Evaluation of Bee Pollen Produced in Wild Mustard (*Sinapis arvensis* L.), Rock Rose (*Cistus criticus*) and Wild Lavender (*Lavandula stoechas*) Fields in Aydın Vicinity as Monofloral Bee Pollen and Determining Some Chemical Properties

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Abstract: In this study, the possibility of producing bee pollens harvested in certain periods from wild mustard (WM), rock rose (RR) and wild lavender (WL) fields, which are common in the region, as single plant bee pollen (BP) was evaluated and some chemical properties were determined in BP collected from these plants. In the research, 4 colonies each were moved to WM fields in Aydın-Efeler, and to the RR and WL fields in Gaffarlar village of Koçarlı district of Aydın province. In these colonies, the plant origins of the BP pellets taken from each group, separated according to their colors, were determined. Pellet weights, moisture contents, crude protein and total phenolic substance amounts were determined in the samples taken from WM, RR and WL BP pellets of defined plant origin. WM, RR and WL pollen pellet weights were 10,42±0,440 mg, 11,69±0,393 mg and 10,40±0,34 mg, respectively. In the same order as the moisture content were determined as 22,10±0,934%, 19,82±0,613%, 20,55±0,667%. In this study, the crude protein contents of WM, RR and WL bee pollen are; 20,5±0,47%; 13,8±2,51%; 16,5±0,95% also in the same order; total phenolic substance values were determined as 21,53±0,514 mg GAE/g, 16,12±0,539 mg GAE/g and 20,31±0,677 mg GAE/g. With this study, it was realized that the BP produced in WM and RR fields in the Aydın precinct can be considered as monofloral BP. The crude protein content and total phenolic substance content of RR bee pollen were found to be lower than the other two plants. It will be possible to establish standards with more comprehensive chemical analyzes on more WM and RR bee pollen samples collected from various locations.

Keywords: Honey bee, monofloral bee pollen, total phenolic content, bee pollen pellet

Aydın Yöresinde Yabani Hardal (*Sinapis arvensis* L.), Laden (*Cistus criticus*) Ve Karabaş (*Lavandula stoechas*) Alanlarında Üretilen Arı Polenlerinin Tek Bitki Poleni Olarak Değerlendirilmesi ve Bazı Kimyasal Özelliklerinin Belirlenmesi

Öz: Bu çalışmada, yörede yaygın olarak bulunan yabani hardal, laden ve karabaş otu alanlarından belirli dönemlerde hasat edilen arı polenlerinin tek bitki poleni olarak üretilebilme olanakları değerlendirilmiş ve bu bitkilerden toplanan arı polenlerinde bazı kimyasal özellikler saptanmıştır. Araştırmada, Aydın-Efeler'de yabani hardal ve Aydın ilinin Koçarlı ilçesine bağlı Gaffarlar köyünde laden ve karabaş otu alanlarına 4'er koloni taşınmıştır. Çalışmada, renklerine göre ayrılmış her gruptan alınan polen peletlerinin bitki orjinleri saptanmıştır. Bitki orjini belirlenen yabani hardal, laden ve karabaş otu polen peletlerinden alınan örneklerde pelet ağırlıkları, nem içerikleri, ham protein ve toplam fenolik madde miktarı belirlenmiştir. Yabani hardal, laden ve karabaş otu pelet ağırlıkları sırasıyla; 10,42±0,440 mg, 11,69±0,393 mg ve 10,40±0,34 mg olarak belirlenmiştir. Nem içeriği yabani hardal %22,10±0,934, laden %19,82±0,613 ve karabaş otu %20,55±0,667, ham protein içeriği yabani hardal %20,5±0,47, laden %13,8±2,51 ve karabaş otu %16,5±0,95 ve toplam fenolik madde değerleri ise sırasıyla; 21,53±0,514 mg GAE/g, 16,12±0,539 mg GAE/g ve 20,31±0,677 mg GAE/g olarak saptanmıştır. Bu çalışma ile Aydın yöresinde yabani hardal ve laden alanlarında üretilecek arı polenlerinin monofloral arı poleni olarak değerlendirilebileceği saptanmıştır. Laden arı poleninin ham protein içeriği ve toplam fenolik madde içeriği diğer iki bitki polenine göre daha düşük bulunmuştur. Farklı yörelerden toplanacak daha çok sayıdaki yabani hardal ve laden arı polen örneklerinde yapılacak daha kapsamlı kimyasal analizler sonucu, bu arı polenlerine ait standart oluşturmak mümkün olacaktır.

Anahtar kelimeler: *Bal arısı, monofloral polen, toplam fenolik madde, polen peleti*

INTRODUCTION

Bee pollen (BP) has been an important food since ancient times due to the quality nutrients it contains. BP is often referred to as "life-giving dust". Bees collect from plant flowers and mix it with nectar and secretions before carrying it back to the hive. In the hive, it is used to meet the colony's vitamin, mineral and energy needs, especially protein. BP contains basic nutrients such as carbohydrates (13-55%), proteins (10-40%), fibrous compounds (0.3-20%) and oils (1-10%) (Villanueva et al., 2002). It also contains flavones, flavonoids, polyphenols, carotenoids, trace elements, growth regulators, hormones, nucleic acids, minerals like P, Ca, Mg, fatty acids, and all amino acids important for human consumption (Almeida-Muradian et al., 2005; LeBlanc et al., 2009; Rzepecka-Stojko et al., 2012; Dominguez-Valhondo et

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al., 2013). For these reasons, BP is described as a perfect food.

Today, beekeeping is done for honey production all over the world, as honey is a traditional food. Production of other bee products is not yet at a level that can be included in statistics. However, BP production comes in second place (Crane, 1990; Bogdanov, 2016). There is also no official data on BP production and trade. However, China is estimated to be the largest producer and is reported to export 2500 tons per year (Lihong, 2009). Other important producing countries include Brazil, Argentina, Spain, France, Germany and Portugal (Bogdanov, 2016). There is no official data on BP production in Turkey. However, it is known that there has been a significant increase in the demand for BP trap hives in recent years. Therefore, it is possible to say that there is an increase in BP production. A significant part of the BP produced is used in bumble bee (*Bombus terrestris* L.) breeding (Gösterit and Gürel, 2014). Although some companies use imported BP, a significant part of them use local BP. Bombus breeders demand to fresh or frozen BP instead of BP containing corn and sunflower. Almost all of the remaining BP is dried and marketed. Mixed bee pollens produced at different times in different regions are offered for consumption.

In general, beekeepers harvest a mixture of BP collected by bees from various plant species. Studies on different plant BPs have shown significant variation in the amounts of essential nutrients and other compounds that give BP its unique quality (Campos et al., 2008). Efforts are being made to develop computer-aided equipment to separate different plant BPs based on BP color and weight differences (Bogdanov, 2016). However, in certain regions and at certain times, mainly monofloral BP can be harvested. The pollen pellets from unique botanical taxon or the ones having single predominant pollen at > 90% frequency or containing no accessory pollen at > 60% frequency are considered as monofloral (Barth et al., 2010).

Many studies have been conducted to determine the BP source plants used by bees and their contents (Baydar and Gürel, 1998; Karaca et al., 2006; Almeida-Muradian et al., 2005; LeBlanc et al., 2009; Barth et al., 2010; Dominguez-Valhondo et al., 2013; Deveci et al., 2016; Bogdanov, 2016). In this study, first of all, the possibilities of producing BP harvested in certain periods from wild mustard, rock rose and wild lavender fields, which are common in the region, as monofloral BP were evaluated and some chemical properties were determined in the BP collected from these plants.

MATERIAL AND METHODS

This study was carried out in 2021-2023 at Aydın Adnan Menderes University, Faculty of Agriculture, Bee and Silkworm Application and Research Unit. As bee material, 12 Anatolian honey bee Muğla ecotype colonies created from queen bees raised in 2021 were used. Then, the colonies were divided into 3 groups and the first group (4) were moved to the wild mustard (WM) area, the other 4 colonies were moved to the wild lavender (WL) area, and 4 bee colonies were moved to the rock rose (RR) area. Bee pollen (BP) traps were operated 3 times a week and BP harvests were made daily. The harvested BP was collected in a nylon bag, place and date information was added and kept in the deep freezer (-20°C) until analysis. During the BP production process, all colonies were fed with sugar syrup twice a week. BPs collected daily from the hives were combined weekly, and bee BPs taken from each batch (approximately 500 BPs) were separated according to their colors and counted, and their proportions in the total BP were calculated (Rabie et al., 1983; Freitas et al., 2013).

BP pellets taken from each group, separated by color, will be mixed with 15 ml of 0,7% physiological saline water in 50 ml falcon tubes with a shaker, and a drop of this mixture will be dropped onto the slide with a disposable 3 ml pasteur pipette (polyethylene) and applied to the coverslip. After being closed, the plant origin of the BP was determined by examining it under a camera light microscope with a 40x/0.65 objective (Lieux, 1972; Sorkun and İnceoğlu 1984).

BP pellets (10 loads) from 10 randomly selected samples from the collected BP samples (WM, RR and WL) were weighed with a scale (Scaltec brand SBC 31 model) with a sensitivity of 0,0001.

Moisture analysis in BP samples was also performed according to the method of Fuenmayor et al., (2014). For this, 3 g BP sample was weighed with a scale with a precision of 0,0001 and heated at 65°C for 24 hours. After heating, the BP was reweighed. Percentage moisture values were calculated by subtracting the post-incubation weight from the initial weight of the BP.

BP samples were manually sorted based on color band appearance, resulting in 100% purity confirmed by a palynological test, and the total nitrogen content of the BP samples was determined by the traditional Kjeldahl method. Nitrogen values were multiplied by a conversion factor of 5,6 to determine total crude protein (Campos et al., 2008).

Total phenolic substances in BP samples were determined using the Folin-Ciocalteu method (Singleton and Rossi, 1965). First, each BP sample (1 g) dissolved in 4 mL of distilled water was mixed using vortex and the solution was mixed with Whatman No. 1 filtered through 2400 µL water, 200 µL undiluted Folin-Ciocalteu reagent and 600 µL sodium carbonate (20% Na_2CO_3) were added to the solution (40 µL). The absorbance was measured at 765 nm after 2 hours of incubation at room temperature (Magalhaes et al., 2010).

Statistical Analysis

Minitab package program was used in the statistical analysis of the data, and comparison of subgroups was made according to Duncan (p<0,05). The statistical model used to analyze the data to determine the effect of plant origin (WM, RR and WL; 1, 2, 3) on bee pollen pellet weights is as follows:

$$y_{ij} = \mu + ai + eij$$

$$\mu = Mean$$
$$a_i = plant\ origin\ effect\ (i = 1, 2, 3)$$
$$e_{ij} = error$$

RESULTS AND DISCUSSION

Distribution and plant origins of bee pollen (BP) collected from wild mustard (WM), rock rose (RR) and wild lavender (WL) fields are given in Tables 1, 2 and 3. In this study, in the BP collected in the WM field between 28 March and 29 April 2021 (Table 1), except for one week (11 April-15 April 2021), the wild mustard BP was generally above 45% and even harvested on 18 April-22 April 2021. Eighty percent of the BP collected consisted of WM bee pollen. Radish BP was added to WM bee pollen as secondary BP. In the BP harvests made in the colonies taken to the same region in previous years, it was observed that WM and radish BPs were predominant. Similary, the hives taken to the Gaffarlar village of Koçarlı mainly collected RR bee pollen (Table 2). This study and our observations made in previous years have shown that it is possible to evaluate WM and RR bee pollen as monofloral BP in the region. However, it is worth noting that colonies taken to the same place for BP production collected BP in different mixtures. For example, in one of the four colonies in the wild mustard area, mainly thistle BP was collected, while in the other three colonies, mainly WM and radish BP were collected.

Table 1. Distribution and plant origins of pollen collected from the WM field.

Bee pollen harvest date	Bee pollen distribution	Plant origin
28 March-2 April	516 (%46,1)	Wild Mustard
	493 (%44,1)	Radish
	91 (%8,1)	Dandelion
	20 (1,77)	-
4-8 April	546 (%52,56)	Wild Mustard
	337 (%32,43)	Radish
	151 (%14.53)	Dandelion
	5 (%0,47)	White Daisy
11-15 April	301 (%40.3)	Wild Mustard
	242 (%32,4)	Radish
	191 (%25,5)	Dandelion
	13 (%1,8)	Wild Vetch
18-22 April	592 (%80,1)	Wild Mustard
	74 (%10)	Radish
	73 (%9,9)	Thistle
25-29 April	336 (%52,9)	Wild Mustard
	112 (%17,6)	Radish
	109 (%17,1)	Thistle
	79 (%12,4)	Hibiscus

In summary, the dominant flower preferences of colonies located in the same place have changed from time to time. Alimoglu et al. (2021) in their study, they named the RR bee pollen (*Cistus* sp.) as monofloral with a rate of 58%, and some bee pollens belonging to the dandelion (48%), willow (64%) and rosaceae family (47%) as monofloral. Researchers have evaluated that there is a significant variation in the BP compositions of the samples obtained mostly from beekeepers in a single province (Samsun), and the reason for this is the diversity of environmental flora at different altitudes where the beehives are located. However, this study has shown that the flower preferences of hives in the same apiary differ from each other.

WL is known as Karabaş or Kargan in Aydın and its surroundings. WL blooms at the end of March in the lowlands of Koçarlı mountain villages of Aydın province, depending on the season, and its flowering continues throughout April and sometimes may extend until the end of May.

According to Table 2, 16-29% of the BP collected between 4 April and 28 April 2022 constituted black clover BP, and in general, bee colonies brought BP from wild vetch, white clover, meadow clover and black clover in the Gaffarlar region. In general, in the BP collected during April, the black grass BP rates varied between 16,6% and 29,05% and did not show dominant BP characteristics. Bee pollen collected from the Gaffarlar vicinity can generally be considered as a mixture (multifloral).

Table 2. Distribution and plant origins of bee pollen collected from the blackgrass field (Gaffarlar/Koçarlı).

Bee pollen harvest date	Bee pollen distribution	Plant origin
4-8 April 2022	180 (%34,35)	Wild Vetch
	156 (%29,77)	White Clover
	101 (%19,27)	Dandelion
	87 (%16,60)	Wild Lavender
11-15 April 2022	196 (%41,41)	Meadow Clover
	155 (%32,77)	Wild Vetch
	96 (%20,29)	Wild Lavender
	26 (%5,50)	White Clover
18-22 April 2022	119 (%27,10)	Wild Lavender
	112 (%25,51)	Meadow Clover
	120 (%27,33)	Wild Vetch
	88 (20,04)	Dandelion
25-28 April 2022	145 (%29,05)	Wild Lavender
	129 (%25,85)	Thistle
	128 (%25,65)	Wild Vetch
	97 (%19,43)	Dandelion

The RR plant is called pamucak or pamukluk in the region. When Table 3 is examined, 41,52% of the BP collected from colonies on 9-13 May 2022 was made up of RR. The rate of RR bee pollen increased throughout May, and 85,53% of the BP harvested on 6-10 June 2022 and 87,32% of the BP collected on 13-17 June 2022 consisted of RR bee BP.

Parameters related to BP pellet weights are presented in Table 4. According to the analysis of variance, the effect of plant type on BP pellet weight was found to be significant ($p<0,05$). In the comparison of subgroups, it was determined that Laden plant differed from the other two plants ($p<0,05$).

In this study, BP pellet weights ranged between 9,02-13,54 mg. These values are based on Bleha et al. (2019) is consistent with the findings. In this study, the average pellet weight of RR bee pollen was 1,2 mg heavier than WM and WL bee pollen. Thakur and Nanda (2020) reported the weight of 1 BP pellet of bee pollen, which they dried in the shade for 2 hours at 28°C±5 degrees, as 8,14±0,17 mg.

The moisture contents of bee pollen collected from each plant are given in Table 5.

Table 3. Distribution and plant origins of bee pollen collected from RR areas (Gaffarlar/Koçarlı).

Bee pollen harvest date	Bee pollen distribution	Plant origin
9-13 May 2022	304 (%41,52)	Rock Rose
	249 (%39,07)	Thistle
	178 (%24,35)	White Daisy
16-20 May 2022	284 (%55,5)	Rock Rose
	150 (%29,3)	White Daisy
	78 (%15,2)	Wild Pear
23-27 May 2022	247 (%51,24)	Rock Rose
	214 (%44,39)	Hibiscus
	21 (4,37)	White Daisy
30 May-3 June 2022	550 (%70,9)	Rock Rose
	175 (%22,6)	Thistle
	50 (%6,5)	-----
6-10 June 2022	585 (%85,53)	Rock Rose
	81 (%11,84)	Chaste Tree
	18 (%2,63)	---------
13-17 June 2022	477 (%87,32)	Rock Rose
	45 (%10,07)	Chaste Tree
	14 (%2,61)	-------
27-30 June 2022	397(%72,46)	Rock Rose
	62 (%11,31)	------
	48 (%8,75)	-------

Table 4. Bee bee pollen pellet weights collected from wild mustard, rock rose and wild lavender (1 load bee pollen).

Sample	WM bee pollen 1 pellet (mg)	RR bee pollen 1 pellet (mg)	WL bee pollen 1 pellet (mg)
1	10,17	12,9	11,8
2	9,38	10,28	12,6
3	10,2	11,75	10,32
4	9,86	11,22	9,58
5	9,48	10,48	10,36
6	9,34	10,85	10,36
7	9,90	13,02	10,23
8	12,51	13,54	9,02
9	9,97	10,25	10,40
10	13,43	12,59	9,36
Min-Max	9,34-13,43	10,25-13,54	9,02-12,6
Mean	10,42±0,440[b]	11,69±0,393[a]	10,40±0,340[b]

p<0,05; Different letters represent different groups

Table 5. Moisture content (%) of bee pollen of wild mustard, rock rose and wild lavender.

Sample	Wild Mustard	Rock Rose	Wild Lavender
1	22,38	20,51	21,69
2	20,36	18,6	19,38
3	23,56	20,36	20,58
mean	22,10±0,934	19,82±0,613	20,55±0,667

Moisture content of BP harvested from colonies; factors such as the season in which the BP is collected, seasonal air temperatures and humidity, and the harvest time of the BP affect it.

In this study, BP moisture contents varied between 18,6-23,56%. In studies, the moisture content of BP was stated to be between 18-30% (Mahfouz, 2016; Spulber et al., 2018; Keskin and Özkök, 2020). Mahfouz et al. (2016) found the moisture rates in BP to be 29,46% for olive, 30,11% for palm tree and 20,73% for orange. In addition, Spulber et al. (2018) determined the moisture rates for monofloral BP (rapeseed, thistle, dandelion, chestnut, lemon, hawthorn and fruit trees) in the range of 16-31%. The moisture contents (18-23,5%) determined in this study are compatible with the literature (Mahfouz, 2016; Spulber et al., 2018). The crude protein contents in BP collected from each plant are given in Table 6.

Table 6. The crude protein contents (%) of bee pollen samples.

Sample	Wild Mustard	Rock Rose	Wild Lavender
1	19,6	15,9	16,3
2	21,2	11,78	18,5
3	20,7	13,8	14,6
mean	20,5±0,47	13,8±2,51	16,5±0,95

In this study, it was determined that the crude protein content of WM bee pollen (20,5±0,47) was higher than the crude protein content of RR (13,8±2,51) and WL bee pollen (16,5±0,95). In studies conducted, De Melo et al., (2018) found the crude protein content in monofloral BP to be between 10,6-33,9%; Spulber et al. (2018) reported a range of 13-24%. In a study, the protein content in *Actinidia* (vine plant species) pellets varied between 15,4% (Liolios et al., 2015) and 18,1% (Tasei and Aupinel, 2008). Also, Spulber et al. (2018) reported the crude protein ratio in dandelion and chestnut BP as 13,76±0,99% and 20,61±1,35%, respectively. Mahfouz (2016) determined the crude protein rates in olive, palm and orange BP as 41,05%, 40,94% and 20,26%. The protein ratio in BP varies between plants, depending on the botanical origin of the collected plant and even the geographical region. The crude protein ratios found in this study are generally compatible with the literature (Liolios et al., 2015; De Melo et al., 2018; Spulber et al., 2018). According to the study conducted by Baloğlu and Gürel (2015); the crude protein ratio reported for WM and RR bee pollen are; it was reported as 21,8% and 11,9%. In this study, similar values were obtained for WM and RR bee pollen (20,5±0,47% and 13,8±2,51%).

The total amounts of phenolic (TPC) substances detected in BP collected from WM, RR and WL plant are presented in Table 7. Phenolic compounds, including flavonoids, serve as natural antioxidants and are very effective in eliminating the harmful effects of reactive oxygen species (Alimoglu et al. (2021). In recent years, the number of studies on TPC contents in multifloral and monofloral BP has increased and become important.

Table 7. Amount of TPC (mg GAE/g) in wild mustard, rock rose and wild lavender bee pollen.

Sample	Wild Mustard	Rock Rose	Wild Lavender
1	22,36	15,08	18,96
2	21,65	16,89	20,89
3	20,59	16,39	21,08
mean	21,53±0,514	16,12±0,539	20,31±0,677

In this study, TPC values ranged between 15,08-22,36 mg GAE/g. Ulusoy and Kolaylı (2014) determined the TPC value between 44,07-124,10 mg GAE/g. in 13 multifloral BPs collected in the Anzer plateau of Rize in summer and autumn. LeBlanc et al. (2009) found the TPC value of BP obtained in the Sonoro desert to be between 15,91 and 34,85 mg GAE/g.; Kroyer and Hegedus (2001) found the TPC value in multifloral BP in Austria to be 7,4–9,7 mg GAE/g; Morais et al. (2011) found TPC between 10,5–16,8 mg GAE/g in 5 BP samples in Portugal; Leja et al. (2007) 12,93–82,43 mg GAE/g. in 12 BP; De Melo et al., (2018) reported the total amount of phenolic substances in 8 different monofloral BP samples in Brazil as 5,6-29,7 mg GAE/g. Kalaycıoğlu et al. (2017), TPC value of Anzer plateau BP is 8,79-11,0 mg GAE/g; chestnut BP between 17,42-17,46 mg GAE/g; they reported the TPC value in oak BP as 7,56 mg GAE/g.. Mosic et al. (2019) reported that the TPC in bee BP samples ranged between 5,60 and 30,24 mg GAE/g.BP, with the lowest TPC level in Fabaceae (legumes) BP, and TPC levels were also low in samples dominated by Sophora BP.

The TPC range found in this study (15,08-22,36 mg GAE/g) is similar to LeBlanc et al. (2009), Leja et al. (2007). In this study, the TPC value determined for RR bee pollen (16,12±0,539 mg GAE/g) was determined by Alimoglu et al. (2021) was found to be similar to the value found (15,73 ± 0,9 mg GAE/g) for RR bee pollen (*Cistus* sp.).

CONCLUSION

Our country is one of the important beekeeping countries with its colony presence and honey production. However, bees can not adequately use their potential for the production of other bee products. The production of other bee products such as royal jelly, bee pollen (BP), propolis and bee venom is not sufficient. BP is the most produced after honey, but there is no official data on BP production and trade. While some of the BP produced is used in bumblebee rearing, the other part is offered for human consumption. However, BP offered for human consumption is generally dried, exposed to sunlight in small bags and sold. It is marketed in an unclear manner at harvest time, which plant's BP it contains, and its nutritional content.

In general, beekeepers offer multifloral BP collected by bees from different plant species, even spring and summer BP, to the market. Daily harvest and post-harvest cold chain preservation of BP is very important. Especially in BP that is not harvested daily, microorganisms and some fungi may develop due to the high humidity rate. When BP is exposed to sunlight for a long time, its colors fade and its smell changes. For this reason, it is very important to harvest BP daily and include it in the cold chain without keeping it waiting. In recent years, prolizidine alkaloids, which are secondary metabolites of some plants, have been found in borage, daisies and some legumes.

In our country, in the Aegean Region, which has Mediterranean climate characteristics, it is possible to produce monofloral BP or multifloral BP in certain regions and periods by staying only in the plains and in the mountains in summer, without moving the colonies to 4-5 places a year for almost 9 months of the year.

With this study, it was determined that the BP produced in the RR areas in the Aydın region can be considered as single plant BP, and that WM bee pollen can be mixed with wild radish BP, but monofloral BP cannot be produced from WL. In addition, the fact that the RR bee pollen pellets are larger than the other two plants shows that the BP efficiency of the RR plant is higher. In this study, WM bee pollen showed higher values in terms of protein and total phenolic substances than RR and WL bee pollen. However, it is possible to establish standard values for this plant BP through comprehensive analyzes on a larger number of BP samples. In order to offer standard BP with a certain content for human consumption as a food supplement, it is possible to create a standard for monofloral BPs by performing physico-chemical analyzes on the same type of plant BP collected from different geographies.

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