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MELLIFERA RESEARCH ARTICLE

Apis mellifera Keeping in Mila District from Algeria: Colony Management and *Varroa destructor* Control Practices

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A B S T R A C T

This study was carried out to evaluate the level of beekeepers knowledge and to determine the current state of Varroa destructor infestation and treatment strategies used for its control among different groups of beekeepers. A questionnaire was also conducted to investigate management practices among 41 beekeepers during April- May 2018 in Mila district, northeastern of Algeria. It was found that 53.65% of beekeepers are between 20 and 40 years old, 46.34% have a secondary school level, and 19.51% have a university level. This is considered a constraint to the development of this activity. Most of the beekeepers have 30 to 100 beehives (41.46%) and often exercise transhumance (80.48%). The renewal of the hive is periodic according to the professional experience and the technicality, the majority of whom are artificial swarming (82.92%). Statistical analysis revealed a large difference in the behavior of apiaries ($p < 0.05$). Losses of colonies are reported by 62% of beekeepers. The mortality in front of the hives is declared by 73.17% beekeepers. For monitoring and screening of varroa infestation, 39.02% of beekeepers never followed up. This screening is often carried out at the end of the season (36.58% after treatment). More than half of beekeepers practicing screening (60.97%) monitor natural mortalities. Thus, 43.90% of these beekeepers examined less than 20% of the colonies. This study visualizes a critical situation of beekeeping in this region, which needs an adequate strategy to develop it.

Keywords: Algeria, beekeeping management, honey bee, sustainable development, *Varroa destructor*

Introduction

The bee constitutes an essential element of the environmental balance in the world as a pollinator of very many plant species. It also has other interests including the production of honey, propolis, royal jelly, and wax. Over the past decade, several testimonies and press articles have reported an unusual weakening and mortality of bee colonies in several countries of the world [1]. Colony Collapse Disorder (CCD) remains poorly understood by scientists and beekeepers and is often unexplained. The health of the bee has become a real challenge to the development of beekeeping and the conservation of this species of multiple interests. The health of the bee has become a real challenge with annual declines and colony losses for more than a decade. Many environmental and chemical factors and biological pathogens can be blamed as the cause of colony loss [2].

The number one suspect for CCD is, without question, *Varroa destructor*, not only in Algeria but also in several countries of the world such as the United

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States, Austria, Luxembourg, Lichtenstein, and Canada [3]. *Varroa* mites are an external parasitic hematophagous of bees [4]. It parasites not only adults but also brood, with a clear preference for the larvae of false bumblebees [3]. Originally parasitic of *Apis cerana*, it has long since started to adapt its life cycle to that of *Apis mellifera* [4]. It sucks the hemolymph of the bee and transmits to it, by the same token, several diseases, such as the virus of deformed wings, the fungus causing plastered brood, and the virus of acute paralysis of bees [5]. In addition, it weakens the bee's immune defenses, making it even more vulnerable to attack by other parasites, bacteria, or fungi. Studies have also shown that the presence of *Varroa* in a colony reduces the weight and life expectancy of bees by 30%. Other research has shown that in parasitized bees, there is a reduction in fatty substances that are used to store proteins, especially useful during the winter period. Beekeepers have several means of combating varroasis, but it is mainly acaricides such as coumaphos and fluvalinate that have proved their worth [6].

In Algeria, there are few studies and surveys on the situation of bee colonies, although, for several years, phenomena of abnormal mortality have often been reported by beekeepers. Several questions are worth asking; How to assess the losses of local bee colonies? What are the risk factors and what can be their interactions with the honey bee about the reported losses?

This study aimed to clarify the existing relationships between the various factors which harm the health of the local honey bee *Apis mellifera* intermissa and interfere with the development of this sector and to provide answers to the previous questions by a field survey in the region of Mila, northeastern Algeria.

Materials and Methods

In determining the number of beekeeping enterprises to be surveyed in the study. Although N is known, indicating the total number of enterprises, in cases where standard deviation and variance values cannot be determined, the following "equality-1" was used, which is included in the Simple Random Sampling and whose details are described by Yamane [7]. Accordingly, in 2018, 41 beekeeping enterprises among 126 enterprises engaged in beekeeping in Mila district were identified and a face-to-face survey was conducted with these owners. The required sample size was calculated according to the following formula:

$$\mathbf{n} = \frac{[\mathbf{N} \times \mathbf{t^2} \times \mathbf{p} \times \mathbf{q}]}{[(\mathbf{N} - \mathbf{1}) \times \mathbf{D^2} + (\mathbf{t^2} \times \mathbf{p} \times \mathbf{q})]}$$

Where;

n = number of samples

N = cluster size

D = accepted or desired sampling error

t = table value

p = the rate to be calculated

q = 1-p

A written questionnaire was chosen as an information evaluating tool on bee diseases from the interviewed beekeepers. The advantage of this method is that it allows collecting a large amount of information in a short time. For the sake of brevity, we have privileged direct questions.

Several axes were developed in the questionnaire and each axis was devoted to obtaining particular information about;

1. The beekeeper (age, school level) and the apiary (number of hives);
2. The conduct of apiaries (type of breeding and renewal of hives);
3. The symptoms observed by the beekeeper on the bees and on the brood, colony losses and their season of observation;
4. The screening and monitoring of varroa infestation;

- Screening practices (yes/no)
- Difficulties to screening and monitoring (timing and practices)
- Monitoring objectives (the need or the evaluation of treatment)
- Different times of screening (beginning (February-March) or end of beekeeping season (end of July-September)

5. The practices and behavior during the detection and monitoring of varroa infestation;

- Screening and follow-up method (Washing bees with alcohol, Uncapping of worker brood, Uncapping of male brood, Natural mortality monitoring of varroa)
- Number of colonies to be screened (Less than 20%, Between 20 and 30%, More than 30%, 100% of colonies)
- Natural mortality monitoring (Greased diaper, ungreased diaper)
- To behave (Depends on number of colonies and infestation rate, Change the treatment strategy, Consider and / or maintain treatment)
- Methods used for control (Division of colonies, Male brood trapping, Use of natural medicinal products, Use of veterinary products).

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The data obtained from the survey are made ready for analysis with Microsoft Excel spreadsheet. Findings are expressed as frequencies and percents for features that can be counted and summarized in two-dimensional tables. The Chi-square test was used in the analytical evaluation.

Results and Discussion

It has been found that most of the beekeepers surveyed are between 20 and 40 years old. Regarding the literacy level, 46.34% of the beekeepers have a secondary school level, and only 19.51% have a university-level. This is considered a constraint to the development of this type of breeding in our country. Most of the beekeepers have 30 to 100 beehives (41.46%) and often exercise transhumance (80.48%). The renewal of the beehive frames is periodic according to the professional experience and the technicality of the beekeepers, the majority of whom do artificial swarming (82.92%) (Tab. 1).

Table 1. Analysis of apiaries and their behavior.

Category	Frequency (%)	P-value	Category	Frequency (%)	P-value
Age			**Type of bees breeding**		
20 to 40 years	22 (53.65)	0.011*	Sedentary	8 (19.51)	< 0.000**
41 to 60 years	12 (29.26)		Transhumant	33 (80.48)	
Over 60 years	7 (17.07)		**Renewal bees approaches**		
School-level			Purchase of swarms	7 (17.07)	< 0.000**
Primary	4 (9.75)	0.018*	Artificial swarming	34 (82.92)	
Medium	10 (24.39)		**Season of losses**		
Secondary	19 (46.34)		Summer	7 (17.07)	0.000**
University	8 (19.51)		Spring	8 (19.51)	
Hives number			Winter and autumn	26 (63.41)	
Less than 30	13 (31.70)	0.009**			
30 to 100	17 (41.46)				
101 to 200	6 (14.63)				
Over 200	5 (12.19)				

*—significant; P-value is significant at $P \leq 0.05$; **— Very significant; P-value is very significant at $P \leq 0.01$

The results show that only 12.19% of beekeepers have more than 200 hives. Most of the beekeepers have secondary activities, and that beekeeping is practiced by a very large number of amateurs, who have a technical level, often, insufficient.

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The technical quality of beekeepers can be considered as one of the causes of disease occurrence as well as its frequency in the different regions. The majority of beekeepers (83%) rely only on artificial swarming to enlarge their apiaries. This reproductive technology has an impact on the sensitivity of hives to certain pathogens [8, 9], as well as on the transmission of diseases [10]. The disease can be transmitted by an alternative between two modes, a vertical transmission between one of the parents of the first generation towards the descendants, this mode of transmission is considered to be the least virulent with little impact on the physical conditions of the host [10], a horizontal transmission can occur between individuals in the same colony and between individuals in different colonies. The latter mode is the most dangerous [11]. The horizontal transmission can be ensured by the transhumance of the apiaries. In this study, it was noted that 79% of the apiaries in the study area are transhumant.

In addition, Chahbar [12] reported that regions, which are characterized by high beekeeping production, are also characterized by a high transhumance frequency. Transhumance (migratory beekeeping) is an important factor in the spread of beekeeping diseases according to Fernandez and Coineau [13].

Losses of colonies are reported by 63.41% of beekeepers, during the winter and fall periods (Tab. 1). These beekeepers recorded the presence of Colony Collapse Disorder (CCD) symptoms with a high rate, including mortality near or in the hives (Tab. 2). The different characteristics of the studied beekeeping farms influence significantly the efficiency of beekeeping management in these farms ($p<0.05$).

The losses of the colonies in the world are considerable. Europe was one of the first continents to worry about excess bee mortalities [2]. The highest mortality rates were observed during the winter period [14]. High winter losses, between 20% and 50%, have been reported in some countries of the world, such as Italy [15]. In contrast, winter loss rates were acceptable in other countries, which were reported at 10% in Bulgaria [16].

Table 2. Bee symptoms and behavioral changes observed by beekeepers.

Symptoms	Frequency (%)
Dead bees in the alveoli	27 (65.85)
Deformed wings	9 (21.95)
Bee aggressiveness	25 (60.97)
Mortality at the bottom of the hive	7 (17.07)
Irregular egg-laying	21 (51.21)
Mortality in front of the hive	30 (73.17)
Cluster mortality	17 (41.46)

Table 3. Analysis of screening and monitoring of varroa infestation.

Category	Frequency (%)	P-value	Category	Frequency (%)	P-value
Screening practice			**Monitoring objectives**		
yes	25 (60.97)	0.071	Assess the need for treatment in winter	05 (12.19)	0.009**
no	16 (39.02)		Assess the need for treatment in summer	12 (29.26)	
Screening and monitoring challenges			Evaluating the effectiveness of a treatment	24 (58.53)	
It takes time	09 (21.95)	0.045*	**Monitoring time**		
We don't know how to do it	17 (41.46)		Early winter	07 (17.07)	0.198
No use	04 (9.75)		Start of season	09 (21.95)	
What is about it?	11 (26.82)		End of the season before treatment	10 (24.39)	
			End of the season after treatment	15 (36.58)	

*—significant; P-value is significant at $P \leq 0.05$; **— Very significant; P-value is very significant at $P \leq 0.01$

In this study, we asked beekeepers about the all four seasons losses, because winter losses alone do not provide a complete picture of annual losses. However, summer losses are low, less than 5%, or higher and vary depending on region and year [15]. In the United States, in 2012–2013, beekeepers who observed the symptoms of Colony Collapse Disorder in their apiaries, with inexplicable bee mortality, lost many more colonies compared to beekeepers who did not observe CCD signs in their apiaries [17].

In Algeria, five bee diseases appear on the list of animal diseases with a compulsory declaration, fixed by executive decrees n ° 95-66 of March 15, 2006, modified and supplemented. These are varroasis, rags, nosemosis, acariasis and infestation of the hive by the beetle *Aethina Tumida*. Despite the absence of real data on colony losses in Algeria, a previous survey revealed that

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most beekeepers reported mortalities of more than 10% in 2011 [18].

In our survey, we highlighted the role of the parasite *Varroa destructor* as an agent mainly suspected in the mortalities observed, according to the signs recorded and the detection of the parasite by beekeepers. *Varroa destructor* has existed in Algeria since 1981 [19]. This mite caused a lot of damage in the apiaries of the country, despite the treatments carried out by beekeepers declaring mortalities of more than 10% [18]. According to a field survey carried out in 2009 by the National Institute of Veterinary Medicine (INMV) of Algeria, varroasis remains one of the main pathologies that affect beekeeping farms. It is widespread in all the regions studied and present in 100% of the sampled hives, followed by nosemosis with a lower number of outbreaks. Other bee diseases remain less reported.

For monitoring and screening for varroa infestation, about 39.02% (n=16) of the beekeepers surveyed never followed up, allowed them, and 26.82% of them do not know what it is (Tab. 3).

This screening is often carried out at the end of the season after or before treatment. A total of 58.53% of beekeepers practiced monitoring to ensure the effectiveness of a given treatment. Also, for 12 beekeepers (29.26%), monitoring is used to assess the need to treat at the start or end of the season (Tab. 3). The uni-varied analysis of the data from the screening procedure showed that the objectives set differ significantly ($p<0.05$).

A good understanding of the population dynamics of *V. destructor* within bee colonies is essential for the development of new methods of pest control and the application of recommended strategies. The first thing to keep in mind is that varroa population's increase throughout the season as soon as the brood is present in the colony, thus allowing the founder females to reproduce. Thus, following the infestation of a new colony by the varroa mite, the latter can grow until reaching a disproportionate population in just a few years [20]. *Varroa* population growth is influenced by the characteristics of the parasite, such as its reproductive capacity and longevity, as well as by its host, including the size of the bee colony, the presence of brood (workers or males), swarming, and hygienic behavior. Other factors, such as the time of year, the climate, and the presence of bee pathogens also influence the development of mites in the colony [20].

Varroa screening allows beekeepers to estimate the population of mites parasitizing a colony and to apply the best suited control strategy to their situation. This is an essential step in pest control in beekeeping, which allows, in particular, to know the level of parasitism in a colony before and after treatment. Thus, precise monitoring and a good knowledge of the levels of infestation are the basis of an adequate integrated pest management strategy.

More than half of beekeepers practicing screening (60.97%) and follow up on natural fall, others (21.95%) have used male brood uncapping to assess the degree of infestation. The two procedures for monitoring natural varroa mortality have been reported in Table 4.

Thus, 43.90% of these beekeepers examined less than 20% of the colonies, on the other hand only 5 beekeepers (12.19%) detected more than 30% of colonies. Generally, *Varroa* screening is best when carried out at least four times during the year [21]. Among the screening methods described in the literature, the alcohol washing method and that of powdered sugar (icing sugar) are the ones that provide the most reliable estimates of varroa populations according to The Honey Bee Health Coalition [21]. These two methods consist of removing phoretic varroa mites from the body of adult bees and counting mites to establish a percentage of infestation (number of varroa mites / 100 bees). The main difference between the two methods is that powdered sugar is not lethal to bees, which means that bees can be returned to the hive after screening. On the contrary, the use of alcohol implies that the bees sampled will be sacrificed.

Giovenazzo [22] having demonstrated that screening by natural fall of varroa is the most precise method to estimate the mites population in colonies. Natural fall on self-adhesive cardboard is the most sensitive of the screening methods in a colony with or without brood [23]. This method does not allow a percentage of infestation to be calculated, however, the natural fall of parasites is strongly correlated with the total number of varroa mites in the colony [24]. It is a simple, precise method and does not require the opening of the colony. On the other hand, the hive requires a major modification (anti-varroa plate) and two consecutive visits. Also, ethanol washing is a method of monitoring bee infestation level. Bees (about 200 bees) are collected from the brood chamber frames and placed in a jar containing 250 mL of ethyl alcohol [25]. This method is inexpensive, fairly precise, and is done in one visit to the apiary. On the other hand, it requires the opening of the colony and the sacrifice of a few hundred young bees.

In Quebec beekeepers, the detection of varroasis is done mainly by the method of natural fall and, to a lesser extent, by the method of washing with alcohol [26]. Other screening methods are sometimes used by beekeepers, but these often prove to be less effective, less precise, or less constant [27, 28]. Among these, note the ether rolling method, which only detects 50 to 60% of varroa mites present, and the examination of the brood of bumblebees, the results of which are difficult to interpret as a percentage of infestation [21].

If the infestation rate is above average, 73.17% of beekeepers plan and/or maintained treatment for varroa mites. Ten of them (24.39%) used natural products such as garlic, thyme, and figs without using chemicals or veterinary drugs. Others beekeepers (63.41%, n=26) used two products; Apivar (sold in the form of resin pads. It is composed of Amitraz (0.5g / strip)) and a second product which is composed of natural herbs and derivatives of vegetable oils which directly affect the life cycle of the parasite called Menthocaros, it is mainly composed of Thymol (26%) and Eycalypyol (22%). No information about the frequency and duration of use of these products was collected. It must be scientifically reasoned and carried out by zootechnical, biotechnical, and medicinal means [1]. The simplest, most effective, and most used treatment at present is the Apivar® [29].

Thus, according to Quebec standards, treatment should be applied if the number of varroa mites per sticky carton is equal or greater than one mite per day, in spring and fall. During the summer, additional treatment is recommended if the daily fall of varroa mites is between 10 and 25 mites and this treatment becomes necessary if the daily fall is equal or greater than 25 mites [30]. Furthermore, although the densities of varroa mites may vary from one colony to another, all the colonies in the same apiary should be treated at the same time and with the same method of control, whether chemical or not [21]. This recommendation aims to avoid the parasite drifting from the untreated colonies to the treated colonies [31].

Most beekeepers (84%) observed symptoms of varroasis during the season (Tab. 4). It seems that beekeepers take significantly different measures and practices ($p<0.05$), which interferes with the income of beekeeping farms and their continuity in terms of sustainable development.

There are beehive management approachs that vary significantly from one beekeeper to another, revealing heterogeneity of beekeeping practice, which influences the sustainability of apiaries in terms of communicable diseases between apiary due to the lack of effective trade and sales control measures. The choice of a varroa control method depends on several factors, including the time of year, the presence of brood or honey spikes, the temperature, the production management (conventional or organic), the products used in subsequent years, etc.

Table 4. Behavior characteristics during screening of *Varroa* infestation**.**

Category	Frequency (%)	P-value	Category	Frequency (%)	P-value
Screening and follow-up method			**To behave**		
Washing bees with alcohol	2 (4.87)	0.000**	Depends on the number of colonies and infestation rate	7 (17.07)	< 0.000**
Uncapping of brood brood	5 (12.19)		Change the treatment strategy	4 (9.75)	
Uncapping of brood male	9 (21.95)		Consider and / or maintain treatment	30 (73.17)	
Natural mortality monitoring	25 (60.97)		**Control methods**		
Number of colonies to be screened			Division of colonies	1 (2.43)	0.001**
Less than 20%	18 (43.90)	0.010*	Male brood trapping	4 (9.75)	
Between 20 and 30%	14 (34.14)		Use of natural medicinal products	10 (24.39)	
More than 30%	5 (12.19)		Use of veterinary products	26 (63.41)	
100%	4 (9.75)				
Natural mortality monitoring					
Greased diaper	32 (78.04)	< 0.000**			
Ungreased diaper	9 (21.95)				

*—significant; P-value is significant at P ≤ 0.05; **— Very significant; P-value is very significant at P ≤ 0.01

Chemicals registered in Canada for the control of varroasis include synthetic acaricides (amitraz, tau-fluvalinate, coumaphos, and flumethrin), organic acids (formic acid and oxalic acid), and essential oil (thymol). Certain plant extracts as well as other organic acids and essential oils have also been tested and demonstrate variable effectiveness against varroa mites, effects sometimes harmful for bee and human health [32, 33, 34].

Oxalic acid is effective on phoretic mites only. To obtain maximum effectiveness, it must be used during a period of absence of brood. So the use of oxalic acid is favored in the regions where there is a stop of the

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laying during the year. This is the case in temperate regions in autumn and winter. Thymol is fat-soluble and it binds and collects in wax. However, it degrades between treatment periods. Bees can withstand the concentration of thymol they emit very well, while it turns out to be very toxic to *Varroa*. These treatments are simple, quick, and effective. They have been the subject of several research studies aimed at testing their effectiveness in different beekeeping conditions. In general, these works show an efficiency which varies between 54% and 98%. The highest efficiencies are obtained when the temperatures are between 15 °C and 25 °C and when the brood is absent [35, 36].

Although the means of combating varroasis are numerous, beekeepers in Mila district prefer to use artisanal devices based on strips impregnated with tau-fluvanilate and amitraz. These strips are introduced into the colonies and left for several months. The active ingredient circulating in the colony is very concentrated at the start, whereas, after a few weeks, there is practically nothing left. There is, therefore, the first overdose, then underdosing [37]. These conditions are known to develop the phenomenon of resistance which has been reported in various countries about several active substances, such as amitraz, flumethrin, fluvalinate, and coumaphos [38]. Due to the unavailability of other approved products on the market, the drawbacks of applying traditional treatments, are linked to their low efficacy and the risk associated with the presence of residues in beehive products [18].

It is now widely recognized that integrated pest management is the best approach to control varroasis in beekeeping. This approach relies on the integration of a set of proactive, non-chemical, and chemical methods, which offers beekeepers the best strategy to control the parasite and limit damage to colonies [21, 24]. Among other things, these tactics are aimed at controlling densities of mites before they threaten the productivity and survival of colonies, rather than responding after the damage has occurred.

An integrated varroa control strategy, therefore, includes the following aspects: 1) frequent and rigorous surveillance of varroa populations to detect colonies requiring control and to assess the effectiveness of the treatments used; 2) the use of cultural and physical practices to curb the growth of varroa populations; and 3) a rotation of the chemicals used which

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takes into account the population dynamics of mites and bees and which minimizes the development of varroa mites resistance to chemical miticides [21].

Conclusions

Modern beekeeping requires precise monitoring of the colonies to be able to decide, in an increasingly difficult and changing context, of the actions to be carried out according to the objectives and orientations of each beekeeper (level of intensification of honey production, time available for tracking bees, costs, etc.). Unfortunately, academics practicing beekeeping in Algeria are relatively few, which represents another constraint that can disadvantage the development of this type of breeding. We have found that beekeeping is practiced by a very large number of amateurs. Consequently, the level of technicality is insufficient and the good beekeeping practices applied are limited. It is very difficult to blame a single cause for bee colonies loss. Risk factors are multiple and often interact. The beekeepers questioned declared the presence of CCD symptoms with high rates.

Screening and monitoring the *Varroa destructor* infestation fits completely into this context and can provide healthier colonies capable of reproducing faster and better survival in winter.

As we have shown previously, it is true that screening takes time and is not totally reliable, but some techniques and tools make it possible to optimize the time spent and to carry out quality monitoring. Degradation of the ecosystem (decrease in honey flora) and climate change influence the development of hive management. All of these threaten the local bee and negatively affect honey production. This forces us to establish national surveys over several years as part of a beekeeping sector observatory, to allow us to obtain rigorous monitoring of loss rates and to try to understand the causes and guide experimental scientific work, through laboratory analyzes of pathogens present in apiaries. Likewise, it is important to carry out toxicological analyzes and to look for residues of all kinds, in particular heavy metals, in the products of the hive, throughout the season.

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Cezayir'in Mila Bölgesi'nde Arıcılık: Koloni Yönetimi ve *Varroa destructor* Kontrolüne Yönelik Uygulamaları

Bu çalışma, arıcıların bilgi düzeyini değerlendirmek ve *Varroa destructor* istilasının mevcut durumunu ve farklı arıcı grupları arasında kontrol için kullanılan tedavi stratejilerini belirlemek amacıyla yapılmıştır. Cezayir'in kuzeydoğusundaki Mila bölgesinde Nisan-Mayıs 2018 döneminde 41 arıcı arasında yönetim uygulamalarını araştırmak için bir anket düzenlenmiştir. Arıcıların % 53.65'inin 20-40 yaş aralığında, % 46.34'ünün ortaokul ve % 19.51'inin üniversite düzeyinde olduğu tespit edilmiştir. Eğitim seviyesi, arıcılığın gelişiminde bir kısıtlama olarak öne çıkmıştır. Arıcıların çoğu 30 ila 100 arı kovanına sahiptir (% 41,46) ve genellikle yaylacılık yapmaktadır (% 80,48). Kovanın yenilenmesi, mesleki tecrübe ve teknik arıcılık bilgisinin seviyesine paralellik göstermektedir ve katılımcıların çoğunluğu (% 82.92) yapay oğul verdirme eğilimindedir. İstatistiksel analiz, arı kovanlarının davranışında büyük bir fark olduğunu ortaya koymuştur ($p < 0.05$). Arıcıların % 62'i koloni kayıpları rapor etmektedir. Arıcıların % 73,17'i ölümlerin kovan önlerindeki ölü arılar şeklinde olduğunu beyan etmektedir. *Varroa* istilasının izlenmesi ve taranması için, arıcıların % 39.02'i hiçbir zaman takip yapmadıklarını ifade etmişlerdir. Taramalar genellikle sezon sonunda yapılmıştır (% 36,58 oranında tedaviden sonra). Tarama yapan arıcıların yarısından fazlası (% 60,97) doğal ölümleri gözlemlemektedir. Dolayısıyla, düzenli tarama yapan arıcıların % 43,90'ının kolonilerin % 20'inden azında tarama yapmaktadır. Bu çalışma ile bu bölgede, koloni yönetimi ve *Varroa destructor* kontrolüne yönelik uygulamaları geliştirmek için uygun bir stratejiye ihtiyaç duyulduğu saptanmıştır.

Anahtar kelimeler: Cezayir, arıcılık yönetimi, bal arısı, sürdürülebilir kalkınma, *Varroa destructor*

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MELLIFERA RESEARCH ARTICLE

Pollen Analysis of Chestnut Honey in Some Provinces of the Black Sea Region, Turkey

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A B S T R A C T

This study presents the pollen analysis of 16 natural chestnut honeys from the Black Sea Region of Turkey. Honey samples were collected from 12 different localities in 2018 around Samsun, Sinop, Kastamonu and Giresun. Honey samples were taken from a height of at least 10 and a height of 859 meters. The pollen analysis was carried out using microscopical analysis. All of the samples collected are monofloral honey.The highest number of pollen was observed in the samples from Bozkurt, Kastamonu in Turkey. The aim of the present study is to characterize the pollen grain association of monofloral honeys harvested in some provinces of the Black Sea Region. A second goal is to make a contribution to the knowledge of the pollen composition of monofloral honeys from northern Turkey, as has been extensively done for monofloral European honeys.

Keywords: melissopalynology, chestsnut honey, pollen analysis, Samsun, Kastamonu, Sinop, Turkey

Introduction

The variety of honey produced in a region depends on the variety of nectar sources in that region. Identification and classification of monofloral honeys are of scientific and commercial importance. Vegetation of the Black Sea Region allows the production of different types of honey. Studies of different types of honey in the Black Sea region have been carried out in previous years [1]. Chestnut honey is a type of honey obtained from the extracts that bees collect from the flowers of this tree during a certain period of the chestnut tree. This honey, which we can obtain thanks to the intensely working bees in June when chestnut trees begin to bloom, is considered among the more valuable honeys as it can be produced less than many other flower honeys.

The main method used in determining the geographical and herbal characteristics of honey is melissopalinology. Melissopalinology is based on microscopic examination of honey sediment. The first melissopalynological Turkey is based on studies in the 1980s [2-6]. Recently, studies identified nectariferous plants through pollen analysis in honey samples from Aegean region [7], Black Sea region [8] and the eastern and south-eastern regions [9]. There are several reports from

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Turkey on the pollen spectrum of honeys [1,10]. All melissopalynological studies have been carried out in different phytogeographical regions of Turkey as reviewed by Öztürk et al. [10].

Melissopalynolgical, sensory and physico-chemical analyses together are needed for establishing the botanical denomination of a honey, because when considered individually, each one has its own limitations. Regarding melissopalynolgical analysis, whereas some honey types need high percentage of pollen to be considered as monofloral (90% for chestnut honey), for others only 15% is sufficient to declare their botanical origin (e.g., *Lavandula* spp.) [11].

In this study, although made with unifloral honey samples, pollen belonging to 23 different plant species was detected. The main purpose of the research is to analyse the pollen collection amount habits of honey bees in chestnut honey samples at different heights of the Black Sea Region.

Materials and Methods

Study Area

The Black Sea region is located in the north of Anatolian lands. The Black Sea climate is classified as humid climates. It is seen in the Black Sea Region and the Black Sea coastal zone of the Marmara Region. Every season of the year is rainy in this climate zone. This is because; winter and summer air masses affecting Turkey, the Black Sea region comes via the Black Sea. Maximum precipitation occurs in autumn and minimum precipitation occurs in spring. Annual rainfall is 1000-1500 mm. The annual average temperature is 13 - 15 0C. In places where the Black Sea climate is effective, natural vegetation is forest due to the high humidity and rainfall. Alpine meadows are seen in places where forests do not grow. The region, which constitutes 18% of the country's territory, is one of the largest regions of the country with its 141,000 square kilometers of land. According to the 2010 census, 7,540,000 people live in the Black Sea Region, mostly in rural areas. This is the region in Turkey, doing more than living in the city where one of those living in rural areas. The fact that the region consists of mountainous areas and rocky shores makes it difficult to establish large cities.

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Figure 1. Black Sea Region of Turkey

Samsun, Sinop, Kastamonu and Giresun, located on the coastline of the Black Sea region, both in terms of the number of honey bee colonies and honey production, it has an important place in the total of the country. Most beekeepers in the region have an average of 200 hives.

Figure 2. Black Sea Region

Vegetation of the Study Area

Lush forests are found on the Black Sea coastline, where every season is rainy and the temperature is sufficient. Climatic conditions caused forests to start from the coast. However, as a result of the destruction by people in order to settle on the coast and open fields, the lower limit of the forests was drawn to 200 - 300 meters in many places.

Broad-leaved forests take place up to about 700 meters from the shore. Beech, chestnut, alder, oak, hornbeam are the main tree species in this belt. There are mixed-leaf forests between 700 and 1500 meters. In this belt, there are beech from broad-leaved, yellow pine and fir from coniferous. There are coniferous forests between 1500 - 2200 meters. The main tree species in this belt are fir, spruce, scotch pine and larch. Under-forest plants are also very rich in these forests located on the slopes of the mountain facing the sea. On the southern slopes of the mountains, forests become sparse due to the decrease in precipitation. Black Sea forests, Turkey constitute about 14% of the forest.

Forest areas along the coasts of this region, which receives abundant rainfall, especially start from sea level and reach 2000 m. It continues to include some maquis (*Santalum* sp., *Arbutus* sp., *Pistacia* sp., *Phillyrea* sp.) at the lower levels in the form of a strip that narrows from place to place, in areas up to heights, in a louder way than the West section in the East. Then, along the northern slopes, 200 m. It is encountered with a generation continuing until.

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This is also the area known as leafy grove forests. Forest areas in the Black Sea Region are 1200 m. It gives a different appearance in the West, Middle and East parts after the. Accordingly, while larch and firs are concentrated on the coast in the West and Central parts, fir, scotch pine and spruce are concentrated in the East. Dry forests dominate the southern slopes of the Black Sea mountains and lower levels on the second rows. There are various types of oak in the lower levels, while the leaves (beech, hornbeam, alder, larch, linden) in the upper levels are shedding from 600-800 meters. The upper limit of forest in the North Anatolian mountains is 2000 m. is around. After this height, the trees disappear. Alpine meadows begin in the field. Black forest in Turkey shows a value of 32%, which is one third of all our forest areas.

Data Analysis

A total 16 natural chestnut honeys samples (Table 1), were collected from non-migratory beekeepers (members of the Samsun Beekeepers Association) which different altitudes in the Black Sea Region during 2018. The preparation of honey samples was carried out according to the standardized method of Loveaux (1970) [12]., Pendleton [13], Silici and Gökçeoğlu [1]. To analysis the pollen content of the honey samples methods outlined in detail by Ozturk et al. [10,14], and Sorkun [15] were followed. Honey samples (500 gr) were collected from different altitudes was put into sterile jars.

After the honey is mixed thoroughly and homogenized, 10gr is weighed and transferred to the tube. 20 ml of distilled water was added to the test tube, as a witness in the tube. 12 542 Lycopodium spp. the tablet containing the spore was thrown. Tubes 10- It was kept in a 45 ° C water bath for 15 minutes. Add basic fuchsine to solution It was centrifuged at 3 500 rpm for 45 minutes. The supernatant portion of the solution in the tubes was poured out. Later, 0.1 ml of 50% glycerine was added to the tube, 0.01 ml of this mixture taken into another tube in which 0.09 ml of 50% glycerine was previously placed. has been transferred and then 0.01 ml of the solution in this tube was taken and examined under a microscope. Each preparation is 18x18 mm^2 starting from the upper left corner. Scanning of the area completely It was examined microscopically with. All pollen in this area has been determined. Counting was made in two preparations prepared from two separate tubes and It has been

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applied to the formula shown below by taking the average.

TPS-10 = (Number of Pollen Counted x 12 542 *) / Counted Lycopodium spp. spores

*Amount of *Lycopodium* spp contained in one *Lycopodium* spore tablet.

Percentage of every pollen type in the pollen sediment was calculated for all chestnut honey samples. Pollen types were allocated to one of four frequency classes; (*) predominant pollen types (>45% of the total pollen content); (**) secondary pollen types (16-45%); (***) important minor pollen types (3-15%); and (****) minor pollen types (<3%) [16] (Table 1). The total amount of pollen grains in a honey sample was determined and the results were compared with the classification proposed by Louveaux et al. [17]. The five classes according to the pollen content in 10 g of honey were: **1**: less than 20.000 pollen grains; **2**: 20.000 to 100.000 pollen grains; **3**: 100.000 to 500.000 pollen grains; **4**: 500.000 to 1.000.000 pollen grains and **5**: over 1.000.000 pollen grains.

Preparation of Pollen Slides from Honey Samples and Identification

For pollen analysis, the pollen preparations were prepared as recommended by the International Bee Research Assosiation (Louveaux et al.1970) [12] and modified by Sorkun and Doğan (2002) [18]. Accordingly, 10 grams of each honey was dissolved in 20 ml of distilled water in the sterile test tube. The solution was centrifuged for 45 min. at 3500-4000 rpm. The supernatant solution was poured and small quantities of each pellet at the bottom of the tubes were mounted with basic fuchsine added glycerin gelatin on permanent glass slides.

For microscopic analysis of the pollen taxa of honey samples, two slides were prepared from each sample. Pollen identification and count were carried out using a light microscope (Zeiss Axiolab) with 400× and 1000× objectives, the latter being used when greater detail was required for the morphological identification. For each honey sample, we counted and analyzed a minimum of 1000 pollen grains. Frequency classes were determined twice for each sample and designated as dominant pollen (>45% of a specific pollen type), secondary pollen (16–45%), important minor pollen (3–15%) and minor pollen (<3%).

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Results

During the research, a total of 23 pollen types belonging to 25 families were identified from 16 chestnut honey samples. Although honey samples are unifloral, the plant taxa (pollen types) in each honey sample varies between 7 and 22 (Table 1). Since the honey samples studied are unifloral, the dominant taxon has been identified as Fagaceae (*Castanea sativa*, *Quercus* sp. *Coronilla* sp. *Trifolium* sp.).

Based on the absolute pollen content per 10 g of chestnut honey samples, % 68.75 (n=11) of the samples were found to belong to Group 1 (<20.000 pollen grains per 10 g honey), %18.75 (n=3) to Group 2 (20.000 to 100.000 pollen grains per 10 g honey) and %12.5 (n=2) Group 3 (100.000 to 500.000 pollen grains per 10 g honey) (Table 1).

The number of pollen grains per 10 g of honey, extended from the "very poor" (<20.000) to the "very rich" category (500.000 to 1.000.000) [19]. In our study, honey samples generally "poor" in grains represented % 68.75 of all the samples (Table 1).

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Table 1. Pollen spectra and TNP 10g values obtained from the honey samples collected from various localities in the Black Region

Sample	Locality	Altitude	Pollen spectra	TNP-10g
Sample 1	Samsun Aycacık	76 m	**Castanea sativa*	136 691
			**	
			***	
			****Quercus* sp., *Platanus* sp. *Artemisia* sp., *Robinia* sp., *Salix* sp. Rocaceae, Poaceae, Pappaveraceae, *Acer* sp., Lamiaceae, Chenepodiaceae/Amaranthaceae, *Taraxacum* sp., Asteraceae, *Xanthium* sp.	
Sample 2	Samsun Aycacık	76 m	**Castanea sativa*	77 713
			**	
			***	
			****Platanus* sp., *Artemisia* sp. *Quercus sp.,* Poaceae, Chenepodiaceae/Amaranthaceae, *Robinia sp.,* Rosaceae, Asteraceae, *Pinus sp.*	
Sample 3	Samsun Çarşamba Ordubaşı Village	300 m	**Castanea sativa*	45 625
			**	
			***Quercus* sp.	
			****Platanus* sp., *Artemisia* sp. *Xanthium sp., Salix sp., Robinia sp., Ulmus sp., Inga,* Asteraceae, Brassicaceae, Apiaceae, Chenepodiaceae/Amaranthaceae, Rosaceae, Fabaceae, Poaceae, *Ailanthus* sp., *Convolvulus* sp., Papaveraceae	
Sample 4	Samsun Terme	10 m	**Castanea sativa*	27 898
			**	
			***Coronilla* sp	
			****Apiaceae, *Rubus sp., Quercus sp., Artemisia sp.,* Papaveraceae, Asteraceae, Brassicaceae,*Trifolium sp., Sambucus sp.Platanus sp., Xanthium sp., Salix sp.,* Rosaceae, Convolvulus *sp.,* Poaceae, *Tilia sp., Plantago sp., Lotus sp.,* Lamiaceae	

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Sample	Locality	Altitude	Pollen spectra	TNP-10g
Sample 5	Samsun Salıpazarı	859 m	**Castanea sativa*	39 246
			**	
			***	
			*****Coronilla* sp., Quercus sp., Poaceae, Artemisia sp. Apiaceae, *Tilia* sp., Asteraceae, *Papaver* sp. *Trifolium* sp., *Robinia* sp., *Rubus* sp., *Xanthium* sp., Rosaceae, *Salix* sp., *Echium* sp., Lamiaceae, Brassicaceae	
Sample 6	Samsun Salıpazarı	859 m	**Castanea sativa*	17 380
			***Salix* sp.	
			***Brassicaceae, *Papaver* sp., *Robinia* sp., *Coronilla* sp., *Quercus* sp.	
			*****Sambucus* sp., *Rubus* sp., Asteraceae, Apiaceae, *Rumex* sp., *Artemisia* sp., *Tilia* sp., *Xanthium* sp.	
Sample 7	Samsun Ayvacık	76 m	**Castanea sativa*	25 873
			**	
			****Trifolium* sp. *Salix* sp.	
			*****Papaver* sp., *Sambucus* sp., *Artemisia* sp. Poaceae, *Tilia* sp., *Quercus* sp., Apiaceae, Asteraceae, Caryophyllaceae, Rosaceae, Brassicaceae, *Xanthium* sp., *Robinia* sp.	
Sample 8	Samsun Salıpazarı	859 m	**Castanea sativa*	99 442
			**	
			***Asteraceae, *Robinia* sp., Lamiaceae	
			*****Echium* sp., *Quercus* sp., , *Trifolium sp., Rosaceae, Salix sp., Artemisia sp., Papaver sp., Tilia sp.*	
Sample 9	Samsun Tekkeköy	240 m	**Castanea sativa*	45 960
			**	
			***Asteraceae, Lamiaceae, *Echium* sp.	
			**** *Quercus* sp., *Trifolium* sp., Rosaceae,*Xanthium* sp., *Salix sp.*, Poaceae, *Artemisia sp.*, *Papaver sp.*, Caryophyllaceae, Chenepodiaceae/Amaranthaceae, *Rumex* sp.	

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Sample	Locality	Altitude	Pollen spectra	TNP-10g
Sample 10	Samsun Salıpazarı	859 m	***Castanea sativa* ** *** *****Echium* sp., *Papaver* sp., *Trifolium* sp., Lamiaceae, Rosaceae, *Salix* sp., *Quercus* sp.,	10 937
Sample 11	Kastamonu Çatalzeytin	74 m	**Castanea sativa* ** *** *****Quercus sp.*, *Trifolium sp.*, *Salix sp.*, *Papaver sp.*, Poaceae, Lamiaceae, Rosaceae, Apiaceae, Asteraceae, *Xanthium sp.*, Chenepodiaceae/Amaranthaceae	52 832
Sample 12	Giresun Yağlıdere	50 m	**Castanea sativa* ** ***Lamiaceae, *Quercus* sp. ****Caryophyllaceae, Apiaceae, *Salix* sp., *Trifolium* sp., *Tilia* sp., Poaceae, *Coronilla* sp., *Robinia* sp., Rosaceae, *Artemisia* sp., *Papaver* sp., Asteraceae	50 470
Sample 13	Kastamonu Günebakan	100 m	**Castanea sativa* ** *** *****Salix sp.*, *Papaver sp.*, Poaceae, *Quercus sp.*, *Trifolium sp.*, Caryophyllaceae, *Robinia sp.*, *Coronilla sp.*, Lamiaceae, *Artemisia sp.*, Apiaceae	133 823
Sample 14	Kastamonu Demirci	220 m	**Castanea sativa* ** *** *****Quercus* sp., *Papaver* sp., *Tilia* sp., Rosaceae, *Trifolium* sp., Caryophyllaceae, Lamiaceae, *Salix* sp., *Robinia* sp.	86 153

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Sample	**Locality**	**Altitude**	**Pollen spectra**	**TNP-10g**
Sample 15	Kastamonu Bozkurt	10 m	**Castanea sativa*	167 772
			**	
			***	
			****_Tilia sp., Quercus sp.,_ Poaceae, _Trifolium sp.,_ Caryophyllaceae, _Rumex sp.,_ Asteraceae, Apiaceae, Betulaceae	
Sample 16	Sinop Erfelek	300 m	**Castanea sativa*	61 457
			**	
			***	
			****_Salix sp.,_ Lamiaceae, _Trifolium sp.,_ Poaceae, _Artemisia sp.,_ Ericaceae	

(*) predominant pollen types (>45% of the total pollen content); (**) secondary pollen types (16-45%); (***) important minor pollen types (3-15%); and (****) minor pollen types (<3%) [16]

Discussion

Pollen content ant the diversity is most important factor to determine the quality level of honey [20]. Pollen present in the dominant and secondary group are primary contributors to the formation of honey while pollen content in quantities less than other pollen are added to the honey generally by external factors such as wind [21]. The dominant and secondary groups determine the honey content and quality. According to Lieux (1972) [22], the diversity of trace and minor groups has always bigger than diversity of dominant group pollen taxa.

Monofloral honeys are predominantly from a single botanical source (numerous plants and contains only one plant source predominantly) it is. Generally, the taste of monofloral honey is specific [23].

In monofloral honey, the sensory properties of nectar of the dominant plant species are intense. It is felt and the contribution of nectar of other plant species it contains is minor or can also be a work [24]. Honey, produced in a natural environment containing different plant species, is never a single plant. It is impossible to control the honey bee behavior in such an environment is generally accepted. So scientifically

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unifloral honey producing is much more difficult than producing multifloral honey [25].

It has been reported that certain types of monofloral honeys are beneficial for human health. Due to its antibacterial properties, such honeys in the treatment of wounds and diseases it is used. Therefore, recently, plant source of monofloral honeys interest in verification is increasing [26].

Melissopalinological analysis is still considered as a suitable method for honey evaluation. Many workers think that acidity and humidity are not the only significant parameters for honey quality; in addition, pollen analysis gives important knowledge about the geographical and botanical origin [27-29, 1], especially- if the plant is an endemic plant [30]. As emphasised by Mandić et al. [31] Europa has more than 100 unifloral honeys but the honeys have local importance and people produce them periodically. Geographical and botanical properties play critical roles about their quality [32]. About 500 plants in Turkey are important nectar and pollen offering beekeeping plants. It is reported that all of this plants are important for beekeeping also 50-60 of them are economically dominant nectar and pollen yields [10, 15].

This study was done with completely monofloral honey samples (Chestnut honey) in the Black Sea Region. The locations of the producers differ in terms of the flora characteristics of both the altitude and the regions where the hives are located.
Although the localities where honeys are collected vary in height, no significant result has been observed in terms of the amount and concentration of pollen in honey. The reason for this can be considered as the vegetation in the vicinity where the beehives are located. The fact that there is no classification of pollen amounts specific to chestnut honey has caused the use of general classification principles instead of making a special classification. However, we know that for honey samples such as chestnut honey, when the pollen concentrations are 90% and above, it is defined as monofloral honey.

Conclusion

Black Sea Region, Turkey's economy in terms of major tea, nuts, a region where the cultivation of major agricultural products such as honey. However, due to the wrong agriculture and energy policies of the governments, the floral features of the region have been at risk of disappearing. In particular, Hydroelectric Power Plant projects will lead to the drying of freshwater resources in the region, which will cause the destruction of natural vegetation. In this case, quality and especially honey production in the region will be compromised.

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Karadeniz Bölgesi (Türkiye)'nin Bazı Yörelerinde Üretilen Kestane Ballarında Polen Analizi

ÖZ: Bu çalışma, Türkiye'nin Karadeniz Bölgesi'nde bulunan farklı şehirlerden 16 doğal kestane balının polen analizini içermektedir. 2018 yılında Samsun, Sinop, Kastamonu ve Giresun çevresinde 12 farklı bölgeden bal örnekleri toplanmıştır. Bal örnekleri deniz seviyesinden en az 10 metre, en fazla 859 metre yükseklikten alınmıştır. Balların mikroskobik analizi Toplam Polen Sayısı (TPS) kullanılarak gerçekleştirildi. Toplanan örneklerin tamamı unifloral baldır. Çalışmada en fazla polen Bozkurt, Kastamonu'dan alınan örneklerde görülmüştür.

Çalışmanın amacı, Karadeniz Bölgesinin bazı şehirlerinde üretilen monofloral balların polen miktarını ve tayinini yapmaktır. İkinci olarak da Avrupa' da yapılan geniş kapsamlı monofloral bal analizlerini, Karadeniz Bölgesi ve ülkemiz için de yaygınlaştırmaktır.

Anahtar kelimeler: melisopalinoloji, kestane balı, polen anlizi, Samsun, Kastamonu, Sinop, Türkiye

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MELLIFERA REVIEW ARTICLE

Pesticide and Pathogen Induced Oxidative Stress in Honey Bees (*Apis mellifera* L.),

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A B S T R A C T

Honey bees represent an important cultural and economic benefit for humans by pollinating wildflowers and crops. Honey bees, like other organisms, face a wide range of environmental stressors throughout their lives. These stress factors disrupt the physiological balance of the organism. During the use and metabolism of oxygen taken into the organism, aggressive molecules known as free radicals are formed and the organism cannot keep these radicals under control and oxidative stress occurs. In such a situation, free radicals attack, oxidize, and degrade healthy cells. This degradation is characterized by the increased production of reactive oxygen species (ROS), by the simultaneous degradation of waste systems. Exceeding the oxidative stress threshold in honey bees causes bee losses on an individual or colony level. Colony losses, which have been increasing day by day due to environmental factors, reveal the importance of studies on the formation, physiology, and prevention of oxidative stress. The most important antioxidant enzymes identified in honey bees are glutathione S-transferase (GST), glutathione peroxidase (GPX), catalase (CAT), and superoxide dismutase (SOD). This review examines the mechanism of oxidative stress and the effects of pesticides and pathogens on oxidative stress in honey bees.

Keywords: Honey bee, *Apis mellifera* L., oxidative stress, reactive oxygen species (ROS), pesticide, pathogen

Introduction

Pollination is a vital ecosystem service required for 76% of global crops and about 87,5% of all flowering plants [1, 2]. The most important pollinator species in worldwide are honey bees (*Apis mellifera*), providing about 50% of the pollination of global products [3]. Honey bees have a long history with humans and are the most domesticated pollinator species globally [4]. Unfortunately, a significant loss in bee population has been observed in recent years. Both bee population loss and colony collapse are caused by climate change, habitat loss, environmental stress factors, the availability and diversity of feeds, as well as particularly insecticide use, parasites, and pathogens [5-13].

Various chemicals (insecticides, drugs, metals, smoke, the abnormal concentration of oxygen, etc.), physical (radiation,

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temperature, noise, vibration, etc.), and physiological (diseases, physical injury, aging, etc.), stressors may cause stress that can disrupt homeostasis. Such a situation is called oxidative stress. Oxidative stress occurs when excessive oxygen radicals are produced in cells that may exceed normal antioxidant capacity, and reactive oxygen species (ROS), are produced simultaneously enhanced by waste degradation. Increasing ROS concentrations cause oxidative damage to proteins, lipids, and nucleic acids; therefore, the functions of cells, organs, or the whole organism by being severely impaired lead to death [14].

The Mechanism of Oxidative Stress

Oxidative stress causes an abnormal ROS (Reactive Oxygen Species), level such as free radicals (hydroxyl, nitric acid, superoxide, etc.), or non-radicals (hydrogen peroxide, lipid peroxide), by damaging cells or tissue, is a general term used to describe the effect of oxidation by damaging specific molecules. Oxidative stress results from an imbalance between the ability to easily detoxify reactive intermediates of a biological system with reactive oxygen production or to repair the damage that has occurred. In other words, oxidative stress is a defect in the balance between ROS (free radicals), production and antioxidant defenses that can cause tissue injury. Oxidative stress occurs when ROS production in a system exceeds the system's ability to neutralize and eliminate them. The imbalance can be caused by production, distribution and/or environmental or behavioral stressors and lack of antioxidant capacity caused by excessive production of ROS. This damage can affect a specific molecule or an entire organism. If not regulated properly, excess ROS can inhibit normal function by damaging a cell's proteins, lipids, carbohydrates, and DNA, which can lead to cytotoxicity, genotoxicity, and even carcinogenesis when damaged cells proliferate; therefore, oxidative stress plays a role in the aging process as well as increasing diseases [15-17].

Reactive Oxygen Species (ROS), Oxidative Damage and Cell Singal

Reactive oxygen species (ROS), are highly reactive molecules composed of various chemical types such as superoxide anion (O2−), hydroxyl radical (•OH), and hydrogen peroxide (H2O2). ROS are

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produced as byproducts of aerobic respiration and various other catabolic and anabolic processes. Mitochondria are the largest producers of ROS in cells, and most part of the mitochondrial ROS is produced in the electron transport chain. Electrons infiltrate directly into oxygen through the electron transport chain and generate short-lived free radicals such as O2−. O2− can be converted into non-radical derivatives such as H2O2, either spontaneously or catalyzed by superoxide dismutase (SOD). H2O2 is relatively stable and membrane-permeable; besides, it can diffuse into the cell and be removed by cytosolic antioxidant systems such as catalase, glutathione peroxidase, and thioredoxin peroxidase. In addition to being produced during cellular metabolism in mitochondria, ROS can be produced in response to different environmental stimuli such as growth factors, inflammatory cytokines, ionizing radiation, UV, chemical oxidants, chemotherapeutics, hyperoxia, toxins, and transition metals. Apart from mitochondrial respiration, a number of cytosolic enzymes can produce ROS. Nicotinamide adenine dinucleotide phosphate (NADPH), oxidases are a group of enzymes associated with the plasma membrane found in various cell types. The function of NADPH oxidases is to produce superoxide from oxygen using electrons from NADPH [18].

ROS after produced, react with lipids, proteins, and nucleic acids that cause oxidative damage to macromolecules. ROS readily attack DNA and produce various DNA lesions such as oxidized DNA bases, abasic regions, and DNA strand breaks, leading to genomic instability [19, 20]. 7,8-dihydro-8-oxo-deoxyguanosine (8-oxo-dG), is one of DNA lesions good characterized and the most common caused by ROS. It is a mutagenic lesion resulting in G: C to T: A transversions. To limit cellular damage caused by ROS, the cells have developed amount of enhancement defense mechanism. DNA lesions produced by ROS are mainly repaired by base excision repair and other DNA repair pathways such as nucleotide excision repair, double-strand rupture repair, and mismatch repair. Additionally, the detrimental effects of ROS can be neutralized through high antioxidant defense pathways that include SOD, catalase, and glutathione peroxidase [18]. Depending on the cell types, ROS has been found to function as signaling molecules in cell proliferation, cellular

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aging or cell death. Many cellular processes being different effects of ROS mediate not only harmful byproducts of ROS but also various signaling pathways [18].

Increased oxidative stress causes many diseases in humans: cardiovascular diseases, cancer, diabetes, neurodegenerative diseases (Parkinson, Alzheimer's disease, paralysis, dementia, epilepsy, etc.), and psychiatric diseases (attention deficit, hyperactivity disorder, autistic disorder, anxiety disorder, bipolar disorder, depression and mood disorders, history of suicide attempt, psychosis, schizophrenia, and sleep disorders). In addition to these diseases, the role of oxidative stress has been well specified for diseases such as alcohol and drug abuse, asthma, chronic obstructive pulmonary disease (COPD), various seizures, hepatitis and liver diseases, rheumatoid arthritis, kidney diseases, and various eye disorders [21].

Oxidative stress can be classified according to density with density scales ranging from physiological oxidative stress (eustress), to toxic oxidative load that damages biomolecules. Various oxidants are produced by endogenous or exogenous sources. Low exposure of oxidant cells and organisms allows specific targets in the use of the redox signal (oxidative eustress; beneficial stress), while high exposure results in disruption of the redox signal and/or damage to biomolecules (oxidative distress). In the 21st century, as Paracelsus addressed in his dictionary, the paradigm "*dose creates poison*" is a viable paradigm for oxidative stress [22, 23] (Figure 1).

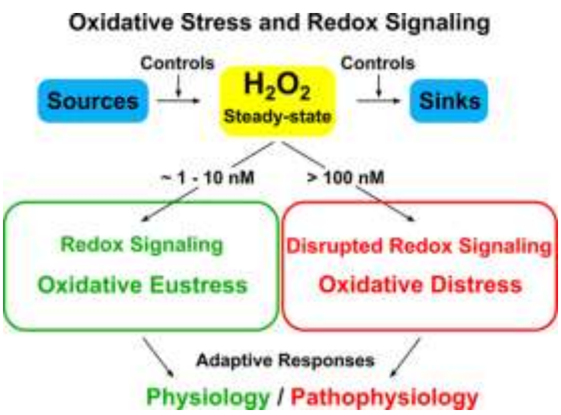

Figure 1. Role of hydrogen peroxide in oxidative stress [24].

Oxidative equivalents used in redox signaling target directly or indirectly regulatory pathways, particularly those addressed by transcription factors. Hydrogen peroxide has emerged as a major redox metabolite that is effective in redox detection, signaling, and redox

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regulation [23-26] (Figure 1). However, nitric oxide, hydrogen sulfide, and peroxynitrite play an important role as superoxide anion radical and single molecular oxygen redox metabolites. In the short term, it acts on the activation of pre-existing enzymes or ion channels, while in the longer-term (hours/day), it is mediated by the activation of gene transcription resulting in changes in enzyme patterns [23].

Oxidative Stress in Honey Bees

While there is a balance between ROS production and antioxidant process under normal conditions, exogenous stress factors (pesticides, heavy metals, biotic infections, etc.), can disrupt this balance and cause more than normal ROS production [27]. There are enzymatic and non-enzymatic defense systems to prevent damages caused by ROS [27, 28]. The most important antioxidant enzymes identified in honey bees include glutathione S-transferase (GST), glutathione peroxidase (GPX), catalase (CAT), and superoxide dismutase (SOD), while non-enzymatic components are glutathione, NAD(P)H, vitamins C and E, albumin, uric acid, and keratin [29-33]. GST is one of the important oxidative stress enzymes related to insecticide resistance. The damage of insecticides entered into the body on the redox balance, oxidative stress, as well as the production of lipid hyroperoxides, can be decreased by GST activities [34].

In *Apis mellifera*, vitellogenin and juvenile hormone are proteins involved in oxidative stress [35]. The life span and oxidative stress levels of bees depend on the levels of hexamerins (Hex), and vitellogenin (Vg), which are the main storage proteins [36,37]. Vg being the reproductive protein plays an important role as an antioxidant, which can explain the resilience in the aging of the worker bees and the queen. [35, 36, 38, 39].

In particular, the hemolymph titer of Vg is directly linked to the survival of acute oxidative stress [36]; the classical antioxidant defenses may be less important to explain the differences in life expectancy between honey bee casts [40]. In contrast, drones may be more susceptible to oxidative stress because they have much lower Vg levels and are haploid [41,42]. The correlation of resistance with the expression of Vg to

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oxidative stress in worker honey bees reveals that Vg can extend the lifespan of the queen and worker bees [43].

Pesticide induced oxidative stress in honey bees

The main causes of honey bee losses are pesticides, poor reared and feeding conditions, weather conditions, and bee diseases. Sudden and intense deaths in bees are usually seen due to poisoning. The cause of poisoning in bees is the agricultural drugs used in plant production as we call pesticides. The areas where plant production and pesticides are used are a common use for bees too. Beekeepers and farmers using pesticides do not have sufficient knowledge and awareness about the effects of pesticides on bees. Farmers using pesticides and beekeepers in the same region are not in contact with relevant public officials who have the necessary knowledge and experience. An inadequate control and legal practices, insufficient knowledge and guidance of the central beekeepers' association and beekeeper unions on pesticide use, the taking insufficient control of the current use of pesticides without considering their toxicity on bees by scientists and experts in universities and research institutes can be listed as the main poisoning reasons with pesticides [44].

In recent years, with the increase of insecticide use in agricultural areas, bees are exposed to these insecticides through direct contact or during the collection of contaminated pollen and nectar. Insecticides cause toxic effects both at the individual level and at the entire colony-level due to carry them to their colonies [31, 33, 45] (Figure 2). In particular, it is observed that the performance of bees is negatively affected, such as the decrease in the foraging instinct and success of bees, lethargy, dizziness, paralysis, abnormal bee behavior, loss of balance, uncontrolled movements, difficulty in finding the hive entrance, as well as incubation production and decrease in disease resistance. On the other hand, the presence of a large number of dead forager bees (500-1000 or more), in front of the hives within an hour (s), or day (s), as a result of the poisoning of the forager bees in the healthy and strong hives is one of the main negative effects of pesticide-induced bee poisoning [31, 46-48].

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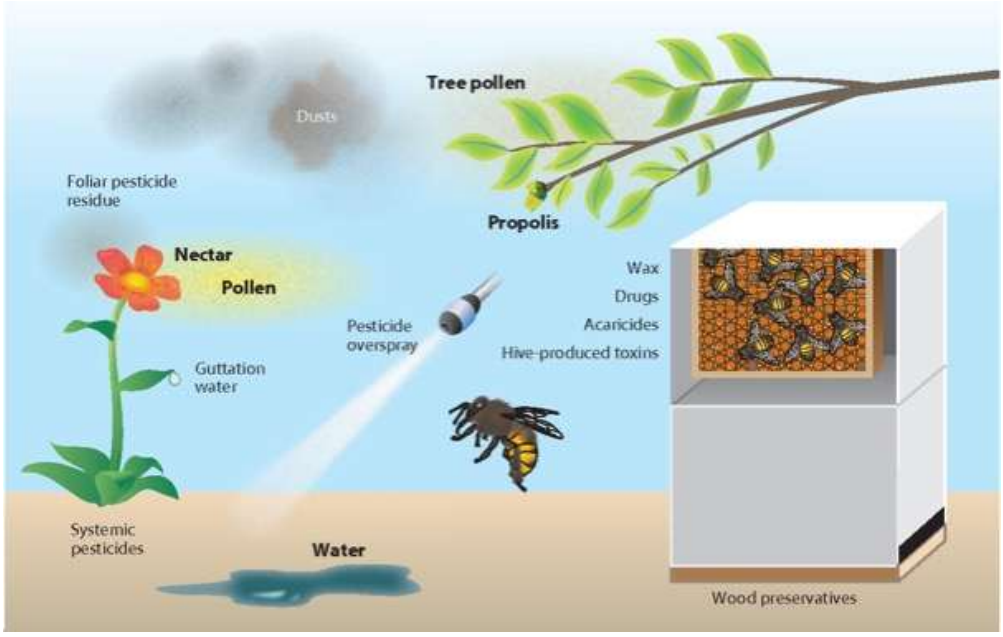

Figure 2. A summary of the different routes by which honey bees may be exposed to potentially toxic pesticides. Materials collected by foraging honey bees are in bold letters [49].

Pesticides are known to cause significant oxidative stress on all insects such as honey bees. Honey bees, on the other hand, can use various mechanisms as a defense against pesticides. A high level of antioxidant enzyme activity is an important marker for honey bees in pesticide-loaded environments [31]. In a study, in honey bees (*Apis dorsata* and *A. cerana*), exposed to pesticides (organophosphorus (OP), pesticide, a synthetic pyrethroid (SP)- cypermethrin and an organochlorinated pesticide (ES)-endosulfan), whether there was a change in SOD, CAT and xanthine oxidase (XOX), antioxidant levels were examined and as a result, it was found that the SOD and CAT levels in honey bee samples collected from regions with high pesticide density were higher than those with low pesticide density [31]. In another study, it was found that chlorpyrifosa, being one of pesticides, exposed by *Apis mellifera* increases lipid peroxidation known to cause oxidative stress/damage in the nervous system [50].

One of the most frequently used pesticides for insect control on plants in the world is imidacloprid, one of the neonicotinoid insecticides [33, 51]. Imidacloprid is a neurotoxin that acts as an antagonist of nicotinic acetylcholine receptors and causes paralysis and death. Old bees with higher antioxidant protection have been reported to be less susceptible to imidacloprid toxicity, so the toxic effect of the pesticide is dangerous in the early stages of honey bees' lives [52]. Nicodemo et al. [53] demonstrated that imidacloprid affects energy production by bees' mitochondria; besides, Nicodemo et al. [54] showed that imidacloprid reduced the level of lipoforin in hemolymph of honey bees. Balieira et al. [33] investigated the effects of imidacloprid on the cellular antioxidant system of honey bees and the potential antioxidant activity of caffeine. They found that imidacloprid increases the activity of GPX and CAT antioxidant enzymes in bee thorax as an indicator of oxidative stress induction. In addition, it is known that the use of caffeine, which affects the antioxidant systems and lifespan of worker bees, in bee nutrition increases the activity of GPX, CAT, SOD and GTS. Caffeine acting as an antioxidant has a preventive effect on the damage caused by insecticides [33]. As in Strachecka et al. [55], Balieira et al. [33] observed that caffeine promotes an increase in the activity of GPX and CAT enzymes. Besides, the addition of 2.0 ng/mL caffeine to imidacloprid has been found to reduce the formation of MDA, which shows antioxidant activity, although the insecticide only causes an increase in GPX activity.

The responses of acetylcholinesterase (AChE), carboxylesterases (CaEs-1-3), glutathione-S-transferase (GST), catalase (CAT), and alkaline phosphatase (ALP), were evaluated in bees exposed to insecticides such as deltamethrin, fipronil, and spinosad and it was determined that fipronil and spinosad induce CAT activity; deltametrine modulates CaE-1 and CaE-2 with opposite effects; spinosad exhibits an induction profile for most biomarkers other than AChE; fipronil does not modulate AChE, CaE-2, or GST and does not increase CAT and CaE-1, but it decreses ALP [56].

Acetylcholinesterase inhibitors, both organophosphate (OP), and methylcarbamate (MC), insecticides act on the nervous system of honey bees by

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inhibiting the activity of acetylcholine esterase (AChE), the enzyme that inactivates the neurotransmitter acetylcholine in central nervous synapses [57]. Both classes of AChE-inhibiting insecticides have an extremely broad toxicity to bees (topical LD50 = 0.018¬¬¬–31.2 µg/bee), [58, 59]. However, highly toxic OPs and subsets of MHs also pose a significant hazard to bees [60]. Coumaphos, a subset of OP, has such low acute toxicity (LD50 = 31.2 µg/bee), that it is used by beekeepers to control Varroa mites [61]. With repeated use, coumaphos reaches concentrations as high as 90 ppm in wax of colonies [62, 63]. The use of coumaphos in colonies is thought to cause increased larval mortality in both queens and workers [64, 65]. It has been determined that larvae reared with a diet containing 8 mg/L coumaphos have a significantly higher mortality rate during development than control larvae [66].

In another study, it was determined that exposure of honey bees to the herbicide atrazine, which is widely used in the laboratory and hive, leads to oxidative stress responses that can endanger the health of bee colonies; in addition, having a general decrease in antioxidant enzyme activities, and changing the relative expression levels of some antioxidant encoding genes after exposure to atrazine, differently were specified [67].

In a study on the development of acetylcholinesterase (AChE), carboxylesterases (CaE1, CaE2, CaE3), glutathione-S-transferase (GST), alkaline phosphatase (ALP), and catalase (CAT), as enzyme biomarkers of exposure to xenobiotics such as thiamethoxam in honey bees, it had been determined that exposure to thiamethoxam has non-lethal effects and alter the activity of CaEs, GST, CAT, and ALP (There was no response for AChE; however, an increase for GST, CAT, and CaE2 and a decrease in CaE1 and CaE3 had been observed. Besides, ALP and CaE3. showed opposite variations in 2.56 ng bee only), [68].

Malondialdehyde (MDA), is a general biomarker for measuring oxidative stress in honey bees. By Simone-Finstom et al. [69], between stationary colonies and migratory beekeeping was carried out among agricultural lands where the probability of exposure to insecticides is high, the life span and oxidative stress levels of honey bees were affected and

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MDA levels of honey bees were measured. It was observed that the level of oxidative damage was lower in adult worker bees reared in the migratory colony environment in the early period of the season and then placed in a stationary environment compared to the worker bees in the migratory colonies throughout their lifespans. While the MDA level increased throughout the season for bees in the stationary colonies, it was observed to remain at a constant level for bees in migratory colonies. The increased exposure of bees to pesticides in agricultural landscapes may explain why an increase in MDA levels.

Abdelkader et al. [70], studied the effects of insecticides on oxidative stress on the sperm of drones. It has been stated that clothianidin shows significant increases in SOD, GP, CAT, and MDA levels. Since the protein content in the sperm of drones exposed to clothianidin is significantly reduced, it has been thought that drones can cause oxidative stress in the spermatozoa, which can affect the semen quality and hence queen fertility.

Oxidative stress, the copes with it of the organism or time to cause its death is associated with how strong the immune system is. Pesticides also cause rapid damage on the honey bee immune system. The immune response can be divided into a humoral response and cellular response. The humoral response generates antimicrobial peptides (AMPs), through activation of the four immune pathways: Toll, immune deficiency pathway (IMD), c-Jun N-terminal kinase (JNK), and Janus kinase/signal transducers and activators of transcription (JAK/STAT). Sublethal pesticide exposure impairs the humoral immune response by reducing the production of AMPs. The cellular immune response is orchestrated through hemocyte function. Hemocytes can facilitate the melanization of pathogens and wounds through activation of prophenoloxidase (PPO), to phenoloxidase (PO), and reactive oxygen species (ROS), as a by-product. In addition, hemocytes can phagocytosis and clear invading pathogens, as well as differentiation into other immune cells. Multiple aspects of the cellular immune response are impaired by sublethal pesticide exposure [71] (Figure 3).

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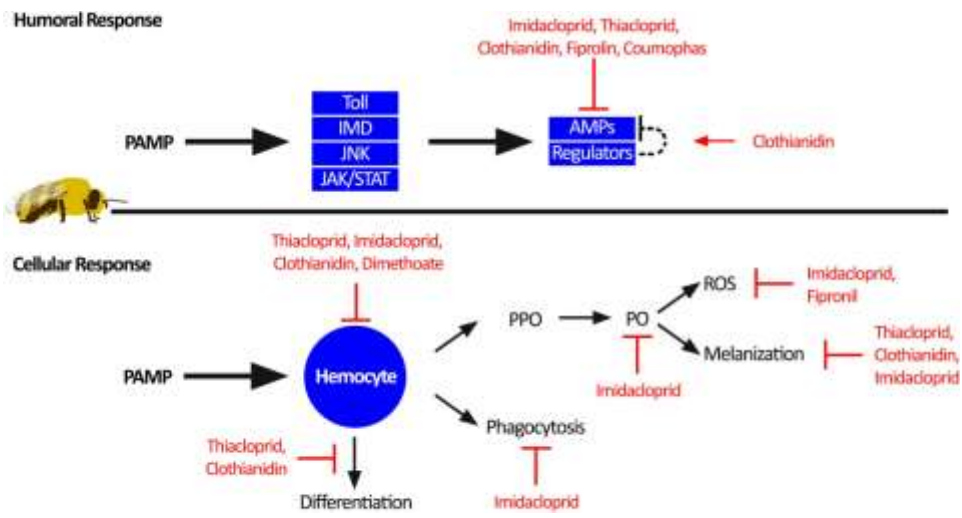

Figure 3. Humoral and cellular response of honey bee immune response against pesticide-associated molecular models (PAMPs), [71].

Considering the destructive effect of pesticides on bees, studies have been initiated on new pesticides and insecticides whose damages are reduced in order to cope up with these effects. Flupyradifurone is the active ingredient of Transform® in Sivanto ™ and sulfoxaflor. Both are relatively new insecticides that have been developed to reduce negative effects on bees when applied to plants. This study was conducted to better understand the potential non-lethal adverse effects of these pesticides on bees. In the experiment, the effects of two pesticides which were applied by a Potter Tower sprayer on nutrient consumption and oxidative stress levels in bees that were exposed to certain application dosages in the field were investigated. In both pesticide applications, a significant difference was observed between the treatment groups and control groups in the amount of sugar syrup and water consumption. The highest mortality rate was observed in bees exposed to Transform® followed by Sivanto ™ exposed bees. Estimates of reactive oxygen/nitrogen species showed significantly increased oxidative stress in both pesticide application groups compared to controls. In addition, caspase-3 protein tests, which are an indicator of the onset of apoptosis, were found to be significantly higher in pesticide administration groups [72].

Pathogen induced oxidative stress in honey bees

Many stressors such as poor nutrition, loss of natural habitat, pesticide exposure, pathogens (i.e., bacteria, fungi, protozoans, viruses), and parasites have caused the big concern for global bee decline and potential economic losses to insect-dependent agricultural crops. Especially pathogens caused colony losses to come in sight due to wrong beekeeping practices such as not doing the control and treatment of *Varroa*, regularly, having an insufficient knowledge on pathogens and their struggle methods, and not following the disinfectant rule in their colonies, etc. Thus, pathogens have been coming big concern on oxidative stress which has an effect on honey bees` lifespan.

The effect of fungus pathogens on oxidative stress

CAT, one of the antioxidant enzymes, is the primary defense against the overproduction of reactive oxygen species (ROS), which can occur after *Nosema ceranae* infection (the obligate intracellular fungi), which is located in the branch of microsporidia, and proliferates in the midgut epithelial cells of honey bees. However, ROS is non-specific and when not sufficiently reduced, it can react with biologically important macromolecules such as lipids, proteins, nucleic acids, and carbohydrates, which can damage the organism and eventually lead to cell death [73]. In the midgut, which is the first barrier to parasite development and oral pesticide exposure, high CAT activity is detected in the queen bee in the presence of *N. ceranae* infection combined with imidacloprid, while a trend towards a decrease in GST activity, a detoxifying enzyme among antioxidant enzymes. This decrease can negatively affect metabolic and detoxification functions. However, the loss of the colony resistance necessary for the survival of the colony under adverse conditions can be observed, as well as the reduction of the queen's lifespan, the reduction of the queen's labor production, and the death of the queen and the workers [74]. Taric et al. [75] measured high CAT activity in caretaker bees collected from commercial colonies with high *Nosema* load and

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Dussaubat et al. [74] had supported the results obtained.

Antúnez et al. [76] stated that consistency was observed between the decrease in Vg expression in worker bees infected with *N. ceranae* [73, 77] and the shortening of the life span of workers whose Vg expression was suppressed. Dussaubat et al. [74] observed that GST activity increased in worker bees with *N. ceranae* infection to tackle with oxidative stress. It has been determined that the titers and antioxidant capacity of Vg, an egg yolk protein that can reduce oxidative stress, increase in infected queen bees.

In a recent study on the differences of commercial and traditional colonies in the parameters of oxidative stress and the prevalence of *N. ceranae*, higher *N. ceranae* prevalence in commercial colonies could be the reflection of observation significantly higher activity of CAT which has an important protective role in insects having high intestinal parasites [76]. At the same time, significantly higher activity of GST was observed in commercial colonies probably due to higher pathogen prevalence measured in the study of Vidau et al. [78].

Another fungus pathogen is *Ascosphaera apis*, an obligate fungal pathogen of honey bee brood and causes chalkbrood disease in honey bee larvae. *A. apis* infection may cause oxidative stress on honey bees larvae [79]. The upregulation of cellular defenses in resisting oxidative damage in honey bees can be provided by the increase in CAT activities as in the other insects such as *Drosophila melanogaster*. Besides the increase in SOD enzymatic activity play an important role to promote oxidative stress resistance [80]. In relation to these antioxidant enzymes activities, decreased CAT, GST, and SOD enzymatic activity in the guts of infected larvae with *A. apis* had been observed significantly by Li et al. [81] compared within the guts of control larvae not infected with *A. apis*.

The effect of Varroa on oxidative Stress

Lipiński and Żółtowska [82] found that antioxidant enzymes SOD, GPX, and ceruloplasmin (CP), were 4 times higher in activity than non-infected prepupae in their study on oxidative stress in drone prepupae infected with the parasitic mite *Varroa*. The other research supported the similar result that SOD and Catalase activities in *Varroa* infested worker pupae

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were almost two times higher than non-infested worker pupae [83]. Gülmez et al. [84] observed that the SOD activity of bees infected with *Varroa* was higher than the non-infected bees. This increase in enzyme activity indicates that superoxidase radicals, which are formed as a result of *Varroa* invasion, activate the host defense mechanism.

The effect of viral diseases on oxidative stress

Łopieńska-Biernat et al. [85] observed that SOD activity was significantly lower in the honey bee group infested with *Varroa destructor* than that infected with Deformed Wing Virus (DWV). It was found that CAT activity was higher in the group infested with *V. destructor* and in the group infected with *N. ceranae* and lower in the group infected with DWV compared to the control group (non-infected group). In the group infected by both *V. destructor* and DWV at the same time, it was observed that CAT activity was lower compared to the groups infected with only one pathogen. GST activity was found to be higher in groups infected with *V. destructor, N. ceranae* and/or DWV compared to healthy bees.

The synergistic effect of pesticides and pathogens on oxidative stress

The combination of pesticides and parasites may cause strong colony losses. Firstly, Ladas [86] have been explained the possible interaction between *Nosema* and pesticides [78]. Alaux et al. showed the synergistic effect of *Nosema* and imidacloprid on the mortality of honey bees; however, any strong connection with the insect detoxification system was observed if did decrease or not [78, 87]. Though, low doses of imidacloprid, one of the common neonicotinoid pesticides, and *Nosema ceranae* infection alone or combined with pesticides caused the increased activity of GST and CAT in the head related to protective response to oxidative stress of honey bees [74]. In addition, the survivorship of queens and worker bees is strongly in dangerous due to exposure of the combination of pesticides and parasites [74]. Another study showed a similiar result that the combination of *N. ceranae* parasite with insecticide fibronil caused a disturbance in the production of ROS and increased oxidative stress; besides, in this combination, the parasite may trigger the increasing of fibronil toxicity on honey

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bees [88].

Several chemical substances as acaricides to eliminate negative impacts on hives by *Varroa* mites also have an impact on the oxidative stress on honey bees. The potential effects of two potent acaricides fluvalinate and oxalic acid on oxidative stress were tested by Rouibi et al. [89] on the adult stages of honey bees to determine the detoxification system for GST and AChE (Acetylcholinesterase). Since GST activity increased in emerged and nurse bees, AChE activity decreased for fluvalinate uses compared with the control group (untreated colonies with fluvalinate). However, in emerged and nurse bees, GST and AChE activity did not show significant differences for oxalic acid uses compared with the control group (untreated colonies with oxalic acid), [89]. A similar effect was reported on increase GST activity in emerged and nurse bees by Loucif-Ayad et al. for treatment with flumethrin and amitraz and by Nielson et al. for treatment with flumethrin [90, 91]. In the recent study, the effects of coumaphos that is the other most commonly used acaracide against *Varroa* mites in parameters of oxidative stress (CAT, SOD, and GST activities), were observed. Since normally in non-infested bees before coumaphos treatment, SOD activity was decreased, SOD activities significantly increased in non-infested untreated bees ($p<0.05$), and infested treated bees ($p<0.0001$), after coumaphos treatment (day 42). On the contrary, in all groups (non-infested untreated bees, non-infested treated bees, and infested untreated bees), except the infested treated bees group (where it declined), after treatment with coumaphos, both CAT and GST activities significantly increased (from $p<0.05$ to $p<0.0001$). Having a high infestation group showed efficacy against *Varroa* mites thanks to using coumaphos that is the reason why treatment decreased oxidative stress to contribute to increasing the colony health [92]. Moreover, a synergy between acaricides (coumaphos), and insecticides (imidacloprid), was demostrated that a mixture of coumaphos and imidacloprid were downregulation of CAT activities as well as inducing higher bee mortality [93].

Conclusion

Oxidative stress is manifested by an imbalance between the production of reactive oxygen species (ROS), and cellular antioxidant defense systems. Free

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oxygen radicals, which are synthesized in small amounts in the organism during normal metabolism and do not harm the organism is overproduced and causes oxidative stress due to some cases such as climate change, habitat loss, environmental stress factors, insecticide use, exposure to parasites and pathogens, exposure to ionizing radiation, and environmental pollution.

In order to prevent pesticide-related deaths in honey bees, it is necessary to gather experts, gather reliable information, scan the literature, examine the crime scene, take the necessary and correct samples, if possible, determine the target pesticides well, send samples to the correct laboratory. In addition, it is an indispensable requirement to have a laboratory that specializes in this field and can follow up-to-date innovations, and have experts working in these laboratories. However, due to the lack of laboratories in Turkey to analyze each group of pesticides and especially clothianidin, imidacloprid, and thiamethoxam from the neonicotinoid group and agricultural products, the analysis in Turkey has not yet been clarified. In addition, in preventing the toxic effects of insecticides that cause oxidative stress, the inclusion of caffeine, which acts as an antioxidant, in the diet which is provided for bees, especially when hives are installed near crops that are attractive to bees, can be an important strategy and can be applied by beekeepers.

Zinc (Zn), the most important ingredient of the antioxidant enzyme Cu/Zn-SOD, may have an important effect on the increase of the concentration of royal jelly thanks to providing the increase of Zn content especially in the hemolymphs of nurse bees. By given it in honey bees` diets, the negative effect of oxidative stress caused by pathogens on the lifespan of bees can be reduced. For this purpose, it is recommended to supplement 30 mg kg-1 Zn in 50% sugar syrup and/or 60 to 75 mg kg-1 Zn into the pollen diet [94].

In national beekeeping, commercial beekeepers tend to transport their honey bee colonies among regional for many months of the year. With this migratory beekeeping, honey bee colonies among agricultural, especially monocultural, landscapes may increase to impose the potential of exposure of colonies with pesticides and pathogens due to high interaction; thus, those reared in a

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commercial operation of migratory beekeeping by increasing oxidative stress on honey bees significantly cause the reduction of lifespans of bees.

Bal Arılarında (*Apis mellifera* L.), Pestisit ve Patojen Kaynaklı Oksidatif Stres

Öz: Bal arıları, yabani çiçek ve mahsullerin tozlaşmasını sağlayarak insanlar için önemli bir kültürel ve ekonomik fayda sağlar. Bal arıları, diğer organizmalar gibi, yaşamları boyunca çok çeşitli çevresel stres faktörleri ile karşı karşıya kalmaktadır. Bu stres faktörleri organizmanın fizyolojik dengesini bozar. Organizmaya alınan oksijenin kullanımı ve metabolizması sırasında serbest radikaller olarak bilinen agresif moleküller oluşur ve organizma bu radikalleri kontrol altında tutamaz ve oksidatif stres oluşur. Böyle bir durumda, serbest radikaller sağlıklı hücrelere saldırır, okside eder ve bozarlar. Bu bozulma, reaktif oksijen türlerinin (ROS), atık sistemlerinin aynı anda bozulması ile arttırılmış üretimi ile karakterize edilir. Bal arılarında oksidatif stres eşiğinin aşılması, bireysel ya da koloni bazında arı kayıplarına neden olmaktadır. Her geçen gün çevresel faktörlere bağlı olarak, artarak oluşan koloni kayıpları oksidatif stresin oluşumu, fizyoloisi ve önlenmesi üzerine yapılan çalışmaların önemini gözler önüne sermektedir. Bal arılarında tanımlanan en önemli antioksidan enzimler glutatyon S-transferaz (GST), glutatyon peroksidaz (GPX), katalaz (CAT), ve süperoksit dismutaz (SOD)'dır. Bu derleme oksidatif stresin bal arılarındaki mekanizmasını ve pestisit ve patojenlerin bal arılarında oksidatif stres üzerindeki etkilerini incelemektedir.

Anahtar kelimeler: Bal arısı, *Apis mellifera* L., oksidatif stres, reaktif oksijen türleri (ROS), pestisit, patojen

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MELLIFERA RESEARCH ARTICLE

SW Nigeria Experience of Impacts of Agricultural Intensification and Climate Change on *Apis mellifera adansonii* Colony Establishment and Health

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A B S T R A C T

Declines of wild bees together with unsustainably high losses of managed colonies and worsening bee health have become global issues. Southwest Nigeria is a tropical rainforest biome. It is one of the most biologically diverse ecosystems, with the changing agricultural development and climate overwhelmingly impacting it. The impact is also significant on beekeeping vis: colony establishment, health and productivity of the native bees the West African honeybees, *Apis mellifera adansonii* Latreille (Hymenoptera: Apidae). This bee was once described as strongly adaptive to the tropical rainforest, productive, hygienic and immuned to pathogenic infections. This study was carried out between December, 2015 to December, 2018 to determine the stress factors associated with colony establishment, health and productivity of the bee colonies. Four states were purposively selected in the Southwest Nigeria. Some beekeepers were selected, sampling and colony observations were made in selected apiaries and laboratory investigations were conducted. Results indicated decline in colony numbers and honey production from 2016 to 2018. Out of 96 inspected colonies, 16 (16.67%) colonies have become weakened or lost due to bee pests and diseases this is greater than losses recorded due to other factors. Similarly, infestation with small hive beetles (SHB) across the region is 82(85.43 ± 0.01%) greater than 67(69.93 ± 2.08) (Mean ± SD) recorded for Galleria mellonella infestation. SHB infestation were significantly different across the states ($P = 0.005$, $p < 0.05$). The mean levels of Gluthathion-S-Transferase (GST) detoxifier chemical signal in the tissues of bees tested in the colonies for the three years were higher than the normal value for bees. The climate change, and the adaptation policy and development such as agricultural intensification programme adopted is a relevant and sustainable mitigation tool but with a pervasive influence on beekeeping, honeybee health, population and productivity.

Keywords: Colony establishment, decline, productivity, pests, climate change, forest

Introduction

The tropical rainforest has been described as the most biologically diverse ecosystem [1]. This biome covers the geographical zone of SW Nigeria that is predominantly modern beekeepers' enclave in Nigeria. The beekeepers use modern hives often than the traditional hives. However, knowledge and practice of modern beekeeping is deficient among the beekeepers [2, 3], honey and beeswax processing remained traditional as generally in the tropics [2, 4]. The bees

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respond to changes in human apicultural practices, environment [5], ecological and climatic factors [6] in Nigeria [7]. The declined populations of honeybees and honey production recorded in many countries are of widespread concern [8, 9], no single factor has been found to account for the incidence, but pesticides and pathogens are known to play important roles [10, 11]. The modern beekeeping methods were supposed to improve colony establishment, sustenance, and honey production because colonies could be managed and manipulated, hives could be opened and examined without undue disturbance; this best described the concept 'modern beekeeping' [8, 9]. But contrary reports from USDA-ARS [12], Watanabe [13], Johansen and Mayer [14] claimed bee colonies were continuously weakened to the point where they succumb to pests and diseases that would otherwise have only minor impacts on their health. The beekeeping practices that agitated the bees are regular colony inspection (without any disturbance), artificial feeding, queen rearing, colony division/splitting, manipulation of colony for pollination, chemotherapy and other treatment of bee diseases, honey harvesting, and some changes in agricultural practices [15]. Although, some of these activities are practice in the tropics despite the wide acceptance of modern beekeeping [16]. Again, climate change and human activities have greatly influenced beekeeping, climate change had resulted in declining floral development, nectar and pollen production affecting colony foraging and development [17]; altering the quality and quantity of the nutrients for honey bees [18]; influencing the honeybee development cycle [5, 19]; the frequency of occurrence and diversity of pests and parasites of the bees [7]; development of migration strategy to escape predation and starvation [5] and; later the same colony returns to recolonize same hive [20, 21]. Similarly, human activities had greatly disturbed the ecosystem. Agricultural practises have resulted in clearing of forest resources for crop production and logging of woods for construction [22]. Dry season bush burning [23] to clear land for farming and cattle grazing had contributed to decline of natural forest and considerably reducing the wild bee population. Agricultural intensification to boost cash and food crop productivity with increasing application of chemical to control insect pests had resulted in poisoning of honeybees [24] and decline of swarms. Similarly, continuous exposure of honeybee to agrochemical applications

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might induce physiological impairment that could affect the bees' health [25], immunity against infections and detoxification of harmful substances they inject [26]. Acetylcholinesterase (AChE) and GSTs are among the enzymes the bees use as biomarkers of chemical toxicity in the environment [27]. GSTs are members of a significant intracellular and multifunctional antioxidant enzyme superfamily that detoxify and protect against oxidative damage caused by reactive oxygen species [28] and catalyse nucleophilic attack in order to bring about detoxification of xenobiotics. Similarly, acetylcholinesterase represents a biomarker of neurotoxicity to chemicals such as pyrethroids [29, 30] organophosphates and carbamate insecticides [28, 31, 32]. Honey bees use these active detoxifying enzyme systems for eliminating harmful substances they come in contact [17, 33, 34]. There is a need to mitigate the effect of xenobiotic exposure on honey bee health and productivity with activities of beekeepers, growers, manufacturers and regulators of agrochemical. In view of multiple factors of environmental conditioning risks, beekeeping management practices, agricultural development, climate change and anthropogenic factors; colonies are continuously exposed to a broad spectrum and highly pathogenic pests, parasites and pathogens that were initially taken as insignificant or non-native to the local bee (Apis mellifera adansonii Latrielle). In Kwara State, Nigeria, pest insurgence had resulted in 15% decline in colony establishment in some Local Government areas [35]. The incidence of Varroa destructor (Acari: Varroidae), 'Korean hypotype' reported by Akinwande et al. [36] in South West Nigeria, recorded an average mite load of 0.01 to 0.055 mites/adult bee. Although, there was no established link between regular complaints of decline in colony establishment in the area and mite festations. The mites feed on bee haemolymph [37, 38] and fat body [39], vector numerous viral pathogens between individual bees and colonies. Although, Shen et al. [40] adjudged Korean haplotype Varroa mites were virulent mostly to the native host, the Eastern honeybee. This race lacks the natural defence mechanism and the mite is capable of wiping off the entire colony within few years of infestation [41, 42]. Wax moths: the greater wax moth *Galleria mellonella* L. and smaller wax moth *Achroia grisella* F. (Lepidoptera: Pyralidae) have been identified as common natural enemies that

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enter the bees' nests in South West Nigeria [7, 35, 43]. Although, these pests, according to May [44] may not affect a strong colony but a weak one that cannot protect its comb, they become susceptible and collapse or abscond. Other terrestrial enemies associated with the honey bees in the tropics are ants (Hymenoptera: Formicidae) and termites (Blattodea: Termitoidea [7, 45]. Harvester ants *Pheidole barbata* and termites *Macrotermes nigeriense* were identified in some colonies in SW Nigeria [7]. Again, the small hive beetle (SHB), *Aethina tumida* Murray (Coleoptera: Nitidulidae), is a pest [46, 47]; and kleptoparasite [48] of bee colonies, it transmits pathogenic viruses [49]. SHBs are mostly recorded in the forest region in SW Nigeria [50] while large African hive beetles *Oplostomus haroldi* and *Oplostomus fuligineus* (Coleoptera, Scarabideae, Cetoniinae) are associated with the savannah in northern Nigeria [51]. Various economic losses have been incurred by beekeepers in Nigeria due to infestation of SHB, these include significantly reduction in colony establishment and productivity [36], and possibly colony collapse [50]. Hence, the objectives of this study are to provide a baseline information on colony loss which is lacking in Nigeria and to determine the stress factors such as climate change, agricultural intensification and detrimental beekeeping practices (anthropogenic factors) associated with the colony loss, health and productivity of the native West African honey bee colonies *Apis mellifera adansonii.*

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Materials and Methods

Study Site

The study area covered the Southwest geographical zone of Nigeria which consists of Lagos, Ogun, Oyo, Osun, Ondo and Ekiti states (Figure 1). The region lies between longitude 2°311 and 6°001 East and Latitude 6°211 and 8° 371 North [52].

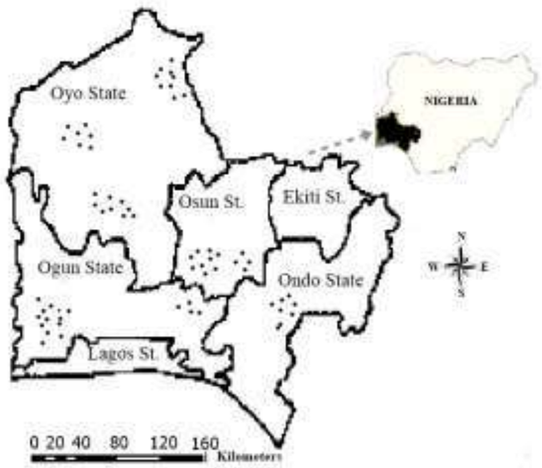

Figure 1. Map of Southwest Nigeria showing areas of study

It is a tropical rain forest biome. Expected temperature throughout the year ranges from 29 ± 5Oº C, relatively high humidity of 70 - 85% and rainfall varies from 2,000 mm [53]. This study was carried out between December, 2015 to December, 2018 to determine the stress factors associated with *Apis m. adansonii* establishment, health and productivity of their colonies. 4 states were purposively selected in the region because of accessibility to information from the large number of beekeepers in the states.

Sample Collection

Sampling and sample collections were carried out in 48 apiaries (n =12/state) randomly selected among the apiaries owned by 179 beekeepers that responded to our requests. In each apiary, 2 framedbar colonies were randomly inspected (n = 96 colonies (24 colonies/state) and sample collections were carried out. Honeybee and brood samples were collected from each colony. The bees were shaken into zip lock bag and sealed, while about 5 x 10 cm pieces of brood combs were cut neatly with knife and wrapped in an absorbent tissue paper. The sampled combs were ensured not containing honey and not wrapped in airtight containers/plastics to prevent condensation which might cause fungi and moulds to grow, thereby, making it difficult to identify the bee pathogens. The knife used was washed thoroughly with water and detergent before reused to avoid cross infestation.Also, each sample in zip lock bag from colony was labelled and immediately placed in ice cubes to inactivate the bees and also preserves the pathogens. The samples collected were taken to the laboratory for analysis.

Colony Observation

Visits and observations were made to therandomly selected colonies early in the morning between the hours of 9:00 am and 12 noon and twice per season in the year (wet and dry seasons) for the three years of study. The colonies selected were those in standard framed bar hives with good top covers, placed in protected shade where rainfall impact is minimal and the quadruped stands supporting the hives immersed in 4 containers of used oil to protect the colonies from predators like lizards and geckos climbing the hives to feed on live and dead bees:

i.Presence of large clusters of dead bees

Observations of large clusters of dead bees

[54] in the hive hollows, in and around the hive entrances and in the hive surroundings within 5 metres radius were conducted in the selected colonies (classified as strong or weak colonies) in the apiaries. Regular observation (once per month) and feedback were sought from the (owner) beekeepers while the researcher visited and conduct similar observation twice per season in the year. Inquiries (through the questionnaire administered) were made of cases of application of agrochemicals in the cultivated land within or nearby the apiary before or during the planting season. The presence of large cluster of dead bees (>1000) was used to confirm pesticide poisoning according to Akratanakul [54].

ii. Presence of pests and parasites

The colonies were opened, pests and parasites present in the hives were collected, identified and recorded, while the bee samples were examined for ectoparasites and pathogens. The hive surroundings were also examined for the presence of pests and predators. This exercise was carried out twice per season in the year (wet and dry seasons) for the 3-year period of study.

Beekeepers' Activities, Colony Establishment and Productivity

Random cluster sampling procedure was used in selecting the professional beekeepers and farmers keeping bees for the study. Each state selected for the study has clusters of local associations of beekeepers and farmers keeping bees (Figure 1). Multiple choice survey questionnaires were administered during the state and cluster groups' meetings, to

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focus group and on social media (WhatsApp) platform. Extensive interviews, contact discussion and seminars were organised. 220 (n =55/state) questionnaires were administered out of which 199 respondents were returned, information received from 179 (81.36%) of the respondents were treated on the following semantic areas: Ecological problems of beekeeping, management practices, pest and diseases management, pesticide poisoning, colony behaviour, colony number and loss, honey and wax production, brood rearing, brood pattern and harvesting. The questionnaire surveys were repeatedly conducted twice annually for the 3-year period (2016 -2018) on the same respondents and subject, to update the information on the semantic areas. The information collected were reviewed and compared to justify the reliability of the instrument used.

Laboratory Analyses of pathogens

i. Microbial test

Bacteriological and mycological examinations of brood comb/bee samples collected from the apiaries were carried out on the same day in the Federal University of Technology, Akure (FUTA) Laboratory. 1.0 gram of the brood/bee samples collected were crushed and sample extracts were made in 10ml sterile distilled water, centrifuged and the extracts obtained were serially diluted in ten tubes. 1ml of aliquot of dilution factors 10-2, 10-4 and 10-6 each were inoculated into molten potato dextrose agar (PDA) containing tetracycline (inhibit bacteria growth), while nutrient agar (NA) and De Ma Ro (MRS) agar were inoculated for bacteria growth. PDA plates were incubated at 30oC for 3 days while NA and MRS were incubated at 37^{o}C for 3 days. The plates were prepared in duplicates and were examined daily for growth. These media were prepared following the manufacturer instructions. Each different colony was subcultured to obtain pure culture and was identified using morphological and biochemical methods as described by Idowu et al. [55]. Colony forming unit (CFU) growth on PDA were counted, sub-cultured on new PDA using streak plate method and identified using staining techniques (Gram's staining techniques) and biochemical tests methods [56]. The cultural characteristics of the isolates were done based on colour, shape, pigmentation and opacity of the colonies. The examination helped to detect the presence of bacteria and not to identify the type.

ii. Viral test

For the viral analysis, bee samples collected in the selected colonies in all the 48 apiaries visited were labeled accordingly. The bees were crashed while still alive into falcon tubes containing about 20 ml RNA-later. The samples were labelled showing date and place of collection. RNA-later was prepared by: 935 ml of autoclaved, MilliQ water; 700 g Ammonium Sulfate; Stir until dissolved; 25 ml of 1 M Sodium Citrate added; 40 ml of 0.5 M EDTA added; adjusted to pH 5.2 using concentrated H2SO4 (about 20 drops = 1 ml); They were stored at room temperature before the samples were sent to the Microbiology Department, FUTA for analyses to detect the presence of viral pathogens.

Tissue homogenate for biochemical analyses

250mg of freshly collected honey bees per sample were weighed with a scale (JS600H-A & GULF) from each sampling bags and placed in a clean thoroughly washed mortar and pestle. The weighed samples were homogenized in 900µl of phosphate buffer (pH 6.5), and centrifuged at 1000rpm for 10 mins with a centrifuge (MSE-MINOR35). All cellular debris were discarded while the supernatants obtained were kept in a refrigerator at 4^{o}C.

Test on levels of detoxification enzymes (Glutathione-S-transferases)

Glutathione-S-transferases (GSTs) levels were estimated using CDNB (1-chloro-2, 4-dinitrobenzene) as substrate [57] in a reaction mixture containing 100 µl of 25mM of (1-chloro-2, 4-dinitrobenzene), 150µl of 20mM reduced glutathione, 500µl of 40mM Phosphate buffer (pH6.5 and 30µl of enzyme). These mixtures were incubated at 20^{o}C for 3 mins and the absorbance was recorded after 3mins at 340nm using UV Visible Spectrophotometer (Jenway 6850). The level of GSTs was reported in (µmol/ml/min). The experiments were replicated three times for each sample. Freshly colonized and healthy colonies from the University Research Farm were used as control against the colonies sampled from the beekeepers.

Statistical Analysis

Differential and inferential statistics were used to process the data. Descriptive analysis was used to process information obtained from questionnaire, factors responsible for decline, and pest/parasite/disease infestation/infections in order to make inference on their impacts while inferential statistics of one-way analysis of variance (ANOVA) were used to establish relationship between GSTs data, percentage infestation, colony and honey production decline across the states and where significant differences existed, the means were compared at $P < 0.05$ significant level established using the New Duncan's Multiple Range Test.

Results and Discussion

Colony Loss and Honey Productivity Decline

Three years (2016-2018) collated information from responses to questionnaires obtained from 160 beekeepers (n = 40/state) selected out of 179 consistent respondents revealed the following: In 2018, the total number of hives owned by the selected beekeepers were 9,371 and number with established colonies inside were 4,513 (48.16%) (Table 1). Annual loss in the region increased from 36.22 ± 6.73% ; 43.32 ± 9.60%; 49.44 ± 8.42% (Mean ± SD) in 2016, 2017, 2018 respectively (Table 1). Colony establishment declined over the years. The decline was significantly different between and within the states in the region ($F_{2,8}$ = 7.012, p= 0.015 ($p < 0.05$).

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Table 1. Colony establishment and honey yield

	2016			2017			2018			Cumulative (2016 - 2018)	
State	No of hives	Established colonies No.(%)	Estd honey yield (Lt/ colony/year)	No. of hives	Established colonies (%)	Estd honey yield (Lt/ colony/year)	No of hives	Established colonies (%)	Estd honey yield (Lt/ colony/yr)	colony loss (%)	decline in honey yield /colony
Osun	684	443 (64.76)	860 (1.94)	897	564 (62.87)	788 (1.39)	1440	802 (55.69)	860(1.07)	35.24; 36.31; 40.12	1.94; 1.39; 1.07
Ondo	712	507 (71.20)	712 (1.40)	1001	571 (57.06)	809 (1.42)	2001	1081 (54.02)	1448(1.34)	28.80; 42.94; 45.98	1.40; 1.42; 1.34
Ogun	908	584 (64.31)	922 (1.57)	1540	970 (62.98)	1314(1.35)	3090	1490 (48.22)	1622(1.09)	35.69; 37.02; 51.78	1.57; 1.35; 1.09
Oyo	1108	698 (54.87)	1229 (1.76)	1689	726 (42.98)	1214(1.67)	2840	1140 (40.14)	1509(1.32)	45.13; 57.02; 59.86	1.76; 1.67; 1.32
Total	3412	2232 (65.41)	3723 (1.67)	5127	2831(55.31)	4125(1.46)	9371	4513 (48.16)	5439(1.21)	36.22; 43.32; 49.44	1.67; 1.46; 1.21

Note : Hives are colonized/colony established by swarms. Number of beekeepers sampled (n = 40/state).

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Similarly, the region experienced an average decline in honey production by 1.66 ± 0.23; 1.46 ± 0.144; 1.21 ± 0.144 kg/colony/year (Mean ± SD) in 2016, 2017, 2018 respectively (Table 1).This decline was not significantly different ($F_{2,8}$ = 3.336, p= 0.082 ($p < 0.05$). There was a weak positive correlation (r = 0.047) between the percentage of colony established and honey yield/colony in all the states (Figure 2).

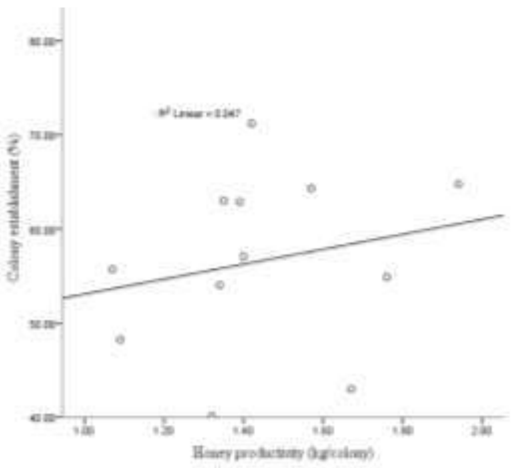

Figure 2 . Correlation between colony loss (%) and decline in honey productivity (kg/colony)

Climate and Agricultural Development Factors

Information obtained on climate change from Nigeria Meteorological Centre (NIMET) and observation reports by the beekeepers during the period of the study included the following: dryness, heat waves and bush fires following the heat waves, late and heavy rainfall, preceded with unusual flooding. These changes impacted beekeeping activities with loss of some plants identified as foraging plants and high infestation levels of pests/parasites/diseases pathogens. Again, during the period, climate change adaptation facilitated modification and intensification of agriculture as different agricultural programmes aimed at improving food production and rural development were embarked upon. Loans were provided exclusively for crop farming with many open lands cultivated. Therefore, 128 (71.5%) out of 179 beekeepers were engaged in agricultural programme of cassava and maize planting that linked agricultural intensification to extensive cultivation of natural wild forest. Decline in number and percentages of colony loss out of 4,513 established colonies of the beekeepers were 25 (0.55%) due to clearing of vegetation; 108 (2.4 %) due to land disputes and 65 (1.45%) damaged by cattle herds, as a result of herdsmen migration to south west in search of greener pasture from drought ravaged savannah north.

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Anthropogenic and Influence of Local Beekeeping Practices

The study revealed the following colony loss due to some human activities reported by the selected beekeepers. Out of total of 4,513 established colonies recorded by the beekeepers, 90 (2.0%) and 29 (0.64%) were lost to theft and damaged through land dispute respectively and 71(1.58%) colonies were lost to poor management. Some beekeeping practices were linked to colony loss and production decline such include traditional placing of hives under shade to reduce the bee aggressiveness and baiting with honey to attract swarms, both practices were observed to attract pests and pathogens, contributing to high infestation and infection levels respectively. Hence, the inspection conducted by the researchers on 96 colonies revealed 16 (16.67%) have become very weak, almost or been lost due to pests and pathogens. Other bad beekeeping practices include: harvesting of all the honey combs and removing the brood and pollen combs, cutting off any part of the brood comb with little store of honey and sharing of equipment were indicted to have negatively affected the established colonies.

Agrochemicals in Use and GST Activation Levels of Bees in Selected Colonies

Beekeepers admitted intentional use of agrochemicals in the surrounding farmland. Agrochemicals indicted include endosulfan (24%), methyl parathion (21%), mevinphos (20%), trithion (16%) and tedion/tetradifon (12%). 7(8.5%) out of 96 colonies observed by the researchers had large clusters of dead bees in the hive hollows, around the entrances and within 5m radius. These colonies were very weak colonies and few later absconded. The mean activation levels of GST in all the tested bee samples in the 96 colonies within and across the 4 states (Figure 3) in 2016 range from 0.074 - 0.087 μmol/min/ml, the activation levels were not significantly different ($F_{3,11} = 1.168$, Sig = 0.363, $p > 0.05$) (Table 3), in 2017, the GSTs activation levels range from 0.082 - 0.094 and also were not significant different in all the colonies within and across the states in the region ($F_{3,11} = 0.201$, Sig = 0.894, $p > 0.05$). Similarly, in 2018, GSTs activation levels range from 0.082 - 0.094 and they were also not significant different ($F_{3,11} = 0.267$, Sig = 0.848, $p > 0.05$). There was a significant difference in rise of GST activation levels ($P < 0.05$) annually in tissues of tested

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bees in colonies within and across the four states (Figure 3). These results indicated the formation and build-up of glutathione conjugate in the bees.

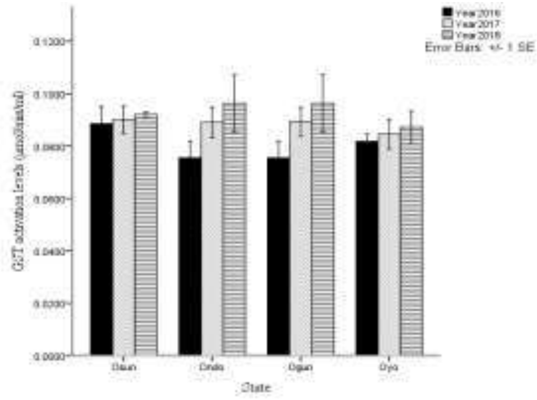

Figure 3. GST levels of sampled colonies from 2016 -2018.

Table 3. Mean GST activation levels for all sampled bees across all the colonies .

Period	Mean GST levels	*S.E*	*F*	*Sig*
Year 2016	.080	.003	1.168	.363
Year 2017	.088	.003	.201	.894
Year 2018	.093	.004	.267	.848

Pests and Disease Pathogens

There were persistent problems of insect pests, predators and parasites in the 96 colonies inspected during the period in all the states (Table 2). 16 (16.67%) colonies out of 96 inspected have become very weak, almost or been lost to pests and diseases. The percentage colonyinfestation for the following insect pestswere recorded: ants (Companotus pennsylvanicus) 62.50 ± 6.25%, greaterwax moth (GWM) (Galleria mellonella) 69.93 ± 2.08%, lesser wax moth (LWM), (Achroia grisella) 61.45 ± 1.82%, spider (Lactrodectus mactan) 34.50 ± 1.47%, termite (Macrotermes militaris) 62.50 ±1.63%, crickets and wasp (Polistes fuscatus) 62.50 ± 1.34%, large hive beetle(LHB) (Hoplostomus fuligenius) 45.98 ± 3.12%, and small hive beetle (SHB) (Aethina tumida) 85.43 ± 0.01% (Table 2). SHB infestation is highly prevalent (χ = 14.15, p = 0.001 (p>0.05) when compared to others (Figure 4). Also, the beekeepers claimed the presence of vertebrates' pests that included rodents, reptiles (e.g. lizards (Agama agama), and amphibians (e.g. toad (Bufo regularis), some birds e.g. woodpeckers and mammals moving around the colonies.

Table 2. Average number and percentage of colonies infested and infected for 3years in each state

States	**Oyo**	**Ogun**	**Osun**	**Ondo**	**Total**
No. colonies inspected	24	24	24	24	96
Pests	Average No. (%) of colonies infested for 3years				Total No. (%)
Ants	13(54.2)	15(62.5)	18(75.0)	14(58.3)	60 (62.5 ± 6.25)
SHB	20(83.3)	16(66.7)	24(100)	22(91.67)	82 (85.43 ± 0.01)
LHB	10(41.7)	12(50.0)	13(54.7)	9(37.5)	43 (45.98 ± 3.12)
GWN	15(62.5)	15(62.5)	19(79.7)	18(75.0)	67 (69.93 ± 2.08)
LWN	14(58.3)	15(62.5)	14(58.3)	16(66.7)	59 (61.45 ± 1.82)
Termites	16(66.7)	18(75.0)	12(50)	14(58.3)	61 (62.50 ± 1.63)
Spiders	7(29.7)	8(33.3)	9(37.5)	9(37.5)	33 (34.50 ± 1.47)
Crickets/Wasps	12(50)	14(58.3)	16(66.7)	18(75.0)	60 (62.50 ± 1.34)
Pathogens	Average No. (%) of colonies infected for 3years				Av/ Total No. (%)
Fungi	19(79.17)	16(66.67)	20(83.33)	18(75.0)	73 (76.04 ± 5.21)
Bacteria	21(87.5)	23(95.8)	22(91.67)	23(95.8)	89 (92.69 ± 0.01)
Virus	23(95.83)	24(100)	22(91.67)	22(91.67)	91 (94.79 ± 2.6)

Note : 24 colonies were inspected every year from 2016 to 2018, average of percentage colonies infested/infected for the 3 years were as shown

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Viral pathogens were detected in 94.79 ± 2.6% of colonies inspected, colony forming units (CFU) of moulds and bacteria were observed in 76.04 ± 5.21% and 92.70± 0.01% respectively in samplesacross all the states. Percentage colony infestation with pests were significantly different across the states ($F_{3,7}$ = 14.228, Sig = 0.033, $p < 0.05$). The decline in colony establishment and honey productivity were unexpected despite the annual increase in the number of hives and colonies possessed by the beekeepers during the survey. The average annual yield of honey per colony plummeted to extremely low level compared to values obtained from other African countries.

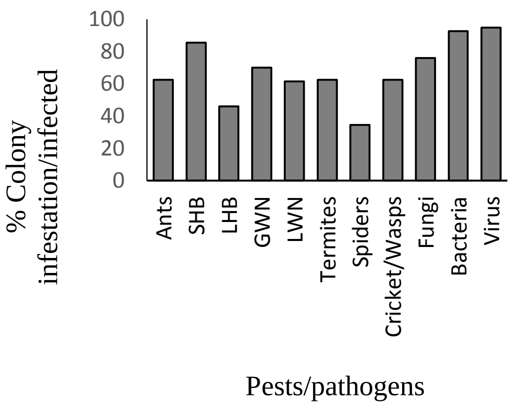

Figure 4. Percentage colony infestation/infected with pests/pathogens in the sampled colonies in the area

Nuru et al. [58] reported in Saudi Arabia, the average annual productivities of colonies to be 6.64 ±5.64 kg and 3.69 ± 2.62 kg honey/colony/annum for box and traditional hives respectively which were still considered low compared to the honey yield (>10kg/colony/annum) recorded in many other countries. In Tanzania, base line survey revealed 15kg of honey per hive annually [59] while Michael [60] recorded colony yields between 30-35 kg/hive/year. When honey production/hive falls below these recommended average productions per year [61], the area is termed unsuitable and many factors including climate change could be posing worrisome implications on the bees and beekeeping. This might be the possible contributors to the decline in honey production, colony establishment, pollination, and a loss of synchronization between pollinator activity and flowering [6, 62]. Although, the West African honey bee species, *Apis mellifera adansonii* has shown adaptive potentials to the tropical climate [43].

Climate change constraints/factors such as decreased precipitation, shift in seasonal rainfall, heat waves, flooding etc due to rising temperatures and heat waves have impacted colony health, survival and colony density [63, 64, 65], reduced plant vigour, delayed and fluctuations in greening, flowering and aging periods, and an overall shortening of the growth [66].

These might have hampered the livelihood of bee population. Annual temperature of 28 +/- 3°C for colony survival is desirable in the tropics [67]. Temperatures above this threshold constrain foraging capacity, reduce colony density and high rate of mortality [63, 64, 65]. The heat wave have produced temperatures that hindered plants growth, reduced foraging, increased colony temperature and swarming tendencies. Rainfall 350 - 700mm [68] wil spur brood production which determines bee foraging and forage availability for nectar and pollen. Late and excessive rainfall with characteristic flooding reported during the period must have increased the colonies vulnerability to diseases and plagues and the flooded or washed away bees reduced the colony population [68]. In the Southwest Nigeria, the yearly short time drought witnessed in the three years of investigation affected bees forage crops and the later appearance of heavy rainfalls characterized by heavy flooding might have also resulted in loss of arable land and wild forage plants.

To reduce the impacts of climate change, it requires adaptation. Adaptation is a phenomenon of reducing vulnerability and increase resilience, limiting the risk of climate impacts on life forms, and seizing the opportunities posed by the climat change. The loss encountered by the beekeepers due to climate change was because of lack of adaptation. Adaptation would have helped the beekeepers to maintain their trade despite the changing climatic conditions. Ozor et al. [69] noted that poor climate change information and farmers' lack of access to weather forecast technologies are major barriers to climate change adaptation among farmers in Southern Nigeria.

Therefore, in Nigeria, vulnerability to climate change is high because adaptation is low and because climate change affected food production and water resources, mitigation effort is tailored towards massive agricultural development or intensification. Agricultural development unlike climate change has short and reversible effects when it is limited only to large scale mono-cropping and absence of pesticides and land degradation. However, reported use of various grower pesticides detected through the heavy presence of GST biomarkers, poison the bees, impair their reproduction, eliminate nectar sources and deplete bees' nesting materials [70], chronic herbicide use may be driving the loss or reduce foraging [71]. With chronic or sub-lethal exposure of bees to these agricultural chemicals, the bee's immune system might be weakened and

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flight impaired, vulnerability to various pathogens and damage to colony health become obvious [9]. Therefore, pesticides are environmental stress on the honeybees, and the bees come into contact with it on the field. According to Gilbert and Wilkinson [33]; Yu [34]; Smirle and Winston [17] the bees actively detoxify and eliminate these chemicals with the enzyme systems which formed the biomarkers in their system when exposed to the chemicals in the environment. Acetylcholinesterase (AChE) and GSTs enzyme activities are among the biomarkers [27]. Therefore, the significant increase in GST activation levels in the tissues of bees in sampled colonies within and across the four states indicated the formation and build-up of glutathione conjugate in the bees. According to Kostaropoulos et al.[72] GST activity i induced by various substances (food quality and certain insecticides). The activity level of this biomarker in the larval and adult stages of honey bees is an evidence of exposure to toxic stress especially synthetic agrochemicals [73, 74].

Changes in land-use and landscape structure from agricultural intensification in addition to climate change, human activities have all impacted seriously on beekeeping activities in the region. Human activities have impacted the landscape through fragmentation, degradation and destruction of natural habitats with key adverse changes for beekeeping and bee population [75]. Mono-cropping has made it increasingly difficult for pollinators to obtain sufficient pollen sources for all their essential amino acids hampering successful larva development. Government empowerment programme in the cultivation of grains and cereals which are staple food of the people might have negatively impacted colony health, these crops have great propensity for pest infestations, facilitating increase in the application of pesticides in farmlands around the apiaries.

The persistent problems of insect pests, Small hive beetles, Large hive beetles, Lesser wax moth, Greater wax moth, Ants; parasites which includes bacteria, fungal spores and predators such as crickets experienced in all the apiaries appeared similar to that experienced elsewhere, Kugonza et al, [76] reported high infestation of greater wax moth, *Galleria mellonella* in all hives placed under shades, however, he had a contrary view on the infestation and distribution of the Small hive beetles, *Aethina tumida* Murray (Coleoptera: Nitidulidae) with respect to shades. Pests and pathogens' infestation that are emerging and increasing in the colonies were due to climate change and host shift as a result of natural habitat destruction.

Conclusion

The dwindling colony establishment, honey production and worsening colony health are connected with environmental and anthropogenic factors. These factors were borne from climate variability and adaptation policies and development. The agricultural intensification and development policy adopted by the government as a relevant and sustainable mitigation tool to climate change, rather than ameliorate the situation, negatively impacted beekeeping and the pollination industries.

Author's contributions

AKL conceived the research, analysed the data and wrote the scripts, AEO carried out the experiment under the supervision of AKL.

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Conflict of interest

None.

Güneybatı Nijerya'da Yoğun Tarımsal Uygulamaların ve İklim Değişikliğinin *Apis mellifera adansonii* Kolonilerinin Gelişimi ve Sağlığı Üzerindeki Etkileri

Öz: Yabani arıların azalması, bal arısı kolonilerinin kayıpları ve bal arılarının kötüleşen sağlığı küresel sorunlar haline gelmiştir. Güneybatı Nijerya, biyolojik olarak en çeşitli ekosistemlere sahip bölgelerden bir tanesidir. Ancak bu bölge, farklılaşan tarımsal uygulamalar ve iklim değişiminin etkileri altındadır. Bu etkiler aynı zamanda *Apis mellifera adansonii* Latreille (Hymenoptera: Apidae)(doğal olarak yayılış gösteren Batı Afrika bal arıları)'nin koloni kurulumu, sağlık ve üretkenliği gibi arıcılık faaliyetleri üzerinde de etki göstermektedir. Bu çalışma, arı kolonilerinin koloni oluşumu, sağlığı ve üretkenliği ile ilgili stres faktörlerini belirlemek amacıyla Aralık 2015 - Aralık 2018 tarihleri arasında gerçekleştirilmiştir. Güneybatı Nijerya'da belirlenen dört eyalette, seçilen arılıklarda örnekleme, koloni gözlemleri ve laboratuvar incelemeleri yapılmıştır. Sonuçlar, koloni sayılarında ve bal üretiminde düşüş olduğunu göstermiştir; kolonilerin arı zararlıları ve hastalıkları nedeniyle kayıpları, diğer faktörlere kıyasla daha yüksektir. Bölgedeki küçük kovan böcekleri (SHB) ile istila, Galleria mellonella istilası için kaydedilen 67'den (69,93 ± 2,08) (Ortalama ± SS) daha büyüktür. Kolonilerde üç yıl boyunca test edilen arıların dokularındaki ortalama Gluthathion-S-Transferase (GST) detoksifiye edici kimyasal sinyal seviyeleri, arıların normal değerlerinden daha yüksek bulunmuştur. İklim değişikliği, uyum politikaları ve tarımsal uygulamalardaki farklılaşmaya paralel gerçekleştirilen programlar arıcılık, bal arısı sağlığı, populasyonu ve üretkenliği açısından da oldukça önemlidir.

Anahtar kelimeler: Koloni kuruluşu; düşüş; verimlilik; zararlılar; iklimdeğişikliği; orman

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MELLIFERA RESEARCH ARTICLE

Contributions to *Bombus* Latreille (Hymenoptera: Apidae) Fauna of Eskişehir Province of Turkey

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A B S T R A C T

In this study, *Bombus* species, which are distributed in Eskişehir province, were considered. The study was conducted in 2020. A total 72 specimens were examined, 11 species were recorded and *B. barbutellus*, *B. rupestris*, *B. laesus* and *B. ruderarius* were reported for the first time from Eskişehir. Besides, the data about their distribution among Turkey are given in addition to the data about the plant taxa on which the specimens were caught. As a consequence, the decreased population trends of some bumblebees of Turkey were discussed.

Keywords: *Bombus*, Bees, Apoidea, Fauna, IUCN, Foraging Plants, Turkey, Distribution

Introduction

Bumblebees (*Bombus* Latreille) contain species that attract everyone's attention because of their large and colorful bodies [1]. They are distributed especially in alpine, subalpine and arctic belts of Palaearctic, Nearctic, Oriental and Neotropic regions [2, 3]. Bumblebees are considered highly efficient pollinators because they show activity even at extreme temperatures due to their thermoregulatory mechanisms [4, 5, 6].

Bombus contains more than 250 species in the world [7]. Turkey is one of the highest species richness in the West-Paleartic region with 48 species [8, 9]. While there is a web-based atlas of bumblebees, faunistic studies especially northwest of Turkey are needed.

Eskişehir is a province in the northwestern Turkey (39°06'N and 40°09'N; 29°58'E and 32°04'E). It locates at the intersection of 3 different phytogeographical regions (Irano-Turanian, Euro-Siberian and Mediterranean), which cause the formation of many different habitat types and an increase in plant diversity. There are about 1400 plant taxa belonging to 96 families in this region. Besides there are 225 endemic

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plant taxa and 34 of them are local endemic. Asteraceae (188 taxa), Fabaceae (145 taxa), Brassicaceae (106 taxa), Caryophyllaceae (86 taxa) and Lamiaceae (84 taxa) families are reported as the most common families for Eskişehir province [10, 11, 12]. Among these, Fabaceae, Asteraceae and Lamiaceae members are known as the most preferred plants by bees. However neither specific plant preferences of *Bombus* species of Eskişehir nor *Bombus* fauna of this province studied before. Because of this fact, in this study we aim to expose *Bombus* spp. fauna of Eskişehir.

Materials and Methods

Field studies were performed in Eskişehir province in 2020. All specimens were captured on plants by sweep net, prepared for collection and deposited in the Apoidea collection of Morphometry Laboratory of Hacettepe University's Department of Biology. The specimens were examined with stereoscopic binocular microscope and were identified according to Aytekin [13, 14], Özbek [15] and Williams et al [7]. Also plants were collected, properly dried and pressed for diagnosis. Plants were identified according to the Flora of Turkey [16, 17].

The species are listed below in alphabetical order within subgenera.

Abbreviations: ♀ : Queen, ☿: Worker, ♂ :Male

Results

In total 72 collected specimens were identified as 11 species from Eskişehir province.

***Bombus* (*Bombus*) *terrestris* (L., 1758)**

Material examined: 11-VIII-2020 Kayakent, Günyüzü (39°18'2.72"N 31°44'50.61"E) 1780 m., 1 ☿, 1 ♂ ; 11-VIII-2020 Büyükdere, Seyitgazi (39°34'50.97"N 30°45'13.74"E) 935 m. 2 ☿☿.

Plants recorded: *Consolida regalis* S.F.Gray subsp. *paniculata* var. *paniculata* (Host) Soo (Ranunculaceae), *Sideritis galatica* Bornm. (Lamiaceae).

***Bombus* (*Megabombus*) *argillaceus* (Scopoli, 1805)**

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Material examined: 10-VIII-2020 Günyüzü 1 ♀; 11-VIII-2020 Kayakent, Günyüzü (39°18'2.72"N 31°44'50.61"E) 1780 m., 2 ♀♀, 1 ♂; 11-VIII-2020 Büyükdere, Seyitgazi (39°34'50.97"N 30°45'13.74"E) 935 m., 3 ♀♀, 2 ♂♂; 22-VIII-2020, Balık Damı, Sivrihisar (39°12'16.20"N 31°39'34.67"E) 799 m. 1 ♀.

Plants recorded: *Consolida regalis* S.F.Gray subsp. *paniculata* var. *paniculata* (Host) Soo (Ranunculaceae), *Sideritis galatica* Bornm. (Lamiaceae), *Cephalaria transsylvanica* (L.) Schrader (Dipsacaceae).

***Bombus* (*Melanobombus*) *lapidarius* (L., 1758)**

ssp. *lapidarius*

Material examined: 11-VIII-2020 İdrisyayla, Seyitgazi (39°23'56.48"N 30°24'42.72"E) 1388 m. 7 ♀♀, 2 ♂♂; 20-VIII-2020 Büyükyayla, Seyitgazi (39°10'53.49"N 30°33'20.64"E) 1138 m. 2 ♀♀.

Plants recorded: *Carduus nutans* L. (Asteraceae), *Dipsacus laciniatus* L. (Dipsacaceae).

***Bombus* (*Psithyrus*) *barbutellus* (Kirby, 1802)**

Material examined: 19-VIII-2020 Hekimdağ (39°54'9.30"N 30°35'48.42"E) 1272 m. 1 ♀.

Plants recorded: *Marrubium parviflorum* Fisch. & Mey. subsp. *oligodon* (Boiss.) Seybold (Lamiaceae).

***Bombus* (*Psithyrus*) *rupestris* (Fabricius, 1793)**

Material examined: 11-VIII-2020 İdrisyayla, Seyitgazi (39°23'56.48"N 30°24'42.72"E) 1388 m. 4 ♀♀.

Plants recorded: *Echium italicum* L. (Boraginaceae).

***Bombus* (*Sibiricobombus*) *niveatus* Kriechbaumer, 1870**

ssp. *niveatus*

Material examined: 11-VIII-2020 Kayakent, Günyüzü (39°18'2.72"N 31°44'50.61"E) 1780 m. 2 ♂♂.

Plants recorded: *Sideritis galatica* Bornm. (Lamiaceae).

ssp. *vorticosus* Gerstaecker, 1872

Material examined: 11-VIII-2020 Kayakent, Günyüzü (39°18'2.72"N 31°44'50.61"E) 1780 m. 2 ♀♀; Odunpazarı (39°45'19.62"N 30°29'50.67"E) 809 m. 2 ♀♀, 1 ♂.

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Plants recorded: *Sideritis galatica* Bornm. (Lamiaceae), *Carduus nutans* L. (Asteraceae), *Syringa vulgaris* L. (Oleaceae).

***Bombus* (*Subterraneobombus*) *fragrans* (Pallas, 1771)**

Material examined: 22/23-VIII-2020 Balık Damı, Sivrihisar (39°12'16.20"N 31°39'34.67"E) 799 m., 2 ♀♀, 3 ♂♂.

Plants recorded: *Cirsium arvense* (L.) Scop. subsp. *vestitum* (Wimmer & Grab.) Petrak (Asteraceae).

***Bombus* (*Thoracobombus*) *laesus* Morawitz, 1875**

Material examined: 11-VIII-2020 Büyükdere, Seyitgazi (39°34'50.97"N 30°45'13.74"E) 935 m. 1♀; 23-VIII-2020 Balık Damı, Sivrihisar (39°12'16.20"N 31°39'34.67"E) 799 m., 3♀♀.

Plants recorded: *Cirsium arvense* (L.) Scop. subsp. *vestitum* (Wimmer & Grab.) Petrak (Asteraceae), *Stachys byzantina* C. Koch (Lamiaceae).

***Bombus* (*Thoracobombus*) *ruderarius* (Müller, 1776)**

ssp. *ruderarius*

Material examined: 11-VIII-2020 İdrisyayla, Seyitgazi (39°23'56.48"N 30°24'42.72"E) 1388 m. 6♀♀.

Plants recorded: *Echium italicum* L. (Boraginaceae).

***Bombus* (*Thoracobombus*) *sylvarum* (L., 1761)**

ssp. *citrinofasciatus* Vogt, 1909

Material examined: 11-VIII-2020 Büyükdere, Seyitgazi (39°34'50.97"N 30°45'13.74"E) 935 m. 1♀; 19-VIII-2020 Hekimdağ (39°54'9.30"N 30°35'48.42"E) 1272 m., 4♀♀; 19-VIII-2020 Yukarısöğüt, Seyitgazi (39°27'6.26"N 30°34'48.99"E) 1089 m., 2♀♀.

Plants recorded: *Carduus nutans* L. (Asteraceae), *Marrubium parviflorum* Fisch. & Mey. subsp. *oligodon* (Boiss.) Seybold (Lamiaceae), *Echium italicum* L. (Boraginaceae).

***Bombus* (*Thoracobombus*) *zonatus* Smith, 1854**

Material examined: 09-VIII-2020 Paşakadın, Sivrihisar (39°29'20.34"N 31°19'25.21"E) 1045 m. 1♂; 09-VIII-2020 Kaymazyayla, Mahmudiye (39°29'6.89"N 31°6'42.89"E) 868 m., 1♀; 11-VIII-2020 Büyükdere, Seyitgazi (39°34'50.97"N 30°45'13.74"E) 935 m., 5♀♀; 11-VIII-2020 Akın, Seyitgazi (39°19'54.86"N 30°31'4.02"E) 1028 m., 1♀; 19-VIII-2020 Hekimdağ (39°54'9.30"N

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30°35'48.42"E) 1272 m., 1♀; 19-VIII-2020 Yukarısöğüt, Seyitgazi (39°27'6.26"N 30°34'48.99"E) 1089 m., 1♀; 23-VIII-2020 Balık Damı, Sivrihisar (39°12'16.20"N 31°39'34.67"E) 799 m., 2♀♀, 1♂.

Plants recorded: *Consolida regalis* S.F.Gray subsp. *paniculata* var. *paniculata* (Host) Soo (Ranunculaceae), *Centaurea solstitialis* subsp. *solstitialis* L. (Asteraceae), *Cephalaria transsylvanica* (L.) Schrader (Dipsacaceae), *Vicia cracca* L. subsp. *cracca* L. (Fabaceae), *Echium italicum* L. (Boraginaceae), *Teucrium orientale* L. var. *orientale* (Lamiaceae), *Marrubium parviflorum* Fisch. & Mey. subsp. *oligodon* (Boiss.) Seybold (Lamiaceae).

Discussion

Faunistic studies [9, 14, 20, 24, 28 – 30, 36] revealed that bumblebees are represented by 13 different species in Eskişehir (Table 1). But some of these records are suspicious because of the discrepancies between faunistic studies. For example, Özsaltık [24] recorded B. alagesianus from Eskişehir but subsequent studies [9, 37] showed that this species distribute in North-east of Turkey (Transcaucasia, Caucasus and North Iran). The discrepancy between these studies can be the result of misevaluation of the specimens that were collected by Özsaltık [24] but we can not be sure without re-examining of the specimens.

Although these previous studies showed that *B. lucorum*, *B. incertus*, *B. armeniacus*, *B. pascuorum*, and *B. pomorum* distribute in Eskişehir, we did not encounter in the related field and other areas. Decreasing in population size can be one of the reasons that we could not observe these species. *B. pomorum* is considered in vulnerable (VU) category whereas *B. armeniacus* in endangered (EN) category in IUCN Red List and their population trend are decreasing [38]. Only one worker *B. pascuorum* was recorded by Özsaltık [24] in Bozdağ, but we did not observe this species in this area. *B. pascuorum* is considered in least concern (LC) category in IUCN but its population trend is also decreasing [38].

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Table 1. *Bombus* spp. recorded in Eskişehir and their IUCN Red List Categories (LC: least concern; NT; near threatened; VU: vulnerable; EN: endangered) [9, 14, 20, 24, 28 – 30, 36, 38].

Species	Previous Studies	This Study	IUCN Red List Category - Population Trend
Bombus (Bombus) lucorum	+	-	LC - Stable
Bombus (Bombus) terrestris	+	+	LC - Increasing
Bombus (Melanobombus) alagesianus	+	-	-
Bombus (Megabombus) argillaceus	+	+	LC - Stable
Bombus (Melanobombus) incertus	+	-	-
Bombus (Melanobombus) lapidarius	+	+	LC - Increasing
Bombus (Psithyrus) barbutellus	-	+	LC - Decreasing
Bombus (Psithyrus) rupestris	-	+	LC - Unknown
Bombus (Sibiricobombus) niveatus	+	+	LC - Stable
Bombus (Subterraneobombus) fragrans	+	+	EN - Decreasing
Bombus (Thoracobombus) armeniacus	+	-	EN - Decreasing
Bombus (Thoracobombus) laesus	-	+	NT - Decreasing
Bombus (Thoracobombus) pascuorum	+	-	LC - Increasing
Bombus (Thoracobombus) pomorum	+	-	VU - Decreasing
Bombus (Thoracobombus) ruderarius	-	+	LC - Decreasing
Bombus (Thoracobombus) sylvarum	+	+	LC - Decreasing
Bombus (Thoracobombus) zonatus	+	+	EN - Decreasing

Distribution map of species, which were recorded in this study, in Turkey were given in Figure 1 [9, 13 - 15, 18 - 36]. The most widespread and abundant species across Turkey is *B. argillaceus*. *B. terrestris*, *B. niveatus*, *B. zonatus* and *B. sylvarum* are also distributed in a wide range of the country. On the other hand *B. fragrans* is one of the rarest and least abundant species. Although Reinig [20], Özsaltık [24] and Özbek [28] recorded *B. fragrans* from different localities in Eskişehir, we only observed this species in one locality – Balık Damı that is one of the protected areas. To encounter with this species is hard since it is considered EN species according to IUCN Red List [38]. On the other hand, although *B. zonatus* is also evaluated under EN category [38], members of this species are more common than *B. fragrans* and observed especially on the edges of agricultural lands. Also *B. zonatus* are quite abundant species all over the country.

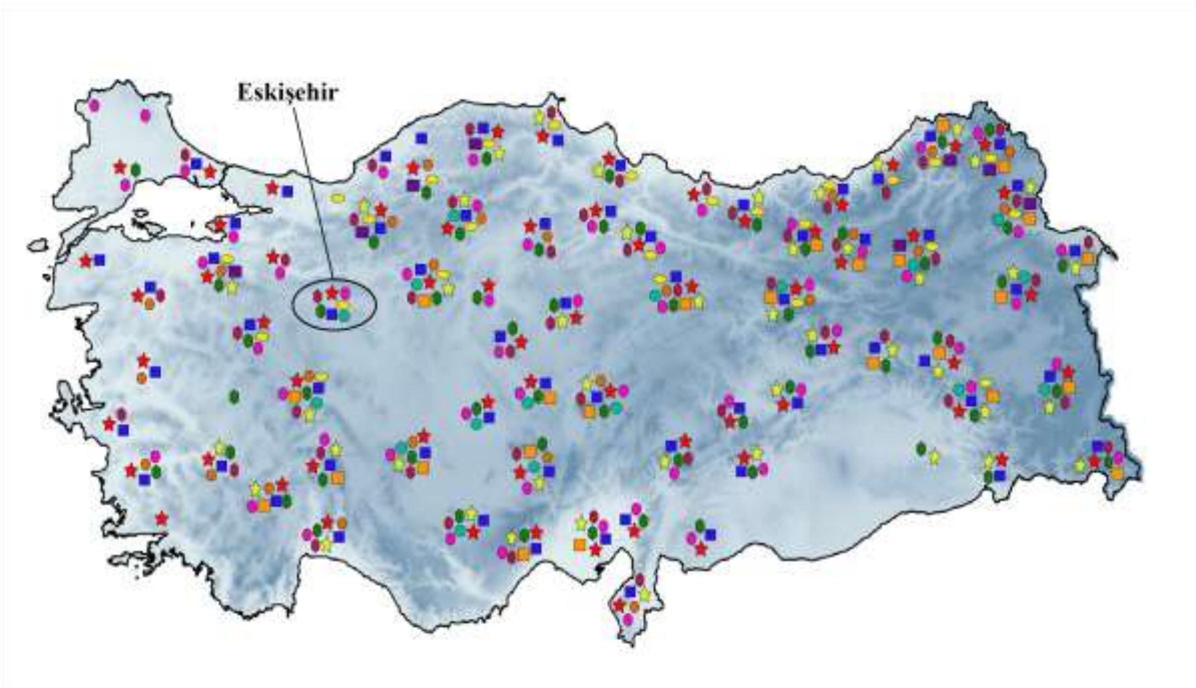

Figure 1. Distributions of *Bombus terrestris* (★), *B. argillaceus* (■), *B. lapidarius* (●), *B. barbutellus* (■), *B. rupestris* (■), *B. niveatus* (■), *B. fragrans* (■), *B. laesus* (■), *B. ruderarius* (☆), *B. sylvarum* (■), and *B. zonatus* (■) in Turkey.

B. barbutellus, *B. rupestris*, *B. laesus* and *B. ruderarius* were reported for the first time from this province in this study. Among these *Bombus* species *B. barbutellus* and *B. rupestris* are classified under *Psithyrus* subgenus whose members are parasitic [7].

Main host of *B. barbutellus* in Europe is *B. hortorum*, *B. ruderatus* and *B. argillaceus* but main host of this species in Turkey is not known [9, 37]. On the other hand, *B. rupestris* was recorded in the locality where its potential host *B. lapidarius* was also recorded [9].

Another new record for Eskişehir, *B. laesus* is a near threatened (NT) species according to IUCN risk category [38]. *B. laesus* was recorded various localities in Turkey with few specimens [28]. Our field observations are in agreement with these findings, only four specimens were found from two different locations, in Eskişehir. Although the *B. ruderarius* is evaluated in the LC category, the population trend of this species is also decreasing [38]. Our field studies support the propositions of IUCN Red List [38] since only four specimens from one locality were found.

As a conclusion, according to previous studies and our current study, *Bombus* spp. is represented by 17 species, four of them are new records, in Eskişehir province. Their most preferred plants are recorded as Lamiaceae and Asteraceae. The population trends in bumblebees give us an emergency signal about the wild bee populations in Europe and Turkey. Such local studies that monitor the bee population trends should be increased in order to minimize the risk of extinctions.

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We thank Dr. Kurtuluş Özgişi for his help during field survey and identification of plant taxa.

Eskişehir *Bombus* Latreille (Hymenoptera: Apoidea: Apidae) Faunasına Katkılar

Bu çalışmada Eskişehir ilinde yayılışı olan Bombus türleri ele alınmıştır. Çalışma 2020 yılında gerçekleştirildi. Toplam 72 örnek incelenmiş, 11 tür tespit edilmiş ve *B. barbutellus*, *B. rupestris*, *B. laesus* ve *B. ruderarius* Eskişehir'de ilk kez kaydedilmiştir. Ayrıca, türlerin Türkiye dağılımı ve üzerinden yakalandığı bitki taksonları da verilmiştir. Sonuç olarak, Türkiye'deki bazı bombus arılarının azalan popülasyon trendleri tartışılmıştır.

Anahtar kelimeler: *Bombus*, Arılar, Apoidea, Fauna, IUCN, Bitki tercihi, Türkiye, Dağılış

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MELLIFERA ERRATUM

Socio-Economic Determinants on The Profitability of Beekeeping Enterprises in Turkey: A Case Study in The Kelkit District of Gümüşhane

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E R R A T U M

Mellifera 2020, 20(1) 28-40 the figures and tables could not be uploaded to the management editorial system (https://dergipark.org.tr/tr/pub/mellifera) because of technical problems. Completed version with tables and figures can be found from the next page. We apologize for this error and any inconvenience it may have caused.

MELLIFERA RESEARCH ARTICLE

Socio-Economic Determinants on The Profitability of Beekeeping Enterprises in Turkey: A Case Study in The Kelkit District of Gümüşhane

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ABSTRACT

The aim of this study is to determine the effects of socio-economic factors on the profitability of the beekeeping enterprises in the province of Gümüşhane in Turkey. The relationship between gross profit and some socio-economic characteristics was investigated, and the effects of socio-economic factors on profitability were analyzed by the decision tree method. The results showed that the socio-economic factors affecting the gross profit of beekeepers were the non-beekeeping income, the production of the other bee products except honey, the beekeeping experience, the number of the hives and the years of education. Additionally, if the beekeeping is performed as a second source of income and with more experience, more education and working with fewer beehives will produce positive results on profitability. For producers who did not have any other income, other bee products provided more gross margin per hive. Therefore, other bee products besides the honey production would increase their profitability. The low amount of the other bee products such as propolis, royal jelly, bee pollen, bee bread (perga), apilarnil, bee venom, etc. were result from some socio-economic factors that had been identified in the research area and lack of training. Interventions should aim at trainings that overcome production, management practices and marketing constraints in the value chain.

Keywords: Beekeeping, decision tree, profitability, socio-economic factors, Turkey

Introduction

Beekeeping is a branch of production that can be carried out with other agricultural activities in rural areas, and it is also one of the most important agricultural activities because of the importance of bee products in the human diet, their use in the pharmaceutical, traditional medicine usage in treatment and the role of bees in improving product quality in crop production.

According to FAO's data on the number of bee colonies, with 9 million 148 thousand colonies, China is in the first place, while the second place is occupied by Turkey with 8 million 331 thousand colonies. China takes part in the first place in the world in terms of the number of colonies with 502 thousand tons in honey production, and it is followed by Turkey (114 thousand tons), the United States (73 thousand tons) and Russia (69 thousand tons) [8]. Although Turkey, both in the number of hives

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and in the production of honey in the world, comes after China, the value of its exports remains relatively low in comparison to other countries.

Although beekeeping may be practiced almost anywhere around the country, the honey yield per hive is still low in Turkey. According to the 2018 data from Republic of Turkey Ministry of Agriculture and Forestry [21], the yield is only 14 kg per hive in Turkey. Despite the increase in the number of beehives over the years in Turkey, the yield per hive has decreased. The yield per hive decreased from 17 kg in 2005 to 13 kg in 2016 [30]. Productivity is closely related to the production technique applied in beekeeping. Due to the lack of technical methods, serious financial losses occur in beekeeping. Achieving technical beekeeping increases the economic value of the activity of beekeeping and ensures that it becomes profitable for the beekeeper.

In the world and Turkey, several studies examining the economic aspects of beekeeping have been carried out so far. Beekeeping techniques in various provinces of Turkey have aimed at solving economic problems, and there are many studies about the significance of beekeeping [2, 5, 6, 7, 10, 11, 13, 14, 17, 20, 25, 26, 27, 28-31].

Despite the significance of the beekeeping enterprises, there was any empirical evidence on potentials and challenges of the beekeeping enterprises in the research area. Republic of Turkey Ministry of Agriculture and Forestry is currently focusing attention on how to increase agricultural production with providing employment opportunities for the local people in rural areas. So, production of the honey and the other bee products in Gümüşhane is important for the national honey market, and this affects a profitable enterprise in this context.

The aim of this study is to determine the effects of socio-economic factors on the profitability of beekeeping farms in the province of Gümüşhane and the relationship between gross profit and some socio-economic characteristics by Decision Tree-CRT algorithm. The socio-economic factors that have an effect on profitability will be identified in this study, and this information will fill the gap in the literature. Furthermore, introducing the optimum type of beekeeping enterprise in Gümüşhane will be a guide for decision-makers and beekeeping enterprises that need such information.

Research Questions

a.What are the socio-economic characteristics of the beekeepers?
b.What are the production characteristics of the beekeeping enterprises?
c.What is the contribution of beekeeping to beekeepers' household income?
d.What is the contribution of beekeeping enterprises to poverty alleviation?

Research Hypothesis

i.There is no significant relationship between selected socio-economic characteristics and poverty status.
ii.There is no significant relationship between beekeeping enterprises' production characteristics and present status
iii.There is no significant relationship between the contribution of beekeeping enterprises and poverty status.

Materials and Methods

The main material of this study was obtained from a survey conducted with beekeeping enterprises. The secondary sources of the study were previous national and international studies and research reports.

Gümüşhane was chosen as the research area where the survey was conducted. There were about 41 thousand hives and approximately 615 tons of honey production in 2018 [21]. When the number of hives and honey production in

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the province of Gümüşhane were analyzed based on the district, the Kelkit district had the largest share of production in the province with approximately 17 thousand hives and 441 tons of honey production (Table 1). Therefore, the Kelkit district was included in this study.

Table 1. Colonies, honey production and honey yield by districts in Gümüşhane

District name	Number of Beekeeper	Number of Hives (piece)	Honey Production (kg)	Honey yield (kg/ colony)
Kelkit	110	17395	441,570	25.38
Şiran	43	5002	37,065	7.41
Centre	161	11803	72,546	6.14
Köse	32	2699	35,838	13.27
Torul	39	2232	15,435	6.91
Kürtün	33	1783	12,230	6.85

Source: Ministry of Agriculture and Forestry, 2018

Although beekeeping was common in the Kelkit district of Gümüşhane, where the survey was conducted, reliable data on the number of hives could not be obtained. For this reason, it was found appropriate to use proportional sampling method in the study. In addition, the fact that this study was carried out with the own financial resources of the researchers and that there was a time constraint in choosing this method.

The sample size was calculated by using the proportional sampling method. In terms of this method, the sample according to the predicted ratio (p) of the population size N is given below [22].

$$n = \frac{Np(1-p)}{(N-1)\sigma^2_{\hat{p}_x} + p(1-p)}$$

n= Sample size

N= Number of beekeepers in the Kelkit district

p= Proportion of beekeepers on an adequate level (0.50 for maximum sample volume)

$\sigma^2{}_{px}$ = Variance of rate

There were 110 registered beekeepers in the Kelkit district in the Bee Registration System (BRS) of the Ministry of Agriculture and Forestry. Beekeepers in the Beekeepers' Association, producers who were not in BRS, beekeepers with fewer than 30 hives and beekeepers who came to Kelkit from outside (migratory) were also included in the study, and as a result, the population size was calculated as 190 producers. According to the proportional sampling method, the sample size was calculated as 60 with a 90% confidence interval and a 10.5% error rate the beekeeping enterprises surveyed were selected randomly. In this study, the effects of socio-economic factors on the profitability of the enterprises were analyzed by the decision tree method in this study. The explanatory variables were the level of education of the producer, age, the beekeeping experience, non-beekeeping income, the size of the producer's household, the type of beekeeping production, bee breeds, number of hives, status, use of consultancy and production of other bee products except honey. Gross profit per hive was used as the dependent (continuous) variable (Table 2).

The gross margin for a beekeeping enterprise is one measure of profitability that is useful for enterprise planning. Calculation of gross margins may be the starting point for construction of cash flow budgets and assessment of the whole farm's profitability. Gross margin profit is the difference between the annual gross income and the variable costs directly associated with the enterprise [9].

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Table 2. Variables used in CRT analysis

The dependent variable	Abbreviation	Explanation
Gross profit per hive (TRY/hive)	gmargin	It was obtained by subtracting the variable costs from the gross production value per hive.
Independent Variables		
Beekeeper's years of education	edu	Illiterate (0), literate (1), Primary school (2), Secondary school (3), High school (4), Pre-degree (5), University (6)
Beekeeper age	age	Number of years
Beekeeping experience	bexp	Number of years
Non-beekeeping income	nbi	1) available, 0) not available
Size of household	hsize	Number of persons
Types of beekeeping	btype	1) migratory, 0) constant
Bee breed in the production	race	1) Caucasian, 0) if not
Number of hives owned	nhive	Number of hives
Produced honey type	honeytype	1) If both pine and flower honey are produced; 0) If only flower honey is produced
Beekeeping training situation	training	1)Yes, 0) No
Counseling situation	advise	if the beekeeper receives counseling from a specialist institution…etc.; 1) Yes, 0) No
Production of other bee products except honey	otherprod	1) yes, 0) no

The Classification and Regression Trees (CRT) algorithm is used to construct decision trees. A decision tree is a classification method consisting of decision nodes and leaf nodes in the form of a tree structure. A decision tree algorithm develops a dataset consisting of categorical and/or numerical data by dividing it into small pieces. In a decision tree, the first node is called the root node, and the other branches are called the decision nodes. A decision node may include one or more branches. According to the contributions of the independent variables in classification of the dependent variable, child nodes are formed. Various algorithms are used to construct the tree. The CRT (Classification and Regression Tree) algorithm is widely used among these algorithms that have been developed. In the CRT algorithm, the contribution of the independent variables to classification of the dependent variable is determined by their importance [3].

Results and Discussion

Socio-Economic Characteristics of Beekeeping Enterprises

The socio-economic characteristics of the beekeeping enterprises were given in Table 3. The average age of the beekeepers was 52 years, their mean years of education were 8.5 years and the period of beekeeping experience was 19 years. This age result explained that beekeeping was maintained by an older generation and did not attract young people enough in Kelkit area. A similar result on the age factor was obtained in the beekeeping study of Affognon et al. [1]. The average age of beekeepers was found as 51. Makri et al. [18] found the mean year of education of the beekeepers was 10 years, and the beekeeper age changed from 40 to 50 years. In the study of Öztürk [26], the average period of education of beekeepers were found to be only 5.35 years that was the lower finding from this study.

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Table 3. Socioeconomic characteristics of beekeeping farms

Items	Min.	Max.	Mean	Standard Dev.
Age of beekeeper (yrs)	28.00	71.00	52.03	12.037
Years of education (yrs)	1.00	15.00	8.50	3.753
Farming experience (yrs)	3.00	50.00	25.00	14.679
Beekeeping experience (yrs)	1.00	50.00	19.28	12.192
Household size (person)	1.00	10.00	4.30	2.036
Number of Family Labors (person)	0.00	4.00	1.18	1.127
Number of hives (number)	22.00	470.00	145.88	134.922
Value of sales of bee products (US$)*	61.50	69.322	13.930	15424, 044

**The average exchange rates between Turkish Lira (TRY) and the US dollar (USD) for 2018 was $1= TRY4.813 (BÜMKO, 2018).*

Approximately 42% of the beekeeping enterprises (25 enterprises) took part in the animal breeding or the crop production other than the beekeeping, the period of their average agricultural experience was 25 years, and the average period of the beekeeping experience was found as 19 years. This average beekeeping experience value was less than 21 years determined by Ceyhan and Canan [35]. On the other hand, in the study performed by Kalanzi et al. [12], 56.3% of the surveyed beekeepers had less than 10 years of beekeeping experience. In this study, the average household size was found as 4 people. However, in the study that was published by Mbah [19]on the topic of the profitability of honey production, the average size of the households was found as 12 persons.

The average number of hives per farm was 146. The mean sales value obtained from bee products in the production period was calculated as US$13930.

The majority of the beekeeping enterprises (66.70%) did not produce other bee products. Only 33.30% of the investigated enterprises produced 1 to 2 other bee products including honey (Table 4). Similar result was obtained in Kebede and Tadesse's [15] study, and the beekeepers (86.4%) reported that they did not produce any bee products apart from honey.

The interviewed producers (66.70%) stated that they did beekeeping as additional activity. On the other hand in the study by Okpokiri et al. [23], 70% of the beekeepers who participated in the survey reported that they took part in honey production as their main source of livelihood. To the study of Ceyhan and Canan [35]; 64% of Turkish beekeepers do the beekeeping as the main source of income. But this result was obtained different in this study. The main reasons for the beekeeping as a second job by the majority of Kelkit beekeepers were that it was easier to produce in comparison to other production activities (crop and animal), they aimed to provide the employment opportunities for the family members, and it was seen as a profitable activity. When we considered the mean age of the beekeepers in the research area, this finding was an expected result. As a result, it was understood that the training activities could be carried out continuously in order to encourage the beekeeping to the target group of the young or middle age groups.

In order to ensure the economic feasibility of beekeeping, it was necessary to defuse the missing technical knowledge of the producers about this production activity. In this context, it

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was important that beekeepers receive basic training in apiculture and seek consultancy from experts during their activities. The findings obtained from this study showed that the level of technical knowledge about beekeeping of the interviewed beekeepers was generally good. As a matter of fact, 83.30% of the beekeepers stated that they participated in a course or a training program on beekeeping in the past. The percentage of the beekeepers receiving consultancy or assistance to obtain technical information on beekeeping was 43.30% at present. However, Kebede and Tadesse [15] showed that the most important problem faced by beekeepers was lack of adequate training on beekeeping.

According to the results, 78.30% of the interviewed producers were members of the Beekeepers Association as it is shown in Table 4. This was a positive result that shows that the producers depended on producer organizations.

Gross Margin Analysis

The variable costs of the beekeeping enterprises were firstly determined in this section. The variable costs associated with honey production per colony were given in Table 5.

The total variable costs included subsequently feed costs (sugar and cake), medication (parasite and disease control), wax foundation, transportation of hives, labor, location rental fees, and packaging of honey, repairs and maintenance, interest on variable costs. The total variable cost per hive was determined US$69.14. Labor cost and feed cost were identified as the significant cost items among the variable costs in this study. In a similar study conducted by Vaziritabar and Esmaeilzade [32] on the profitability of apiculture in the Karaj region of Iran, the variable cost per hive was found as about US$60.10. Variable costs were obtained as US$18.53 per hive in the study by Aydın et al. [34] and as US$94.25 in the study by Adanacıoğlu et al. [33]. These results showed that the beekeeping enterprises' operating costs was higher in the research area. According to these results in order to increase the economic performance of the beekeeping enterprises, the feed and the labor cost had to be reduced.

Table 4. Beekeeping activities, knowledge and skills of the producers

Items	Frequency	Percentage
Do you produce other products apart from honey?		
Yes	20	33.30
No	40	66.70
Is beekeeping your main job?		
Yes	23	38.30
No	37	61.70
Have you received any training on beekeeping?		
Yes	50	83.30
No	10	16.70
Do you receive consultancy assistance to get technical information on beekeeping?		
Yes	26	43.30
No	34	56.70
Are you a member of the Beekeepers Association?		
Yes	47	78.30
No	13	21.70

The gross revenue and variable costs associated with honey production are given in Table 6. While the gross revenue per hive was US$124.22, the total variable costs per hive was calculated as US$69.14 in the beekeeping enterprises. Therefore, the gross margin was calculated to be US$55.08 per hive.

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Table 5. Variable costs of beekeeping farms (US$ per hive)

Items	Cost	%
Feed costs (sugar and cake)	21.65	31.31
Medication (Parasite and disease control)	0.99	1.43
Wax foundation	6.95	10.05
Transportation of hives	7.53	10.89
Labor costs	26.60	38.47
Location rental fees	1.20	1.73
Repairs and maintenance	0.70	1.01
Packaging of honey (jar)	1.19	1.72
Interest on variable costs	2.33	3.36
Total Variable Costs	**69.14**	100.00

*The average exchange rate between Turkish Lira (TRY) and the US dollar (USD) for 2018 was US$1= TRY4.813 (BÜMKO, 2018).

Table 6. Gross margin in beekeeping farms (2018)

	Value (US$ **per hive)**
Gross Revenue (1)	124.22
Total Variable Costs (2)	69.14
Gross Margin (1-2) (3)	55.08

Analysis of The Effects of Socio-Economic Factors on The Profitability

In this section, the effects of socio-economic variables on the gross profit obtained by beekeeping enterprises were shown by the decision tree method. In this context, the effects of the producer's education years, age, beekeeping experience, non-beekeeping income, the size of the producer's household, the type of beekeeping, the bee breed used in production, the number of hives, the type of honey produced, the status of receiving training on beekeeping and the effects of the production of other bee products except honey on the gross profit were analyzed. As a result of the CRT algorithm that was used, the non-beekeeping income of the beekeeper, the beekeeping experience of the producer, the production of other bee products except honey and the number of hives were found to be more effective than the other factors. Whereas among the evaluated predictors, only two ones "age of enterprise" and "non-beekeeping income" were effective in the study of Aksoy et al. [2].

The non-beekeeping income of the beekeepers was found to be the most effective. The mean gross profit per hive for the producers who had non-beekeeping income was found to be higher (Node 1= US$58.28 (TRY280.54) than the producers who did not have non-beekeeping income (Node 2= US$23.92 (TRY115.17). On the other hand, it was seen that beekeeping experience was important for beekeepers with non-beekeeping income.

According to a single beekeeper with less than 1.5 years of experience in beekeeping, the experience variable was subdivided into sub-categories, and the gross profit was found to be lower among the beekeepers with little experience. The gross profit of the producer was found as US$61.05 (TRY293.86) (Node 4). According to the results of Kutlu [17] on determination of socio-demographic and economic factors that affect honey production, an increase in the beekeeping experience of beekeepers had a positive effect on honey production. The same finding was reached in the study by Onuç et al. [24]. They found that the professional experience of the beekeeper was an important factor. In our study, in addition to honey, production of other bee products was found to be a significant factor for the producer. The mean gross profit per hive for the producers who produced other bee products was US$53.99 (TRY259.89) (Node 6), while the mean was US$4.60 (TRY22.14) (Node 5) for the producers who did not.

As another variable, the number of hives was found to be effective on the producers with more beekeeping experience. The gross profit for the producers with less than 98 hives was US$70.59 (TRY339.77) (Node 7), and for those with more than 98, this was US$36.25 (TRY174.50) (Node 8). The years of education was an effective factor for the producers with a low number of hives. The producers with more

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education years had more gross profit per hive. The gross profit for producers who had more than 5 years of education was US$129.16 (TRY621.67) (Node 10), whereas, for those who had less than 5 years of education, it was US$62.95 (TRY303) (Node 9) (Figure 1-Table 7).

Table 7. Descriptives of Regression Tree

Node	Mean	Std. Deviation	N	Percent (%)	Predicted Mean	Parent Node	Primary Independent Variable		
							Variable	Improvement	Split Values
0	217.15	246.11	60	100.0	217.15				
1	280.54	258.94	37	61.7	280.54	0	nbi	6464.319	Yes
2	115.17	187.33	23	38.3	115.17	0	nbi	6464.319	No
3	-199.00	0	1	1.7	-199.00	1	bexp	3939.115	<=1.5
4	293.86	249.43	36	60.0	293.86	1	bexp	3939.115	>1.5
5	22.14	121.36	14	23.3	22.14	2	otherprod	5160.812	No
6	259.89	184.02	9	15.0	259.89	2	otherprod	5160.812	Yes
7	339.77	245.23	26	43.3	339.77	4	nhive	3287.787	<=97.50
8	174.50	230.17	10	16.7	174.50	4	nhive	3287.787	>97.50
9	303.00	230.49	23	38.3	303.00	7	edu	4491.566	<=5.0
10	621.67	180.49	3	5.0	621.67	7	edu	4491.566	>5.0

The factors that affected gross profit per hive and their importance values were shown in Table 8 and Fig. 2 (importance and normalized importance values of the independent variables). Among these factors, the non-beekeeping income of the producer was determined as the first and 100% effective factor on gross profit. In a similar study by Aksoy et al. [2], the age indicator was a 100% effective factor. Production of other bee products than honey (79.8%), the producer's beekeeping experience (76.7%), number of hives (75.2%), the producer’s education years (69.5%), the producer’s age (34.8%), the size of household (18.6%), beekeeping type (15.6%) and honey type (13.6%) followed these. However, the variables on the producer’s age, size of household, beekeeping type and honey type were not included in the decision tree diagram.The results showed that the socio-economic factors affecting the gross profit of the beekeeping farms were the income of other bee products except honey, the beekeeping experience of the beekeeper, the number of hives and the education year.

In the regression tree analysis, it was found that the beekeepers who had non-beekeeping income had more gross profit per hive than the beekeepers who did not. However, it was determined that the beekeepers who had non-beekeeping income, those with fewer hives, and those with high education levels made higher gross profits. The results of this study showed that, if beekeeping was performed as a second job, it was expected that more experience, high education and fewer beehives would have positive results on profitability. In this situation, we might state that, as the activity of beekeeping was carried out as a second job by the majority of beekeeping enterprises, a high number of beehives would limit the effective management of the hives.

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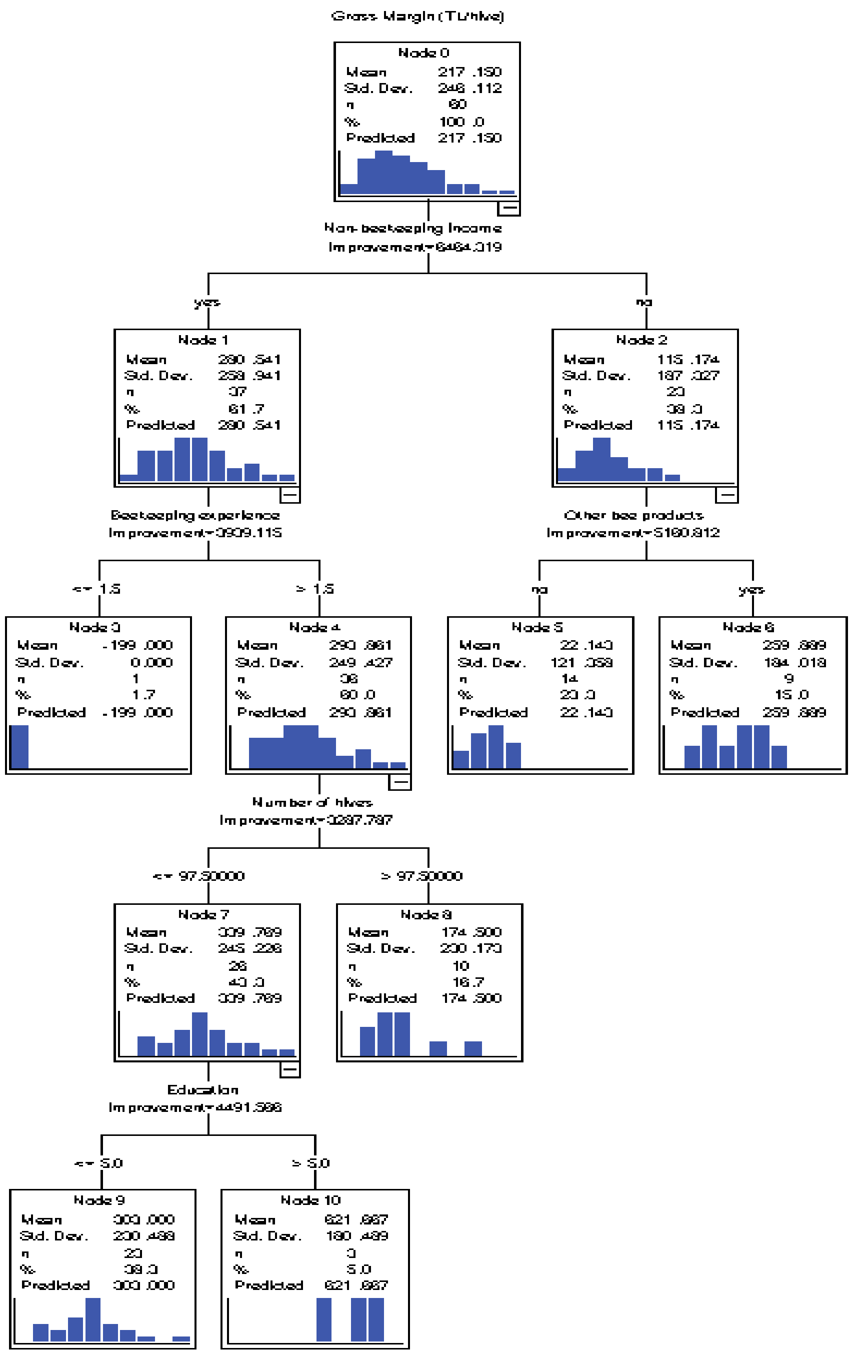

Figure 1. The Regression Tree diagram for factors affecting gross margin

According to the findings, non-beekeeping income beekeepers who produced other bee products than honey had more gross profit per hive. Unfortunately, other bee products than honey were not widely known. There are many products such as propolis, royal jelly, bee pollen, bee bread (perga), apilarnil, and bee venom. All these products may be used

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effectively in the world in apitherapy and alternative medicine. Without doubt, all the bee products could be used effectively to make more profit by the beekeeping enterprises in the research area. Therefore, production of the other bee products as a side activity to the primary honey production in Gümüşhane would increase the profitability of the beekeeping enterprises.

Table 8. Importance values of independent variables

Independent Variable	Importance	Normalized Importance
Non-beekeeping income	6464.319	100.0%
Other bee products	5160.812	79.8%
Beekeeping experience	4957.838	76.7%
Number of hives	4863.936	75.2%
Education	4491.566	69.5%
Age	2248.444	34.8%
Household size	1201.132	18.6%
Types of beekeeping	1006.818	15.6%
Honey type	881.405	13.6%

*Growing Method: CRT, Dependent Variable: Gross Margin (TRY/hive)

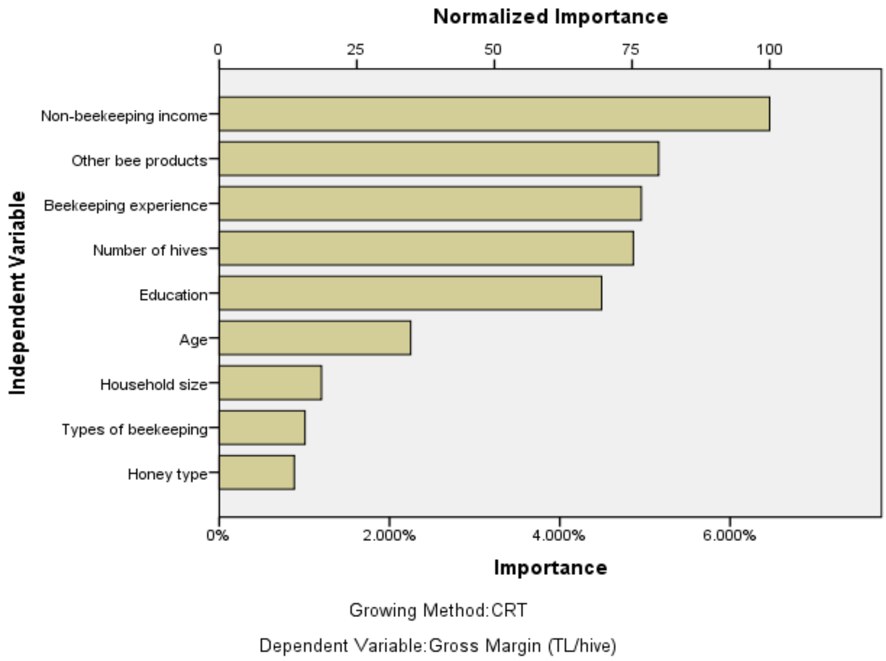

Figure 2. Importance and normalized importance of independent variables

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Conclusion

In conclusion, despite having adequate advantages such as the natural resources, the gross profit, and the yield in the research area, the number of beekeeping enterprises are still low. This is due to some insufficient management practices and lack of adequate training. In this context, aiming to improve the beekeeping management and the increasing profitability through identifying the socio-economic factors, providing the training courses, improving the marketing bee products except honey will be very vital to all the governmental and non-governmental organizations. Organizations are essential areas of intervention to utilize the management practices and the training.

Beekeeping in Kelkit area should be promoted to improve the employment and as a main income with the young/middle aged local people. Additionally, further study need to be conducted for improving the technical efficiency of the beekeeping enterprises.

Türkiye'de Arıcılık Işletmelerinin Karlılığına İlişkin Sosyo-Ekonomik Belirleyiciler: Gümüşhane'nin Kelkit İlçesinde Uygulamalı Bir Çalışma

Öz: Bu çalışmanın amacı, Gümüşhane ilinde arıcılık işletmelerinin karlılığına etki eden sosyo-ekonomik faktörleri belirlemektir. Brüt kar ile bazı sosyo-ekonomik özellikler arasındaki ilişki araştırılmış ve sosyo-ekonomik faktörlerin karlılık üzerindeki etkileri karar ağacı yöntemi ile analiz edilmiştir. Elde edilen sonuçlar göstermiştir ki, arıcıların brüt kârını etkileyen sosyo-ekonomik faktörler sırasıyla arıcılık dışı gelir, bal hariç diğer arı ürünlerinin üretimi, arıcılık deneyimi, kovan sayısı ve eğitim yılıdır. Ayrıca, yüksek arıcılık deneyimi, yüksek eğitim seviyesi, az kovan sayısı ve arıcılığın ek gelir olarak yapılması faktörlerinin arıcılık işletmelerinin karlılığını olumlu etkileyeceği saptanmıştır.

Bal hariç propolis, arı sütü, arı poleni, arı ekmeği (perga), apilarnil, arı zehiri,...gibi diğer arı ürünlerinin düşük miktarda üretilmesinin nedeni, araştırma alanında tespit edilen bazı sosyo-ekonomik faktörlerden ve yetersiz eğitim etkinliklerinden kaynaklandığı anlaşılmaktadır. Bundan dolayı, bal üretim değer zincirinde yapılması gereken müdahaleler; üretim, yönetim uygulamaları ve pazarlama kısıtlamalarının üstesinden gelecek şekilde hedeflenmelidir.

Anahtar Kelimeler: Arıcılık, Karlılık, Karar ağacı, Sosyo-ekonomik faktörler, Türkiye

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