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CONTENTS

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RESEARCH PAPER

Determination of Some Yield Characteristics of Hungarian Vetch Varieties and their Evaluation as Bee Pasture

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Abstract

This study was carried out to determine some yield characteristics of Hungarian vetch varieties and to evaluate them as bee pastures based on bee-plant relationship. Eight Hungarian vetch varieties were used in the study. In Bingöl province, where the research was carried out, flowering of Hungarian vetch started on April 18. Counts started on April 23 and ended on May 11. Hungarian vetch remained blooming for about three weeks in Bingöl province. An average of 14.9 bees per m2 visited the Hungarian vetch and the bees remained on the flower for an average of 9.0 seconds. It has been determined that Hungarian vetch has an average of 377 flowers per m2, the natural plant height is 38.7 cm, the fruit per plant is 21.3, the seed per fruit is 4.0, the seed yield is 22.5 kg/da, and the thousand-seed weight is 33.0 g. Also, it was observed that the Hungarian vetch reached the highest natural plant height by April 30, the number of bees visiting the plant was high on May 04-07, the bees stayed on flower for a long time when the plant bloomed the most. It was seen that Kansur and Efes varieties are distinguishable for the features such as the number of bees per m2, the duration of the bee staying on flower, and the number of flowers, while the features such as natural plant height, seed yield and thousand-seed weight, are distinguished in Akçalar, Efes and Tarm Beyazı varieties.

Introduction

Vetch, an annual legume forage crop, is used as a source of roughage and concentrated feed in animal nutrition, and is also known as plants grown in different agricultural systems to increase the fertility of the soil. Vetch are known as important fodder crops because of their high grass yield and high nutritional value. There are about 150 species of vetch (*Vicia*) growing all over the world (Serin & Tan, 2008). In Turkey, natural vegetation is very rich in terms of vetch species. Hungarian vetch is an annual, whitish yellow flowered vetch species whose cultivation has become widespread in our country in recent years. Hungarian vetch is an native plant of Central Europe, the Balkans, the Danube countries and the Eastern Mediterranean Region. It is cultivated in a wide area from Spain to Asia Minor to the Caucasus and from the Lower Balkans to Central Europe (Balabanlı et al., 2009; Hashalıcı et al., 2017). Hungarian vetch takes the first place among the vetch varieties in terms of winter resistance. In addition, Hungarian vetch is resistant to drought and is cultivated even in arid conditions. For this reason, it can be planted in arid conditions in winter and can benefit greatly from early spring precipitation (Serin & Tan, 2008). Hungarian vetch, which can produce 350-450 kg/da of hay under arid conditions, contains 15-17% crude protein (Balabanlı et al., 2009). When Hungarian vetch is used in

crop rotation systems, it increases soil fertility, prevents the formation of foot stones, uses water economically, and does not tire the field. Therefore, it can be used successfully in fallow areas. There is a good forage production opportunity with Hungarian vetch planting in regions with an annual precipitation of more than 400 mm and in the bottom areas (Serin & Tan, 1999). Many plant species continue their generation by pollination. Pollination is the pollination of flowering plants and can also be defined as the name of the transfer "from the stamen to the pistil". While it was thought that pollination was provided by wind only before, as a result of scientific studies, it was determined that insects are effective and mostly bees (*Apoidea*) and the importance of studies on bee plant pollination has increased (Benedek, 1996). Honey bees are important insects that contribute to the fertilization of plants and increase the product while collecting pollen and nectar from plants in order to meet their nutritional needs (Çankaya & Korkmaz, 2008). Pollen collected by honey bees ensures the development of the glands that secrete royal jelly in worker bees and is used as the sole protein source of baby bees (Kutlu et al., 2005).

Pollen, which is a source of protein in the nutrition of honey bees, is obtained only from natural flora. With this study, it is desired to investigate whether Hungarian vetch flowers, which are thought to be a good food source for honey bees, will be preferred by honey bees as a source of nectar and pollen in areas where flora is insufficient. Therefore, in this study the relationship between Hungarian vetch and bee was discussed and it is aimed to plant Hungarian vetch to support the plant population in Bingöl ecological conditions, to evaluate Hungarian vetch varieties as bee pasture, and to determine some yield and yield elements of Hungarian vetch varieties.

Materials and Methods

Material

Hungarian vetch varieties named Akçalar, Aygün, Budak, Doğu Beyazı, Efes, Kansur, Sarıefe, and Tarm Beyazı were used as plant material in the research. The research was carried out at Bingöl University, Agricultural Application and Research Center. This area is 15 km away from the city center of Bingöl, located at the coordinates of 38º 32' 41.85" N and 40º 32' 25.58" E and its height above sea level is 1080 m.

Climatic Characteristics of the Research Area

The annual average temperature in Bingöl is 12.1ºC. In January and February, the average temperature is below zero, and July and August are the hottest months. The annual total precipitation of Bingöl province is 948.4 mm. The most precipitation is received during the winter months. July and August are the months with the least rainfall.

Soil Characteristics of the Research Area

According to the soil analysis, the soil structure is clayey-loamy, slightly acidic (pH: 6.26), salt-free (0.014%), organic matter content is low (1.09%), slightly calcareous (0.41%), potassium content is low (18.27 kg/da) and phosphorus ratio was found to be medium (7.60 kg/da).

Method

In the study, some yield characteristics of Hungarian vetch varieties were examined and the possible chance of using Hungarian vetch as a bee pasture was evaluated. The trial was established on October 02, 2020, with each application plot in 6 rows, the distance between rows 40 cm and the length of each row 20 m. The results were made on an area of 1 m^2 determined in the parcels in three replications. The first bloom was seen on April 18, 2021. With the increase in the flowering rate of the plot, observations began to be taken as of April 23. On six different days, 23 April, 27 April, 30 April, 04 May, 07 May and 11 May, at 9:00 in the morning, 12:00 in the afternoon and 15:00 in the afternoon (Tansı & Kumova, 1999; Bakoglu & Kutlu, 2006; Kutlu et al., 2018) the number of bees per m^2 was determined as the average of these three different times for five minutes at three different times. The duration of stay of the bees in the flower was determined by keeping the time in seconds with the help of the chronometer. The natural plant height was measured in cm on six different days, with 10 plants in each replication. Again, data were obtained by counting the number of flowers per m^2 and taking the average at six different times. After the flowering phase was over, the number of fruits per plant, the number of seeds in the fruit were obtained, and 1 m^2 of land was harvested from each plot, the seed yield obtained from this area, the seed yield by hand threshing and the thousand-seed weight data were obtained.

Analysis of variance was applied to the obtained data with the help of JMP statistical package program. The differences of the means were compared with the LSD test at the 0.05 level.

Results and Discussion

In the study, characteristics such as the number of bees visiting Hungarian vetch cultivars per m^2, the duration of the bees in flower, the number of flowers per m^2, plant height, fruit per plant, seeds per fruit, seed yield and thousand grain weight were investigated.

1- The Number of Bees Per m^2 Determined at Different Counting Times of Hungarian Vetch Varieties

In this study, the number of bees that visited Hungarian vetch varieties per m^2 at different times is given in Table 1. In the table, it is observed that the

difference between Hungarian vetch varieties and the number of bees visiting per m^2 at different times is statistically significant. According to the table, the highest number of bees per m^2 on average was determined on 07 May. On April 23, the lowest number of bees per m^2 was determined. In terms of variety, the highest number of bees was determined in Efes and Kansur varieties, and the lowest number of bees in Sarıefe, Aygün, and Akçalar varieties. The number of visiting bees per m^2 was determined as 14.9 as the average of the varieties and different times. The flowering date of Hungarian vetch varieties, the air temperature and the number of rainy days in April and May affect the number of visiting bees per m^2.

Table 1. The number of bees per m^2 determined at different counting times of Hungarian vetch varieties (units)

Varieties	Time of Count						
	23 April	**27 April**	**30 April**	**04 May**	**07 May**	**11 May**	**Average**
Akçalar	8.5	6.0	9.3	17.2	20.0	8.5	11.6 c**
Aygün	4.2	4.7	13.5	21.2	16.0	5.0	10.8 c
Budak	3.2	8.5	13.3	19.0	31.7	5.3	13.5 bc
Doğu Beyazı	6.5	11.7	19.0	17.5	34.8	3.3	15.5 b
Efes	4.7	7.0	5.5	23.2	43.0	42.0	**20.9 a**
Kansur	6.3	12.8	22.2	30.8	38.7	15.2	**21.0 a**
Sarıefe	5.7	8.0	15.8	17.2	14.8	1.3	10.5 c
Tarm Beyazı	5.7	10.2	11.7	28.3	34.8	4.8	15.9 b
Average	5.6 e**	8.6 de	13.8 c	21.8 b	**29.2 a**	10.7 cd	**14.9**

**: $P \leq 0.01$

2- The Duration of the Bees in Flower at Different Counting Times of Hungarian Vetch Varieties

In the study, the duration of the bees in flower in Hungarian vetch varieties and at different times is given in Table 2. In the table, it is seen that the difference between Hungarian vetch varieties and the duration of the bees in flower at different times is statistically significant. According to the table, the maximum duration of inflorescence was determined on May 04. In terms of variety, the maximum duration of inflorescence was determined in Akçalar and Kansur varieties, and the lowest duration in flowering was determined in Sarıefe variety. As the average of the varieties and different times, the duration of the bees in flower was determined as 9.0 seconds. Air temperature, cloudy or rainy weather on the dates of the observations were effective on the duration of the bees' staying on flower.

Table 2. The duration of bees in flower of Hungarian vetch varieties at different counting times (seconds)

Varieties	Time of Count						
	23 April	**27 April**	**30 April**	**04 May**	**07 May**	**11 May**	**Average**
Akçalar	6.7	7.1	9.3	16.4	10.6	11.7	**10.3 a***
Aygün	7.3	6.3	10.3	11.2	10.7	8.9	9.1 abc
Budak	10.0	6.5	11.8	10.0	5.8	7.8	8.7 abc
Doğu Beyazı	6.8	8.5	11.8	13.5	8.5	3.0	8.7 abc
Efes	10.2	6.4	5.5	8.5	6.3	10.8	8.0 bc
Kansur	7.9	5.9	15.7	11.2	8.5	11.1	**10.1 a**
Sarıefe	7.8	5.7	9.8	12.4	8.0	2.1	7.6 c
Tarm Beyazı	7.9	7.5	9.6	14.9	7.2	10.7	9.6 ab
Average	8.1 c**	6.7 c	10.5 b	**12.3 a**	8.2 c	8.3 c	**9.0**

*: $P \leq 0.05$, **: $P \leq 0.01$

3- The Number of Flowers Detected Per m^2 of Hungarian Vetch Varieties at Different Counting Times

The number of flowers determined per m^2 in Hungarian vetch varieties and at different times in the study are given in Table 3. In the table, it was observed that the difference between the Hungarian vetch varieties and the number of flowers per m^2 at different times was statistically significant. According to the table, the highest number of flowers per m^2 was determined on May 04. April 23 was the date when the lowest number of flowers per m^2 was seen. In terms of variety, it was determined that the highest number of flowers per m^2 was in Kansur cultivar and the lowest number of flowers per m^2 was in Akçalar cultivar. The average number of flowers per m^2 at different counting times of the cultivars was determined as 377.

Table 3. Number of flowers per m^2 of Hungarian vetch varieties at different counting times (pieces)

Varieties	Time of Count						
	23 April	**27 April**	**30 April**	**04 May**	**07 May**	**11 May**	**Average**
Akçalar	26	164	313	558	429	185	279 d**
Aygün	41	164	395	710	405	138	309 cd
Budak	48	188	250	773	425	151	306 cd
Doğu Beyazı	56	313	421	1013	656	105	427 ab
Efes	18	43	110	710	720	1105	451 ab
Kansur	49	170	359	1024	670	690	**494 a**
Sarıefe	48	273	455	843	395	291	384 bc
Tarm Beyazı	55	350	455	656	520	188	371 bcd
Average	43 e**	208 d	345 c	**786 a**	528 b	357 c	**377**

**: $P \leq 0.01$

4- Natural Plant Height in Hungarian Vetch Varieties

In the study, the data about the natural plant heights in Hungarian vetch varieties and at different times are given in Table 4. It was observed in the table that the difference between Hungarian vetch varieties and natural plant heights determined at different times was statistically significant. According to the table, the highest natural plant height was determined on 30 April, 04 May, 07 May and 11 May. In terms of variety, it was determined that the highest natural plant height was determined in Akçalar variety. The average natural plant height of the cultivars at different times was determined as 38.7 cm.

Table 4. Natural plant heights of Hungarian vetch varieties according to different counting times (cm)

Varieties	Time of Count						
	23 April	**27 April**	**30 April**	**04 May**	**07 May**	**11 May**	**Average**
Akçalar	32.7	39.2	63.2	39.1	38.9	42.2	**42.6 a****
Aygün	32.6	37.8	38.1	46.0	39.0	41.0	39.1 bcd
Budak	26.1	33.0	41.1	38.2	39.3	39.9	36.3 d
Doğu Beyazı	32.0	40.2	42.5	41.5	41.2	40.5	39.7 abc
Efes	24.9	35.3	34.4	39.2	37.9	45.6	36.2 d
Kansur	27.9	32.3	37.5	40.7	39.1	40.5	36.3 d
Sarıefe	36.1	35.3	39.4	46.7	46.3	46.2	41.7 ab
Tarm Beyazı	29.0	41.9	35.8	38.8	39.4	43.1	38.0 cd
Average	30.2 c**	36.9 b	**41.5 a**	**41.3 a**	**40.1 a**	**42.4 a**	**38.7**

**: $P \leq 0.01$

5- Number of Fruits Per Plant, Number of Seeds Per Fruit, Seed Yield And Thousand-Seed Weight in Hungarian Vetch Varieties

In the study, the number of fruits per plant, number of seeds per fruit, seed yield and thousand-seed weights of Hungarian vetch cultivars are given in Table 5. Among these characteristics, the difference between the cultivars in terms of seed yield and thousand grain weight was found to be statistically significant. According to the data obtained, the highest seed yield was determined in Efes variety (76.9 kg/da), and the highest thousand kernel weight was determined in Tarm Beyazı variety with 38.6 g. In the study, it is seen that there is no statistical difference between the cultivars in terms of the number of fruit per plant and the number of seeds per fruit. The number of fruits per plant was determined as 18.0-25.8 with an average of 21.3, while the number of seeds per fruit was determined as 3.7-4.6 with an average of 4.0.

Table 5. Number of fruits per plant, number of seeds per fruit, seed yield and thousand seed weights in Hungarian vetch varieties

Varieties	Fruits per plant (unit)	Number of seeds per fruit (unit)	Seed yield (kg/da)	Thousand seed weights (g)
Akçalar	25.8	3.7	23.0 bc**	38.3 ab*
Aygün	22.4	4.6	4.9 e	26.3 d
Budak	21.9	4.3	8.0 e	29.1 d
Doğu Beyazı	20.2	3.7	18.2 cd	31.5 bcd
Efes	22.9	4.0	76.9 a	37.0 abc
Kansur	20.9	4.3	26.9 b	32.4 abcd
Sarıefe	18.4	3.8	6.3 e	30.9 cd
Tarm Beyazı	18.0	3.8	15.9 d	38.6 a
Average	**21.3**	**4.0**	**22.5**	**33.0**

*: $P \leq 0.05$, **: $P \leq 0.01$

The number of flowers in plants is very important in terms of both seed yield and nutrition of bees. It is generally known that plants with more flowers, are visited more frequently by bees according to the nectar status in the flowers of the plant (Özyiğit & Bilgen, 2003). Since pollination will increase during these visits by bees, more seeds are obtained from plants.

A study was conducted by Tansı and Kumova (1999) on the possibilities of using some forage crops (Broad bean, rapeseed and phacelia) as a bee pasture and on the determination of seed yields. They reported that the average flowering period of 3 years during the flowering periods of the plants in the parcel consisting of all three plants was 54 days, the number of flowers varied between 16.6-746 pieces/m² and the bee density varied between 1-64 bees/m². They also determined that the seed yield increased significantly in rapeseed and phacelia plants that were visited by honey bees.

Kumova et al. (2001), in their study to determine the preference of honey bees in phacelia cultivars, reported that they made flower and honey bee counts in the plant once a week at three different points of each cultivar determined in the plot during the flowering period. In the censuses made in the research, the average number of flowers in Turan 82, T-98/1 and T-98/2 cultivars was 1077.60 ± 231.43, 971.10 ± 283.06 and 1021.10 ± 403.57 units/m^2, and the average number of honey bees was 68.10 ± 17.30, 62.36 ± 14.93 and they determined that they were 62.23 ± 21.57 pieces/m^2 and the difference between varieties was insignificant.

In the study conducted by Özyiğit and Bilgen (2003) to determine the effect of different cutting times on yield and agricultural characteristics of some legume forage crops in Antalya lowland conditions, it was reported that the first flowering plant was the forage pea and was initially visited by bees, but almost no bees came to this plant after the grass pea bloomed. The researchers determined the number of flowers per plant and the number of bees per m^2, 41.33 and 13.67 in common vetch, 86.33 and 20.00 in Anatolian clover, 81.62 and 5.00 in melilot, 12.33 and 10.33 in sainfoin, 42.33 and 10.67 in hairy vetch, 16.33 and 12.33 in grass pea, 13.33 and 2.33 in field pea. In addition, researchers reported that they observed a dense bee population in Anatolian clover despite the flowering of plants in the natural environment.

Kızılşimşek and Ateş (2004), in their study to examine the flowering course of phacelia at different sowing times in Kahramanmaraş conditions and to determine the possibilities of using it as a bee pasture, the number of flowers during the flowering period was between 61-1662 pieces/m^2, and the number of flowers in 5 minutes visited the flowers in m^2. They reported that the number of bees varies depending on the number of flowers and they found an average of 7 per square meter when the flowers were very few, and 119 pieces/m^2 when the flowers were plentiful.

In the study conducted by Bakoğlu and Kutlu (2006) to determine the effects of different row spacing on some agricultural characteristics in phacelia in Bingöl irrigated conditions, the number of flowers was determined as 8982.23 pieces/m^2, the number of bees as 116 pieces/m^2, and the highest values were observed in 50 cm row spacing.

Kuvancı and Deveci (2010), in their study to evaluate facelia, sainfoin and clover plants according to honey bee preference, found that the most visited plant by honey bees in terms of plant preference was phacelia with 71.8 units/m^2 that followed by sainfoin with 55.9 units/m^2 and reported that the alfalfa plant, with 1.5 units/m^2, is much less preferred next to phacelia and sainfoin.

In the study conducted by Tuncer (2014) to determine the vegetative properties of phacelia at different nitrogen doses (2.5, 5.0, 7.5 and 10.0 kg/da); it has been reported that N doses have a significant effect on bee visitation, the highest bee visitation occurs at the N4 dose during the 50% flowering period, and the lowest bee visitation occurs at the N1 dose. Researchers have determined that the number of bees visiting the flowers in the plant varies between 18.17-24.33 pcs/m^2, including the control.

Kuvanci et al. (2016) in the study conducted to determine the importance of Alexandria clover and phacelia in plant preference of honey bees, it was declared that the average number of flowers in plants was 33600 per Alexandria clover and 9500 per m^2 in phacelia. In addition, it was reported that the bee visits to the flowers in the plants within 5 minutes were 72.74 units/m^2 in the phacelia plant, 53.90 units/m^2 in the Alexandria clover, and the visits made during the day were at noon in both plants.

Hashalıcı et al. (2017) obtained the highest main stem length of 75.5 and 76.3 cm from Aegean Beyaz-79 and Budak varieties in the first year of Hungarian vetch planting. In addition, they determined the highest main stem length of 53.0, 55.3, 56.7 and 56.9 cm in the second year in Tarm Beyazı-98, Anadolu Pink-2002, Aegean Beyazı-79 and Budak varieties. Tenikecier et al. (2020) determined the main stem length of Hungarian vetch genotypes as 51.60 cm in the 50% flowering period in the first year and 55.52 cm in the 50% flowering period in the second year in the study they carried out for two years. Bakoglu et al. (2004) reported that they achieved an average plant height of 46.20 cm in their study on the adaptation of some Hungarian vetch lines and varieties to Bingöl dry conditions. Sayar et al. (2012) reported the plant height as 52.3-63.1 cm in the study they conducted with 12 Hungarian vetch genotypes in Kızıltepe conditions of Mardin province. In the study of Çaçan et al. (2021) the highest plant height (93.3 cm) at the first planting time was reported in their study in which they examined the yield, quality and nutrient content of Hungarian vetch according to different sowing times. It is seen that the natural plant heights obtained in these studies are similar to the natural plant heights obtained in other studies, but are lower than the findings measured as the main stem length.

Erdoğdu et al. (2016) reported that the seed yield was between 53.7-76.5 kg/da in their study on the forage and seed yields of Hungarian vetch lines and varieties in Eskişehir conditions. Albayrak et al. (2011) investigated the effects of 2 row spacing (17.5 and 35.0 cm) and 4 sowing norms (4, 6, 8 and 10 kg/da) applications on the forage yield and quality of Hungarian vetch in Isparta conditions, and according to the data obtained in their two years study, They declared that the seed yield is between 53-98 kg/da in average.

Bakoğlu et al. (2004), in their study on the adaptation of some Hungarian vetch lines and varieties to Bingol dry conditions, reported that the highest number of pods per plant in the Ege Beyazı variety was 9.37 and the average of the varieties was 7.65. In addition, they reported that they found the highest number of seeds in the pod (3.50 units) from line 16, and the Ege Beyazı variety with the highest number of seeds per plant (26.76 units).

Uzun et al. (2004) investigated the effects of four different sowing rates on seed yield and yield components in four Hungarian vetch lines planted in winter. Statistically significant differences were observed between the Hungarian vetch lines, which were examined according to the two-year data they obtained, in terms of all measured characteristics except 1000 grain weight. In the study, the number of pods per plant was 27.7-43.9, the number of seeds per plant was 94.1-171.2, the weight of 1000 seeds was 36.2-37.3 g, and the seed yield was 50.5-140.3 kg/da.

Conclusion

This study was carried out to determine some yield characteristics of Hungarian vetch varieties and to evaluate them as bee pastures; the highest number of bees per m^2 visited Hungarian vetch varieties was on 07 May. The maximum duration of stay of the bees and the number of flowers per m^2 were observed on May 4, the highest natural plant height was observed on May 11, and the differences between the groups were found to be statistically significant. In addition, in terms of seed yield, Efes cultivar and Tarm Beyazı cultivar showed the highest yield according to thousand seed weight characteristics and statistically differences were observed between cultivars. In this study, there was no difference between cultivars in terms of the number of fruits per plant and the number of seeds per fruit.

As of April 18, the first flowers have started to appear in Hungarian vetch in Bingöl conditions. Counts started on April 23 and flowering ended on May 15. Hungarian vetch stayed on flower for about 3 weeks in Bingöl conditions. The highest number of bees visiting the plant was on 07 May. It has been determined that bees stay on flower longer between 30 April and 04 May. The date of May 11 was when the plant reached its highest plant height.

As a result, when Hungarian vetch was evaluated as bee pasture, it was concluded that Kansur and Efes in terms of bee-plant relationship, Akçalar, Efes and Tarm Beyazı varieties are ideal for Bingöl conditions in terms of natural plant height, seed yield and thousand-seed weight. Considering the time in the flowering period of Hungarian vetch, it was concluded that bees can benefit from this plant significantly after the winter season and therefore it can be used as a bee pasture until the first

half of May in Bingöl conditions.

Ethical Statement

Not applicable.

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Author Contributions

The authors contributed on an equal footing and there was no conflict between the authors.

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RESEARCH PAPER

Determination of Antioxidant Capacities with the Phenolic and Flavonoid Contents of Royal Jelly Mixtures

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Abstract

Royal jelly, which is used in functional foods due to its antioxidant capacity, has started to be widely used. In this study, the antioxidant capacities and phenolic contents of royal jelly mixtures were determined. Total phenolic substance contents (TPC) of royal jelly mixtures were specified using the Folin-Ciocalteu procedure, total flavonoid substance contents (TFC) by aluminium chloride colourimetric method, phenolic compound contents with LC-MS/MS and antioxidant activity with 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. TPC ranged from 0.6 to 38.2 mg GAE/100 g, and the mean value was determined as 11.08 mg GAE/100 g. TFC ranged from were ranged from 0.1 to 30.06 mg QE/100 g and a mean value of 9.2 mg QE/100 g, and DPPH ranged from 4.4 to 93.0 mg TE/mL and a mean value of 29.11 mg TE/mL. TPC, TFC and DPPH activities were highest in S6 and S7 samples, and lowest in S1 and S2 samples. Caffeic acid phenethyl ester was the highest yielding phenolic compound in all samples, and Luteolin was the lowest yielding compound. The highest phenolic compound contents were determined in the S6 and S7 samples. As a result, it has been observed that food products containing bee products are rich in phenolic contents and have high antioxidant properties. TPC, TFC, DPPH activity and phenolic contents were found to change as the type and ratio of bee products in the food changed.

Introduction

The oxidation reaction, which occurs as a result of the interaction between the oxygen in the air and the food components, can often cause undesirable problems such as colour, taste and odour changes and a decrease in nutritional value. The substances that can be found naturally in the food that prevent these undesirable problems or that can be added externally are defined as antioxidant substances (Köksel, 2007). For example; Royal jelly is food with natural antioxidant properties. The amount of antioxidant substances in the content of bee products varies depending on the plant source from which the nectar is produced, seasonal and environmental factors (Kılıç Altun & Aydemir, 2021; Spilioti et al., 2014). In addition to these, in addition to enzymes such as catalase, glucose oxidase, and peroxidase, the antioxidant feature of bee products is obtained from phenolic acids (benzoic, ferulic, coumaric and caffeic acid), flavonoids, tocopherols, carotenoids and vitamins such as thiamine, ascorbic acid and riboflavin originates (Khalil et al., 2012). It has been stated that the amount of phenolic substances contained in bee products and their antioxidant properties are related, and the antioxidant properties increase due to the increase in the total amount of phenolic substances (Alzahrani et al., 2012).

Natural diets rich in flavonoids and phenolic content with antioxidant effects have increased the interest in nutrition and food science in recent years. Natural phenolic and flavonoid compounds are secondary metabolites holding an aromatic ring with at least one hydroxyl group (Köksel, 2007). Phenolic compounds because their hydroxyl groups can directly contribute to the antioxidant effect, and some even stimulate the synthesis of endogenous antioxidant

molecules in the cell (Gomes et al., 2010; Kahraman et al., 2010). Research reports show that phenolic compounds prevent metal inactivation, free radical inhibition in biological systems, peroxide decomposition and oxidative disease burden (Özmen & Akın, 2006).

Beekeeping, which has been carried out without the need for much capital since ancient times, is one of the most important agricultural activities worldwide, which provides a return in a short time with honey and other bee products (Bölüktepe & Yılmaz, 2008). As a result of beekeeping, which is one of the oldest agricultural activities in the world in the historical process, various bee products such as honey, beeswax, pollen, royal jelly, bee venom and propolis are obtained. These products, which are used as foodstuffs, are also widely used in the treatment of many diseases.

Bee products have been used as a natural medicine, immune booster and antioxidant in order not to be exposed to the chemical effects of drugs for the protection of health and the treatment of diseases, and have been preferred by people since ancient times. Today, various bee products such as honey, pollen, royal jelly, propolis and foods containing bee products appear in markets, advertisements and breakfast rooms as functional foods. In this case, the nutritional values, nutritional contents, and product characteristics of bee products come to the fore. High total phenolic and flavonoid contents in bee products are associated with high nutritional and nutritive values and antioxidant capacity. Royal jelly is the special food of the queen honey bee larva and is a special bee product secreted from the hypopharyngeal and mandibular glands of worker honey bees between the sixth and twelfth days of their lives (Haydak, 1970; Pavel et al., 2011). Today, royal jelly is included in many functional foods due to its many benefits. This study aimed The aim of this study was to determine the antioxidant capacities and phenolic compositions of mixtures containing royal jelly.

Materials and Methods

Collection of Royal Jelly Mixtures

In this research, 7 samples of commercial royal jelly mixes were selected from products offered in pharmacies and marketplaces in Türkiye (Erzurum) in 2022. While the samples were being collected, care was taken that they were not from the same brand or the same content. The contents of the product are based on the information written on the packaging of the products. The purchased royal jelly mixtures were brought to the laboratory in their original packaging, protected from sunlight, and immediately analyzed.

Determination of total phenolic content (TPC)

The TPC of royal jelly mixtures was specified using the Folin-Ciocalteu procedure (Singleton & Rossi, 1965). Firstly, 12 µL of each royal jelly mixture was mixed with methanol and was added to 60 µL of 2.5 mL of 0.2 mol/L Folin-Ciocalteu solution for 10 minutes. Afterwards, 48 µL Na_2CO_3 solution (7.5% w/v) was supplemented with this reaction and incubated for 30 minutes at room temperature. The absorbance measurements were performed at 760 nm. Results were read as gallic acid equivalent (µM Gallic acid/g dry weight).

Determination of Total Flavonoid Content (TFC)

The aluminum chloride colorimetric method was altered from the method analyzed by Chang et al (2002). The royal jelly mixture sample was mixed with methanol (10 µL) and then added with 3 µL $NaNO_2$ solution (5% w/v) with 40 µL of distilled water. 5 minutes later, 3 µL of 10% (w/v) aluminum chloride solution was added. In the final stage, 20 µL of NaOH was reacted after 5 minutes. The absorbance was measured at 415 nm after 15 minutes of incubation at room temperature. Results were stated as quercetin equivalent (µM Quercetin/g dry weight).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

Heretofore, different procedures have been applied to survey the antioxidant activity of royal jelly samples. The antioxidant activity of royal jelly was estimated by the determination of DPPH (2,2-diphenyl-1- picrylhydrazyl), by the method of Liu et al. (2008) and Takım, 2010. The stock solution of 0.6 mM DPPH in methanol was equipped. The working solution was acquired by diluting the stock solution with methanol to get an absorbance of 1.1 at 517 nm. The reagent mixtures in the 96-well plates occurred of a sample (7.5 µL) and DPPH stock (150 µL) was dissolved in methanol and protected in a dark place for 30 minutes at room temperature. The absorbance was read at 517 nm against methanol as blank. The results were expressed as Trolox equivalent as antioxidant capacity (TEAC) (mg TE/mL dry weight).

Extraction Stage of LC-MS/MS Analysis

The royal jelly mixtures extraction for LC-MS/MS analysis was carried out using the method of Necip et al. (2021). 25 g of royal jelly mixture was diluted with 5X of acidic water (pH 2 with HCl) and filtered. The filtrate was then poured into a glass column loaded with Amberlit XAD-4 rosins. Therefore, phenolic compounds stayed in the column, and other polar compounds were washed with a hydrated solvent. Then the washing step was carried out. The sediment was melted in 5 mL of water and extracted with diethyl ether. The extracts were concentrated in degraded pressure at 30°C in a rotary evaporator. The dried residue was reacted with 0.5 mL of methanol and passed through a 0.45 µm membrane filter ready for LC-MS / MS analysis.

Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS) Analysis of Phenolic Compounds

Analysis of the phenolic compounds was done with a UHPLC apparatus connected to a dual MS device. 15 different phenolic compounds were analyzed in the royal jelly mixtures. These compounds were epicatechin, caffeic acid, t-cinnamic acid, apigenin, resveratrol, luteolin, quercetin, chrysin, hesperetin, rhamnetin, pinocembrin, caffeic acid phenethyl ester, p-hydroxybenzoic acid, p-coumaric acid, ferulic acid (Necip et al., 2021).

Extraction Stage of LC-MS/MS Analysis

All data were analyzed statistically using SPSS 24.0 (SPSS Inc., Chicago, IL, USA) software. Results are given as mean ± standard deviation (SPSS, 2017).

Results and Discussion

Antioxidant capacity, TPC, and TFC results are given in Table 1. and Figure 1. TPC ranged from 0.6 to 38.2 mg GAE/100 g, and the mean value was determined as 9.2 mg GAE/100 g. Flavonoids, a significant subclass of polyphenols, were also analyzed entirely in the royal jelly mixtures, the results ranged from 0.1 to 30.06 mg QE/100 g and a mean value of 11.08 mg QE/100 g. DPPH ranged from 4.4 to 93.0 mg TE/mL, and a mean value of 29.11 mg TE/mL (Table 1).

TPC, TFC and DPPH activities were highest in S6 and S7 samples, and lowest in S1 and S2 samples. The highest TPC, TFC and DPPH activities in the 6th and 7th samples may be due to the high propolis content and the carob molasses content. Although the 3rd sample contains a higher percentage of honey and propolis than the S5 sample, it was observed that the antioxidant

Table 1. The number of bees per m^2 determined at different counting times of Hungarian vetch varieties (units)

No	Sample content	Total flavonoid (mg QE/100 g dry weight)	Total phenolic (mg GAE/100 g dry weight)	DPPH (mg TE/mL dry weight)
S1	royal jelly(3%) honey(95.5%) propolis(1.5%)	0.1±0.1	0.9±0.3	2.1±0.4
S2	royal jelly(1.5%) honey (97.5%) propolis (1%)	0.1±0.1	0.6±0.2	1.6±0.9
S3	royal jelly (3%) honey (95.5%) propolis (1.5%)	2.9±0.8	3.7±0.3	7.4±1.4
S4	royal jelly (1.5%) honey (97.5%) propolis (1%)	0.8±0.1	2.4±0.1	4.9±0.9
S5	royal jelly (5%) honey (89 %) propolis (1%) ginseng (5%)	1.7±0.2	10.9±0.1	26.7±1.0
S6	royal jelly (12%) harnup molasses (30%) propolis (18%) polen (30%) cacao (10%)	30.6±0.2	20.9±7.4	68.1±2.1
S7	royal jelly (24%) harnup molasses (15%) propolis (36%) polen (25%)	28.2±14.1	38.2±5.1	93.0±8.2

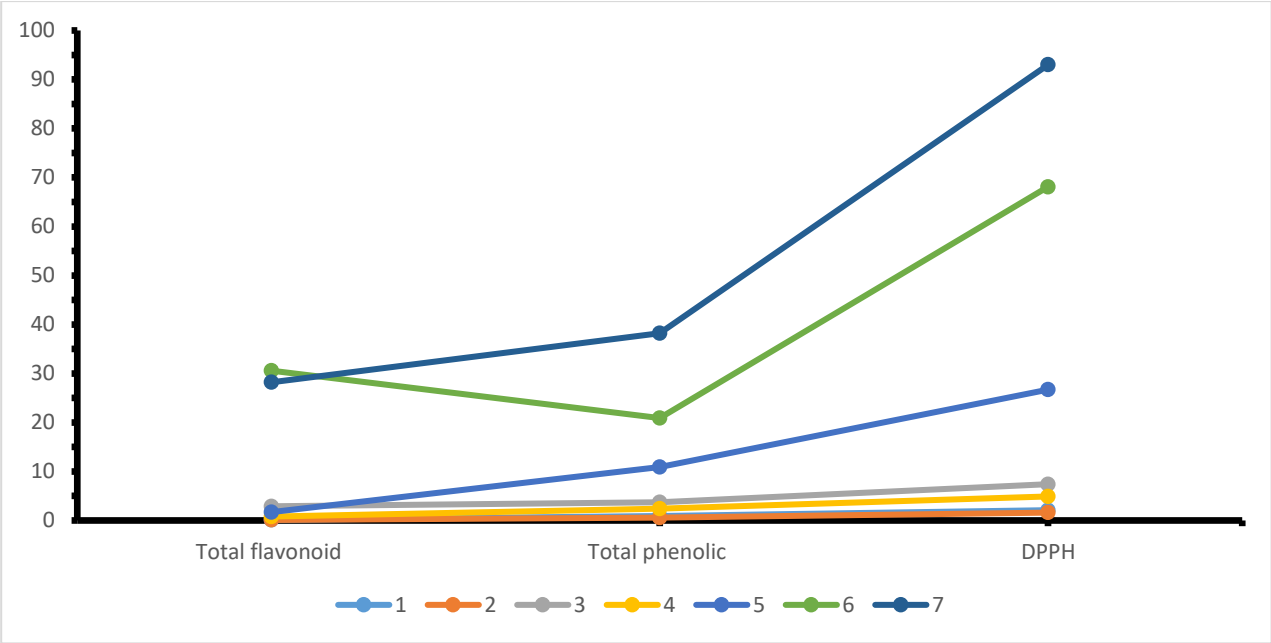

Figure 1. Total phenolic, total flavonoid and DPPH values of the samples

activity was very high in the S5 sample. This may be due to the ginseng (5%) contained in the 5th sample. It was determined that as TPC and TFC amounts of all samples increased, DPPH activities also increased.

The phenolic compound profile and amounts of the samples are given in Table 2. Caffeic acid phenethyl ester was the highest yielding phenolic compound in all samples, and Luteolin was the lowest phenolic compound. The highest phenolic compound contents were determined in the S6 and S7 samples. The phenolic compound contents of the samples were in harmony with the total phenolic and total flavonoid contents, and the total phenolic and total flavonoid contents of the samples with high phenolic content were also high. In addition, phenolic compound content was compatible with DPPH activity, and samples with high phenolic content were also found to have high DPPH activities.

According to the results of the study, TPC, TFC, DPPH activity and phenolic content were found to be variable among the samples. In fact, this difference and

Table 2. Phenolic compund profile of the samples

Phenolic compound	S1	S2	S3	S4	S5	S6	S7
Epicatechin	4.9±0.1	5.6±1.2	12.8±1.4	6.5±1.3	36.2±2.6	91.2±3.6	130.1±2.3
Caffeic acid	21.2±0.6	25.7±2.4	46.4±3.2	29.8±2.1	109.5±3.7	312.7±8.7	528.7±8.9
t-Cinnamic acid	1.2±0.1	1.3±0.3	2.6±0.2	1.4±0.4	9.6±1.1	29.6±2.3	37.7±1.5
Apigenin	9.1±0.3	9.8±0.7	11.6±1.2	10.6±0.8	59.3±3.0	184.2±6.4	218.0±5.8
Resveratrol	0.8±0.4	1.1±0.1	1.6±0.3	1.4±0.2	5.7±0.2	16.9±2.3	27.9±2.1
Luteolin	0.7±0.1	0.9±0.1	1.2±0.1	1.1±0.1	4.1±0.4	12.5±1.2	14.6±1.1
Quercetin	2.9±0.3	3.8±0.8	6.6±0.9	4.0±0.1	27.0±1.7	67.5±23	85.9±2.1
Chrysin	165.1±2.9	179.8±6.4	207.2±5.8	187.0±3.7	742.3±11.2	1613.5±20.2	2431.6±12.5
Hesperetin	7.7±0.8	8.2±1.0	11.8±1.4	8.9±1.4	41.2±4.2	161.0±4.3	194.8±5.3
Rhamnetin	8.2±.0.4	12.0±1.2	13.9±1.5	9.7±1.0	53.2±4.8	150.4±4.8	186.1±4.1
Pinocembrin	22.2±1.2	23.1±2.8	35.9±2.2	25.9±2.1	123.0±5.8	323.8±6.5	409.4±4.8
Caffeic acid phenethyl ester	246.5±4.7	254.4±6.3	424.7±8.4	260.9±4.9	1131.0±19.1	4753.1±38.6	5501.1±15.5
p-Hydroxybenzoic acid	2.2±0.2	2.8±0.8	5.4±1.2	3.0±0.2	12.8±1.1	40.9±2.4	60.3±1.2
p-Coumaric acid	15.4±1.3	16.1±1.2	16.4±1.0	16.2±0.8	55.4±2.3	151.9±4.3	206.6±2.2
Ferulic acid	20.0±1.1	20.4±2.3	32.3±2.6	21.3±1.1	171.5±4.9	337.4±3.8	431.5±3.8

variability is an expected result. Because the types and proportions of bee products contained in the samples are different. It is already known that the level of antioxidant capacity of bee products is closely related to their chemical composition. It was determined that TPC, TFC, DPPH activity and phenolic content increased as the ratio of royal jelly increased in the samples. In addition, it was determined that the 6th and 7th samples containing pollen had higher TPC, TFC, DPPH activity and phenolic content than the samples without pollen. Similar to this result we found, Ulusoy (2010) reported in a study that pollen contains 10-20 times more total phenolic substances than honey. Based on the literature data, it is reported that propolis has the strongest antioxidant property among all examined bee products, and also contains high amounts of TPC and TFC. Propolis is followed by bee pollen and royal jelly, respectively (Kocot, 2018). In our results, it was determined that the samples with high propolis content had increased TPC, TFC, DPPH activity and phenolic content. It can be said that the TPC, TFC, DPPH activity and phenolic content of the samples are variable, as well as the geographical location, climate and vegetation of the region where the bee product used in the samples was collected (Bakkaloğlu & Arıcı, 2019).

As a result of the literature research, no studies have been found on products similar to the one we have done. However, there are studies related to TPC, TFC, DPPH activity and phenolic contents of bee products. The results we found in the samples are similar to the results found in studies on bee products. Saroğlu (2018), showed that the total phenolic content of 9 different types of honey, pollen, and propolis samples from the Bayburt region for honey, pollen and propolis, respectively; 6.32-18.21, 769.4±17.7-547.64±15.43, 1564±178-7206±120.8 mg GAE/100 mg. Ülgen (2017) reported that the total phenolic content of honey samples was 70.60-212.06 GAE/100 g, and antioxidant activity of 38.30-138.28 mg AAG/g in the study he conducted to determine the bioactive properties of medicinal honey produced in Türkiye. Kalelioğlu Akbulut (2019) reported that the total phenolic content of various honey extracts is between 28.067-84.400 mg GAE/g, the phenolic content of pollen extracts is 471.567 and 1.151 mg GAE/g, the phenolic content of royal jelly extracts is 48.233-113, 40 mg GAE/g. Özdal (2017) reported that the total phenolic amount of propolis is between 6.18±0.36-157.25±12.2 mg GAE/g EEP and the total flavonoid amount is between 10.24±0.33-261.61±13.6 mg QE/g. Tabatabaei (2017) reported that the total phenolic content of pollen is between 21.23-27.66 (mg GAE/g), the flavonoid amount is between 03.72 and 4.97 (mg QE/g), DPPH activity is between 77.93 and 69.49% inhibition.

Yıldız (2011) reported that the total phenolic content of pollen is between 13.68-28.87 mg GAE/g and its antioxidant capacity is between 68.04-82.31 mM TEAC/g. Balkanska et al. (2017) The total phenolic content of royal jelly is 11.66 – 36.73 mg GAE/g RJ, Pavel et al. (2014) found that the total phenolic content of royal jelly was 14.56-39.90 mg GAE/g, Ceksteryte et al. (2016) reported that the total phenolic content of royal jelly varied between 10.7 ± 0.03 mg GAE/g.

As a result of our literature review, we could not find a study in which the types and amounts of phenolic compounds were analyzed in royal jelly mixtures or similar foods. However, many studies have been carried out to determine phenolic compounds in many bee products (Bayram et al., 2018; Coşkun et al., 2018; Kılıç Altun S., & Aydemir M.E., 2020). Kılıç Altun & Aydemir (2020) reported that they detected 21 phenolic compounds in propolis collected in different regions of Anatolia in their study and the compounds they detected the highest was Quercetin (14.49 ppm) and Hydoxycinamic acid (16.85 ppm). According to Bayram et al. (2018) reported that 64 propolis samples from different places of Hakkari exhibited a wealthy chemical substance in flavonoids, including furocoumarins and coumarins. Sorucu (2019) reported that propolis is opulent in phenolic acids such as caffeic acid, ferulic acid, gallic acid, and flavonoids such as pinocembrin, galangin, rutin, and apigenin. On the other hand, Coşkun et al. (2018) reported that they detected phenolic compounds such as naringenin, galangin luteolin, hesperetin, apigenin in 86 different propolis samples collected from 25 provinces in Türkiye. In addition, the researchers concluded that pinocembrin, chrysin is mainly found in Turkish propolis. In our study, pinocembrin and chrysin were detected in all samples.

As a result of the literature review, it has been seen that the phenolic contents of bee products vary widely. It was reported that the major biologically dominant components of propolis are flavonoids (flavanones and flavones), phenolic acids, and their esters. It has been reported that red propolis is characterized by many flavonoids (liquiritigenin, formononetin, pinobanksin-3-acetate, rutin, pinocembrin, luteolin, quercetin, isoliquiritigenin and, daidzein) (Salatino & Salatino, 2018; Graikou et al., 2016). The major biologically dominant components of bee pollen vary. The compounds of bee pollen are benzoic acid derivatives - p -hydroxybenzoic acid, syringic acid, gallic acid, vanillic acid and protocatechic acid - as well as caffeic acid and their glycerol esters, cinnamic acid derivatives - p - coumaric acid, and ferulic acid. Other more complex derivatives such as rosmarinic acid, amide derivatives of hydroxycinnamic and ferulic acids, and dihexoside are also reported (de Florio Almeida et al., 2017; Mohdaly et al., 2015; Sun et al., 2017). It was reported that the major biologically dominant components of royal jelly were flavanones (naringenin, hesperetin, isosacuranetin), flavones (chrysin, luteolin glucoside acacetin, apigenin and its glycoside), flavonols (kaempferol glucosides and isorhamnetin) and isoflavones (troloids) (López-Gutiérrez et al., 2014).

The majority of these phenolic compounds reported in bee products were also detected in our study. The fact that bee products contain such a variety

of compounds can be explained by the geographical location of the region where they are collected, the season in which they are collected, the climate and the different vegetation.

Conclusion

As a result, it has been observed that food products containing bee products are wealth in phenolic contents and have high antioxidant properties. TPC, TFC, DPPH activity and phenolic contents were found to change as the type and ratio of bee products in the food changed. The highest TPC, TFC, DPPH activity and phenolic contents were found in products containing propolis and pollen. According to the results of our study, it was concluded that foods containing bee products (honey, propolis, bee pollen and royal jelly) are natural agents that can counteract the effects of oxidative stress, which is the main factor underlying many diseases.

Ethical Statement

Not applicable.

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Author Contributions

Serap KILIÇ ALTUN: Conceptualization, Data curation, Writing – original draft, Writing – review & editing, Project administration

Mehmet Emin AYDEMİR: Conceptualization, Formal analysis, Funding Acquisition, Investigation, Methodology

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RESEARCH PAPER

Investigation of the Mineral and Heavy Metal Contents of Propolis Additive Ice Cream

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Abstract

In this study, it was aimed to add a functional food property to the ice cream, which is a popular food, by adding propolis. At the same time, study aims to provide a widespread consumption potential to propolis which is impossible to be consumed in raw form and whose benefits and functional properties not known by consumers. 6 groups of ice cream containing control group and 0.1%, 0.2%, 0.3%, 0.4%, and 0.5% propolis powder were obtained from prepared ice cream mix. Mineral substance analysis to propolis sample and ice cream mix groups applied. According to the results, the addition of propolis had no effect on mineral and heavy metal amounts of ice cream mix groups. Propolis-added ice cream is thought to have the potential to offer new functional food to consumers of all ages.

Introduction

Propolis is a natural resinous substance created by bees by collecting from some parts of plants, plant buds and plant secretions (Ghisalberti, 1979). It has a unique aromatic fragrance and different colors that vary according to its source and maturity (Brown, 1989). More than 300 different compounds have been identified in propolis, which has a very complex chemical structure. Its chemical composition is 50% resin, 30% wax, 10% essential oils, 5% pollen and 5% other organic components. Compounds such as phenolic compounds, esters, flavonoids, terpenes, beta-steroids, aromatic aldehydes, alcohols, sesquiterpenes, stilbene terpenes, and caffeic acid phenyl ester (CAPE) are some of the organic components that propolis contains (Yucel et al., 2017). B1, B2, B6, C and E vitamins and silver, cesium, mercury, lanthanum, antimony, copper, manganese, iron, calcium, aluminum, and vanadium elements were detected in propolis (Deblock-Bostyn, 1982). Components of propolis change depending on factors such as climate, secretion source, environmental factors (Chen & Wong, 1996).

In recent years, the positive properties of propolis in terms of human health have been a popular field of study. It has been reported in the literature that propolis has antibacterial, antiviral, antifungal, antitumor, anti-inflammatory, cytostatic effect, and antioxidant activity. In addition, many studies have shown the benefits of using propolis against some conditions such as dermatological, ear nose throat, gynecological and stomach diseases etc. (Marcucci, 1994). In this study, the effect of adding propolis powder to ice cream in terms of heavy metal and mineral content was investigated.

Materials and Methods

Material

Raw propolis that used in this study was collected from apiaries of Apiculture Research Institute. It was extracted with 70:30 (V:V) ethanol: water solution and was lyophilized for obtaining propolis powder.

63.07% milk, 16% sucrose, 15.17% cream, 5.17% skimmed milk powder, 0.3% emulsifier, and 0.3%

stabilizer were used for preparing ice cream mix. Control, ICM1 (0.1% propolis powder containing ice cream mix), ICM2 (0.2% propolis powder containing ice cream mix), ICM3 (0.3% propolis powder containing ice cream mix), ICM4 (0.4% propolis powder containing ice cream mix) and ICM5 (0.5% propolis powder containing ice cream mix) ice cream mixes were prepared for analysis.

Methods

1- Mineral and Heavy Metal Analysis

Propolis samples and ice cream mix samples were weighed 0.2-0.5 g according to Nordic Committee on Food Analysis (NMKL 186:2007). 2 mL nitric acid (65%) and 0.5 mL hydrogen peroxide (30%) were added and wet combustion was performed in the Milestone Ethos Easy microwave digestion system. The solution obtained after wet burning was completed to 5 mL with distilled water and heavy metal and mineral matter analysis was performed in ICP-MS (Thermo Scientific IcapQ).

2- Statistical Analysis

Analysis data were analyzed statistically using SPSS statistical software and the results were presented as "mean ± standard deviation". Analysis of variance was performed (ANOVA) for determination of statistical differences between ice cream types.

Results and Discussion

In the study, mineral substance and heavy metal contents of powder propolis added to ice cream types were investigated. The mineral content, a feature of propolis that has not been studied much, contributes to its healing effects (González-Martín et al., 2015). The heavy metal content of propolis can increase through the solvents used and the contamination during the extraction procedure (Tosic et al., 2017). Heavy metal and mineral substance analysis results of propolis are given in Table 1.

Ice cream, like other dairy products, is a food product rich in mineral content. Minerals have important functions in the human body. Dairy products present a particularly significant amount of Ca element to the human body in a form that can be used in biological functions (Erkaya et al., 2012). Heavy metals can easily contaminate food through water, air and soil. For this reason, their involvement in the food chain has negative effects on human health (Conficoni et al., 2017). In the study, the effect of the difference in propolis ratio between ice cream mix groups on the element content was investigated.

Table 1. Results of heavy metal and mineral content analysis of propolis

Elements	(mg/kg)	Elements	(mg/kg)
B	4.119±0.014	Ni	0.515±0.003
Na	32.77±0.112	Cu	0.711±0.007
Mg	215.9±6.856	Zn	9.372±0.037
Al	2.245±0.006	As	0.035±0.000
Si	63.46±0.123	Se	0.016±0.000
K	1250.5±33.62	Sr	0.423±0.011
Ca	201.9±3.802	Mo	0.025±0.000
V	0.036±0.000	Sb	0.008±0.000
Cr	0.303±0.003	Ba	0.089±0.002
Mn	1.958±0.018	Pb	0.041±0.001
Fe	11.90±0.231		

Macro element quantities of ice cream mixes are as shown in Table 2. Because of the statistical analysis, it was determined that there was no significant difference between the groups in terms of the amount of macro elements ($P > 0.05$).

Table 2. Macro element values in ice cream mix

	Macro Elements (mg/kg)			
Ice Cream Types	Na	Mg	K	Ca
Control	768.8±11.0	148.8±5.80	1318.0±27.8	1286.9±64.8
DM1	794.8±42,7	158.7±33.8	1181.9±60.4	1374.4±33.2
DM2	724.4±23.2	141.1±39.1	1072.7±63.6	1253.2±32.6
DM3	724.1±36.4	164.7±20.1	964.4±54.7	1406.7±54.2
DM4	774.4±37.7	162.0±11.4	1242.1±43.9	1389.1±89.6
DM5	798.9±12.8	176.32±5.1	1333.2±41.2	1511.0±13.9

Trace element amounts of ice cream mix groups are shown in Table 3. It was determined that there was no statistically significant difference between the trace element values of the groups (P>0.05).

Heavy metal contents of ice cream mixes are as shown in Table 4. There was no statistically significant difference between the heavy metal contents of the groups (P>0.05).

Table 3. Trace element values in ice cream mix

Element	Trace Element (mg/kg)					
	Ice Cream Types					
	Control	DM1	DM2	DM3	DM4	DM5
B	1.10±0.20	1.30±0.00	1.10±0.50	1.10±0.10	1.20±0.10	1.20±0.08
Si	69.7±0.40	67.9±6.60	66.7±12.2	57.4±9.70	62.5±2.90	64.8±5.01
V	0.05±0.00	0.04±0.00	0.04±0.02	0.03±0.00	0.03±0.00	0.03±0.01
Se	0.04±0.00	0.04±0.01	0.03±0.01	0.03±0.01	0.04±0.00	0.04±0.00
Sr	0.42±0.04	0.42±0.02	0.41±0.09	0.36±0.00	0.33±0.00	0.36±0.03
Mo	0.08±0.03	0.07±0.01	0.08±0.04	0.08±0.01	0.07±0.03	0.08±0.01
Sb	0.01±0.00	0.004±0.0	0.01±0.00	0.004±0.0	0.002±0.0	0.003±0.0
Ba	0.08±0.00	0.09±0.01	0.08±0.02	0.09±0.01	0.09±0.00	0.10±0.11

Table 4. Heavy metal contents of ice cream mixes

Element	Heavy Metal (mg/kg)					
	Ice Cream Types					
	Control	DM1	DM2	DM3	DM4	DM5
Al	0.76±0.07	0.54±0.02	0.73±0.39	0.62±0.28	0.71±0.07	0.88±0.30
Cr	0.23±0.05	0.17±0.01	0.17±0.11	0.17±0.02	0.15±0.01	0.20±0.06
Mn	0.07±0.00	0.07±0.01	0.09±0.03	0.10±0.01	0.01±0.01	0.15±0.03
Fe	13.8±1.26	14.2±2.18	13.7±3.84	12.1±3.53	15.0±0.12	13.4±3.07
Cu	0.07±0.01	0.08±0.00	0.09±0.01	0.08±0.01	0.08±0.01	0.09±0.00
Zn	2.11±1.13	1.57±0.73	1.29±0.20	2.30±0.02	1.83±0.12	2.59±0.70
As	0.003±0.0	0.003±0.0	0.003±0.0	0.003±0.0	0.003±0.0	0.003±0.0
Pb	0.012±0.0	0.013±0.0	0.019±0.0	0.015±0.0	0.011±0.0	0.015±0.0
Ni	0.05±0.00	0.048±0.0	0.048±0.0	0.052±0.0	0.046±0.0	0.048±0.0

The determination of Na and Ca values of the macro elements of propolis powder used in the study was determined by Tosic et al. (2017) lower than the values were found to be high. When the trace element contents of propolis powder were compared with the results obtained by Tosic et al. (2017), conducted a similar study, Fe, Zn, Cu, B, Mn, Ni, Cr and Se contents were found to be lower, while the Si content was found to be higher. Considering the heavy metal contents, the Fe, Zn, Cu, Cr, Ni and Pb values of the propolis powder are compared to the values obtained by González-Martín et al. (2015) in their study with 91 propolis samples was found to be low. Since the composition of propolis is affected by many factors such as the source from which it is obtained, seasonal effects, and extraction method, it is thought that there are great differences between the element values in the studies.

Conficoni et al. (2017) investigated the heavy metal content of industrial production ice cream and ice cream samples produced by small-scale producers. They found the amount of As below 0.02 mg/kg in all their samples. It coincides with the As values obtained in this study. Cr and Pb heavy metal values in small-scale producer ice cream samples were 0.128 and <0.02 mg/kg, respectively; found it to be 0.179 and 0.056 mg/kg, respectively, in industrial production ice cream. The Cr element values found in our study correspond to these values. Industrial production ice cream Pb value is higher than the values obtained in our study.

Erkaya et al. (2012) produced ice creams by adding ground cherries in different concentrations to the formulation and investigated the element content of the ice creams they produced in their studies. In their elemental analysis, the quantities of Ca, K, Mg, Na, Fe, Mn, Ni and Zn elements were determined as 1200-1900, 1600-2100, 600-700, 150-200, 9.78-23.77, 0.23-0.42, 1.05-1.64 and 59.54 mg/kg, respectively. The amount of Ca and Fe calculated in this study corresponds to the amounts calculated in our study. The amounts of K, Mg, Ni and Zn are higher than the amounts we found, while the amounts of Na and Mn are higher in the ice creams we produce. When the element analysis was examined in general, it was seen that the addition of propolis did not have a statistically significant effect on the mineral and heavy metal content of ice creams. The reason for this is that the powdered propolis was added at a low rate.

Conclusion

The addition of propolis powder to ice cream had no significant effect on the mineral contents of ice cream due to propolis added at very low concentrations. Also there was no negative effect of propolis addition regarding to heavy metal content. Propolis content varies depending on region and sessional conditions. By adding propolis at different concentrations and from different sources to ice cream, obtaining a new functional food can be possible in future works.

Ethical Statement

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Author 1: Methodology; Investigation; Analysis; Original Draft, Formal Analysis

Author 2: Conceptualization; Supervision.

Author 3: Analysis; Conceptualization

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RESEARCH PAPER

Detection of *Varroa destructor* Mite and *Nosema* spp. in Bee Samples From Bulgaria

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Abstract

This study is focused on the investigation of honey bee samples for the presence of the two most common and widely distributed honey bee parasites. In a two-year period during 2020-2021, 185 bee samples were tested. All samples were examined by morphological and light microscopic methods. The obtained results showed that 32.43% of bee samples were infested with *Varroa destructor.* The degree of infection in the bees ranged from 0.5% to 60%. Spores of *Nosema* spp. were established in 25.40% of samples with a degree of invasion in the range from $3x10^5$ to $26x10^6$ per bee. Mixed infections of both parasites were observed in 32.43% of the samples. Negative samples were with the lowest value of 9.74%.

Introduction

The managed honey bee (*A. mellifera*) considerably contributes not only to crop pollination, but and many wild flowering plants (Botías et al., 2013; Hristov et al., 2020a). Maintaining the health of honey bee colonies is of great importance for pollination and agriculture sustainability. Crop yields are influenced by the density and quality of honey bee colonies placed in fields, groves, and orchards (Calderone, 2012; Chatterjee, 2021; Lowe et al., 2021).

Beekeeping is facing many challenges. On the one hand the impact of the increasingly unfavorable for the bees as environmental conditions (climate change, lack of feed, insufficient variety of food, environmental pollution, pesticides etc.). On the other hand, many honey bee pathogens and pests have a significant impact on honey bee health and survival (Neov et al., 2019; Hristov et al., 2020b). The ectoparasitic mite *Varroa destructor* contributes to the higher levels of bee losses around the world (Ramsey et al., 2019; Chen et al., 2021). Climate change induces longer periods of brood rearing in honey bee colonies and foraging because of longer warm seasons. A longer brood period means more *Varroa* reproduction cycles and may lead to an increase in mite populations (Le Conte et al., 2010; Beaurepaire et al., 2017). Also, the *Varroa* mite potential to act as vectors of honey bee associated viruses (Barroso-Arévalo et al., 2019). Several bee viruses, including Acute Bee Paralysis Virus (ABPV), Black Queen Cell Virus (BQCV), Chronic Bee Paralysis Virus (CBPV), Deformed Wing Virus (DWV), Kashmir Bee Virus (KBV), Sacbrood Virus (SBV) and Israeli Acute Paralysis Virus (IAPV), have been documented to be aggravated and transmitted by *Varroa* mites (Annoscia et al., 2019; Erban et al., 2019; Roberts et al., 2020). Nosemosis caused by the microsporidia *Nosema apis* and *Nosema ceranae* are among the most common pathogens affecting adult honeybees. Especially, *Nosema ceranae* infection is associated with annually honey bee losses worldwide (Rubanov et al., 2019; Shumkova et al., 2021). One of the main reasons for the high losses of bee colonies are varroosis and nosemosis, the most prevalent and damaging infection diseases of honeybees, which cause large losses in beekeeping. For this reason, this study aimed to evaluate the presence of *Varroa destructor* and *Nosema* spp. in honey bee samples in different regions of Bulgaria.

Materials and Methods

In a two-year period (2020-2021), in the private laboratory "Primavet - Sofia Ltd." and in the Department of Experimental Parasitology, IEMPAM-BAS were tested 185 bee samples from apiaries, located in different regions of the country. The private laboratory "Primavet - Sofia Ltd." surveyed 94 samples for diagnosis, mainly from bee colonies with some pathological symptoms such as abnormal behavior, depopulation of beehives, weakness and high losses of colonies. In the Department of experimental Parasitology were tested microscopically 91 bee samples for monitoring of *Varroa destructor* and *Nosema* spp. infestation rate. Bee samples were taken personally by beekeepers or a veterinarian. In the case of dead bee colonies, the method for diagnosing varroosis was by examining the debris generated by the bees themselves, where fallen/dead mites can be found. For investigation of nosemosis samples of older worker honey bees were taken from the hive entrance or from external frames if the weather does not permit flight conditions. For investigations of varroosis from colonies with some pathological problems, beekeepers selected any uncapped brood frame and check that the queen is not present and take a sample of 300 bees using a jar (with a mark at the level of 100 mL of water, which is the volume occupied by 300 bees) by sliding it up and down so that the bees tumble in. The beekeepers then store the samples until they are sent by cooling at 4°C for 15 minutes or by freezing to –18°C for 5 minutes.

Identification of *Varroa destructor*, and *Nosema* spp. were performed according to instructions described by OIE Terrestrial Manual (2021) and OIE Terrestrial Manual (2013), respectively. Diagnostic methods used to detect *Varroa destructor* morphological identification by alcohol wash method (Oliver, 2020) and to proof spores of *Nosema* spp., included light microscopic (×400 magnification) according to Fries et al. (2013).

Investigation for *Varroa destructor* Mite

The dead bees from the bee samples were flooded with industrial alcohol and stirred continuously for around 5-10 minutes. Then the contents were placed of the sieve on a bright plate, where the mites can be easily identified and counted. The percentage of a mite infestation level was calculated by the following formula:

% *V. destructor* = (Number of foretic mites/number of adult bees) × 100

The results can be expressed as a percentage of infestation, dividing the number of mites dislodged by the number of bees in the sample and then multiplying by 100.

Investigation for Spores of *Nosema* spp.

The abdomens of sixty bees of each sample were obtained in 60 mL of distilled water. Smears of the suspension were made on a glass slide. They were air-dried, ethanol-fixed and stained with Giemsa's stain (10% in 0.02 M phosphate buffer) for 45 minutes. *Nosema* spp. spores had a distinctive appearance, with thick unstained walls and an indistinct blue interior, without visible nuclei. To quantify the average infection level spores were counted and were calculated per bee as the abdomens of 60 individuals are macerated in 5 mL of water using a mortar and pestle and 50 mL of water was added for a total volume of 1 mL per bee (5 mL is added later). When tissue pieces have become quite fine, the suspension was filtered through two layers of muslin (thin loosely woven cotton fabric) in a funnel leading to a graduated centrifuge tube. A second 5 mL of water was used to rinse the pestle, swirl around the inside of the mortar and pour through the subsample in the funnel. When the suspension appeared to be homogenous after shaking, a sample was taken to fill the calibrated volume under the cover-slip of a haemocytometer (blood cell counting chamber). After a few minutes, the spores will have settled to the bottom of the chamber. *Nosema* spp. spores appear transparent but with a very distinct dark edge and are 5–7 µm long and 3–4 µm wide. They were counted using a magnification of ×400. The number of spores in each square was counted. The whole grid consists of 3 × 3 large squares, separated by triple lines. Each large square is further subdivided into 16 smaller squares subdivided by double lines, in total 144 squares. The spores are counted in the smaller squares with the area of 1/25 mm^2. When the counting is completed, the number of spores per bee in the sample can be calculated according to the formula:

$$\mathbf{Z=\alpha/\beta\times\delta\times 250\ 000}$$

Where

Z = spore numbers per bee

α = total number of spores counted

β = number of squares counted

δ = dilution factor

The number 250 000 was used because the volume in each counted square was 1/250 000 mL and the equation uses the average number of spores per counted square.

Results

Results from the private laboratory "Primavet - Sofia Ltd." have shown in Fig.1. It can be seen that 32.98% of bee samples were infested with *Varroa destructor.* The degree of invasion in bees was in the range of 0.5% to 60%. Spores of *Nosema* spp. were established in 26.60% of samples with a degree of infection ranged from $3x10^5$ to $26x10^6$ per bee. Mixed infections of both pathogens were observed in 28.72% of the analyzed samples, while the negative samples were 11.70%.

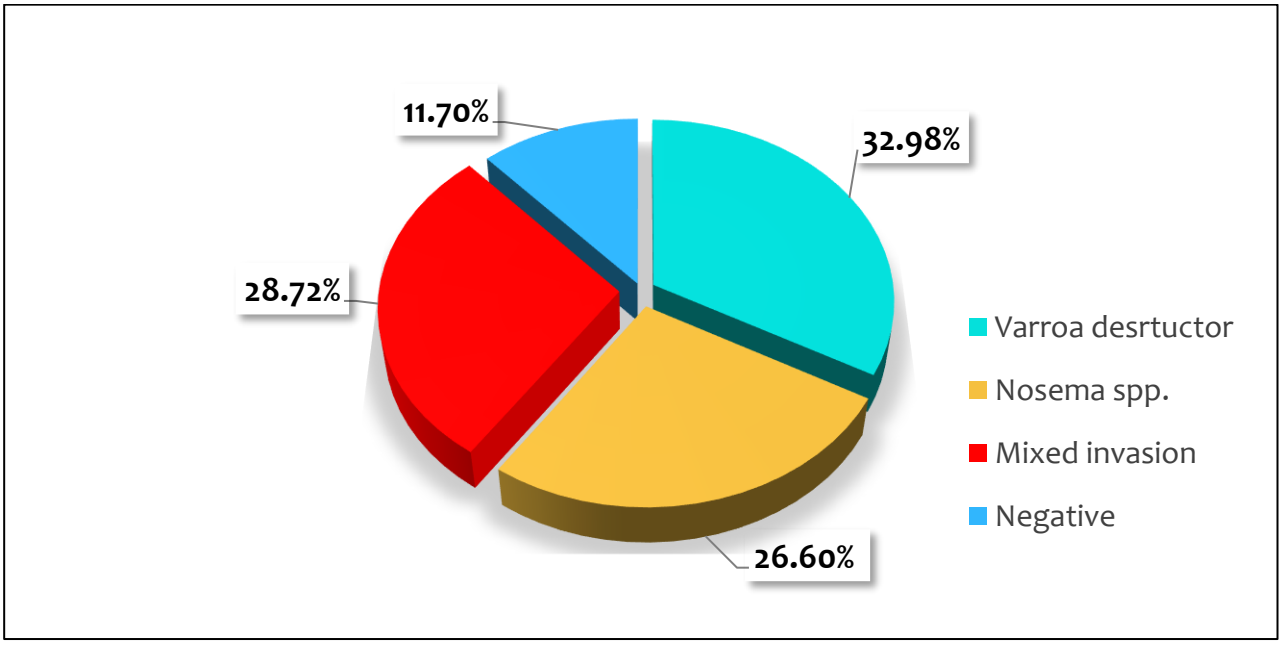

Figure 1. The results of investigated bee samples from the private laboratory "Primavet"

Results from ninety-one investigated samples in the experimental laboratory (IEMPAM – BAS) (Fig. 2) showed the highest percentage of mixed infections (36.26%), samples infested with *V. destructor* were 31.87%, and spores of *Nosema* spp. were identified in 24.18% of honey bee samples. Negative samples were only 7.69%.

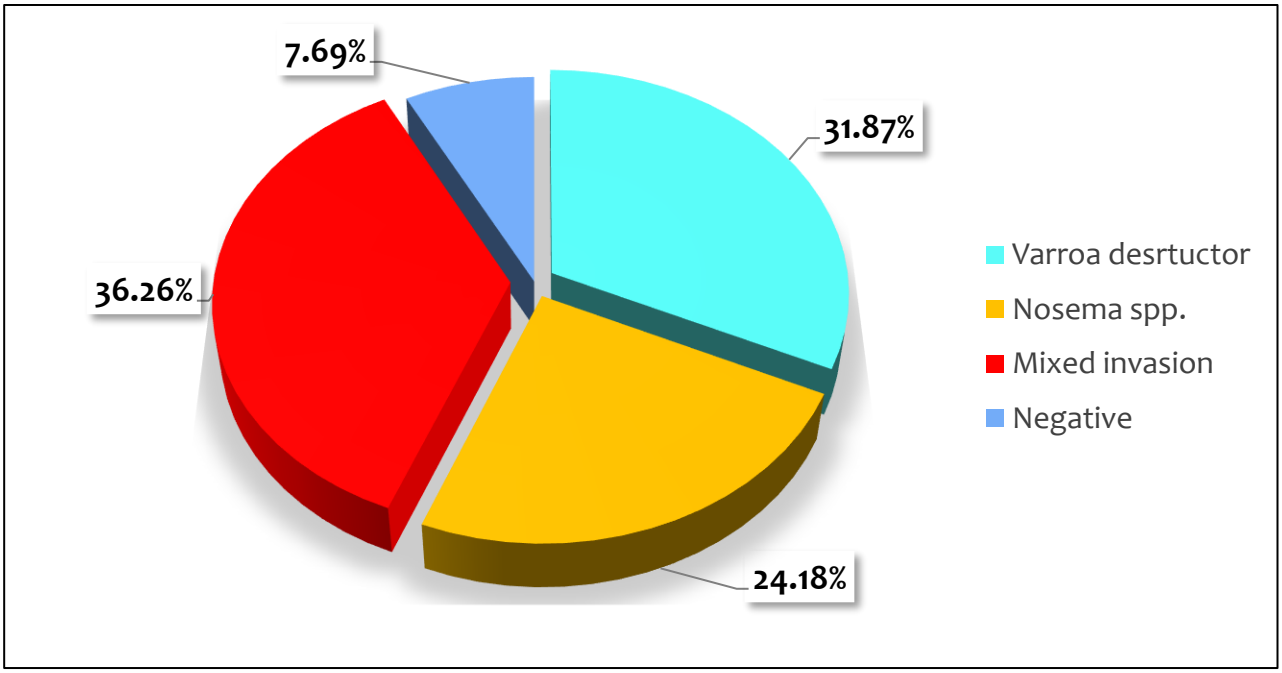

Figure 2. The results of investigated bee samples from the experimental laboratory

Figure 3 presents the obtained results from tested honey bee samples from different regions in the country.

The majority of samples were obtained from the Pleven, Sofia, Sofia-province, Pazardzhik, Ruse, Razgrad, Veliko Tarnovo, Stara Zagora, Blagoevgrad and Burgas regions. The large number of samples obtained from a given area is not always in direct correlation with high percentage of positive for *V. destructor* or *Nosema* spp. samples from the same area. The highest percentage of positive samples for *V. destructor* was found in Targovishte (100%), Blagoevgrad (71.4%), Stara Zagora (71.4%), Sofia-city (55.6%), and the lowest infestation rate was observed in Sofia province (12.5%), Pazardzhik (14.3%) and Ruse (20%). In some regions there was no detected *Varroa* positive samples (Gabrovo and Lovech) (Fig. 3). The highest percentage of positive samples for *Nosema* spp. was found in Lovech (100%), follow by Gabrovo (75.0%), Veliko Tarnovo (62.5%), Razgrad (50.0%) and Ruse (40.0%). The *Nosema* spp. infection did not observe in Vidin and Targovishte. The mixed invasion was observed most often in samples sent by beekeepers from Sliven, Pazardzhik, Sofia-region and Ruse (Fig. 3).

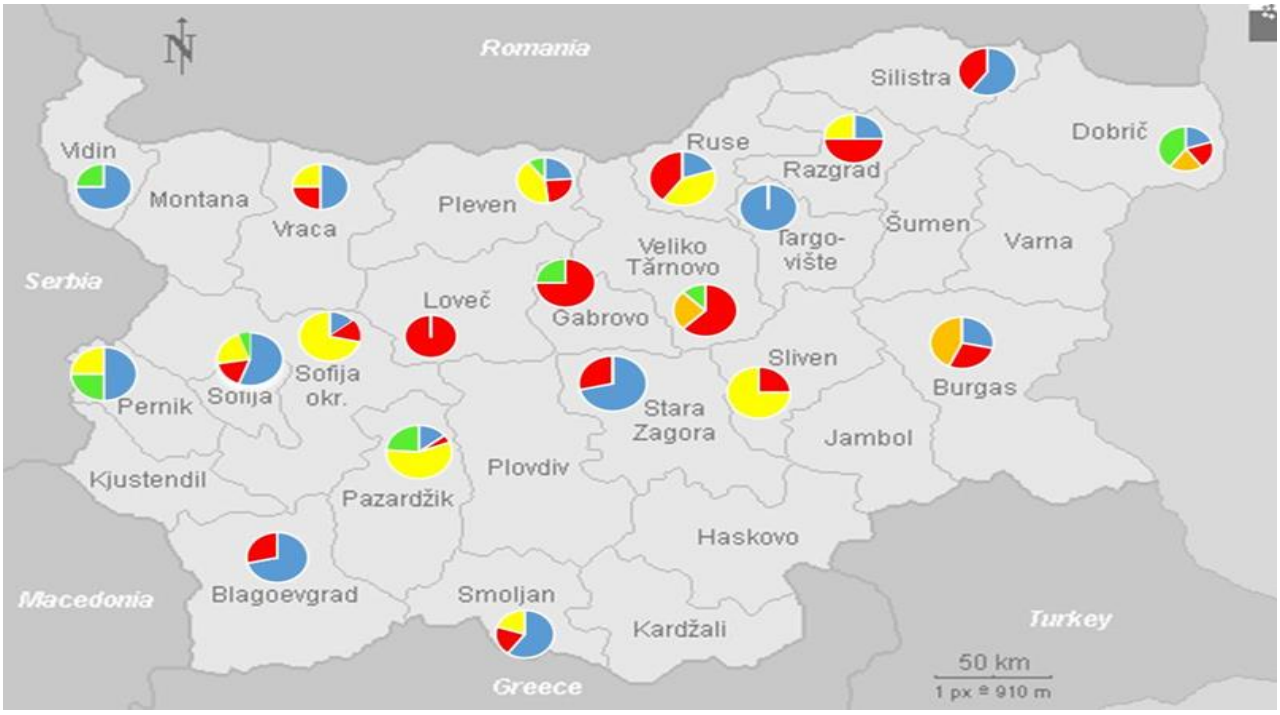

Figure 3. Distribution (%) of *Varroa destructor* mite and *Nosema* spp. in honey bee samples from different locations in Bulgaria.

*****Blue color - honey bee samples positive for *V. destructor*; red color- honey bee samples positive for *Nosema* spp.; yellow color - mixed invasion, green color - negative samples.

Discussion

The ectoparasite mite *Varroa destructor* has caused severe damage to populations of the European honeybee, *Apis mellifera* worldwide in recent years. In our studies, we found a very high percentage of bee colonies infested with both *Varroa destructor* mite and *Nosema* spp. The data are not representative for the whole country due to the different number of samples obtained from the various areas. The highest rate of *V. destructor* infestation was observed in plain regions of the country (Targovishte, Stara Zagora and Vidin) (Fig. 3). One possible explanation is associated with significantly longer honey bee brood rearing in the plain regions compared to mountainous parts – from early spring (February) to late autumn (October). This gives an opportunity to the prolonged influence of the mite on honey bee colonies.

The obtained results showed that *Nosema* spp. infection also prevailed in the plain regions in the country (Gabrovo, Lovech and Razgrad). In the plain regions compared to the mountainous, the transport of infected honey bees and/or by the increased mobility of people, goods and livestock are observed much more often. Thus, it is a possible explanation of higher *Nosema* spp. infestation rate in the plain regions of the country. In our preliminary studies, we found that most of the tested samples of dead bees were infested with *Nosema* spp. (Salkova et al., 2015; Salkova et al., 2016a; Salkova et al., 2016b). The results of our previous research showed the predominant part of the research the samples showed the presence of *V. destructor* and *N. ceranae* in Central and Northeastern Bulgaria. In addition, in the regions observed in 2020, 37% of the samples show mixed infestation of varroosis and nosemosis, 33.3% - only varroosis and 14.8% - only

nosemosis (Salkova et al., 2022). According to the Bulgarian Food Safety Agency (BFSA), the results of an epizootological survey in different areas of the country in 2020 showed that bee colonies are most often affected by varroosis and nosemosis, while in the previous year varroosis predominated as a cause of mortality in honey bee colonies.

The reason of this result might be connected with beekeeper's under estimate *Varroa* infestation levels in their apiaries, and often beekeepers' management of infestations is failing. In order to control the level of *Varroa destructor* infestation, beekeepers must start control treatment when monitoring and controlling varroosis before the number of mites is high enough to cause significant harmful effects on the bee colony. As is the case for mite population dynamics, damage thresholds are highly variable and depend on the interaction between genotype and environment together with beekeeping management practices and the time of year, resulting in substantial differences between regions (Rosenkranz et al., 2010). In recent years, the presence of mite resistance against certain groups of acaricides is becoming all too common in manly areas of the world. To effectively control of varroosis, beekeepers should be interested in new veterinary medicinal products (VMPs) and check for resistance to the product used in their apiary. A reliable and harmonized diagnosis is crucial to ensure the quality of surveillance and research results.

Conclusion

In conclusion, we can say that our study has shown a prevailing higher percentage of infested with *Varroa destructor* mite bee samples than samples, positive for *Nosema* spp. in our country. We should also note the relatively high percentage of samples with mixed infection. A small percentage of the tested samples were negative.

Ethical Statement

Not applicable.

Funding Information

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Author Contributions

Prof. Kalinka Gurgulova tested the bee samples sent to the private laboratory of "Primavet".

Chief Assist. Delka Salkova tested the bee samples in the laboratory of Experimental

Parasitology for monitoring of bee parasites and has prepared the manuscript.

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RESEARCH PAPER

Traditional Forest Beekeeping and Its Challenge in Benishangul Gumuz Regional State, Ethiopia

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Abstract

The study was conducted to assess the status of traditional forest beekeeping and related challenge faced by the beekeepers in three representative districts of Benishangul Gumuz. Through the systematic random sampling method, 167 households were selected and data were collected through a semi-structured interview schedule. The collected data were analyzed using descriptive statistics, chi-square test, and T-test of Statistical Packages for Social Sciences (SPSS Version 23). The result shows as compared to the backyard and other beekeeping systems traditional forest beekeeping (71.30%) was still the major type of honey bee colony management in the region. During honey harvesting, 43.71% of beekeepers harvest any hive product by throwing the hive from the long tree and collecting the entire available hive product at night time of the day. Beekeepers' response shows that the factors were no different (P>0.05) across the district to keep honey bee colonies traditionally in the forest area. The result further indicates the participation of females in the beekeeping sector is still very low level in the study area (10.2%). In conclusion, in the study area due to forest beekeeping practice the honey bee population, diversity, and the hive product highly declined and the beekeepers still have not benefited from the sector. So, to decrease traditional forest beekeeping practice further activities must be done by the government and a research center on the integrated improved forest beekeeping, awareness creation, and on reducing honey bee race aggressiveness behavior.

Introduction

Ethiopia has a huge potential for beekeeping production because of its endowment with diversity in climate and vegetation resources for beekeeping (Kidane, 2014). The result of the Central Statistical Agency revealed that a total of about 6.99 million hives are estimated to be found in the rural and pastoral sedentary areas of Ethiopia (CSA, 2013). Of these total hives, 95.89 percent are traditional hives. Ethiopia has a share of around 23.58 and 2.13% of total African and world honey production, respectively (Workneh & Ranjitha, 2011). An early study by Kassaye (1990) shows Ethiopia is the leading honey producer in Africa and the tenth largest honey producing country in the world. Oxfam Canada's study (2008), indicates there were 166 736 traditional and 682 modern beehives available in Benishangul Gumuz regional state.

Adequate forage availability coupled with favorable and diversified agro-climatic conditions in Ethiopia creates environmental conditions conducive to the growth of over 7000 species of flowering plants which have supported the existence large number of bee colonies in the country (Beyene & David, 2007). Beekeeping is one of the agricultural sub-sectors that most suits the rural poor people being simple and relatively cheap to start, as it requires a very low level of inputs such as labor, capital, and knowledge (Gemechis et al., 2012). In Ethiopia, 95% of beekeepers follow the traditional method of beekeeping practice with no improved techniques or technology and the beekeeper still has very traditional knowledge and skill of honey and beeswax production (Belie, 2009).

Over 150 000-200 000 km^2 of the west and south-west region of Ethiopia is infested by Tsetse fly (Tikubet,

2000). Benishangul Gumuz regional state is part of south-west Ethiopia, endowed with vast arrays of livestock resources and it is an area of highly trypanosomiasis stricken, it has an estimated tsetse infestation area of about 31 000 km^2. Much of this land is potentially productive but its full economic development is being denied because of the impact of trypanosomiasis. This situation retarded the livestock sector other than beekeeping development and societal livelihood improvement.

Over 60% of Benishangul Gumuz is covered with forest, including bamboo, eucalyptus and rubber trees, incense and gum forests as well as indigenous species (Kassa et al., 2015). This high coverage of the forest and honey bee flora creates an opportunity for the majority of beekeepers in the study areas to practice traditional forest beekeeping followed by backyard beekeeping. In Benishangul Gumuz traditional forest beekeeping is practiced by placing hives in the forest on very tall trees for catching swarms and honey harvesting. Its disadvantages are lack of close follow-up and during the honey harvesting period as the beekeeper drops down the hive from the tree, it damages the honeybee colony and reduces the honey bee colony population. It is also dangerous for the beekeeper to climb a tall tree at the night (HBRC, 2004). A study by Abebe et al. (2016) indicates during honey collection from traditional hives, beekeepers in Benishangul Gumuz regional state of Ethiopia remove all combs and destroy a colony.

In Benishangul Gumuz even in the traditional beekeeping system still, the sector is one of the economically important sub-sector for income generation activity. Observation shows as compared to other regional states Ethiopian beekeepers in the region widely practiced traditional forest beekeeping.

In the region majority of beekeepers purposively prepare one side fully closed and the other side moveable to cover a short-length traditional hive about 1-1.5 m in length and 30-50 cm in width to hang easily on the tall tree at the time of the flowering season (Fikadu, 2018). Those colony management systems in the region have different problems for regional beekeepers to manage honey harvesting, quality and quantity of honey, disease, pest and predator controls, and inspecting the colony in general.

Bambasi, Homosha, and Mao-Komo districts of Benishangul Gumuz regional state are believed to be potential areas for beekeeping development as they have good climatic conditions and diversified bee flora. Moreover, in these areas, the improved beekeeping sector creates huge job opportunities for youth and women in particular. But traditional forest beekeeping system in the region generally and specifically in the study area only participates in traditionally experienced persons who climb long trees to hang and harvest honey from the traditional hive. This type of beekeeping has highly reduced the participation of youth and women in the beekeeping sector. So, the region to get benefit from the sector and save the honey bee colony population must transform from traditional forest beekeeping to improved beekeeping by introducing modern beekeeping practices. At this time there is no data about the regional forest beekeeping practice status and challenge. Therefore, this study aims to assess the status of traditional forest beekeeping and related challenge in the beekeeping sector in the study area.

Materials and Methods

Description of the Study Area

This study was conducted in three districts of the Benishangul Gumuz regional state, namely Bambasi, Homosha, and Maokomo. The study areas were selected based on honey bee colony potential and road access. Assosa town Benishangul Gumuz capital city located 670 km west of Addis Ababa. Bambasi is located about 40 km south of Assosa, Maokomo is located about 105 km south of Assosa town and Homosha is located about 30 km west of Assosa town. The regional state is located between geographical coordinates: 9°30'N-11°39'N latitude and 34°20' E to 36°30' E longitude with altitudes ranging from 580 to 2730 m above sea level. Mean annual rainfall and temperature in the region range between 700 to 1450 mm and 21 to 35°C respectively (AMS, 2008). Benishangul Gumuz regional state has high forest cover in Ethiopia and possesses around 20% of the national forest areas (Bessie et al., 2016).

Data Type, Source, and Data Collection Techniques

Through purposive sampling techniques select three districts (Bambasi, Homosha, and Maokomo) based on accessibility to the road, and the population of the honey bee colony. In this study, a semi-structured questionnaire was prepared and administered to collect information from the randomly identified beekeepers. For this study, both primary and secondary data were used. To collect primary data systematic random sampling techniques were employed to select household heads, while secondary data was collected from the livestock and fishery agency and extension workers of respective study districts. A formal survey was conducted by frequent visiting during a time of honey production season. And also, to get a general overview of the beekeeping system an informal survey was conducted with key informant farmers, extension workers, and the district agricultural office interview.

A pre-tested semi-structured questionnaire was used to interview the selected beekeepers. The interview was held on their respective farms using a

translated local language. The questionnaire covered a large range of variables which include demographic characteristics, resource holdings, beekeeping system and management practices, honey production system, honey pests and predators, and challenges of beekeeping.

Information about the beekeeping system and the factors affecting the beekeeping system was collected through semi-structured interviews with 167 beekeepers. The semi-structured questionnaire, observation, in-depth interviews, and focus group discussions with key informants and extension agents were held in each district.

To obtain secondary data, reviewing different books, thesis papers, dissertations, magazines, and journals were reviewed to acquire in-depth information that was more related to the beekeeping system. The survey data was done from January 2020 up to June 2020.

Data Analysis

The data collected from beekeepers were analyzed using descriptive statistics through SPSS (Version 23). Percentages, frequency, T-test, and chi-square test were used to describe socioeconomic characteristics and beekeeping management. A T-test is used to assess the age, experience, and the honey bee colony status of the beekeepers. The Chi-square test was used to determine differences in percent frequencies of nominal data. Rank index calculation was also employed to identify economically important major pests and predators and constraints for honey bee production in the study area (Musa et al., 2006). The level of significance was set at 5%.

Results and Discussion

Socio-demographic Characteristics of Respondents

In the study areas, the participation of females in beekeeping activity was significantly lower (10.2%) than males (89.8%). This may be due to the beekeeping system of the region is difficult for females to work in the forest area. A similar result was also observed by Fikadu (2018) in Wombera district of Benishangul Gumuz from the total respondent beekeepers, 98% were male and 2% were female. A study by Bogale (2009) affirmed in Ethiopia beekeeping is the man's job.

Of the total respondent 34.7% of beekeepers there is no formal education. This lower level of education in the study area may affect the adoption of improved honey bee technology. A study by Mulatu et al. (2021) indicates the use of modern beekeeping technology is a direct relationship with education level. The respondent household head in the study area on an average of 12.41 years of experience in beekeeping (Table 1).

Table 1. Household characteristics

Household head		Frequency	Percent (%)
Sex	Male	150	89.8
	Female	17	10.2
	Total	167	100.0
Education Status	Illiterates	58	34.7
	Read and write	17	10.2
	Elementary	73	43.7
	Grade 9-10	17	10.2
	Grade 11-12	1	0.6
	Higher education	1	0.6
	Total	167	100

	N	Mean	SD	Min	Max
Age	167	40.55	12.7	18	71
Beekeeping Experience	167	12.41	9.34	1	40

N: Number of respondent, SD: Standard deviation, Min: Minimum, Max: Maximum

Beekeeping System

The result indicates traditional beekeeping in the forest system was perceived predominant practice in the Benishangul Gumuz regional state of Ethiopia (Table 2). The beekeeping system no difference (P>0.05) across the study districts. About 71.3% of the beekeepers in the study area were managing honey bee colonies in the forest area locally known as Berha. The finding of this study is in line with those of Amssalu et al. (2004) and Workneh (2011) who reported that beekeeping practice in south and southwestern Ethiopia is predominantly traditional. The study by Abebe et al. (2016) also shows in Benishangul Gumuz most beekeepers hang their traditional hives upon trees in the forest or homestead area until honey harvesting season. In general, beekeepers' response indicates hang a traditional hive on the selected tree in the forest area is a major practice in the study area to catch swarms, absconded, and migratory colonies. Whereas the backyard and under the roof still practice very low in the study area at 22.8% and 1.2% respectively.

Table 2. Honey bee colony management

	Traditional beekeeping system (%)				
Districts	**Backyard**	**Forest**	**Other**	**X^2**	**P- value**
Bambasi (N=57)	24.60	71.90	3.50	5.44	0.24
Homosha (N=58)	29.30	65.50	5.20		
Mao Komo (N=52)	13.50	76.90	9.60		
Total (N=167)	22.80	71.30	6.00		

N: Number of respondents per district, * and ** are significant at *P*< 0.05 and 0.01 respectively.

Traditional Forest Beekeeping System

The survey result shows that the reasons to keep honey bee colonies traditionally in the forest area are no different (*P*>0.05) across the district (Table 3). The beekeeper's response shows figuratively lack of awareness of improved beekeeping practice was the greater factor to keep honey bee colonies traditionally in the forest area (Figure 1). The second reason is due to the *A. m. scutellata* (*Apis mellifera scutellata)* race's highly aggressive behavior that is difficult to keep in the homestead area. A study on the aggressiveness of the *A. m. scutellata* race by Amssalu (2002) indicates that the honey bee race found in the lowland parts of Ethiopia is more aggressive from September through November. And another study by Collins et al. (1988) also shows that *A. m. scutellata* honey bees had high defensive behaviors. Moreover, other reasons also play a huge role in beekeepers practicing traditional forest beekeeping like lack of pest and predator control mechanisms and higher availability of flora species in the forest area.

Table 3. Reasons for traditional forest area beekeeping

	Districts (%)					
Variables	Bambasi (N=41)	Homosha (N=38)	Mao Komo (N=40)	Total (N=119)	**X^2**	**P-value**
Aggressiveness behavior of bees	31.70	18.40	20.00	23.50	5.19	0.75
Flora species availability	14.60	28.90	17.50	20.20		
Lack of awareness of improved beekeeping practice	19.50	26.30	27.50	24.40		
To prevent pests and predators	14.60	10.50	12.50	12.60		
To catch swarm	19.50	15.80	22.50	19.30		

N: Number of respondents per district, * and ** are significant at *P*< 0.05 and 0.01 respectively.

Figure 1. Traditional hives hanged in the forest area from a long tree during the time flowering season

Honey Bee Colony Status

Benishangul Gumuz regional state compared to other regional states of Ethiopia found a higher colony population per beekeepers (Table 4). The respondent beekeepers contain on average 12.98 colonies in the study area. The high number of colonies in our study area may be related to a wide range of honey bee flora species, forest areas, agricultural land, and water resource availability. Moreover, the beekeeper's response shows that hanging a large number of traditional hives on a selected tree in the forest area is an alternative means to increase the honey yield per harvesting season. A different study shows in another region of Ethiopia like Oromia regional state in the Arisi zone beekeeping potential area on average 5.68 colonies per respondent (Gebiso, 2015) and also in Tigray regional state Ahferom district among the major honey-producing districts average number of beehives with bee colonies for the total sample smallholder beekeepers was around 5 (3 traditional and 2 improved) with a minimum of 1 and maximum of 13 beehives (Gebremichael & Gebremedhin, 2014).

Table 4. Colony ownership status

Colony	N	Min	Max	Mean	SD
Total number of colony	166	0.00	75.00	12.98	14.70
Honeybee colony in traditional hives	164	0.00	125.00	11.50	16.41
Honeybee colony in transitional hives	159	0.00	5.00	0.37	3.97
Honeybee colony in box hive	160	0.00	20.00	0.91	2.16

N: Number of the respondent, SD: Standard deviation

Traditional Hive Management

In the study area, traditional hives were collected after honey harvesting from the forest area due to different reasons (Table 5). The response shows beekeepers collecting traditional hives after the honey harvested is the major one (49.1%) for hives without colonies. This study is in line with Serda et al. (2015) most of the local beehives are hung on a high tree during the time of the honey flow period, and collect traditional hives after honey harvest for the second honey flow period. This traditional forest beekeeping type of hive management in the study area causes huge colony losses in each production year. Moreover, the respondent's response shows that work at the time of hive hanging on the tree, honey harvesting at night time, and other beekeeping activities are very risky to beekeepers.

Table 5. Traditional hive management

Reasons for hives without colony	Frequency	Percent
Absconding due to pests and predators	70	41.91
Migration	15	9.00
Hives collected after honey harvest	82	49.10
Total	167	100.00

Honey Production System

The result indicates still in Benishangul Gumuz the beekeepers practice traditional forms of honey collecting by the destruction of the entire colony when the honey was harvested. The result shows that 47.9% of beekeepers harvest comb containing sealed honey and 43.71% of beekeepers by throwing the hive from the long tree and collecting the entire available hive product at night time of the day (Table 6). After harvesting all the hive product mix without extraction and storing for household consumption and marketing purpose. In the study area at the time of the honey flow period, huge colony death and migration occur because beekeepers harvest the entire available hive product and collect the hive for the next honey flow period. This type of honey harvesting still highly affects the honey quality, quantity, and honey bee colony population itself. The honey bee colony in the study area has very low productivity and poor quality of bee products which is the major economic impediment for rural beekeepers (Nuru, 1999). Group discussion participant response indicates due to the destruction of the colony at the time of honey harvesting in the region year to year the honey bee colony population is highly reduced.

Table 6: Kind of hive product harvested

Woreda		Kind of comb harvested					X^2	P-value
		With nectar	With pollen	Sealed honey	Any available at the hive	Total		
Bambasi	N	3	1	31	22	57	6.75	0.34
	%	30.00	25.00	38.80	30.10	34.10		
Homosha	N	6	1	22	29	58		
	%	60.00	25.00	27.5	39.70	34.70		
Mao komo	N	1	2	27	22	52		
	%	10.00	50.00	33.80	30.10	31.10		
Total	N	10	4	80	73	167		
	%	6.00	2.40	47.90	43.70	100.00		

N: Number of respondents per district, * and ** are significant at *P*< 0.05 and 0.01 respectively.

Honey Bee Pests and Predators

The major honey bee pests and predators in the study areas are indicated in Table 7. The ant was the most important pest in Bambasi and Mao Komo districts. But in the Homosha district, spider highly affects the honey bee colonies. Generally, in the study area ants, spiders, honey badgers, birds, and hive beetles greater effect on the beekeeping sectors. This result is in line with the findings of Ejigu et al. (2009), who reported that ants, honey badgers, bee-eater birds, wax moths, spiders, and beetles were the most harmful pests and predators in order of decreasing the importance of beekeeping in Amhara region of Ethiopia. Another study in Benishangul Gumuz by Abebe et al. (2016) also showed that ants, honey badger, wax moth, small hive beetle, and spider were frequently occurring pests and predators. Sample household response indicates that the first solution to prevent colonies from pests and predators is hanging on trees in the forest area is the preferable site for beekeepers. Research Centre produces different technology to prevent the incidence of ants on honey bee colonies but due to the regional beekeeping system still, the technology is not in practice by most of the regional beekeepers.

Table 7. Major honeybee pests and predators in each district

Pests/Predators	Bambasi		Homosha		Mao Komo	
	Index	Rank	Index	Rank	Index	Rank
Ant	0.38	1	0.22	2	0.25	1
Birds	0.06	5	0.04	7	0.11	3
Hive beetles	0.08	4	0.10	3	0.09	5
Honey badger	0.12	3	0.06	6	0.21	2
Lizard	0.04	6	0.09	4	0.05	6
Spider	0.23	2	0.40	1	0.10	4
Wax moth	0.08	4	0.08	5	0.10	4
Monkey	0.01	7	0.01	8	0.09	5

Challenge of Beekeeping Development

The major challenges of keeping honeybees in the study areas are indicated in Table 8. Honeybee pests and predators (28.9%) were the most important challenges of keeping honeybees for beekeepers. The survey result also shows indiscriminate utilization of agrochemicals huge problem in a honey bee colony. Moreover, in the study area lack of extension supports for an improved honey bee colony production system is one of the major challenges for beekeeping.

Table 8. Challenges of beekeeping in the study areas

Challenge	Beekeepers	
	N(Index)	Rank
Shortage of beekeeping materials	40(0.078)	5
Death of colony	3(0.005)	12
Drought	7(0.010)	10
Marketing	10(0.020)	9
Beekeeping skill	18(0.036)	8
Lack of credit facility	0(000)	14
Low-quality beekeeping materials	4(0.006)	11
High cost of beekeeping materials	28(0.038)	6
Disease, pest, and predators	97(0.289)	1
Shortage of bee forage	15(0.003)	13
Reduction of honey bee colonies	24(0.038)	7
Indiscriminate application of agro-chemicals	65(0.158)	2
Lack of extension support	67(0.147)	4
Absconding and migration of colony	74(0.149)	3

N: Number of respondent

Conclusion

Even if the major beekeeping system is traditional forest beekeeping in the region the sectors still play a vital role by creating a variety of assets for the beekeepers in the study areas. However, households have not sufficiently benefited from the beekeeping industry. Traditional forest beekeeping found in the study area is highly practiced and huge colony losses during the time of honey harvesting time and quantity of honey. The study further shows still the regional beekeepers practice traditional forms of hive and honey collecting by the destruction of the entire colony when the honey was harvested. The participation of youth and women in beekeeping activities is also at a very low level. Majorly, honey bee race aggressiveness and poor awareness about improved beekeeping practices lead the beekeepers to work in the forest area. In the area of pests and predators ants are the major challenge for beekeepers. Generally, pests and predators, indiscriminate application of agrochemicals, absconding and migration of colonies, and lack of extension support are the major constraints that undermine the beekeeping practice in the study area. Further activities must be done by a Research Centre on reducing honey bee race aggressiveness behavior. Moreover, emphasis must be given to women and youth to participate in beekeeping activities and create job opportunities. Finally, in the region strengthening the extension system on improved beekeeping practice system is the major one especially to save the colony from distraction at the time of honey harvesting time.

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Ethical Statement

The ethical statement does not apply to this study and does not involve any animal that requires approval from the ethical committee

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Author Contributions

The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

Statements and Declarations

The authors declare that they have no known competing interests or personal relationships that could have appeared to influence the work reported in this paper.

Conflicts of Interest

The authors have declared that no competing interests exist.

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