*, (1978) procedure. Melissopalynological examinations were done to determine both pollen grains and fungal spores for each feeding group and districts. According to the microscopic analyses the dominant pollen types were determined as *Astragalus*, *Cistus*, Poaceae, *Verbascum*, *Echium*, *Berberis*, *Artemisia*, *Plantago*, *Vicia*, *Onobrychis*, Cichorioideae, *Astragalus* pollen grains were most frequent in glucose and control groups, *Cistus* pollen grains were widely represented in sucrose and bee feed groups in both study area. Dominant fungal spore types were determined as *Aspergillus / Penicillium*, Urediniospores, *Cladosporium* and *Myrotechium*. *Aspergillus / Penicillium* spores were dominated almost all samples by varying degrees. Analysis of pollen grains and fungal spores is useful instrument for determining the botanical, geographical and ecological sources of honey. This paper could be guide to beekeepers for selecting convenient apiary domains and appropriate feeding methods for qualitative honeys and the study is also help agriculturists for increasing the crop yield, the bees could be diverting to less-preferred plants during forages by sugar manipulation in order to cultivation improving.

Key words: Honey, Pollen, Fungal spores, Malatya, Turkey.

Introduction

Honey is a bee product, occurred by the treatment of nectar, with invertase enzyme secreted by salivary glands in honeysacs. Honey bees visit various flowers for collecting nectar and pollen to produce honey. The chemical composition of honey varies depend on its botanical and geographical origin. Simple carbohydrates (fructose and glucose) consist 82.4 % of total carbohydrates and amino acids, vitamins, minerals are also included in honey content (Jeffrey & Echazarreta, 1996; Hermosin *et al.,* 2003; Khan *et al.*, 2007). The different botanical and geographical origins supply the honey various sense as color, aroma, bitter-sweet-sour-sour to sweet-mixture taste and nutritional constitution (Ball, 2007; Suntiparapop *et al*., 2012; Chanchao, 2013; Agussalim *et al*., 2015; Agussalim *et al.*, 2019). Blossom honey could be divided into two groups. Monofloral honey is originated from one dominant species; polyfloral honey contains various plant taxa nectaries and pollen grains.

Proximately 60.000 worker bees take part in colonies of *Apis mellifera* L. and the huge number of individuals mean large amount of food requirement for nutrition, survival, development and health of the colony (Southwick & Heldmaier, 1987; Abou-Shaara, 2017). For such great colonies nectar is alone not sufficient, therefore beekeepers feed the colonies with different sugars (Abou-Shaara, 2017). Phloem liquid containing glucose, fructose and saccharose and the nectar obtain from this fluid (De la Barrera & Nobel, 2004). Inverted sugar (fructose and glucose) feeding could be more suitable for bees not to expend their biological sources because bees break down the saccharose into glucose and fructose during take nutriment but while this catabolic activity they deceive energy with the ratio 23.0 % (Zaboenko, 2000; Ceksteryte & Racys, 2006).

However if honeybee colonies are manipulated by sugar feeding they increase their visits for pollen because the honeybees ensure their sugar request from feeding as a consequence they do not need nectar but pollen requirement is proceed for development (Free, 1965; Gemeda *et al*., 2018). This situation is beneficial for plant pollination in that not only the rising of foraging quantity but also the diversity of plant visits increase thus the plant pollination ratio ascends due to bee contact to stigmas, not nectaries mostly at the base of flowers (Free, 1965; Gemeda *et al*., 2018). Herewith the manipulation could be use by people to increase the pollination of their own selected plants.

Honey is used for medicinal purposes by Egyptians, Greeks, Romans and Chinese traditionally for many years (Pasupuleti *et al*., 2017). It's nutritional and health beneficial properties make honey also popular nowadays in many cultures. For marketing quality of honey should be determined for have knowledge about its nutritional content. Determining mere chemical components is not enough for qualification of honey for all that knowing botanical, geographical and ecological origin is essential. Pollen grains give an idea about botanical and geographical origin however specifying of fungal spores give a chance to us make a decision about ecological origin of honey (Seijo *et al.*, 2011). Fungal spores are involved in honey either by the nectar sucking or by secondary contamination (Pérez-Atanes *et al*., 2001). Thus the spore collection could be active or passive way by honeybees (Parish *et al.*, 2020). To the former

researches fungal spores considered to be used as nutriment when the pollen diet is insufficient (Shaw, 1990; Parish *et al.*, 2020). Especially honey bee workers, feeding with fungal spores together with pollen grains, have longer span of life (Parish *et al*., 2020).

Melissopalynological searches are useful process for define the sources of honey. These studies have conducted by researchers for years around the world (Herrero *et al.*, 2002; Tatlidil *et al*., 2005; Ramos & Ferreras, 2006; Seijo *et al.*, 2011; Ramírez-Arriaga *et al*., 2011; Fagúndez, 2016; Gunes *et al.*, 2017; Bandeira & de Novais, 2020; Pavlova *et al.*, 2021; Radaeski & Bauermann, 2021). In this study we aimed to determine botanical, geographical and ecological sources of honey and the differences between two study areas. Besides we conducted this study on 3 different feeding methods for indicate the effect of sugar manipulating on pollen preferences of honeybees.

Materials and Methods

Study Area: Battalgazi and Dogansehir are districts of Malatya city and take parts in East Anatolian region of Turkey. Battalgazi is placed in eastern (38°42'47" N, 38°36'59" E) and Dogansehir district is in southern (38°09'48" N, 37°87'91" E) Malatya (Fig. 1). The city is floristically rich by the elements of 3 phytogeographic region as Irano-Turanian (42.81 %), Mediterranean (7.82 %) and Euro-Siberian (3.84 %) (Karakuş, 2016). Malatya city is located south-east of Anatolian Diagonal and the endemism ratio is 21.1 % and the most abundant families of native flora are Asteraceae, Fabaceae, Brassicaceae, Lamiaceae, Poaceae (Karakuş, 2016).

Malatya Mountains are constituted by the south branch of South-eastern Toros Mountains and Dogansehir Plain is the largest lowland area of the city (Yakar *et al*., 2014). Karakaya Dam borders Battalgazi and the altitude decreases from approximately 900 m to less than 700 m in this area (Arslan & Hayli, 2007).

Feeding methods: Glucose, sucrose and bee feed were used to determine the effect of different feeding methods on pollen variety in honey for beekeeping. The Caucasian race, *Apis mellifera caucasica* L. colonies supplemented with different sugars. Colonies were fed ad libitum (the supplement sugar types was added when it run out and the bees can reach at need) with plastic bee hive feeder (25X48X3cm) placed in hives. For Sucrose group (S) the syrup was prepared with commercial "crystallized granulated sugar", by the ratio 1:1 water. For Glucose group (G) commercial "glucose syrup" (Brix 82,DE 37, Dextrose 14, Maltose 12) was diluated with water by the ratio 1:1 and For Bee Feed group (BF) "Pasteurized Bee Feed Syrup" (sucrose; 30-36%, glucose; 27-30%, fructose % 37-40, dry matter 72% ± 2) was given to the colonies without any process. The Control group (C) were left the bees for visiting the flowers. Every feeding group, including control group, had 5 beehives and arranged side by side at the same location.

Palynological methods: The initial colonies were set up 22/05/2018 in Battalgazi and Dogansehir districts and the honey was collected on 30/08/2018 from four different fed hives. In Malatya honey collection could be done only one time in a year, month August, because of the arid climatic features of the city. The 8 samples were prepared according to the methodology of Louveaux *et al.*, (1978). Every feeding and control group were represented with 5 hives in both location and the pollen samples were prepared as 2 investigation material. Consequently glucose (G), sucrose (S), bee feed (BF) and control groups (C) were studied by 8 samples for each feeding methods (Fig. 2). The 10 gr of each honey dissolved in distilled water at 45°C and centrifuged at 3500 rpm for 10 minutes. Then supernatant was discarded. Basic fuchsine glycerine gelatin was added to the sample. Pollen examinations were carried out by Nikon Eclipse E100 microscope, 40X approchment and counted 300 pollen grain for each sample (Fig. 3). For fungal spore examinations same samples examined 100X approchment immersion object and scanned whole area over 22X22 cover glass.

The pollen descriptions were done according to Erdtman (1952; 1969), Wodehouse (1965), Aytuğ (1967), Charpin *et al*., (1974), Faegri & Iversen (1975). Besides, during the study the two areas were visited for collecting the native flora specimens and then this specimens were made reference pollen samples accordingly Wodehouse (1965) method. The morphological spore identification was based on Domsch *et al.*, (1980), St-Germain & Summerbell (1996), Ellis and Ellis (1998), Grant Smith (2000), Watanabe (2002) and our references.

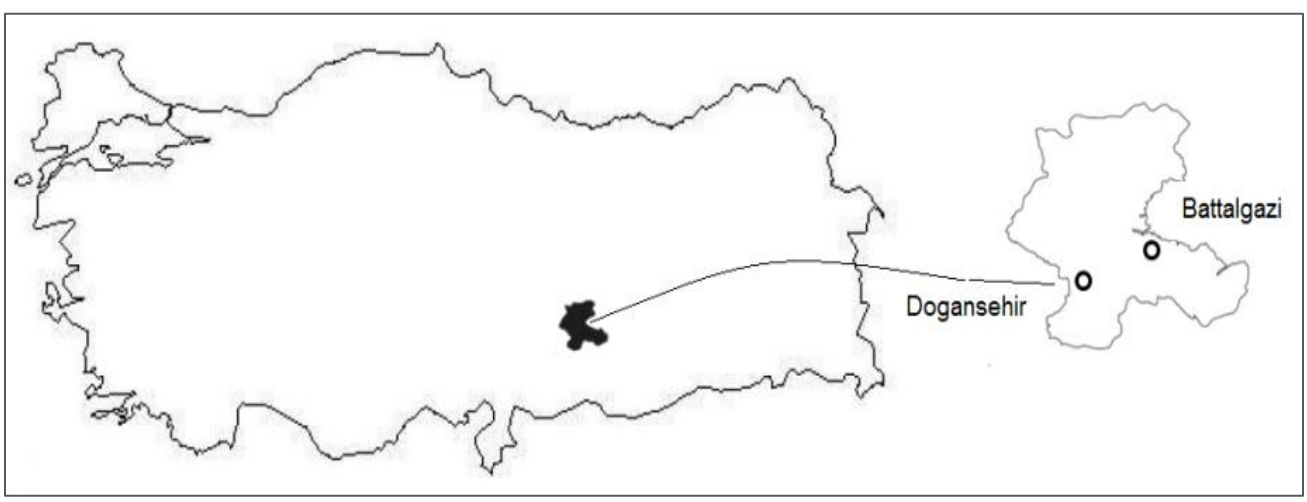

Fig. 1. Location of Malatya City (marked 2 study areas).

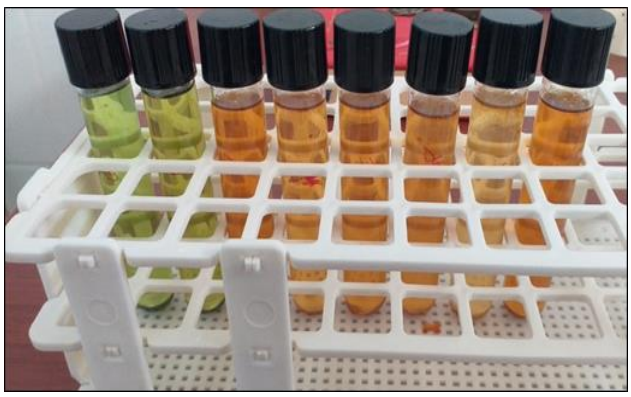

Fig. 2. Honey samples: In order; Bee Feed Group, Sucrose Group, Glucose Group, Control Group (Battalgazi-Dogansehir).

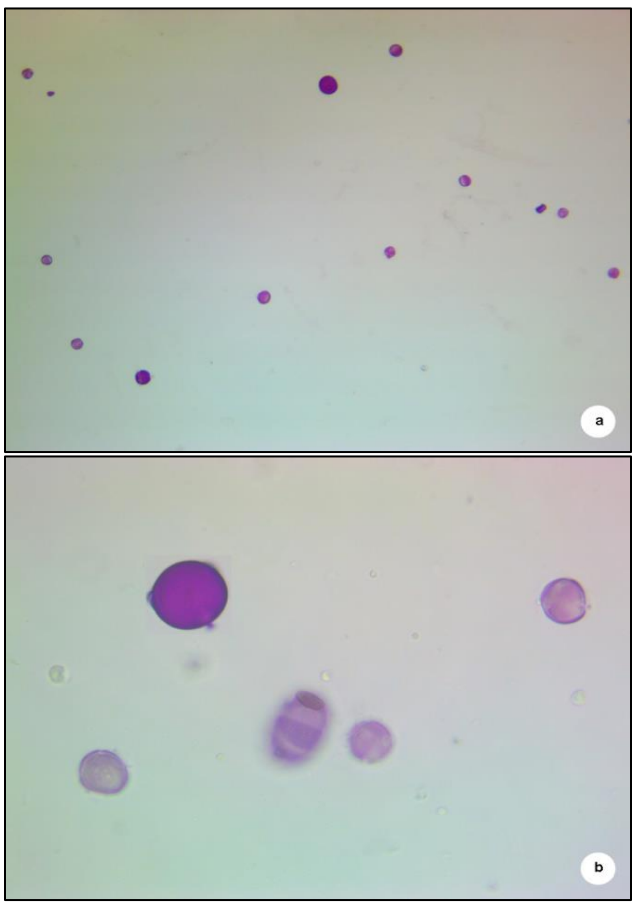

Fig. 3. A section of microscopic view a) X10 approchment, b) X40 approchment.

Results

The study was conducted during 2018 summer season (between end of May-end of August), in Battalgazi and Dogansehir districts of Malatya by a control group and 3 different feeding groups; glucose, sucrose and bee feed.

Pollen Analysis

In Battalgazi district *Cistus* (25.67%), *Astragalus* (16.33%), Poaceae (15.00%), *Verbascum* (9.67%) and *Echium* (7.67%) pollen grains were the most frequent taxa for glucose group (Fig. 4, Table 1). For sucrose group *Astragalus* (24.67%), *Cistus* (14.67%), *Verbascum* (12.53%), *Plantago* (9.33%) and Poaceae (8.00%) were dominant taxa (Fig. 4, Table 1). *Cistus* (37.00%), *Astragalus* (13.00%), *Verbascum* (10.33%), *Berberis* (8.67%) taxa were arranged according to the value for bee feed group (Fig. 4, Table 1). For control group the most widely represented taxa were mainly ranged as *Astragalus* (16.67%), *Cistus* (12.67%), *Artemisia* (12.00%), *Verbascum* (12.00%), *Plantago* (10.00%), *Echium* (7.33%) (Fig. 4, Table 1).

In Dogansehir district for glucose group the most frequent taxa in honey were identified as *Cistus* (20.67%), *Astragalus* (14.33%), *Vicia* (12.67%), *Echium* (7.00%), *Verbascum* (6.67%), *Plantago* (6.33%), *Onobrychis* (5.33%) (Fig. 5, Tab 1). For sucrose group *Astragalus* (29.33%), *Cistus* (19.67%), *Vicia* (12.00%), *Verbascum* (9.33%) were dominant taxa (Fig. 5, Table 1). For bee feed group *Cistus* (20.67%), *Astragalus* (19.33%), *Vicia* (9.00%), *Verbascum* (8.67%), *Berberis* (8.00%), Cichorioideae (6.00%) taxa were investigated frequently (Fig. 5, Table 1). *Astragalus* (47.00%), *Cistus* (11.00%), *Vicia* (7.00%), *Echium* (5.67%) taxa were represented as most widely for control group (Fig. 5, Table 1). 30 taxa belong to 20 family were determined in both districts' honey samples. In Battalgazi Geraniaceae family, in Dogansehir Apiaceae family was not defined in the pollen preparations (Table 1).

A pollen frequency classification could be made while determining the botanical origin of the honey. If the percentage of pollen > 45% , the frequency could be accepted as "very frequent"; the percentage of pollen between 15−45, it could be named as "frequent"; 3−15% was accepted as "few"; 1−3% could be accepted as "very few"; if the percentage of pollen taxa was below 1% it could be admitted as "detected" (Barth, 2005) (Table 2).

Any pollen types accounted over % 45 in honey samples, the honey is accepted as monofloral honey (Louveaux *et al.*, 1978). In our study Dogansehir control group contained *Astragalus* pollen 47% ratios for this reason the honey could be called as *Astragalus* honey (Fig. 5, Tables 1 and 2).

Fungal spore analysis

In honey samples 19 types of fungal spores and hypha fragments were determined. In Battalgazi glucose group the dominant fungal spore types were found as *Aspergillus / Penicillium* (39.58%), Urediniospores (29.17%), *Cladosporium* (9.38%); sucrose group Urediniospores (49.46%), *Aspergillus / Penicillium* (18.28%), bee feed group *Asperillus / Penicillium* (36.36%), Urediniospores (29.75%), control group similarly with bee feed group by different percentage *Aspergillus/Penicillium* (49.22%), Urediniospores (32.81%) (Fig. 6, Table 3).

In Dogansehir for glucose group Urediniospores (73.88 %) and *Aspergillus / Penicillium* (10.07 %) were observed as dominant fungal spore types. For sucrose group Urediniospores (27.85%), *Aspergillus / Penicillium* (17.72%), *Cladosporium* (11.39%), Myrotechium (7.59%) determined as most widely represented taxa. For Bee Feed group Urediniospores (38.37%), *Aspergillus / Penicillium* (27.91%) and for control group *Aspergillus / Penicillium* (63.87%), Urediniospores (14.19%) types determined frequently (Fig. 7, Table 3).

Table 1. The pollen spectra comparison according to feeding methods for each two locality.

Battalgazı								Dogansehır							
Glucose	**%**	**Sucrose**	**%**	**Bee Feed**	**%**	**Control**	**%**	**Glucose**	**%**	**Sucrose**	**%**	**Bee Feed**	**%**	**Control**	**%**
Cistus	25.67	*Astragalus*	24.67	*Cistus*	37.00	*Astragalus*	16.67	*Cistus*	20.67	*Astragalus*	29.33	*Cistus*	20.67	*Astragalus*	47.00
Astragalus	16.33	*Cistus*	14.67	*Astragalus*	13.00	*Cistus*	12.67	*Astragalus*	14.33	*Cistus*	19.67	*Astragalus*	19.33	*Cistus*	11.00
Poaceae	15.00	*Verbascum*	12.33	*Verbascum*	10.33	*Artemisia*	12.00	*Vicia*	12.67	*Vicia*	12.00	*Vicia*	9.00	*Vicia*	7.00
Verbascum	9.67	*Plantago*	9.33	*Berberis*	8.67	*Verbascum*	12.00	*Echium*	7.00	*Verbascum*	9.33	*Verbascum*	8.67	*Echium*	5.67
Echium	7.67	Poaceae	8.00	*Plantago*	3.67	*Plantago*	10.00	*Verbascum*	6.67	*Onobrychis*	4.00	*Berberis*	8.00	Boraginaceae	3.67
Plantago	4.00	Boraginaceae	2.67	*Artemisia*	3.00	*Echium*	7.33	*Plantago*	6.33	*Papaver*	3.67	Chicorideae	6.00	*Verbascum*	3.67
Papaver	3.33	*Echium*	2.33	Chicorideae	2.67	Poaceae	3.00	*Onobrychis*	5.33	*Echium*	3.33	*Papaver*	4.33	Lamiaceae	2.67
Asteraceae	2.67	*Berberis*	2.33	Lamiaceae	2.33	*Papaver*	2.67	Boraginaceae	4.00	*Berberis*	2.67	*A. hippocastanum*	4.00	Chicorideae	2.00
A.hippocastanum	2.00	*Artemisia*	2.00	Asteraceae	1.67	*Berberis*	2.67	*Artemisia*	3.67	*Plantago*	2.33	*Onobrychis*	3.33	*Papaver*	2.00
Berberis	1.67	Chicorideae	2.00	*Dianthus*	1.67	*A.hippocastanum*	2.00	*Papaver*	2.33	*A. hippocastanum*	1.67	Asteraceae	2.33	*Trifolium*	2.00
Artemisia	1.33	*A.hippocastanum*	1.67	*Papaver*	1.67	Asteraceae	2.00	Asteraceae	2.00	*Artemisia*	1.67	*Plantago*	2.00	*Plantago*	1.67
Lamiaceae	1.33	Fabaceae	1.67	Boraginaceae	1.33	*Dianthus*	1.67	Lamiaceae	2.00	Chicorideae	1.33	*Trifolium*	1.67	*Artemisia*	1.33
Chicorideae	1.00	*Anchusa*	1.33	*Calystegia*	1.33	Fabaceae	1.67	*A. hippocastanum*	1.67	*Anchusa*	1.00	*Dianthus*	1.33	*Tilia*	1.33
Dianthus	1.00	Apiaceae	1.33	*Onobrychis*	1.33	*Urtica*	1.67	Poaceae	1.67	Boraginaceae	1.00	*Artemisia*	1.00	*A. hippocastanum*	1.00
Onobrychis	1.00	*Medicago*	1.33	*Trifolium*	1.33	Boraginaceae	1.33	*Anchusa*	1.33	*Calystegia*	1.00	*Echium*	1.00	Asteraceae	1.00
Anchusa	0.67	*Papaver*	1.33	*A.hippocastanum*	1.00	*Medicago*	1.33	*Fabaceae*	1.33	*Trifolium*	1.00	Fabaceae	1.00	*Calystegia*	1.00
Boraginaceae	0.67	*Quercus*	1.33	*Echium*	1.00	Chicorideae	1.00	*Medicago*	1.00	Fabaceae	0.67	Poaceae	1.00	*Anchusa*	0.67
Fabaceae	0.67	*Urtica*	1.33	*Medicago*	1.00	*Quercus*	1.00	Geraniaceae	0.67	Asteraceae	0.33	*Anchusa*	0.67	*Dianthus*	0.67
Quercus	0.67	*Calystegia*	1.00	*Quercus*	1.00	*Tilia*	1.00	*Quercus*	0.67	Cup/Tax	0.33	Geraniaceae	0.67	Fabaceae	0.67
Apiaceae	0.33	Cup/Tax	1.00	*Urtica*	1.00	Apiaceae	0.67	*Berberis*	0.67	*Dianthus*	0.33	Lamiaceae	0.67	*Medicago*	0.67
Calystegia	0.33	*Onobrychis*	1.00	*Anchusa*	0.67	*Calystegia*	0.67	*Trifolium*	0.67	Geraniaceae	0.33	Boraginaceae	0.33	Cup/Tax	0.33
Cup/Tax	0.33	Asteraceae	0.67	Apiaceae	0.67	Cup/Tax	0.67	*Urtica*	0.67	*Hedysarum*	0.33	*Calystegia*	0.33	Geraniaceae	0.33
Hedysarum	0.33	*Dianthus*	0.67	Cup/Tax	0.33	*Hedysarum*	0.67	*Calystegia*	0.33	Lamiaceae	0.33	Cup/Tax	0.33	*Hedysarum*	0.33
Medicago	0.33	*Hedysarum*	0.67	Fabaceae	0.33	Lamiaceae	0.67	Chicorideae	0.33	*Medicago*	0.33	*Hedysarum*	0.33	*Onobrychis*	0.33
Pinus	0.33	Lamiaceae	0.67	*Hedysarum*	0.33	*Onobrychis*	0.67	Cup/Tax	0.33	*Pinus*	0.33	*Medicago*	0.33	*Pinus*	0.33
Rosaceae	0.33	Rosaceae	0.67	*Pinus*	0.33	Rosaceae	0.67	*Dianthus*	0.33	Poaceae	0.33	*Pinus*	0.33	Poaceae	0.33
Tilia	0.33	*Trifolium*	0.67	Poaceae	0.33	*Trifolium*	0.67	*Hedysarum*	0.33	*Quercus*	0.33	*Quercus*	0.33	*Quercus*	0.33
Trifolium	0.33	*Vicia*	0.67	Rosaceae	0.33	*Anchusa*	0.33	*Pinus*	0.33	Rosaceae	0.33	Rosaceae	0.33	Rosaceae	0.33
Urtica	0.33	*Pinus*	0.33	*Tilia*	0.33	*Pinus*	0.33	Rosaceae	0.33	*Tilia*	0.33	*Tilia*	0.33	*Berberis*	0.33
Vicia	0.33	*Tilia*	0.33	*Vicia*	0.33	*Vicia*	0.33	*Tilia*	0.33	*Urtica*	0.33	*Urtica*	0.33	*Urtica*	0.33
Geraniaceae	0.00	Geraniaceae	0.00	Geraniaceae	0.00	Geraniaceae	0.00	Apiaceae	0.00	Apiaceae	0.00	Apiaceae	0.00	Apiaceae	0.00

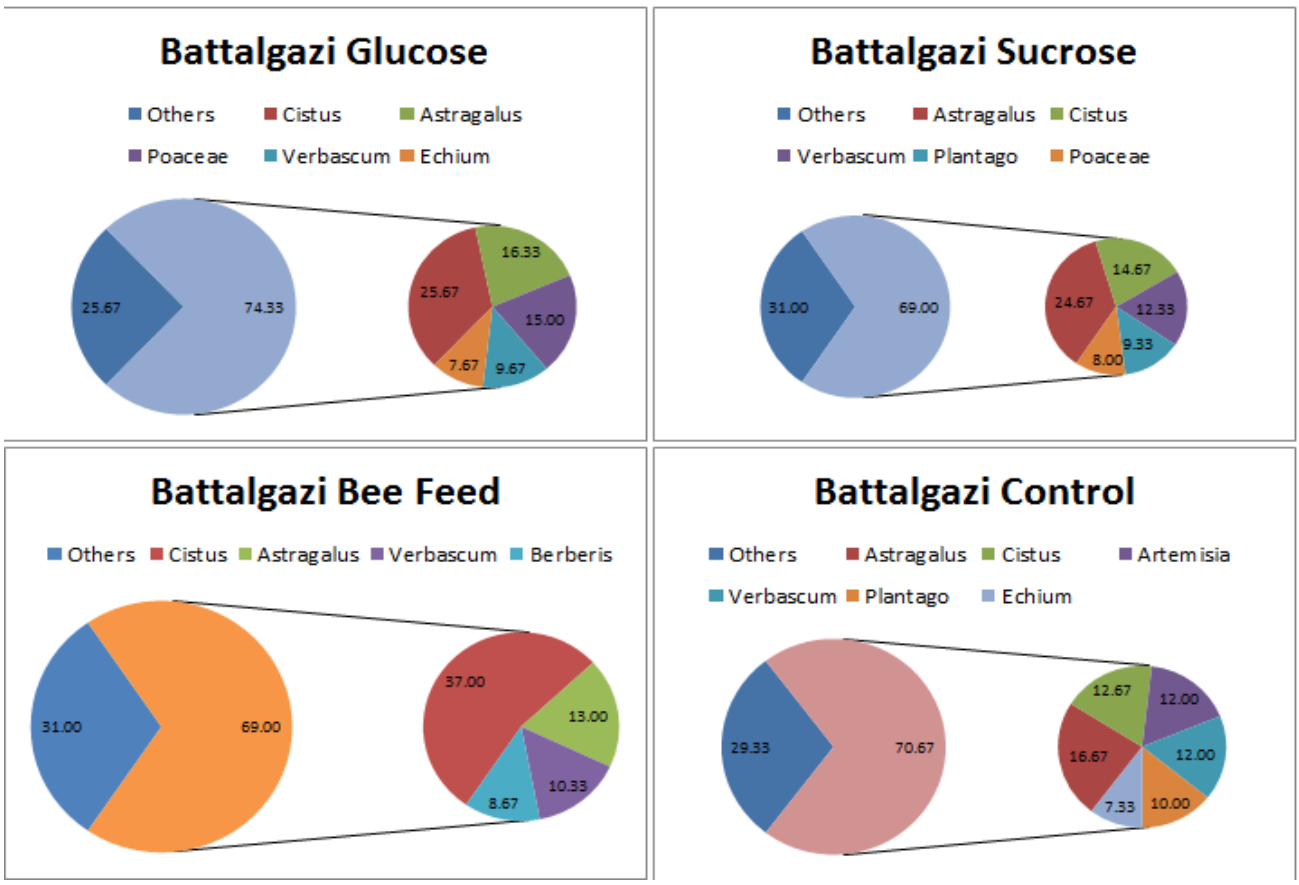

Fig. 4. Dominant pollen taxa (%) for different feeding groups in Battalgazi.

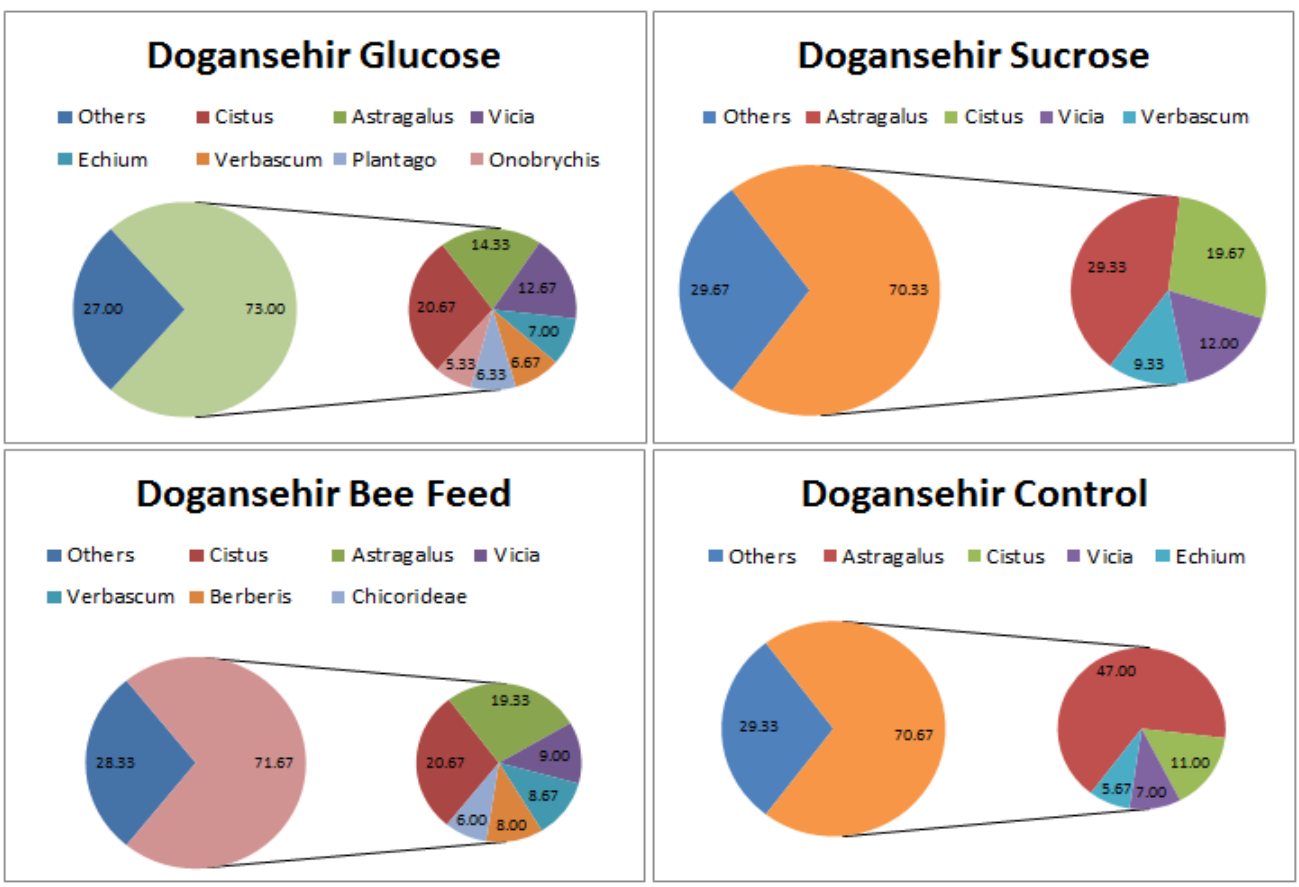

Fig. 5. Dominant pollen taxa (%) for different feeding groups in Dogansehir.

Table 2. Pollen frequency in RJ samples: =very frequent, =frequent, =few, =very few, detected.

	Taxa	BG	BS	BBF	BC	DG	DS	DBF	DC
1.	*Aesculus hippocastanum*								
2.	*Anchusa*								
3.	Apiaceae								
4.	*Artemisia*								
5.	Asteraceae								
6.	*Astragalus*								
7.	Boraginaceae								
8.	*Calystegia*								
9.	Chicorideae								
10.	*Cistus*								
11.	Cupressaceae/Taxaceae								
12.	*Dianthus*								
13.	*Echium*								
14.	*Fabaceae*								
15.	Geraniaceae								
16.	*Hedysarum*								
17.	Lamiaceae								
18.	*Medicago*								
19.	*Onobrychis*								
20.	*Papaver*								
21.	*Pinus*								
22.	*Plantago*								
23.	Poaceae								
24.	*Quercus*								
25.	Rosaceae								
26.	*Berberis*								
27.	*Tilia*								
28.	*Trifolium*								
29.	*Urtica*								
30.	*Verbascum*								
31.	*Vicia*								

Discussion

The feeding method comparatively melissopalynological study was conducted at summer season of 2018 in two different districts of Malatya (Eastern Turkey). The honey samples investigated palynologically for pollen grains and fungal spores. According to the microscopic analyses the dominant pollen types were determined as *Astragalus* , *Cistus*, Poaceae, *Verbascum*, *Echium*, *Berberis*, *Artemisia*, *Plantago*, *Vicia*, *Onobrychis*, Cichorioideae. *Astragalus* and *Cistus* pollen grains were found in all samples with a ratio of over % 5 (Figs. 4-5, Table 1). By microscopic analyses of fungal spores *Aspergillus / Penicillium*, Urediniospores, *Cladosporium* and *Myrotechium* were defined as dominant types. Former ones were determined in all samples as most frequent taxa (Figs. 6-7, Table 3).

According to similar studies about botanical and geographical origins of honey; in Portugal *Lavandula*, *Echium*, *Eucalyptus* (Aira *et al.*, 1998), In Antalya-Turkey Apiaceae, Asteraceae, Fabaceae, Lamiaceae, Rosaceae families (Silici & Gokceoglu, 2007), In Mardin- Turkey *Hedysarum* sp., *Carduus* sp., *Melissa officinalis*, *Gossypium hirsitum*, *Paliurus spina-christi*, *Salix* sp., *Pimpinella anisum* (Cenet *et al.*, 2017), in Ethiopia Fabaceae, Asteraceae, Lamiaceae families (Bareke & Addi, 2019), in Iran *Astragalus*, *Xeranthemum* / *Achillea*, *Eryngium*, *Prosopis*, *Pyrus* / *Prunus*, *Onobrychis* / *Alhagi*, *Centaurea*, *Cousinia* / *Centaurea*, *Plantago* and *Solanum* (Khansaritoreh *et al*., 2021) were the dominant plant taxa to pollen analysis.

Apidae members prefer *Astragalus* genus flowers because of papilionaceous form (Green & Bohart, 1975). This form causes the pollination is happening sternotribic as the bee lands on the keel and for sucking the nectar presses under the standard during the forage (Faegri & van der Pijl, 1971; Kozuharova & Firmage, 2007). For Dogansehir Control group *Astragalus* pollen grains constituted 63.87 % of total pollen count in honey for this reason the honey could be called as *Astragalus* honey (Fig. 5, Tables 1-2). Not only DC group but also in BC, BS and DS groups *Astragalus* pollen grains were found as most frequent taxon (Figs. 4-5, Table 1). The dominancy of *Astragalus* pollen in honey samples could be related with relatively abundant flowers of this taxon in the area (Green & Bohart, 1975). However the glucose ratio of most *Astragalus* species is more than to other monosaccharides according to rare studies about *Astragalus* sugar content; so the bees would prefer directly supplying glucose from this taxon instead of sucrose hydrolysing (Ebrahimzadeh *et al.*, 2000; Niknam & Lisar, 2004).

Table 3. The fungal spore spectra comparison according to feeding methods for each two locality.

Battalgazi							Doganschir						
Glucose	%	Sucrose	%	Bee Feed	%	Control	%	Glucose	%	Sucrose	%	Bee Feed	%	Control	%
Asp/Pen	39,58	Urediniospores	49,46	*Asp/Pen*	36,36	*Asp/Pen*	49,22	*Asp/Pen*	73,88	Urediniospores	27,85	Urediniospores	38,37	*Asp/Pen*	63,87
Urediniospores	29,17	*Asp/Pen*	18,28	Urediniospores	29,75	Urediniospores	32,81	Urediniospores	10,07	*Asp/Pen*	17,72	*Asp/Pen*	27,91	Urediniospores	14,19
Cladosporium	9,38	*Cladosporium*	4,30	*Myrotechium*	4,13	*Agrocybe*	1,56	*Cladosporium*	1,87	*Cladosporium*	11,39	*Coprinus*	4,65	Ascomycetes	3,87
Hypha	3,13	Ascomycetes	3,23	*Alternaria*	3,31	Ascomycetes	1,56	*Alternaria*	1,49	*Myrotechium*	7,59	*Myrotechium*	3,49	*Cladosporium*	3,87
Rhizopus	3,13	*Ganoderma*	3,23	*Cladosporium*	3,31	*Coprinus*	1,56	*Ascomycetes*	1,49	*Coprinus*	3,80	*Alternaria*	2,33	Hypha	1,94
Ascomycetes	2,08	Hypha	3,23	Hypha	2,48	Hypha	1,56	*Ganoderma*	1,49	*Gyromitra*	3,80	Ascomycetes	2,33	*Agrocybe*	1,29
Myrotechium	2,08	*Myrotechium*	3,23	*Nigrospora*	2,48	*Myrotechium*	1,56	Coprinus	1,12	*Nigrospora*	3,80	*Cladosporium*	2,33	*Alternaria*	1,29
Smut	2,08	*Nigrospora*	3,23	*Rhizopus*	2,48	Torula	1,56	Smut	1,12	*Agrocybe*	2,53	*Ganoderma*	2,33	*Bipolaris*	1,29
Agrocybe	1,04	*Gyromitra*	2,15	Smut	2,48	*Xylaria*	1,56	*Agrocybe*	0,75	Ascomycetes	2,53	Hypha	2,33	*Fusarium*	1,29
Alternaria	1,04	Smut	2,15	*Agrocybe*	1,65	*Alternaria*	0,78	*Bipolaris*	0,75	*Fusarium*	2,53	*Rhizopus*	2,33	*Nigrospora*	1,29
Bipolaris	1,04	*Agrocybe*	1,08	*Coprinus*	1,65	*Bipolaris*	0,78	*Gyromitra*	0,75	*Ganoderma*	2,53	Smut	2,33	*Torula*	1,29
Coprinus	1,04	*Alternaria*	1,08	*Ganoderma*	1,65	*Cladosporium*	0,78	Hypha	0,75	Hypha	2,53	*Agrocybe*	1,16	*Coprinus*	0,65
Ganoderma	1,04	*Bipolaris*	1,08	*Gyromitra*	1,65	*Ganoderma*	0,78	*Nigrospora*	0,75	Smut	2,53	*Bipolaris*	1,16	*Ganoderma*	0,65
Gyromitra	1,04	*Coprinus*	1,08	*Torula*	1,65	*Gyromitra*	0,78	*Rhizopus*	0,75	*Torula*	2,53	*Fusarium*	1,16	*Gyromitra*	0,65
Nigrospora	1,04	*Fusarium*	1,08	*Xylaria*	1,65	*Nigrospora*	0,78	*Torula*	0,75	*Alternaria*	1,27	*Gyromitra*	1,16	*Myrotechium*	0,65
Torula	1,04	*Rhizopus*	1,08	Ascomycetes	0,83	*Rhizopus*	0,78	*Trichotechium*	0,75	*Bipolaris*	1,27	*Nigrospora*	1,16	*Rhizopus*	0,65
Xylaria	1,04	*Torula*	1,08	*Bipolaris*	0,83	Smut	0,78	*Fusarium*	0,37	*Rhizopus*	1,27	*Torula*	1,16	Smut	0,65
Fusarium	0,00	*Trichotechium*	0,00	*Trichotechium*	0,83	*Venturia*	0,78	*Myrotechium*	0,37	*Trichotechium*	1,27	Trichotechium	1,16	*Venturia*	0,65
Trichotechium	0,00	*Venturia*	0,00	*Venturia*	0,83	*Fusarium*	0,83	*Venturia*	0,00	*Xylaria*	1,27	*Venturia*	1,16	*Trichotechium*	0,00
Venturia	0,00	*Xylaria*	0,00	*Fusarium*	0,00	*Trichotechium*	0,00	*Xylaria*	0,37	*Venturia*	0,00	*Xylaria*	0,00	*Xylaria*	0,00

Cistus species transfer their pollen grains primarily by bees (Talavera *et al*., 1993). Whereas *Cistus* is less abundant in area the pollen percentage of these taxa would be high intensity because of large flowering period of different *Cistus* species' overlapping (Torné-Noguera *et al*., 2016). *Cistus* flowers attract pollinators with yellow and purple pigments, a polished surface and an excessive quantity of yellow stamens (Glover, 2011). In our study during glucose and bee feed groups' examinations *Cistus* pollen grains were found as dominant in both study areas (Figs. 4-5, Table 1). According to HPLC analysis on *Cistus* the percentage of fructose is higher than percentage of glucose (Guimarães *et al.*, 2009; Liolios *et al.*, 2018). In glucose feeding the deficiency of fructose would be replace by *Cistus* pollen grains. Bee feed ailment is an approximately equal proportions mixture of fructose, glucose and sucrose. In Doganschir Bee Feed group the *Cistus* and *Astragalus* pollen percentages were determined closely. The differentiation over the proportions of these two genera in Battalgazi Bee Feed group could be related with the bee preferences of other three feeding groups (Glucose, Sucrose and Control).

According to"effective pollination" hypothesis *Verbascum* is so attractive for bees with its taller stem and strong terminal preponderance (Gross & Werner, 1978; Aarssen, 1995; Lortie & Aarssen, 1999). Other dominant taxon *Echium* nectar and pollen grains are collected by honey bees and this taxon is accepted bee plant because of its nutrient merit (Chwil & Weryszko-Chmielewska, 2011). Also the violet-blue colour of *Echium* flowers and pollen grains make charmfull this plant for pollinators (Maurizio & Grafl, 1969; Prabucki, 1988; Chwil & Weryszko-Chmielewska, 2011). *Vicia* flowers adapted to entomophily and pollen grains provide the honey bees basic nutrient necessities for development (Bond & Poulsen, 1983; Somerville, 1999). It is generally accepted that *Plantago* is anemophilous plant but bees collect *Plantago* pollen for a supplementary food as well as high nutritious pollen grains (Sabugosa-Madeira *et al.*, 2008). For Bee Feed Groups in each district *Berberis* pollen is one of our dominant pollen types and biochemical studies carried about *Berberis* unifloral honey (Nazarian *et al*., 2010). *Artemisia* is both enthomophilous and anemophilous taxon in Asteraceae that was found in our study among dominant taxa (Parihar *et al.,* 2009). *Onobrychis* has very attractive coloured papilionaceous flowers for pollinators and pollination is mostly done by *Apis mellifera* (Goplen *et al*., 1991; Bhattarai, 2016).

According to Gemeda *et al.*, (2018) by sugar manipulation, the honey bees could be canalized to less preferred plants in this way crop plants' pollination will be improved and the amount of product will increase. In our study Poaceae pollen grains were dominant (15.00%) in Glucose Group in Battalgazi. The percentage of Poaceae was quite low in other feeding groups and control group. Also in Doganschir district Poaceae pollen grains proportionally high in Glucose Group compared to others. In that case it could be acceptable if we manipulate the honeybees with glucose sugar Poaceae pollination will raise relatively. The different ratios of two districts for Poaceae pollen grains could be explain by the disparity of plant abundance. Another crop plant *Onobrychis* pollen grains were determined in our study. Pollen grains percentages of this taxon were higher in three feeding groups than control group. Sugar manipulating had been beneficial for *Onobrychis* as well as Poaceae.

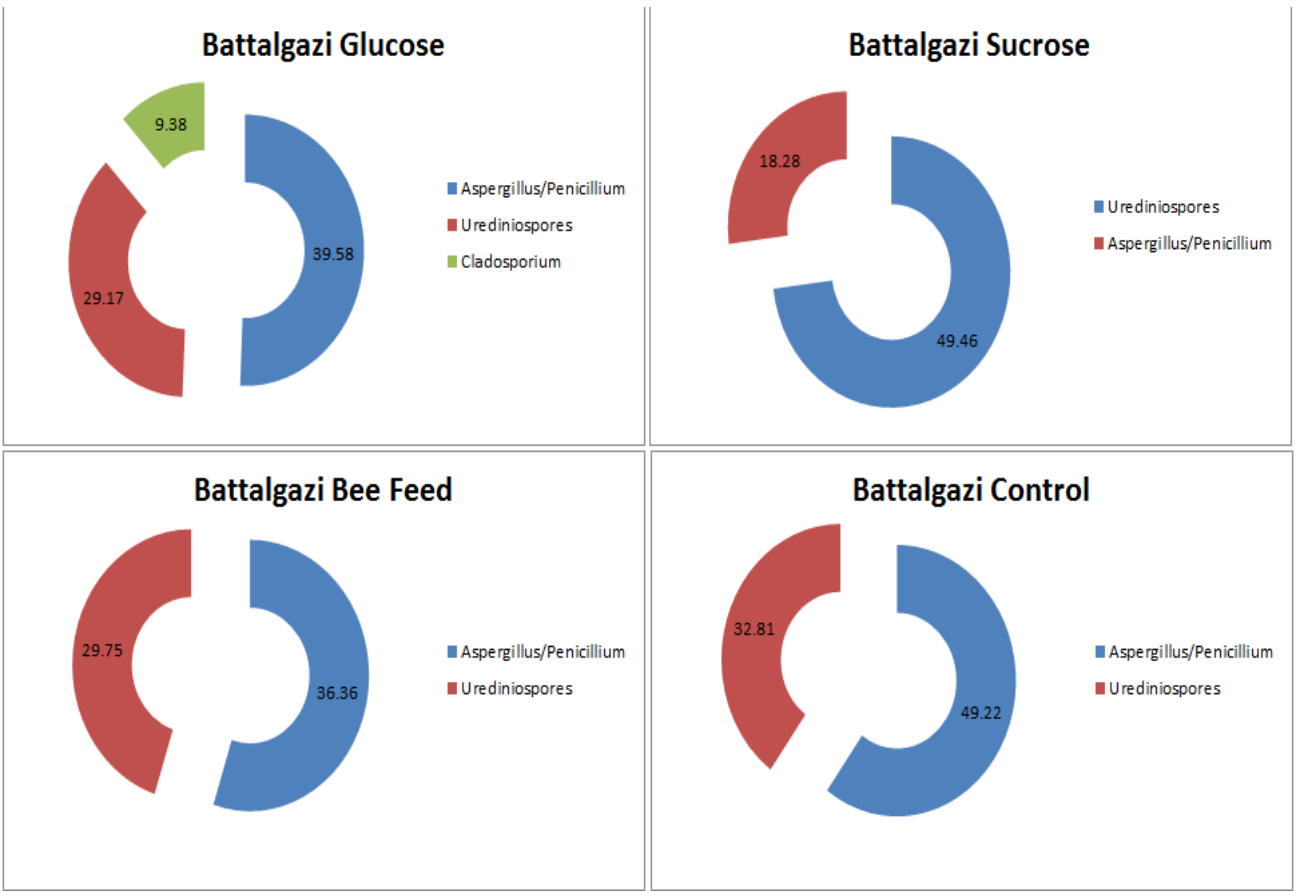

Fig. 6. Dominant fungal spore taxa (%) for different feeding groups in Battalgazi.

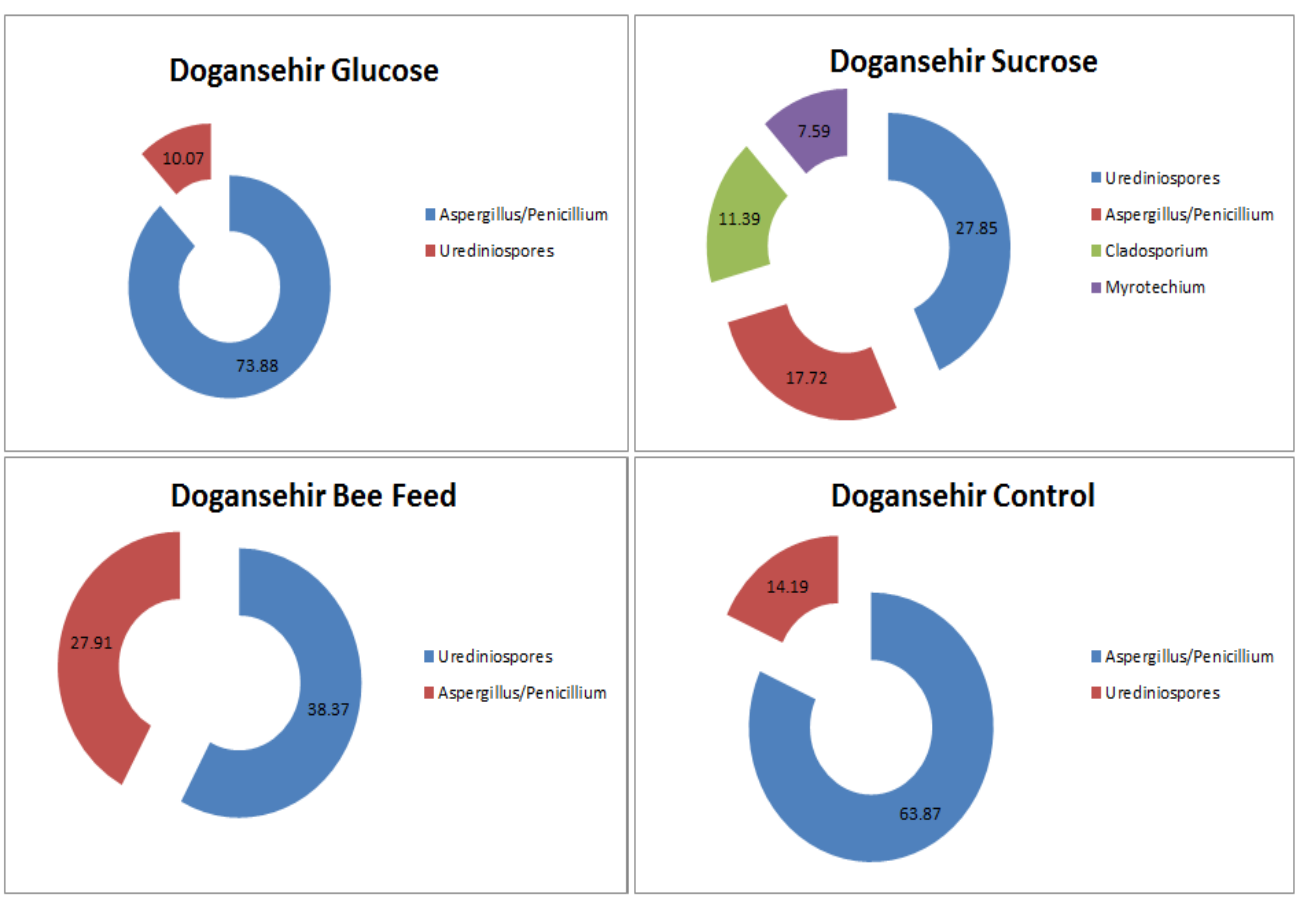

Fig. 7. Dominant fungal spore taxa (%) for different feeding groups in Dogansehir.

The examinations of fungal elements in honeys are concentrating in Spain. *Alternaria*, *Cladosporium*, *Drechslera*, *Geotrichium*, *Phithomyces*, *Stmphylium*, *Torula*, *Ulocladium* and *Ventura* were observed in honeydews of Spain (Terrab *et al.*, 2019). *Cladosporium*, *Metschnikowia*, *Leptosphaeria*, *Stemphylium*, *Urediniospores* were found dominant in NW-Spain (Seijo *et al*., 2011), *Cladosporium*, *Penicillium* / *Aspergillus*, Basidiospores were identified frequently in Galicia-Spain honey samples (Pérez-Atanes, 2001).

Cladosporium, Aspergillus / *Penicillium* genera are substantial nourishment for worker honey bees and have the effect of ascending longevity (Gilliam, 1997; Parish, 2020). These genera's spores are found in air pollution and they are accepted as parasitic fungi of honey (Pérez-Atanes, 2001). *Cladosporium* is a saprotrophic genus and its spores found plenty amounts in outdoor air (Magyar *et al*., 2016).Urediniospores were determined as dominant spore taxa in our study like Seijo *et al.*, (2011) study. Uredinales are also parasitic and they exist on anthers of flowers (Barth, 1989). *Uromyces* sp. is one of toxic effectual Urediniospores but when they mixed with multisource pollen grains, collected by honey bees, the toxicity of this fungal spore decreased and the nutritional value of hive nourishment is increased (Schmidt *et al*., 1987; Parish, 2020).

Determination of pollen and fungal spore grains in honey is a beneficial instrument to have knowledge the honey's geographical, botanical and ecological origins. Besides the pollination preferences of honey bees could be define by melissopalynological analysis. By these examinations the quality of honey arises and the standards could be increased by choosing right fields to set up the apiaries. However sugar feeding converts the pollen preferences of honeybees. This could be utilized for obtain qualitative honeys and increase the crop fertility in agricultural areas.

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