MELLIFERA RESEARCH ARTICLE

# Investigation of Pollinator Species of Order Hymenoptera in Kastamonu University Campus

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#### **ABSTRACT**

Pollination is defined as the transfer of male gametophyte, called pollen to the stigma's of the female organ of seeded plants . The pollination has vital importance for the formation of fruit and the development of fertile seeds. While some plants have the ability to self-pollination, the vast majority of plants require a carrier vector for pollination. In this study, it was aimed to determine the pollinator diversity in the campus area of Kastamonu University and to determine pollen diversity on pollinator vectors. For this purpose, a field study was carried out in the campus area in May and October 2016, the collected samples were identified and the pollen on them was determined. Among the samples collected in May as a result of the study, 7 species / subspecies [Xylocopa violacea (Linnaeus, 1758), Apis mellifera anatolica (Maa, 1953), Ceratina sp., Bombus terrestris (Linnaeus, 1758), Megachile sp., Polistes dominula, Phygadeuon sp.] Belonging to 4 families of the Hymenoptera family (Apidae, Ichnemonidae, Megachilidae, Vespidae). Five species/subspecies [Ceratina sp., Sphecodes sp., Symmorphus sp., Apis mellifera anatolica (Maa, 1953), Bombus terrestris (Linnaeus, 1758)] belonging to 3 family members of the Hymenoptera family (Apidae, Halictidae, Vespidae). To determine pollen variety collected taxa were washed with alcohol and the pollen and spores slides prepared. Pollen grain of 40 taxa and spores of 6 taxa identified and amount of they counted from this slides.

Keywords: Pollination, Hymenoptera, pollen, entemogami

#### Introduction

Pollination, which is one of the conditions required for fruit and seed formation in flowering plants, is defined as the transfer of pollen grains from the male to the stigma of the female organ in different ways, primarily insects [1,2,3]. While some plants have the ability of self-pollination, the vast majority of plants require a carrier vector for pollination. As a carrier vector sometimes water and wind are used, while the vast majority of flowering plants are pollinating with animals. Mollusk, birds, mammals (bats, some apes, etc.)

and insects are the most important pollinator vectors. Pollinators are a very important contributor to natural and agricultural ecosystems by providing both biological diversity and increasing yield and quality in crop production [3,4]. The vast majority of bees who act as pollinators in natural and cultural areas are social and solitary bees [4].

The Hymenoptera group is one of the most important insect order, including all of the most known insect as ants, bees, bugs, leaves and woody weevils. Hymenoptera is an insect group

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that contains at least 115000 defined species and shows a holometabol metamorphosis. They take their names because of they have two pairs of membranous wings. Of more than 250 thousands flowering plant species spreading around the world, it is known that about 20 thousand were visited by bees [5, 6]. The bees visit the flowers to collect nectar and pollen. They use nectar for source of carbohydrates and pollen is more for a source of protein [7].

They need carbohydrate, protein, fat, minerals, vitamins and water for development, growth, maintenance-nutrition and incubation activity. While flowers and secretory nectars are used for source of honey carbohydrate needs, pollen meets all the remaining nutritional needs of bees. While carbohydrates and water are sufficient for the survival of adult honey bees, larvae needs to pollen, which is the source of the proteins, lipids, minerals and vitamins needed for the growth and development of its [8,9].

According to Güler and Çağatay [10], the use of honey bees and bombus bees in organic farming and alternative production methods has been increased in recent years and some species belonging to the family Megachilidae have been found to be more effective in the pollination of As results of this some plants. studies Creightonella Cockerell 1908, Chalicodoma Lepeletier 1841, Megachile Latreille 1802, Coelioxys Latreille 1809, Osmia Panzer 1806, Chelostoma Latreille 1809, Hoplitis Klug 1807, Heriades Spinola 1808, Anthocopa Lepeletier 1825, Archianthidium Mavromoustakis 1939, Paraanthidium Friese 1898, Anthidiellum Cockerell, 1904, Rhodanthidium Isensee, 1927, Pseudoanthidium Friese 1898, Icteranthidium Michener 1948 and Anthidium Nurse 1902, bees species determined

as pollinator.

About 65% of the world's crop production which including wheat, corn, rice pollinated with wind while 35% of agricultural production need to animal pollination [11]. It is stated that more than ¼ of the world's agricultural production depends on the pollinating functions of bees for better seed and fruit formation [12]. It is stated that the yield of many fruits and vegetables such as oranges, apples, peaches, apricot, plums, cherries, tomatoes, melons, squashes, grapes, olives and cucumbers which are estimated to be worth over 100 billion dollars worldwide is related to the pollination by bees [4].

It is estimated that more than 20,000 bee species with insects and vertebrates pollinator number on the earth over 400,000. Plants provide many awards for pollinators, and a special relationship between plant and pollinator is developed. It is stated that sometimes a special and dependent relationship is established between plant and pollinator, as in the case of the genus of Yucca (Agavaceae) and their pollinator moth species Tegeticula Zeller 1873, while many plant species are attracted to a large number of pollinators to make them become pollinated [4]. Kandori [13] determined that 45 insect species belongs to 5 order visited Geranium thunbergii Siebold ex Lindl. & Paxton flowers in the 2-year study and 11 of them were primer pollinators.

In this study, it was aimed to determine the species of Hymenoptera which is play a role as pollinator in the campus area of Kastamonu University, to determine the pollen type and amount of the pollinators.

## **Material and Methods**

## **Collection of Pollinator Samples**

In May and October 2016, a field study was carried out at Kastamonu University Kuzeykent campus in order to determine the types of pollinators. On the campus, flowering plants grown naturally and grown for landscaping were examined and insects seen on the flowers were catched and picked in 50 ml tubes. On the tubes, the date, collection location and label information of the host plant are written. Insects with falcon tubes were brought to the laboratory and kept in 10 ml of 70% alcohol.

## **Identification of Pollinator Species**

The identification of the Hymenoptera specimens was carried out on specimens taken in 70% alcohol for pollen analysis and on samples which killed with ethyl acetate and prepared as museum material. The wing venation, mouthparts, compound eyes, antennae, legs, colors of the samples to be diagnosed were examined under the Leica APO S8 stereomicroscope Identification made according to previous literature [14, 15, 16, 17]

#### Determination of Pollen Variety and Amount

To determine pollen type and amount pollinator samples were shaken and the pollen they carried on them was passed through to the alcohol and the alcohol was transferred in to into 15 ml tubes than centrifuged for 10 min at 4000 rpm. After centrifugation, the top alcohol is removed and the bottom sediment is mixed with the vortex, then dropped onto the clean slides, the alcohol is slightly heated until it is evaporated. On dried sample, glycerin-gelatin with safranin stain was dropped and covered with coverslip. Determination of amount and variety of pollen was examined by Leica DM3000 Digital Imaging system

after 1 day incubation of slides for staining. The pollen type and their amount on the specimens was determined by scanning the entire area of the slides and the literature on pollen morphology was used for identification of pollen type [18, 19, 20, 21].

## Results

According to the study conducted in May and October 2016, 32 samples of Hymenoptera were collected and 9 taxa belonging to 9 genre of 5 families (Apidae, Halictidae, Ichnemonidae, Megachilidae, Vespidae) were determined (Figure 1). As a result of investigated slides, prepared from collected bee taxa, pollen of 40 taxa and spores of 6 fungi taxa identified and their amount is demonstrated in Table 1 and 2.

#### APIDAE

#### APIS Linnaeus, 1758

## Apis mellifera anatolica Maa, 1953

Samples of *A. melifera* bee collected on *Taraxa-cum officinalis* in May.

Pollen grain of *Acer* sp., Anthemidae, Boraginaceae, Caryophyllaceae, Cupressaceae, *Cyperus* sp., Graminaceae, Lamiaceae, *Lapsana* sp., *Morus* sp., *Pinus* sp., *Plantago* sp., Rosaceae sp., *Rumex* sp., *Salix* sp., *Senecio* sp., *Scirpus* sp., *Syringia* sp., *Taraxacum* sp., *Trapogon* sp., *Tussilago* sp. and *Urospermum* sp. taxa were identified and counted on collected *A. mellifera anatolica* speciemen in May. Fungi spores of *Alternaria* sp. and *Puccinia* sp. were also observed on the same pollinator.

Pollen grains of *Brassica* sp., *Lapsana* sp. and *Daucus* sp. taxa were observed from the specimens of *A. mellifera anatolica* collected in October and fungi spore belonging to *Puccinia* sp. was identified (Figure 2, Table 1 and 2)



Figure 1. Identified bee pollinator collected from Kastamonu University campus. A; Apis mellifera anatolica, B; Bombus terrestris, C; Ceratina sp., D; Xylocopa violacea, E; Sphecodes sp., F; Phygadeuon sp., G; Megachile sp., H; Polistes dominula, I; Symmorphus sp. (Photo: Talip Çeter)

## **BOMBUS Latreille, 1802**

#### Bombus terrestris (Linnaeus, 1758)

B. terrestris specimens were collected from Lamium sp. in May, and from Trifolium sp. in October.

Pollen of Cupressaceae, *Lamium* sp., Liliaceae, Pinaceae, *Populus* sp., Rosaceae and *Syringia* sp. taxa were observed from the specimens of *B. terrestris* collected in May. The pollen of *Lactuca* sp., *Trifolium* sp. and spores of *Pleospora* taxa

were counted from the specimens collected in October (Figure 3, Table 1).

## CERATINA Latreille, 1802

## Ceratina sp.

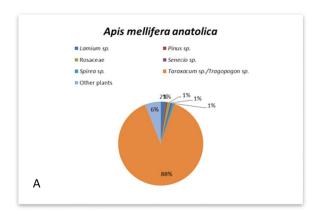
Pollen of *Lamium* sp., *Taraxacum* sp., *Anthemis* sp. and spores of *Alternaria* were observed from specimens of *Ceratina sp.* collected in May, while *Brassica* sp., *Lapsana* sp., *Plantago* sp. pollen were observed on specimen collected in October (Figure 4, Table 1 and 2).

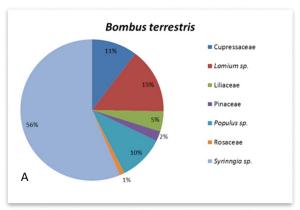
**Table 1.** Pollen types and amount of plant taxa observed on pollinator bee taxa collected from Kastamonu University campus.

	May 2016							October 2016				
	Xylocopa violacea	Ceratina sp.	Megachile sp.	Polistes dominula	Phygadeuon sp.	Apis mellife	Bombus terrestris	Ceratina sp.	Sphecodes sp.	Symmorphus sp.	Apis mellife	Bombus terrestris
	olacea	·	şp.	ninula	<i>1</i> sp.	Apis mellifera anatolica	restris	·	šp.	s sp.	Apis mellifera anatolica	restris
Aesculus sp.	2											
Acer sp.						3						
Alnus sp.	1											
Anthemidae			10			5						
Betula sp.	4											
Brassicaceae										1		
Brassica sp.								5			31	
Boraginaceae						7						
Caryophyllaceae						3						
Chenopodiaceae										4		
Cupressaceae	15		5		6	13	9					
Cyperus sp.						5						
Daucus sp.											4	
Echium sp.	3		4									
Fabaceae										2		
Graminaceae	7		6		3	9						
Lactuca sp.												5
Lamium sp.	280	89	75			35	13					
Lapsana sp.						8		63			18	
Liliaceae	2				1		4					
Malva sp.									4			
Morus sp.						7						
Pinaceae							2					
Pinus sp.	9			1		15						
Plantago sp.	3					6		1	1			
Populus sp.	8						9					
Quercus sp.	65											
Rosaceae	120					20	1					
Rumex sp.				2		4						
Salix sp.	3				1	10						
Senecio sp.					9	15						
Scirpus sp.						4						
Spirea sp.	40					15						
Syringa sp.	7					11	49					
Taraxacum /Tragopogon sp.	20	2			3	1687						
Tilia sp.	1	1						1				
Trifolium sp.	1							1				65
Tussilago sp.						5		1				
Urospermum sp.	1	1				2		1				
-: 55p 4: 5p.		1	1	l	1		l	L	l		l	

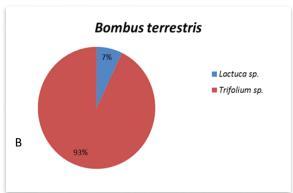
**Table 2.** Fungi spore types and amount observed on pollinator bee taxa collected from Kastamonu University campus.

	May 2016						October 2016				
	Xylocopa violacea )Linnaeus, 1758)	Ceratina sp.	Megachile sp.	Phygadeuon sp.	Apis mellifera anatolica	Bombus terrestris	Sphecodes sp.	Symmorphus sp.	Apis mellifera anatolica	Bombus terrestris	
Alternaria sp.	6	1		1	8			1			
Drechslera sp.							1				
Epicoccum sp.			1								
Pleospora sp.	3		1							1	
Puccinea sp.					11				1		
Venturia sp.	2										



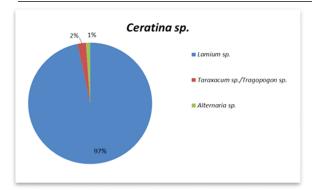






**Figure 2.** Pie graphs of pollen observed on *A. mellifera anatolica*. **A;** May, **B;** October.

Figure 3. Pie graphs of pollen observed on B.terrestris. A; May, B; October.



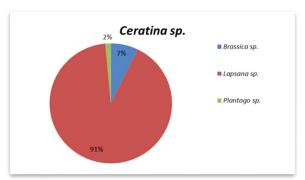


Figure 4. Pie graphs of pollen observed on *Ceratina sp.* A; May, B; October.

## XYLOCOPA Latreille, 1802

## Xylocopa violacea (Linnaeus, 1758)

The specimen of *X. violacea* were collected from *Lamium* sp.

Pollen grain of *Populus* sp., *Quercus* sp., *Pinus* sp., Liliaceae, *Pinus* sp., *Plantago* sp., *Alnus* sp., *Echium* sp., Graminaceae, Cupressaceae, *Lamium* sp. Rosaceae, *Salix* sp., *Spirea* sp., *Syringia* sp., *Taraxacum* sp. and *Tilia* s p. plant taxa and spores of *Alternaria* sp., *Pleospora* sp. and *Venturia* sp fungi taxa were observed from the specimens collected in May (Figure 5, Table 1 and 2). In the field study of October, the specimen of this pollinator was not collected.



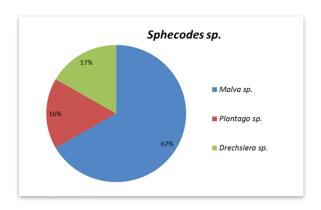
**Figure 5.** Pie graph of pollen observed on *X. violacea* in May

#### **HALICTIDAE**

## SPHECODES Latreille, 1805

## Sphecodes sp.

In the field study conducted in May, the example of the pollinator was not collected. Pollen grains of *Malva sp.* and *Plantago sp.* and spores of *Drechslera* were identified from the *Sphecodes sp.* specimens collected in October (Figure 6, Table 1 and 2).



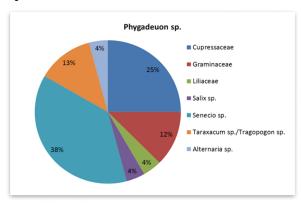
**Figure 6.** Pie graph of pollen observed on *Sphecodes sp.* in October.

#### **ICHNEMONIDAE**

#### PHYGADEUON Gravenhorst, 1829

## Phygadeuon sp.

Pollen of Cupressaceae, Graminaceae, Liliaceae, Salix sp., Senecio sp. and Taraxacum sp. taxa and spores of Alternaria were identified and counted from the specimens of Phygadeuon sp. collected in May, (Figure 7, Table 1 and 2). In the field study conducted in October, the specimens of this pollinator were not collected.



**Figure 7.** Pie graph of pollen and spores observed on *Phygadeuon sp.* in May

#### **MEGACHILIDAE**

#### MEGACHILE Latreille, 1802

#### Megachile sp.

Pollen grains of *Echium* sp., Graminaceae, Lamiaceae, Anthemidae, Cupressaceae and fungi spores of *Pleospora* sp. and *Epicoccum* sp. were identified and counted from the specimen of *Megachile sp.*, collected in May, (Figure 8, Table 1

and 2). In the field study conducted in October, the specimen of this pollinator was not collected.

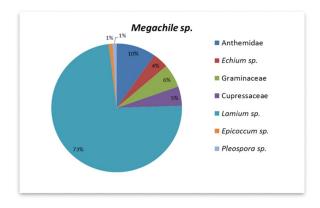


Figure 8. Pie graph of pollen observed on *Megachile sp.*in May.

#### **VESPIDAE**

#### POLISTES Latreille, 1802

#### Polistes dominula (Christ, 1791)

Pollen grains of *Rumex* sp., and *Pinus sp.* were observed from the specimen of *P. dominula* collected in May (Figure 9, Table 1 and 2). In the field study conducted in October, the specimen of this pollinator was not collected.

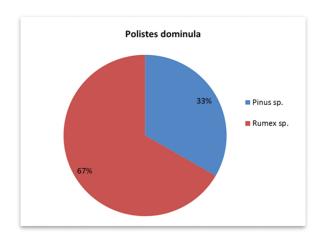


Figure 9. Pie graph of pollen observed on *Polistes dominula* in May.

#### SYMMORPHUS Wesmael, 1836

## Symmorphus sp.

In the field study conducted in May, the specimen of this pollinator was not collected. Pollen grains of *Brassica* sp., Chenopodiaceae, Fabaceae and spores of *Alternaria* sp. were observed from the specimen of *Symmorphus* sp. collected in October (Figure 10, Table 1 and 2).

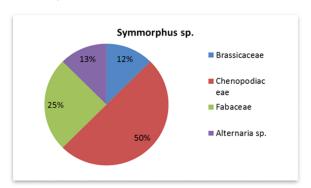


Figure 10. Pie graph of pollen observed on *Symmorphus* sp. in October.

#### Discussion

As a result of this study carried out to determine Hymenoptera species visited flowers of wild and cultivated plants in Kastamonu University campus, 9 taxa belonging to 5 families of the Hymenoptera group were identified. 40 different plant taxa and 6 different fungal spores were identified and counted as results of investigations of slides prepared by washing the bees specimens with 70 % alcohol.

Honey bee has a type of leg called collector leg. Special collecting organs are found in the legs and abdomens of pollen carriers and worker bees.

Hairs present in the body of bees play an important role in pollination. It is stated that a honey bee can visit 50-1000 flowers at a distance of 1-3 km during a visit between 30 minutes and 4 hours. Meteorological factors such as rainfall, wind speed and temperature are said to be very effective in pollination activities of honey bees. It is stated that Honeybees rarely fly at temperatures below 13 °C or at wind speed over 24-32 km/h [4]. Honeybees are considered to be a very important primer pollinator not only because of the production of honey and honey wax, but also they pollinate more than 100 commercially important agricultural plants and numerous wild plants [22, 23, 24].

In our study, on *A. melifera anatolica* sample collected in May, fungal spores of 2 taxa and pollen of 22 taxa were identified among them 90% belonged to *Taraxacum* sp./ *Tragopogon* sp. On the samples collected in October, the amount of pollen and number of taxa were less as compared to May. From specimen collected in October pollen of *Brassica sp., Lapsana* sp., *Daucus* sp. and fungi spores *Puccini* sp. were observed. This difference, as mentioned in the above studies, suggests that the decrease in number of plants during the pollination period and the negative effect on the decreasing temperature value in October, when the number of plants in the pollina-

tion period is high and the weather conditions are favorable in May. It reveals that honey bears play a pollinating role in both seasons, although at different levels.

It is stated that, only about 15% of over 100 species of plants which produce the world's food sources are pollinated by domesticated bees and 80% are pollinated by wild bees and other pollinators [2, 25]. For this reason, wild bees that are outside honey bees are of great importance for pollination. One of the most important of these wild bee species is bumble bees. Bombus bees are very valuable pollinator insects that play a role in the pollination of many plants of great importance to humanity and are a wide spreading area in the world. These bees, which have a fairly flashy, attractive and colorful appearance, are generally more bulky and fuzzy, stronger and more spoiled than the honeybees (A. mellifera). Thanks to their long tongues, they can visit the deep-scented flowers and work in low temperatures, under bad weather conditions and in low light to pollinate the flowers. At the same time they can pollinate 6-8 times more flowers per minute than honey honeys [26].

In the study, pollen of 7 taxa were found on *B. terrestris* species in May. In October, pollen of 2 taxa were observed. In month of May, 56% of total pollen belongs to *Syringia* sp. and 93 % of

total pollen observed in October belongs to *Trifolium* sp. The pollen found on *B. terrestris* mostly belongs to the tubular or papillionide type flowers.

Most *Ceratina* members are solitary while some are living socially. Species which belong to the genus *Ceratina* are generally small in size (2-3 mm), so they are thought to be very less effective in pollination [27]. Inouye [28] stated that small-sized bees are not important particularly for pollinating large flowers.

In this study, pollen of 2 taxa and fungal spore of 1 taxa were identified from the specimen of *Ceratina* sp. in May and pollen of 3 taxa were identified In October. The observed pollen was found to be 97% belong to *Lamium* sp. in the month of May While 91% belong to *Lapsana* sp. in the month of October. All of these Pollen belongs to herbaceous plants.

Xylocopa violaceae, known as violet carpenter bees, is a common species of Europe. They usually make their nests in the dead woods. The adults hibernate in the tunnels they open into the trees and usually wake up in April-May from their hibernation. Female bees place their eggs together pollen balls in separate sections of nests. Due to their large sizes, they are very effective in pollination. They fly quickly around the trees, especially around the upper branches, carrying out pollina-

tion in the flowers around them [27]. There are a number of studies emphasize these bees as an important pollinators of Fabaceae and Lamiaceae species with asymmetric flowers [29, 30]. In our study, pollen of 18 taxa and 3 fungal spores were identified on the *X. violacea* species in May. The identified pollen mostly belongs to the Lamiaceae. This taxa is followed by Rosaceae, *Quercus* sp., *Spirae* sp. taxa respectively.

Halictidae species are common in almost all over the world. Species are generally dark colored with metallic color differences. All species of the Halictidae family are fed with pollen and have an important role in pollination [15]. Pollen belonging to *Malva* sp. and *Plantago* sp. taxa and 1 fungi taxa were observed on the *Sphecodes* sp. specimens collected during the October. In the field study conducted in May, the specimen of this pollinator was not collected.

Species from Vespidae family, such as Vespa orientalis L., Vespula vulgaris L., Vespula germanica (Fabricius), V. rufa (L.) (Vespinae), Polistes associus Kohl, P. bischoffi (Weyrauch), P. gallicus L., P. dominulus Christ), P. nimpha (Christ) (Polistinae) and some Eumeninae species were found visiting the flowers of fruit trees. P. dominula species are fed with nectar and pollen of flowers to meet their energy needs. Their contribution to pollination is very limited because they

do not have the ability to retain pollen grains of body hair [27]. In this study, pollens of *Rumex* sp. and *Pinus* sp. were detected. It is estimated that these pollen grains were infected from the *Pinus* pollen found in the atmosphere at the time of the bee harvest, or from the *Rumex* plant that they visited for hunting.

Phygadeuon sp. are the insects that live as parasites on other insects and hold an important place in the biological struggle. In this study, pollen of 6 taxa and 1 fungus spore were found on sample collected in May.

Megachilidae is the second largest bee family in the world with over 4000 species defined [31, 32]. These solitary bees are ecologically and economically important for pollination of both natural and cultivated plants. For example, *Megachile rotundata* plays a leading role in the pollination of alfalfa plants worldwide [31, 33, 34]. In our work, pollen of 5 taxa and 2 fungal spores were identified on the Megachile sp. species collected in May. Pollen of 3 taxa were observed on the species *Symmorphus sp.* collected in October.

As a result, the Apidae family appears to be the most effective family in Hymenoptera order in terms of pollination. Vespidae family members were found to be the least effective families in pollination according to pollen numbers they carried.

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Kastamonu Üniversitesi Kampüs Alanındaki Tozlaştırıcı Hymenoptera Takımına Ait Türlerin İncelenmesi

ÖZ

Tozlaşma, tohumlu bitkilerde polen olarak adlandırılan erkek gametofitin dişi organın stigması üzerine taşınması olarak tanımlanmaktadır. Tozlaşma meyve oluşumu ve verimli tohumların gelişmesi için hayati öneme sahiptir. Bazı bitkiler kendi kendini dölleme yeteneğine sahipken bitkilerin büyük çoğunluğu tozlaşma için taşıyıcı bir vektöre ihtiyaç duymaktadır. Bu çalışmada Kastamonu Üniversitesi kampüs alanında tozlaştırıcı çeşitliliğinin saptanması ve tozlaştırıcı vektörlerin üzerindeki polen çeşitliliğinin belirlenmesi amaçlanmıştır. Bu

amaçla 2016 Mayıs ve Ekim aylarında kampüs alanında arazi çalışması gerçekleştirilmiş, toplanan örnekler teşhis edilmiş ve üzerinde tasıdıkları polenler belirlenmiştir. Çalışma sonucunda Mayıs ayında toplanan örnekler arasında Hymenoptera takımının 4 familyasına (Apidae, Ichnemonidae, Megachilidae, Vespidae) ait 7 tür/alttür (Xylocopa violacea (Linnaeus, 1758), Apis mellifera anatolica Maa, 1953, Ceratina sp., Bombus terrestris (Linnaeus, 1758), Megachile sp., Polistes dominula, Phygadeuon sp.) tespit edilmiştir. Ekim ayında toplanan örnekler arasında ise Hymenoptera takımının 3 familyasına (Apidae, Halictidae, Vespidae) ait 5 tür/alttür (Ceratina sp., Sphecodes sp., Symmorphus sp., Apis mellifera anatolica Maa, 1953, Bombus terrestris (Linnaeus, 1758)) tespit edilmiştir. Toplanan bu taksonların alkolle yıkandıktan sonra yapılan incelemelerinde 40 taksona ait polene ve 6 taksona ait mantar sporuna rastlanmıştır.

Anahtar Kelimeler: Tozlaşma, Hymenoptera, polen, entemogami

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