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MELLIFERA EDITORIAL GUEST

Varroa Mites: Beekeeper Enemy Number 1.

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Published November 17, 2016

Honey bees are dying. High rates of losses have been reported in Europe, North and South America, Asia, and Africa – virtually everywhere bees are kept. There are many theories about what the underlying cause or causes of these losses are; poor nutrition, pesticides, diseases and parasites are a few of the most commonly mentioned. While all probably play some role, either on their own or in combination, there is growing consensus that one - the parasitic varroa mite, plays a large, if not the largest, role in the high rates of colony mortality experienced in many places around the world.

Figure 1. Ventral view of adult female varroa mite.

Varroa mites (Figure 1) were originally a parasite of the Asian honey bee, but began to parasitize the European honey bee when European honey bees were introduced to Asia in the last century. Since then varroa mites have spread to honey bee colonies in Europe in the 1970's and to United States in 1987. The mites are now established in all regions of the world except for small islands and Australia. Ten years after varroa's introduction in the US, these mites had nearly wiped out all the feral colonies in the US. Without management, varroa mites kill most colonies within a year or two. It is imperative that all beekeepers have varroa management strategies in place to prevent high losses in their operations.

Know your enemy: *Varroa* Biology.

Varroa mites are related to ticks. Like ticks, *Varroa* cannot complete their lifecycle without their host. Right before a worker or drone bee brood cell is capped, a female varroa mite crawls inside and hides in the brood food at the bottom of the cell. As the larvae transitions into a pre-pupa the female mite bites a small hole in its host's body. It feeds from that hole keeping it open so that her offspring can feed there as well. Like honey bees, mites can lay both fertilized and unfertilized eggs. Fertilized eggs become females, while unfertilized eggs become males. A female mite will first lay an unfertilized egg, and her son will then mate with his sisters as they reach maturity. A female mite will lay many eggs. While most will hatch, few will be fully mature when the adult bee emerges. Only those female mites that are fully

mature when the worker bee or drone emerges will survive. This is likely one of the reasons why varroa mites prefer drone brood – drone pupa take a couple of days longer to mature, so more of the mite's offspring will have time to mature and survive when the drone bee emerges. On average, a female mite will have just one surviving female offspring while the same mite infesting a drone cell will have twice as many surviving offspring. All male offspring and immature female mites will die when the bee leaves the capped cell.

Feeding by the varroa mites on brood weakens the bees leading to smaller, shorter lived drones and workers. Feeding mites can also transmit viruses into their host. Deformed Wing Virus is one of the most common and easily identified of these viruses. Bees heavily infected with deformed wing virus particles often emerge as adults with non-functioning wings. While bees with overt symptoms of deformed wing virus die in several days, many bees can have low levels of infection and show no signs that they are sick. If beekeepers note high numbers of worker bees with deformed wing virus, they likely have very high varroa levels and need to put in place a treatment plan to reduce varroa mite populations right away.

Figure 2. *Varroa* mite on thorax of a worker bee (center right).

While on adult bees, varroa mites appear to show a preference to attach to nurse bees, presumably because this makes it easier for them to find the right aged brood cell to invade and reproduce. When beekeepers examine frames of bees in heavily infested colonies they may see some mites on the top of thorax of some bees (Figure 2). However, most mites wedge themselves under the overlapping plates that make up the bees abdomen (Figure 3). The only way beekeepers can know the level of infection in their colonies is to sample and test their mite levels. Instructions for how to quantify mite levels in a colony are found in a recent Varroa management guide that is free for download (Interactive Varroa Guide PDF).

As outlined in this guide, mite populations can increase quickly, doubling every month. Mite populations can climb from near undetectable levels to levels that are thought to harm colonies in several months.

Figure 3. *Varroa* mite's wedged underneath abdominal plates of a worker bee.

How bad are *Varroa* in managed colonies?

In the US, the National Honey Bee Disease Survey has been collecting samples from colonies across the country for over 5 years. Nearly every sample contained varroa mites, so other than a few beekeepers that live on some small Hawaiian islands, all US beekeepers should consider their colonies infested. The same is probably true in all other places varroa are present as well. Considering how wide spread varroa are it is worrying that less than half of small scale beekeepers (those who manage fewer than 50 colonies) treat for varroa. Untreated colonies will almost certainly die in a year or two. Sadly, deciding not to treat does not only affects colony survivorship in untreated operations; those decisions have a collective effect on all beekeepers. Choosing not to reduce populations of mites in small managed bee yards allows for mites to

spread to other colonies. Weak, untreated colonies are robbed by other bees. Robber bees return to their own colonies with mites and the virus complex associated with them. The decision of a nearby beekeeper not to treat reduces the effectiveness of your own treatments and management practices.

There are many options available to beekeepers to help manage varroa mite populations. An excellent summary of management plans is freely available in the aforementioned Interactive Varroa Management Guide.

There is little doubt that varroa is beekeeper enemy number 1. All responsible beekeepers need to have a varroa management plan in place if they hope to keep their colonies alive. Even if colony survivorship is not a priority, managing mite populations is still critically important so that beekeepers don't negatively impact their neighbor's colonies. We don't let dogs or cattle walk around with untreated tick infections why should we think of bees any differently?

VARROA AKARLARI: ARICININ 1 NUMARALI DÜŞMANI

Bal arıları ölüyor. Avrupa, Kuzey ve Güney Amerika, Asya ve Afrika'da olmak üzere arıların bulunduğu her yerde yüksek oranlarda kayıplar kayıt edilmiştir. Bu kayıpların altında yatan neden veya nedenler ile ilgili pek çok teori bulunmaktadır; yetersiz beslenme, pestisitler, hastalık ve parazitler bunlar arasında en sık bahsedilenlerdir. Tek başlarına veya birlikte hepsinin büyük ihtimalle rolü bulunsa da, parazitik varroa akarının tüm dünyada görülen koloni ölümlerinde büyük rol oynadığı konusunda fikir birliği bulunmaktadır.

MELLIFERA RESEARCH ARTICLE

An *In vitro* Study on Antimicrobial and Antioxidant Activity of Propolis from Rize Province of Turkey

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ABSTRACT

In this study antibacterial, antifungal and antioxidant activities of propolis samples from the Rize province of Turkey in different solvents were investigated. A total of fifteen microorganisms belonging to Gram-positive *(Bacillus cereus, Bacillus licheniformis, Bacillus subtilis, Listeria monocytogenes, Micrococcus luteus, Staphylococcus aureus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus salivarius)*, Gram-negative bacteria *(Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella enteritidis)* and a fungi *(Candida albicans)* were studied using a disc-diffusion and minimal inhibition concentration (MIC) methods. Ethanol extracts of propolis (EEP), acetone extracts of propolis (AEP), ethyl acetate extracts of propolis (EAEP) and methanol extracts of propolis (MEP) showed the highest antimicrobial activity against *S. mutans, L. monocytogenes, M. luteus, B. licheniformis and C. albicans.* While dimethyl sulfoxide extracts of propolis (DMSOEP) has the weakly activity against some test organism. The most sensitive microorganisms to propolis were *E. coli, B. licheniformis, S. mutans, L. monocytogenes and B. cereus* in the test microorganisms.
Finally, according to the results shown by GC-MS, at least one substance was dissolved in EAEP and also by us, was found to have the highest antioxidant effect in the EAEP and AEP and the highest antimicrobial effect in the AEP.

Key words: Antimicrobial activity, antioxidant activity, propolis, GC-MS

Introduction

Propolis, a resinous substance collected by *Apis mellifera* bees from various plant sources and mixed with secreted beeswax, is a multifunctional material used by bees in the construction, maintenance, and protection of their hives [1-3]. Propolis is a complex resinous bee product with a physical appearance that varies widely, depending on many factors. The color may be cream, yellow, green, light or dark brown. Some samples have a friable, hard texture, while other samples may be elastic and gummy. Bees use propolis for diverse purposes, one of them is to seal the openings in the hive. A medicine containing vaseline and propolis (propolisin vasogen) was used for wound treatment during the Boer war [4,5]. Propolis composition is extremely complex. The main constituents are beeswax, resin and volatiles. The insects secrete beeswax,

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while the latter two constituents are obtained from plants. Distinction of flora from one origin to another provide variable source of propolis for bees and also cause color changes. The main visited plant species are poplar (*Populus* spp.), beech (*Fagus sylvatica*), horse chestnut (*Aesculus hippocastanum*), birch (*Betula alba*), alder (*Alnus glutinosa*) and various conifer trees [6]. The most favorable poplar species are *Populus alba*, *Populus nigra* and *Populus tremula* [7,8]. Similarly other plants used for the production of propolis are *Eucalyptus* species and *Baccharis dracunculifolia* [9,10].

In the present study, we investigated the antibacterial, antifungal and antioxidant activities of acetone, ethyl acetate, ethanol, methanol, dimethyl sulfoxide and water extracts of propolis samples.

Materials and methods

Propolis samples and preparation of extracts

Crude propolis samples were collected from Rize province of Turkey during October and November 2006. The samples were stored in air-tight glass containers in dark at -20°C until they were used. Propolis extracts were prepared by stirring 30 g samples in 150 mL of 95 % ethanol, acetone, ethyl acetate, methanol, water and dimethyl sulfoxide for a week at 4°C respectively. The extracts were filtered through 45 µm membrane filter, and then the solution was dried with an evaporator. The crude extracts were stored at -20°C until used.

Test strains and culture media

Strains of bacteria and fungi were obtained from ATCC (American Type Culture Collection, Rockville, Maryland), NCTC (National Collection of Type Culture, England), NRRL (Agricultural Research Service, United States of America), RSHE (Refik Saydam Hıfzısıhha Institute, Turkey). Antimicrobial activities of propolis extracts in different solvents were assayed against *Bacillus cereus* ATCC 11778, *Bacillus licheniformis* NRRL B-1001, *Bacillus subtilis* NRRL B-209, *Candida albicans* ATCC 25922, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 5041, *Listeria monocytogenes* NCTC 5348, *Micrococcus luteus* NRRL B-1018, *Proteus vulgaris* NRRL B-123, *Pseudomonas aeruginosa* NRRL B-2679, *Salmonella enteritidis* ATCC 13076, *Staphylococcus aureus* ATCC 6538, *Streptococcus mutans* RSHE 676, *Streptococcus pneumoniae* ATCC 10015 and *Streptococcus salivarius* RSHE 606. The species of bacteria were grown in Mueller Hinton Agar (Merck) and Mueller Hinton Broth (Merck). *C. albicans* was grown in Sabouraud Dextrose Broth (Difco) and Sabouraud Dextrose Agar (Oxoid). The concentrations of bacterial suspensions were adjusted to 10^8 cells/ml, while those of fungal suspensions to 10^7 cells/ml.

Antifungal and antibacterial assay

Antibacterial and antifungal activities were measured using the method of disc diffusion on agar plates [11]. In order to test antibacterial and antifungal activity, the fractions of propolis samples were dissolved in six different solvents. For bacterial Mueller Hinton Agar medium (Merck 20 ml) and Sabouraud Dextrose Agar (Oxoid 20 ml) for fungus were poured into each 150 mm petri dishes. All bacterial strains were grown in Mueller Hinton Broth medium (Merck) for 24 h, at 37°C and *C. albicans,* was grown in Sabouraud Dextrose Broth (Difco) at 27°C for 48 h. Growth was adjusted to OD (600 nm) of 0.1 by dilution with Mueller Hinton Broth medium (Merck) for bacteria and for

fungi Sabouraud Dextrose Broth (Difco). Suspension (100 µL) with approximately 10^8 bacteria and fungi per millilitre was placed in petri dishes, over agar and dispersed. Then, 6 mm diameter sterile blank discs (Oxoid) were placed on agar to load 15 µL of each propolis samples (20 mg/mL). One hundred units of nystatin was used as a positive control for fungus, ampicillin and cephazolin obtained from a local pharmacy and alcohol as a negative control for bacteria. Inhibition zones were determined after incubation at 27°C for 48 h. The study was conducted in three replicates. All measurements were done in triplicate.

Minimum inhibition concentration (MIC)

The agar dilution method, described by Vanden Berghe and Vlietinck was used for antibacterial screening with slight modifications. Instead of 96 well plates, 24 well tissue culture plates (Corning) were used [12]. The crude extracts were dissolved in 95% ethanol and physiological Tris buffer (Amresco) 1:4 and mixed with an equal amount of 3% agar solution at 45°C to a final concentration of 4, 2, 1 and 0,5 mg of extract/ml. An amount of 400 µl from the solutions was transferred into each well of the tissue culture plate (Corning). After solidification each well was inoculated with 10µl of freshly prepared bacterial suspension of 10^8 bacterial/ml and incubated at 37°C for 24 h. For bacteria ampicillin and cephazolin obtained from a local pharmacy, were used at 4, 2, 1 and 0,5 mg/ml (1 g/mL stock) as positive control, for fungus nystatin and 95% alcohol was used as negative control. The bacterial and fungal growth were assessed by a stereo microscope after the incubation period. All the assays were performed in triplicate.

GC-MS analysis

Propolis, grated after cooling, was extracted for 24 h with 95% ethanol (1:10, w/v) at room temperature. The extract was evaporated to dryness. About 5mg of the residue was mixed with 50 µl of dry pyridine and 75 µl bis (trimethylsilyl) trifluoracetamide, heated at 80 °C for 20 min and analyzed by GC-MS. The GC-MS analysis was performed with a Shimadzu Gas Chromatograph 2010 Plus linked to Shimadzu 2010 mass spectrometer system equipped with a 23m long, 0.25mm id, 0.5 mm film thickness HP5-MS capillary column. The temperature was programmed from 100 to 310 °C at a rate of 5 °C/min. Helium was used as a carrier gas, flow rate 0.7 ml/min. Split ratio 1:80, injector temperature 280 °C, ionization voltage 70 eV. The identification was accomplished using computer searches on a NIST98 MS data library. In some cases, when identical spectra have not been found, only the structural type of the corresponding component was proposed on the basis of its mass spectral fragmentation. If available, reference compounds were co-chromatographed to confirm GC retention times. The components of propolis extracts in different solvents were determined by considering their areas as percentage of the total ion current. Some components remained unidentified because of the lack of authentic samples and library spectra of the corresponding compounds [13].

Ferric Reducing/Antioxidant Power Assay

For ferric reducing/antioxidant power (FRAP) used in the determination of total antioxidant activities, the improved TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) is preferred [14, 15]. The activities of the samples were determined as micromolar FRAP value, which was obtained by using ascorbic acid in the 62.5–1000 µM range to prepare the calibration plot (Figure 1). Briefly,

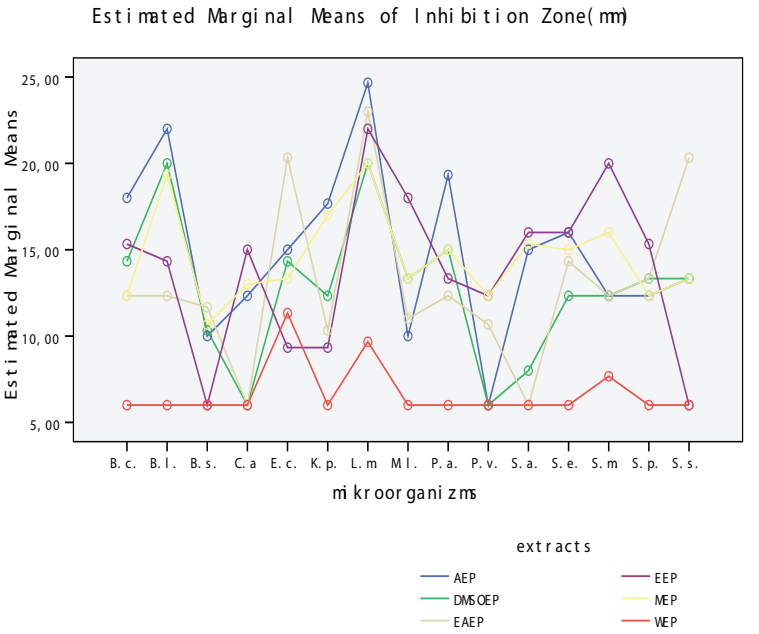

Figure 1. Profile Plot (Results of antimicrobial screening different solvent of Rize province propolis extracts determined by the agar diffusion method)

100 µL sample of same concentration for all the samples was mixed with 3.0 mL FRAP reagent (prepared by mixing acetate buffer, TPTZ, and FeCl3.6H2O solutions), and the absorbance was read at 595 nm against water blank at the end of 20 min incubation period. FRAP values were obtained by multiplying the µM concentration of ascorbic acid corresponding to the absorbance of the sample from calibration graph by two, the stoichiometric factor.

Statistical analysis

The data were analyzed by using SPSS for Windows (v.15.0). The differences between the means of the inhibition zones were tested with one-way variance analysis followed by Tukey's HSD test. The results were evaluated in the confidence limit of 0.05.

Results and Discussion

In the present study, the antimicrobial activity of acetone, ethyl acetate, ethanol, methanol, dimethyl sulfoxide and water propolis extracts from the Rize province of Turkey were investigated. The antibacterial and antifungal activity of propolis extracts in different solvents were initially evaluated by the disc diffusion method against nine Gram positive, five Gram negative bacteria and one fungus strain. The six tested compounds exhibited relatively strong antibacterial and antifungal activity. The results obtained in the disk diffusion assay regarding the growth inhibition zones of the tested microorganisms are shown in Table 1. In generally, methanol extract of propolis (MEP) samples more or less exhibit inhibitory action on the test organisms, but the samples showed a strong inhibitory effect on the growth of *L. monocytogenes* and *B. licheniformis* (20-19 mm/15 µl inhibition zone), among bacteria.

Table 1. Zones of inhibition (mm) showing the antimicrobial activity of propolis extracts

	Tested microorganisms mean zone of inhibition in mm ± standart derivation														
Extracts type	E.c	S.a.	S.s.	K.p.	S.e.	S.p.	B.c.	L.m.	S.m.	B.l.	M.l.	B.s.	P.a.	P.v.	C.a.
EAEP	20.33±1.57	6.00±0.00	20.00±1.00	10.33±0.57	14.33±0.57	13.00±1.00	12.00±0.00	22.66±0.57	12.33±0.57	11.66±0.57	11.33±0.57	11.00±0.00	12.33±0.57	10.33±0.57	6.00±0.00
EEP	9.00±1.00	16.00±0.00	6.00±0.00	8.66 ±0.57	16.00±0.00	15.33±1.52	15.00±0.00	22,33±0.57	20.00±1.00	14.33±0.57	18.00±1.00	6.00±0.00	13.00±0.00	12.00±0.00	15.33±0.57
AEP	15±0.00	14.66 ±0.57	13.00±2.00	17.00±1.00	16.00±0.00	12.00±1.00	17.33±1.15	24.33±0.57	11.67±0.57	21.66±0.57	10.00±1.00	10.00±0.00	19.33±0.57	6.00±0.00	12.00±1.00
MEP	13±0.00	14.67 ±0.57	13.00±0.00	17.00±0.00	15.00±3.00	11.67 ±0.57	12.00±0.00	20.33±0.57	16.33±0.57	19.33±0.57	13.00±1.00	9.67±0.57	15.00±0.00	12.00±01.00	13.00±0.00
DMSO	13.66±0.57	8.33±0.57	6.00±0.00	11.66±0.57	12.00±0.00	13.00±1.00	13.66±0.57	20.33±0.57	12.00±0.00	20.00±0.00	13.00±1.00	10.00±0.00	15.33±1.52	6.00±0.00	6.00±0.00
WEP	10.66±0.57	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	9.33±0.57	7.33±0.57	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
Means of column	13.67g	10.94c	11.83d	11.77d	13.22fg	11.83d	12.66ef	19.88ı	13.27fg	15.50h	11.88de	8.77a	13.50g	8.72a	9.72b
Ampicillin	15	10	10	13	35	28	27	25	25	30	35	36	28	28	NT
Cephazolin	15	10	10	10	36	22	23	32	30	25	35	38	24	28	NT
Nystatin	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	15
70% ethanol	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00

SD: standard deviation, 6.00 : no inhibition, NT: Not tested, Microorganisms: *S. salivarius* RSHE 606, *S. aureus* ATCC 6538, *K. pneumonia* ATCC 5041, *E. coli* ATCC 25922, *S. enteridis* ATCC 13076, *S. pneumonia* ATCC 10015, *B. cereus* ATCC 11778, *L. monocytogenes* NCTC 5348, *S. mutans* RSHE 676, *B. licheniformis* NRRL-B1001, *M. luteus* NRRL- B1018, *B. subtillis* NRRL-B209, *P. aeruginosa* NRRL-B2679, *P. vulgaris* NRRL-B123 and *C. albicans*ATCC25922

On the other hand, antifungal activity was shown against *C. albicans* (13 mm/15 µl inhibition zone). However, ethanol extract of propolis (EEP) samples exhibited inhibitory action on the *C. albicans*. Moroever, the samples showed a strong inhibitory effect on the growth of *S. mutans, L. monocytogenes* and *M. luteus* among bacteria. EEP did not show activity only against *S. salivarius* and *B. subtilis.* The crude propolis sample obtained from (AEP) samples showed antibacterial and antifungal activity (24-18 mm/15 µl inhibition zone) against the *L. monocytogenes, B. cereus, P. aeruginosa* and *B. licheniformis*. whereas, no activity was observed against *P. vulgaris.* However, this extract showed weak antifungal activity against *C. albicans.*

Water of propolis (WEP) samples did not form an inhibitory zone against any of the microorganisms tested except for *S. mutans, L. monocytogenes* and *E. coli.* It showed weak antibacterial activity (7-11 mm/15 µl inhibition zone) against these bacteria. Dimethyl sulfoxide of propolis (DMSOEP) samples did not exhibit inhibitory action on the *S. salivarius and P. vulgaris.* However, DMSOEP samples weakly exhibited inhibitory action on *S. aureus,* and *B. subtilis*. At the same time, the samples highly showed inhibitory effect on the growth of *M. luteus,* and *L. monocytogenes* among bacteria but did not show antifungal effect on *C. albicans*. On the other hand, ethyl acetate of propolis (EAEP) samples weakly exhibited inhibitory action on *P. aeruginosa, P. vulgaris* and *B. subtilis* but the samples showed a strong inhibitory effect on the growth of*, L. monocytogenes, E. coli* and *S. salivarius* but did not show antifungal effect on *C. albicans* and *S. aurues.* Evaluation of MIC s of different solvent propolis extracts from the Rize province of Turkey by means of agar dilution experiment method is reported in Table 2.

Table 2. Antimicrobial activity of propolis extracts

Extracts Type	Tested Microorganisms Minimal Inhibitory Concentration (MIC) in mg/ml												
	E.c.	*S.a.*	*S.s.*	*K.p.*	*S.e.*	*S.p.*	*B.c.*	*L.m.*	*S.m.*	*B.l.*	*M.l.*	*B.s.*	*P.a.*	*P.v.*	*C.a.*
EAEP	1≤	2≤	1≤	4≤	2≤	2≤	2≤	1≤	4≤	4≤	4≤	4≤	2≤	4≤	-
EEP	4≤	2≤	-	4≤	1≤	1≤	1≤	1≤	1≤	2≤	1≤	-	2≤	4≤	1≤
AEP	2≤	2≤	2≤	1≤	1≤	1≤	1≤	1≤	4≤	1≤	4≤	4≤	1≤	-	4≤
MEP	2≤	2≤	2≤	1≤	2≤	2≤	2≤	1≤	2≤	1≤	4≤	4≤	1≤	4≤	4≤
DMSOEP	2≤	4≤	-	-	4≤	2≤	1≤	1≤	4≤	1≤	4≤	4≤	1≤	-	-
WEP	-	-	-	-	-	-	-	4≤	-	-	-	-	-	-	-

- : no inhibition, Microorganisms: *S. salivarius* RSHE 606, *S. aureus* ATCC 6538, *K. pneumoniae* ATCC 5041, *E. coli* ATCC 25922, *S. enteritidis* ATCC 13076, *S. pneumoniae* ATCC 10015, *B. cereus* ATCC 11778, *L. monocytogenes* NCTC 5348, *S. mutans* RSHE 676, *B. licheniformis* NRRL-B1001, *M. luteus* NRRL-B1018, *B. subtillis* NRRL-B209, *P. aeruginosa* NRRL-B2679, *P. vulgaris*NRRL- B123 and *C. albicans* ATCC 25922

Table 3. Chemical composition of propolis extracts (% Total ion current, GC-MS)

Compounds	WEP	EPE	EAEP	APE	MEP	DMSOEP	
	%TIC						Total line
Aliphatic Acids							
Oleic acid, trimethylsilyl ester **IUPAC name** trimethylsilyl (E)-octadec-9-enoate		2.41	2.89	2.86			8.16
9-Octadecenoic acid (Z)-, ethyl ester **IUPAC name** ethyl (E)-octadec-9-enoate		0.99					0.99
9,12-Octadecadienoic acid (Z,Z)-, trimethylsilyl ester **IUPAC name** trimethylsilyl (9Z,12Z)-octadeca-9,12-dienoate		0.54					0.54
Octadecanoic acid **IUPAC name** Octadecanoic acid			0.11				0.11
Octadecanoic acid, trimethylsilyl ester **IUPAC name** trimethylsilyl octadecanoate			1.33				1.33
2-Monostearin trimethylsilyl ether **IUPAC name** 1,3-bis(trimethylsilyloxy)propan-2-yl octadecanoate		0.50	0.18	1.78			2.46
Octadecanoic acid, 9,10,18-tris[(trimethylsilyl)oxy]-, methyl ester **IUPAC name** methyl 9,10,18-tris(trimethylsilyloxy)octadecanoate			0.31				0.31
Propanoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester **IUPAC name** trimethylsilyl 2-trimethylsilyloxypropanoate					0.65		0.65
Propanoic acid, 2-(aminooxy) **IUPAC name** 2-(aminooxy) propanoate						0.39	**0.39**

Propanoic acid, 2-methyl-2-[(trimethylsilyl)oxy]-,trimethylsilyl ester **IUPAC name** trimethylsilyl 2-methyl-2-trimethylsilyloxypropanoate			0.17		1.70		1.87
1H-Indole-3-propanoic acid, 1-(trimethylsilyl)-, methyl ester **IUPAC name** methyl 3-(1-trimethylsilylindol-3-yl)propanoate			0.25				0.25
alpha.-(2,4,5-trichlorophenoxy) propionic acid, n- butyl ester **IUPAC name** butyl 2-(2,4,5-trichlorophenoxy)propanoate		0.51	0.12				0.63
4-hydroxyphenylpropionic acid-ditms **IUPAC name** trimethylsilyl3-(4-trimethylsilyloxyphenyl) propanoate			0.84				0.84
ethyl ester of 3-trimethylsilyl-propionic acid **IUPAC name** Ethyl3-(trimethylsilyl)propanoate					0.37		0.37
Hexadecanoic acid, ethyl ester **IUPAC name** ethyl hexadecanoate			0.20				0.20
Hexadecanoic acid, trimethylsilyl ester **IUPAC name** trimethylsilyl hexadecanoate		1.77	2.93	0.70			5.40
Butanoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester **IUPAC name** trimethylsilyl 4-trimethylsilyloxybutanoate					1.09		1.09
Butanoic acid, 3-[(trimethylsilyl)oxy]-, methyl ester **IUPAC name** methyl 3-trimethylsilyloxybutanoate			0.24				0.24
Dodecanoic acid **IUPAC name** Dodecanoic acid			0.15				0.15
Dodecanoic acid, trimethylsilyl ester **IUPAC name** trimethylsilyl dodecanoate			0.17				0.17

Aromatic Acids							
Benzenepropanoic acid, trimethylsilyl ester **IUPAC name** trimethylsilyl 3-hphenylpropanoatepenylpropanoic acid			0.15				0.15
Benzeneacetic acid, 2,4,5-tris[(trimethylsilyl)oxy]-, trimethylsilyl ester **IUPAC name** 2,4,5-Tris[(trimethylsilyl)oxy]benzeneacetic acid trimethylsilyl ester				4.93			4.93
Benzeneacetic acid, 3-methoxy-.alpha.,4-bis[(trimethylsilyl)oxy]-, ethyl ester **IUPAC name** ethyl 2-(3-methoxy-4-trimethylsilyloxyphenyl)-2-trimethylsilyloxyacetate			0.20				0.20
Cinnamic acid, p-(trimethylsiloxy)-, trimethylsilyl ester **IUPAC name** trimethylsilyl (E)-3-(4-trimethylsilyloxyphenyl)prop-2-enoate		1.25	0.58				1.83
Cinnamic acid, 3,4-dimethoxy-, trimethylsilyl ester **IUPAC name** trimethylsilyl (E)-3-(3,4-dimethoxyphenyl)prop-2-enoate		0.69	0.52				1.21
Cinnamic acid, 3,4-bis(trimethylsiloxy)-, methyl ester **IUPAC name** methyl (E)-3-[3,4-bis(trimethylsilyloxy)phenyl]prop-2-enoate			0.41				0.41
Cinnamic acid, p-methoxy-, trimethylsilyl ester_ **IUPAC name** trimethylsilyl (E)-3-(4-methoxyphenyl)prop-2-enoate			0.35				0.35
caffeic acid-tms-ether **IUPAC name** trimethylsilyl (E)-3-[3,4-bis(trimethylsilyloxy)phenyl]prop-2-enoate		1.97	2.64				4.61

trans-Caffeic acid, triTMS **IUPAC name** trimethylsilyl (E)-3-[3,4-bis(trimethylsilyloxy)phenyl]prop-2-enoate	7.15		0.37	23.56			31.08
ferulic acid-tms ether **IUPAC name** trimethylsilyl (E)-3-(3-methoxy-4-trimethylsilyloxyphenyl) prop-2-enoate		2.32	1.03	2.01			5.36
isoferulic acid-tms ether **IUPAC name** trimethylsilyl (E)-3-(4-methoxy-3-trimethylsilyloxyphenyl) prop-2-enoate			0.40				0.40
Benzoic acid **IUPAC name** Benzoic acid			0.33				0.33
Benzoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester **IUPAC name** trimethylsilyl 4-trimethylsilyloxybenzoate			0.21				0.21
Benzoic acid, 3-methoxy-4-[(trimethylsilyl)oxy]-, trimethylsilyl ester **IUPAC name** trimethylsilyl3-methoxy-4-trimethylsilyloxy benzoate			0.15				0.15
Benzoic acid trimethylsilyl ester **IUPAC name** Trimethylsilyl benzoate		0.95	0.48		1.94		3.37
ethanol, 1-(methylencyclopropyl)- **IUPAC name** 1-(2-methylidenecyclopropyl)ethanol	1.12						1.12
Ethanol, 2-(9-octadecenyloxy)-, (Z)- **IUPAC name** 2-[(Z)-octadec-9-enoxy]ethanol			0.10				0.10
3,7-Dioxa-2,8-disilanonane, 2,2,8,8-tetramethyl- **IUPAC name** 5-[(trimethylsilyl)oxy]- tms-glycerol	36.89	2.98	0.30	3.66			43.83
Benzeneethanol **IUPAC name** 2-phenylethanol			0.12				0.12
Total	45.15	18.50	18.89	39.50	6.03	1.25	129.32

The ethanol of propolis (EEP) samples required an MIC of 1 mg/ml for *S. salivarius, E.* coli and *L. monocytogenes* while all propolis samples required an MIC of 4 mg/ml for *B. subtilis*. Only the methanol of propolis (MEP) samples required an MIC of 1mg/ml for *E. coli*. Lower MIC values (1.4 mg/ml) were required against *E. coli*, however none of WEP samples were required against all pathogens. WEP, the other extracts of all propolis samples were active on microorganisms. The most sensitive microorganism to propolis was *E. coli* in the gram-negative group and *Streptococcus mutans* in the gram-positive group. The least sensitive microorganism was *Streptococcus salivarius.*

A control test ran with standard antibiotics revealed that propolis samples from the Rize province of Turkey had a similar or greater inhibitory effect on *S. mutans*, *B. licheniformis L. monocytogenes, M. luteus*, and *C. albicans* growth. According to the results, it may be concluded that, in general, Gram-positive bacteria and fungus were more susceptible to all of propolis samples antibacterial action than Gram-negative bacteria. De novo synthesis of water-insoluble glucan is essential for the adherence of *Streptococcus mutans* and other oral microorganisms to the tooth surface, forming a barrier that prevents the diffusion of acids produced by the bacteria [16]. Extensive screening for biologically active compounds from natural sources with these effects has been performed. For example, except for the water extract of propolis (WEP), the other propolis inhibited the growth of *S. mutans*. Similar results have been reported in other studies, which support our findings that propolis is mainly active against Gram-positives [17,18]. The antimicrobial activity against all pathogens was evaluated. EEP, AEP, EAEP and MEP showed the highest antimicrobial activity against *S. mutans, L. monocytogenes, M. luteus, B. licheniformis* and *C. albicans* While DMSOEP had the weak activity against some test organisms. Except for *S. mutans*, WEP was not effective against all pathogens. However it has been reported that EEP is effective on Gram-negative bacteria at higher concentrations [19]. These results indicated that acetone extracts of all propolis samples were more active than the DMSO extracts of the same samples. However, our findings are not similar to those of other researchers, who found differences [20]. Our results are similar to Ugur and Arslan's results. According to Hegazi et al.[21]; The propolis samples show different antimicrobial activity due to it is complex resinous bee product with a physical appearance that varies widely, depending on many factors. This propolis is known as a healer and used for the treatment of various diseases in humans. Several compounds have been identified in propolis, and three distinct chemical groups have been reported to be present: (i) flavonoid aglycones, (ii) cinnamic acid derivatives, and (iii) terpenoids [22-24]. Flavonoids have been considered as the main biologically active compounds in propolis [1, 25, 26]. In our opinion, the qualitative and quantitative composition of propolis plays an important role in their biological activity.

Several studies regarding antimicrobial activity of propolis ethanolic extracts showed a positive correlation between flavonoid content and antibacterial properties of propolis [27]. The composition of raw propolis depends upon the plant source, bud exudates of different trees, generally *Populus* in the temperate zone [28]. Propolis contains wide variability of active compounds (flavonoids and phenolic acids). Variations in the flavonoid content of propolis are mainly attributable to the

difference in the preferred regional plants visited by honeybees [29]. Numerous researchers have reported that caffeic acids, flavonoids and phenolic esters are the main biologically active compounds in propolis [30-32]. However, our samples were found to be active against the gram negative bacteria. This activity can be a synergism between flavonoids, apigenin, chrysin, and/or other components in raw propolis samples. Besides, 5-[(trimethylsilyl)oxy]-tms-glycerol=36.89 value is ignored because it is bacteriostatic glycerol, and total column of acetone=39.50 are considered as the greatest value. Consequently, acetone of propolis extract showed the highest antibacterial effect.

The extracts (100 μL) were treated with FRAP reagent (3.0 mL), and the absorbance values measured at 595 nm after a 20 min incubation period were used to calculate the FRAP values from a calibration graph prepared with ascorbic acid (Figure 2). The corresponding ascorbic acid concentration value was multiplied by two to express the antioxidant capacities as μM FRAP (Figure 3). A higher FRAP value reflects higher antioxidant capacity. Thus, all the propolis extracts showed much higher antioxidant power in comparison to the standard antioxidant Trolox (500 μM). While the aqueous extract had the lowest activity, acetone extract showed the highest, an approximately 55 fold activity range. In order to show the relationship between the total extract table antioxidant content with the solvent polarity, the dielectric constant of the extraction solvents were plotted against the FRAP values obtained (Figure 4). Dielectric constant is an indicator of solvent polarity. As the solvent polarity increased, the total extracted antioxidants decreased as evident from lower FRAP values. Care must be taken that no solvent with practically nonpolar nature, such as hexane, was used in the tests; the lowest polarity solvent extracts may also be expected to show lower FRAP values. Finally, in our test, we found the highest antioxidant effect in the ethyl acetate extract of propolis and acetone extract of propolis.

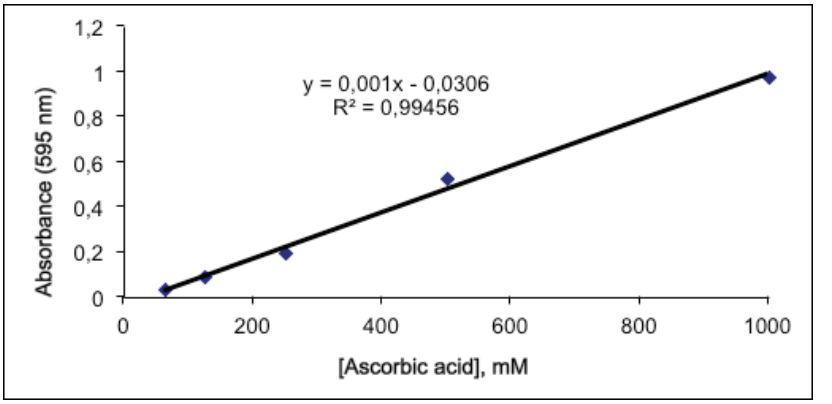

Figure 2. Calibration curve for FRAP test, prepared by ascorbic acid concentration plotted against the absorbance value measured at 595 nm in FRAP assay.

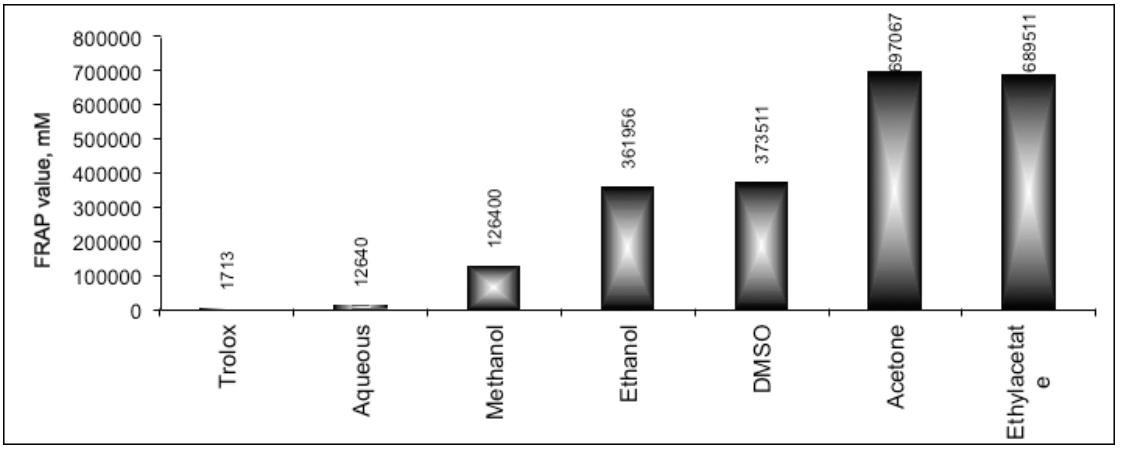

Figure 3. FRAP values of the extracts and the standard antioxidant Trolox (500 μM). Aqueous extracts were diluted 20 fold, and the others 400 fold in the assay, and the FRAP values were calculated by multiplying with the dilution factor.

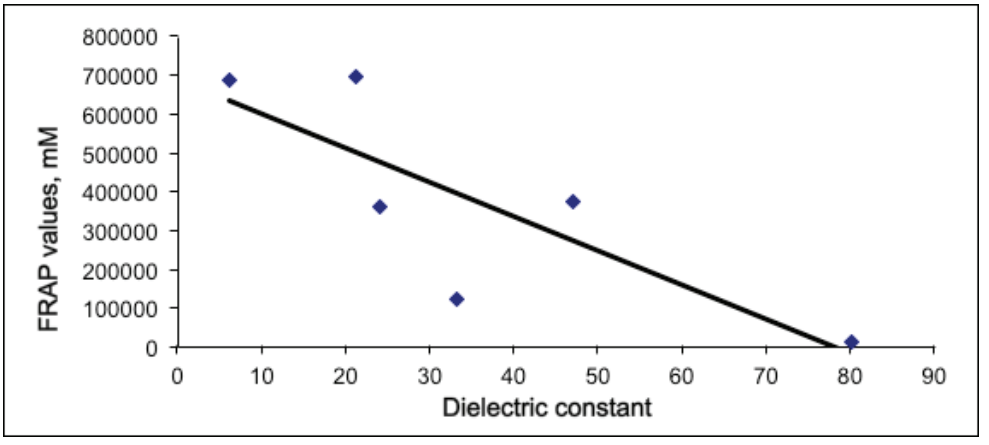

Figure 4. FRAP values of the propolis extracts plotted against the dielectric constantas of the extraction solvent

Türkiye'de Rize İlinden Elde Edilen Propolisin Antimikrobiyal ve Antioksidan Aktivitesi Üzerine İn Vitro Çalışma

ÖZ

Bu çalışmada Türkiye'nin Rize ilinden toplanan propolis örneklerinin farklı solventlerde antibakteriyal antifungal ve antioksidan aktiviteleri araştırılmıştır.Gram pozitif bakteriler *(Bacillus cereus, Bacillus licheniformis, Bacillus subtilis, Listeria monocytogenes, Micrococcus luteus, Staphylococcus aureus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus salivarius)*, Gram negatif bakteriler *(Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella enteritidis)* ve bir maya *(Candida albicans)* olmak üzere toplamda 15 mikroorganizma disk difüzyon ve minimum inhibisyon konsantrasyonu (MİK) yöntemi kullanilarak incelenmiştir. Propolisin etanol ekstraktı (EPE), propolisin aseton ekstraktı (APE), propolisin etil asetat ekstraktı (EAPE) ve propolisin metanol ekstraktı (MPE) *S. mutans, L. monocytogenesis, M. luteus, B. licheniformis ve C. albicans*'a karşı en yüksek antimikrobiyal aktivite göstermiştir. Dimetil sülfoksit Propolis Ekstraktı (DMSOPE) bazı test mikroorganizmalarına karşı zayıf aktivite göstermiştir. Propolise en duyarlı mikroorganizmalar *E. coli B. licheniformis, S. mutans, L. monocytogenes ve B. cereus* olmuştur.

Anahtar Kelimeler: Antimikrobiyal aktivite, antioksidan aktivite, propolis, GC-MS

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MELLIFERA RESEARCH ARTICLE

Pollen Analysis of Honeys From Beypazarı District of Ankara Province (Turkey)

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ABSTRACT

Pollen analysis was performed in honey samples collected from 17 different regions within Beypazarı district of Ankara province in Turkey. Among 17 honey samples, it was found that 9 samples were unifloral honey and the remaining 8 samples were multifloral honey. The honeys obtained in this region yielded, pollen grains of total 24 taxa, including 11 families and 13 genera. The most dominant taxon was *Astragalus*. As a result of the analysis, it was found that *Astragalus* was the principal source of nectar and pollen for honeys obtained in this region.

Keywords: Honey, pollen analysis, TPN-10 g, unifloral honey, multifloral honey

Introduction

According to the Turkish Standards Insti tution, honey is a sweet food, which comes from collecting the nectar of flowering plants or various substances excreted by some of homopterans. This sweet product occurs as a result of its composition being altered in the bodies of honeybees (*Apis mellifera*) and stored in honeycombs and maturing in this area. Honey contains different sweetness such monosaccharides fructose and glucose. Honey may vary in color from pale yellow to dark brown, and it can be crystallized entirely or partly after a while. The taste, aroma or the color of honey depend on various factors including the origin and the kind of the plant, the valotile oil from the flowers of the plants giving smell to the honey. The essential oil also gives smell to the flowers [1]. According to [2], the smell, taste, color and its look depend on plants types. The presence of the different kinds of components in honey than nectar shows that the bee adds some more elements to it, while it seasons honey in its stomach [3]. The chemical composition, physical specifications, and the pollen contents give its value to the honey. These specifications vary according to the geographic and botanic origin the honey where it is produced. Melisopalinologic

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analysis is the way to determine the botanic origin of honey [4]. Honey is hygroscopic and it gets humidity from the weather. The color, clarity, thickness, smell, taste, and the speed of crystallization create its physical specifications. Honey is one of the basic foods, which is both anti-inflammatory and anti-bacterial. The first pollen-analysis on honey studied by [5] specialized on agricultural-chemistry. The first analysis in Turkey is studied by Qustuani on a East-Blacksea honey sample in 1966 [6]. Melisopalinology helps to find the floristic origin of honey. That is the reason why pollen-analysis is used. The pollens inside the honey are identified and the plant taxa to which these pollens belong are determined. This takes an active part in determining the nectar-sources, the geographic origin, the quality, and classifying of honey. The importance of the pollen-analysis rises more and more in many countries in recent years and the researchers try to determine the quality of the honey produced in their countries.

Some examples can be given here; [7] made pollen-analysis of Northeast Buenos Aires honey. [8] made a research in India, and analyzed the honey of Andhra Pradesh region. [9] analyzed East Godovari Region Honey. [10] did Para Stat Honey in North Brasil. [11] determined the pollen-spectrum of the northeastern Himalaya Honey. [12] made quantitative pollen-analysis of the typical Sardinian honey. [13] searched microscopic pollen-analysis of *Apis dorsata* (rock bee) honey collected in the Nallamali forest of Andhra Pardesh. Pollen-analyses are still studied on different kinds of honey in Turkey as in the world (e.g. [14,15,16,17,18,19,20,21,22). The main aim of the present study is to determine vegetal and geographic origin of honey collected from several localities of Beypazarı region in Ankara.

Materials and the Methods

17 kinds of honey samples were used in the study. They were collected from Beypazarı district and its villages in Ankara (Turkey) in September and October 2012. Pollen preparations have been made according to the methods accepted after evaluation of eight European honey institutes standards [23,24,25]. Pollen preparations were analyzed with a Nikon Eclipse E400 microscope, and an immersion-objective was used by shooting of microphotographs.

The entire 18x18 mm cover glass surface has been scanned and the pollens on the surface have been identified. Several reference books [26,27,28,29,30] and the pollen reference collection of Hacettepe University in the Department of Biology were used during the identification processes.

Pollen grains determined in honey samples under examination were divided into 4 main groups according to the pollen spectra. It was considered that pollen amount constituting ≥45% of the whole was dominant, pollen amount between 16% and 44% was secondary, and pollen amount between 4% and 15% was minor, and pollen amount lower than 3% was trace [24,25,31,32]. It was reported that total pollen number in 10 g of honey (TPN-10 g) can be used as a criterion for differentiating artificial and pure honey [33]. In this study, the TPN value in 10 g of honey using the *Lycopodium* spores was also defined. Honey samples examined were divided into 5 main groups in terms of their TPN-10 g values. Accordingly, Group I included honeys with TPN-10 g value lower than 20 000, Group II included honeys with value of 20 000-100 000, Group III included

honeys with value of 100 000-500 000, Group IV included honeys with value of 500 000-1 000 000, and finally, Group V included honeys with value of over 1 000 000 [24].

Discussion

Taxon diversity in honey samples is always less in the dominant group, and always more in the rare group [35].

It has been determined that the honey of Beypazarı is mostly multifloral, and it has been notified that the nectar of honey comes from the plants which include pollens of dominant and secondary groups [36].

Following our analysis, *Astragalus* has been determined as the main nectar and pollen source for the local honey from Beypazarı.

Brassicaceae family has been stated as the second taxon. Apiaceae, Asteraceae, Boraginaceae, Brassicaceae and Lamiaceae are the main taxa source for local honey.

Conclusion

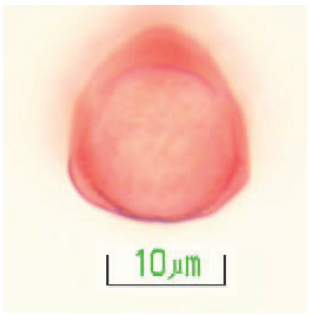
According to pollen analysis of the Beypazarı honey samples, 24 taxa from 20 families have been identified.

Most of these taxa belong to the families Apiaceae, Asteraceae, Boraginaceae, Brassicaceae, Fabaceae, Lamiaceae, Rosaceae, Myrtaceae, and Scrophulariceae (Figure 1, Table 1). It has been determined, that the one taxon with most pollens in local honey is *Astragalus* of Fabaceae family (Table 1). Taxon diversity is always less in the dominant group, and always more in the rare group [35]. This result is also supported by our study. In terms of total pollen quantity (TPN-10 g), the standard honey samples are; Akkaya Village Honey is 2; Kemeres Village honey is 3; Akyazı Village honey ise 4; Tekkeköyü Village honey is 9; Geyikpınar Village honey 10; and Dudaş Village honey is 16. Honey samples with less pollen are; Kapullu Village Honey is 1; Kırbaşı Village honey is 5; Oymaağaç Village honey is 7; Uşakgöl Village honey is 8; Kırbaşı Village honey is 11; Harmancık Village honey is 12; Dikmen Village honey is 13; Başören Village honey is 15; Kargı Village honey is 17 (Table 1). It has been determined that the honey samples from Beypazarı is mostly multifloral. It has been notified that the nectar of the honey, comes from the plants, including pollens of the dominant and secondary groups [36] As the conclusion of our analysis in 9 samples, *Astragalus* pollens are the dominant ones. Taxon which includes pollens as secondary are; Brassicaceae in 9 samples; *Astragalus* in 8 samples; Asteraceae in 4 samples; *Hedysarum* in 2 samples*; Echium, Linaria,* Portulacaceae, each, in 1 sample. After grouping the honey from Beypazarı according to their TPN-10 g quantities; 2 of them are honey with full of pollens (TPN 500 000-1 000 000), 6 of them with normal pollen level (TPN 20 000-100 000), and 9 of them are poor in pollen (TPN <20 000). Harmancık Village honey with sample Nr.12 is the one with the least amount of pollen. Kuyumcu Tekkeköy honey with sample Nr. 14 is the one with the most amount of pollen (TPN-10 g has been determined as 608 751).

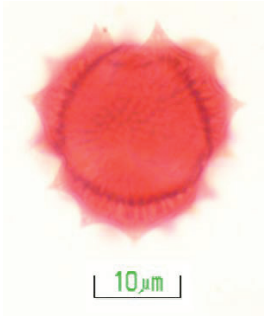
Table 1. *Pollen spectra and TPN-10 g values obtained from the honey samples collected from various localities in the Beypazarı district of Ankara province (*Dominant pollen, **Secondary pollen, ***Minor pollen, ****Rare pollen)*

Honey sample number	Locality	Pollen spectra	TPN-10 g
1	Kapullu Village	*Astragalus **Echium ****Hedysarum,* Lamiaceae, Rosaceae, *Vicia* ****Asteraceae, *Centaurea,* Cupressaceae/Taxaceae, *Elaeagnus, Linaria,* Moraceae, Pinaceae	10 798
2	Akkaya Village	*Astragalus **- ****Echium, Hedysarum,* Rosaceae, *Vicia* ****Brassicaceae, *Cistus, Eucalyptus,* Lamiaceae	66 346
3	Kemeres Village	*- **Astragalus, Hedysarum, Linaria ***Asteraceae, Brassicaceae, *Echium,* Lamiaceae ****Apiaceae, Betulaceae, *Cistus, Eucalyptus, Taraxacum, Vicia*	20 273
4	Akyazı Village	*- ***Astragalus*, Brassicaceae ***Asteraceae, *Echium, Hedysarum,* Rosaceae, *Vicia* ****Apiaceae, *Cistus,* Lamiaceae, Moraceae, *Plantago, Taraxacum*	27 948
5	Kırbaşı Village	*Astragalus **- ***Brassicaceae, *Echium, Hedysarum,* Rosaceae, *Vicia* ****Apiaceae, Betulaceae, *Cistus, Eucalyptus,* Lamiaceae	8 692
6	Akkaya Village	*Astragalus **Asteraceae ***Plantago **** Apiaceae, *Hedysarum*, Lamiaceae	472 358
7	Oymaağaç Village	*- ** Asteraceae, *Astragalus,* Brassicaceae ***Apiaceae, *Cistus, Hedysarum, Plantago* ****Betulaceae, *Echium,* Lamiaceae, Rosaceae, *Salix, Vicia*	4 879
8	Uşakgöl Village	*Astragalus **Brassicaceae ***Asteraceae, Rosaceae, *Vicia* ****Apiaceae, *Echium, Hedysarum,* Lamiaceae, *Rumex, Salix*	3 435

Honey sample number	Locality	Pollen spectra	TPN-10 g
9	Tekke Village	*Astragalus **Brassicaceae ***Asteraceae, Rosaceae ****_Eucalyptus, Hedysarum, Linaria, Rumex, Salix_	20 788
10	Geyikpınar Village	*Astragalus **Asteraceae ***Apiaceae, Brassicaceae, _Linaria_ ****_Hedysarum_, Rosaceae	44 699
11	Kırbaş Village	*Astragalus **Brassicaceae ***Asteraceae, _Echium, Hedysarum_ ****Apiaceae, Lamiaceae, _Linaria_, Rosaceae, _Rumex, Salix, Vicia_	9 503
12	Harmancık Village	*- **Astragalus, Hedysarum ***Brassicaceae, Lamiaceae ****Apiaceae, Portulacaceae, Rosaceae, _Salix_	3 417
13	Dikmen Village	*Astragalus **Brassicaceae ***Apiaceae, _Hedysarum,_ Rosaceae ****_Eucalyptus,_ Lamiaceae, _Linaria, Vicia_	16 112
14	Kuyumcu Tekke Village	*- **Asteraceae, _Astragalus_, Portulacaceae ***Apiaceae, Brassicaceae, Chenopodiaceae/Amaranthaceae, _Cistus_ ****_Eucalyptus, Linaria,_ Rosaceae	608 751
15	Başören Village	*- **_Astragalus,_ Brassicaceae ***Apiaceae, _Hedysarum,_ Lamiaceae, Rosaceae, _Vicia_ ****Asteraceae, _Eucalyptus, Linaria,_ Pinaceae, _Plantago_	4 915
16	Dudaş Village	*- **_Astragalus_, Brassicaceae ***Apiaceae, _Eucalyptus, Hedysarum, Linaria,_ Rosaceae, _Vicia_ ****_Echium,_ Lamiaceae, _Salix_	20 273
17	Kargı Village	*- **_Astragalus,_ Brassicaceae ***_Echium, Hedysarum,_ Lamiaceae, _Linaria_ ****Apiaceae, _Eucalyptus,_ Moraceae, _Vicia_	7 743

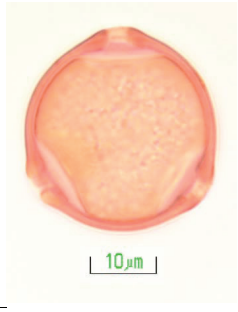


Apiaceae

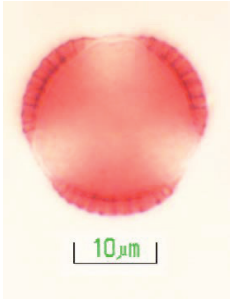


Asteraceae

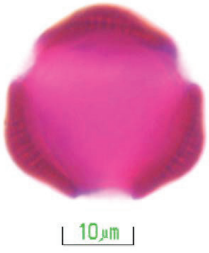
Astragalus



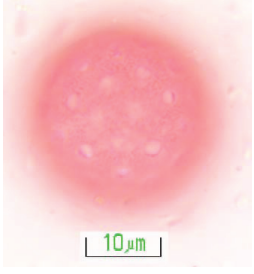
Betulaceae



Brassicaceae



Centaurea



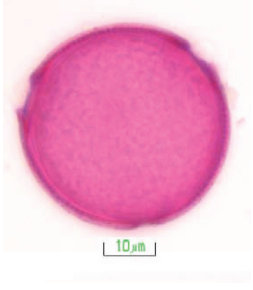


Figure 1. Microphotographs of pollen grains were found within the Beypazarı honey samples

Ankara İli Beypazarı İlçesinin (Türkiye) Ballarında Polen Analizi

ÖZ

Ankara ili Beypazarı ilçesinin 17 farklı yöresinden toplanan bal örneklerinde polen analizi yapılmıştır. 17 bal örneğinden 9 tanesi unifloral, 8 tanesi ise multifloral bal olarak tespit edilmiştir. Yöre ballarında 11'i familya ve 13'ü cins düzeyinde olmak üzere toplam 24 taksonun poleni teşhis edilmiştir. Polenlerine dominant miktarda rastlanan taksonun Astragalus olduğu belirlenmiştir. Yapılan analizler sonucunda, Astragalus'un yöre balları için başlıca nektar ve polen kaynağı olduğu tespit edilmiştir.

Anahtar Kelimeler: Bal, polen analizi, TPS-10 g, tek çiçek kaynaklı bal, çok çiçek kaynaklı bal

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MELLIFERA RESEARCH ARTICLE

On the Potential Pest Risk of the Hive Beetles *Oplostomus fuligineus* and *Oplostomus haroldi* (Coleoptera: Scarabaeidae) to European Beekeeping

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ABSTRACT

The hive beetles *Oplostomus fuligineus* Olivier, 1789 and *Oplostomus haroldi* Witte, 1880 (Coleoptera: Scarabaeidae) predate on Apis mellifera Linnaeus, 1758 brood and are considered pests in southern and eastern Africa respectively. However, little is known about their biology and potential to expand to other geographic areas. This study uses a Pest Risk Analysis (PRA) scheme to assess the hazard the two Scarabid beetles possess to European beekeeping. The risk of entry and establishment in the European Union is evaluated as is their potential economic impact. For this purpose, the information about the species ecology, current distribution and Apis mellifera pest status are revised. It is concluded that, although the pest status of the species may be limited in Africa, it may be prudent to implement measures that would avoid the future possible introduction of the pest into Europe. Our knowledge on *Oplostomus fuligineus* and *Oplostomus haroldi* biology are minimal. It is vital that more information on the Scarabids ecology, especially on its possible alternative hosts and control, is gathered in order to prevent their introduction to Europe.

Keywords: *Oplostomus fuligineus*; *Oplostomus haroldi*; pest risk assessment; European Union; honey bee; *Apis mellifera*

1. Introduction

The honey bee (*A. mellifera*) is considered a key pollinator of a range of agricultural and horticultural crops [1]. However, recently elevated bee colony mortality is being observed around the world [2, 3]. The causes of such declines are challenging to identify [4], but pests and diseases are among the key threats to bees.

An important risk to beekeeping is presented by exotic pests. These may have little effect on the host within their native range, but may prove devastating when introduced and established outside their native range. Among the many examples, *Aethina tumida* Murray, 1867 (Coleoptera: Nitidulidae) which is a minor importance in Africa became a major pest

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of *A. mellifera* in America and Australia when it was introduced there [5]. The ectoparasitic mite *Varroa destructor* Anderson & Trueman, 2000 (Acari: Parasitiformes: Varroidae) presents a similar case, it is native to *Apis cerana* Fabricius, 1793 in Asia, but today causes serious honey bee colony losses around the world [6]. In order to prevent further introductions of alien bee pests, it is important to identify potentially dangerous species in advance. Then, regulations aiming at preventing the species establishment should be implemented.

The hive beetles *Oplostomus fuligineus* and *Oplostomus haroldi* (Coleoptera: Scarabaeidae) predate bee brood in southern and eastern Africa respectively, and are considered pests in the area. Despite this, so far little work aimed at preventing the pest spread to other continents. In order to assess the danger possessed by exotic species, Pest Risk Analysis (PRA) is carried out. PRA schemes evaluate if a pest should be regulated or measures should be taken against it. PRA schemes are frequently used in plant protection science to justify the implementation of phytosanitary measures. For the purpose of evaluating the risk *O. fuligineus* and *O. haroldi* possess to beekeeping in the European Union, the PRA framework developed by the European and Mediterranean Plant Protection Organisation (EPPO) [7] was adapted for these two coleopterans. In addition, the biology of the beetles was revised.

2. Material and Methods

The scheme developed by EPPO [7] consists of two parts. In the first one, a set of yes/no questions are answered to establish whether the species has characteristics of a quarantine pest, the second part consists of a set of 48 questions that are answered by grading from 1 to 9, to explore the entry and establishment potential of the species and to estimate their potential economic impact. In this study, only the second part of the scheme was used. The aim of this paper is not to urge legislation change regarding the beetles, but objectively assess the risk of entry and establishment of *O. fuligineus* and *O. haroldi* in the EU and the risk the beetles pose to the beekeeping industry.

Answering the 48 EPPO questions about the risk of the species entry and establishment requires assigning 1-9 point scores to the questions. Low scores mean low or unlikely impact, while high scores mean high or likely impact. Answering these questions relays on a professional judgement of the expert [8]. In order to make the judgement more objective, the guidelines developed by MacLeod & Baker [9] were followed.

3. Results and Discussion

3.1 Biology of *Oplostomus fuligineus*

The black hive beetle (also known as the Large hive beetle or the Large African hive beetle) (*O. fuligineus*) is known to be a pest of the honey bee (*A. mellifera*) since the early 1900s in the south of Africa. Inside beehives, it feeds on open or freshly capped bee brood, stored pollen and honey.

Depending on the time of the year, usually one to six eggs are laid in a batch. White oval eggs are laid in soil or dung and take on average 7.9 days to hatch. The larval stage takes on average 33 days, larvae are able to feed on dung, compost and soil. The pupal stage usually takes 25 days. The adults live for a few months under laboratory conditions [10].

The black adult beetles occur in beehives from October to late May. The imago stage probably overwinters outside hives. Adults feed preferentially on young bee brood and do not accept old brood under laboratory

conditions. To a smaller extent, stored honey and pollen is accepted [10, 11]. The adults can survive only with honey and pollen under laboratory conditions, but the fecundity of the beetles drops dramatically. The infestations of bee hives range from a few to more than 700 individuals, but on average about 65 beetles can be found in a hive (see [10]). The beetles damage both small and large batches of brood [11] thus weakening or even killing the colony [12].

The species is known from the South African Republic, Botswana and Namibia. Recently, the beetle was reported from Kenya for the first time [10-13].

3.2 Biology of *Oplostomus haroldi*

The hive beetle *O. haroldi* is a bee pest from eastern Africa, where it consumes bee brood, stored pollen and honey [14].

The beetles mate inside the hive. The females then leave the hive to lay eggs. The eggs are laid in small batches of 1-3 into soil or dung under laboratory conditions. Moist soil is preferred for oviposition. Eggs hatch into a curved larva in about 12 days. The larval stage takes on average 68 days. The pupal stage lasts about 31 days. The adults live for two to six months [14].

The beetle has a similar diet as *O. fuligineus,* consisting of brood and also stored pollen and honey [11].

The species is known from Kenya and Tanzania. In Kenya, its distribution range does not overlap with the one of *O. fuligineus*, indicating different environmental preferences. It seems that *O. haroldi* preferred coastal areas and highlands [11]. *O. haroldi* has been recorded from bee colonies from 11 to 1100 metres above sea level [13]. Nevertheless, the lack of large-scale biogeographical surveys means that the full ecological preferences of the species are not known in detail.

3.3 Hosts

There is limited knowledge on alternative host of *O. fuligineus* and *O. haroldi*. Both species are known to occur in beehives in southern and eastern Africa in high numbers; however the host spectrum may be more diverse. More information on the alternative hosts of the hive beetles is necessary to prevent its introduction into Europe.

Dung or soil is required for *O. fuligineus* development. Under laboratory conditions, adults accept grapes and figs and feed on the flowers of *Acacia karoo*. In field, the large hive beetle is rarely reported from flowers, but has been caught in traps with banana bait. Apart from feeding on the brood of *A. mellifera,* it has been observed feeding on the brood of the paper wasps *Polistes smithii* (Hymenoptera: Vespidae) and *Belonogaster* ssp. (Hymenoptera: Vespidae) [10].

Detailed information about alternative hosts of *O. haroldi* is lacking. Initial attempts to rear *O. haroldi* on banana failed [14].

3.4 The Potential for Entry and Establishment in the EU

To assess the likelihood of the hive beetle´s entry and establishment in the EU, the second part of the EPPO PRA scheme [7] was adopted. The original EPPO scheme is assesses the pest risk to plants, thus some of the questions were modified to better suit the nature of the honey bee pests. The scores and comments on each of the 48 questions are provided in Tab. 1.

The following section discusses the most relevant findings of the pest risk assessment.

Given the long developmental time of the beetle and a quiet high longevity, it is possible that *O. fuligineus* and *O. haroldi* that are

known to develop in soil or dung could be imported into Europe via horticultural trade. This is probably the way the Asian predatory wasp (*Vespa velutina*) was introduced into Europe. The wasp now presents a serious problem to the beekeeping industry in much of Western Europe, where it spread so far [15]. Theoretically, the beetle may also be imported with live bees and beekeeping supplies. This was the way *Aethina tumida*, a serious bee pest, was introduced to Portugal in 2004, but luckily eradicated [16]. Alternatively, the beetles may be accidentally introduced with a paper wasp's nest on board of ships. *O. fuligineus* prefers warmer areas [10] and may thus become established in warmer regions of Europe. On the other hand, *O. haroldi* may become established in the highlands and by coastal areas.

The scheme also takes previous interceptions of the organism into account. However, despite the high likelihood of *O. fuligineus* entry and its potential for establishment in warmer regions of Europe, to the author's knowledge, this species was never intercepted in a European port. This may be because *O. fuligineus* and *O. haroldi* are not listed separately in annual statistics [17].

3.5 Potential Economic and Ecological Impact in the EU

To assess the likelihood of the hive beetles´ entry and establishment in the EU, the second part of the EPPO PRA scheme [7] was adopted, results are displayed in Tab. 1. It is unknown what the impacts on the beekeeping industry may be, if the beetle would become indigenous to Europe. At a number of occasions, European bee stock proved to be more prone to parasitism by the mite *Varroa destructor* or the beetle *A. tumida* than the African bee stock [5, 19]. It is possible, that outside their native range, the hive beetles may become more destructive. If anything, the two hive beetles would become an important stress factor to European bees. In addition, controlling the beetles would exert a cost to the beekeeping industry.

Limited published data exist on the control of the hive beetles. The small hive beetle (*A. tumida*) is controlled, among others, by the use of "beetle traps". Similar traps could be developed for the two Cetoniids studied herein. In the future, Integrated Pest Management (IPM) programs for *O. haroldi* may include the use of biopesticides and odour-baited traps. During a laboratory rearing, some larvae died due to a fungal infection, the fungus was later identified as *Metarhizium anisopliae* [14]. Also, developing an odour-baited trap seems as a viable option for controlling the beetle [13], should it become established in the EU.

3.6 Discussion

From what is known on the biology of *O. fuligineus* and *O. haroldi* it seems it is quite similar between the two species. A number of Coleopterans are known to occur in beehives. In Africa, lower numbers of the Scarabid beetles *Anisorrhina flavomaculata*, *Diplognatha gagates, Poecilophila hebrae* and *Pachnoda rufa* or *Dischista cinta* (the last two species are hard to distinguish) were reported from beehives [10]. In light of the present findings, it is urged that their pest status is revised.

Although the true impact of the hive beetles on European beekeeping is debatable, it is important to prevent their introduction and establishment. It will be crucial to monitor and report the interceptions of these pests in the European Union. It is not clear, which part of the EU is the most susceptible to *O. fuligineus* and *O. haroldi* establishment. It is advised that future studies use Species

Table 1. The PRA scheme [7] adapted and filled-in for *Oplostomus fuligineus* and *Oplostomus haroldi* potential pest status in the EU.

Entry and Establishent Potential	*O. fuligineus*	*O. haroldi*	Comment
How many pathways could the pest be carried on?	4	3	The smaller score for *O. haroldi* results from a lower number of known host species and a smaller distribution range.
How likely is the pest to be associated with the pathway at origin?	5	4	It is likely that the hive beetles may be exported via soil, with bee products or live bees etc.
Is the concentration of the pest on the pathway at origin likely to be high?	6	6	Depending on the pathway. It is almost certain the beetles would be exported with bee-related supplies, but less likely with soil.
How likely is the pest to survive the existing commercial practices?	8	8	The recently new records from within Africa suggest its ability to survive transport practices.
How likely is the pest to survive or remain undetected during existing porcedures?	7	7	No detection practices aiming specificaly at these beetles exist. Given the possibility of transport via soil, it may be undetected.
How likely is the pest to survive in transit?	7	7	The larvae can be transported in soil. Adults would only survive in the presence of bee products, bee brood, fruit or flowers.
How likely is the pest to multiply during transit?	1	1	Not possible, the transit lenght exeeds the development length.
How large is movement along the pathway?	2	2	The commodities may not be imported to every EU country.
How widely is the commodity to be distributed through the PRA area?	7	7	Once the goods have arrived, they may be distributed anywhere along the EU.
How widely spread in time is the arrival of different consigments?	3	3	The commodities are likely to be imported in different times of the year.
How likely is the pest to be able to transfer from the pathway to a suitable host?	6	6	*O. fuligineus* reporduction requires bee brood, *O. haroldi* reproduces in bee colonies. The honey bee is widespread through the EU.
Is the intended use of commodity likely to aid introduction?	1	1	Not relevant for the hive beetles commodities.
How many hosts are present in the PRA area?	1	1	The main host would be the single species A. mellifera.
How extensively are the hosts present in the PRA area?	8	8	The honey bee is kept throughout most of the EU.
If an alternate host is needed to complete the life cycle, how extensively are such hosts distributed in the PRA area?	X	X	No alternate hosts are required for completing the life cycle. Not relevant, therefore not scored.
If a vector is needed for dispersal, how likely is the pest to become associated with a suitable vector?	X	X	Not relevant, therefore not scored.
Has the pest been recorded on protected host alswhere?	X	X	Not relevant, therefore not scored.
How likely are feral hosts to be significant in the dispersal or maintance of the populations?	9	9	Wild bee colonies may host the pest. Feral bee colonies are widespread along the EU and are hard to control.

Entry and Establishent Potential	*0. fuligineus*	*0. haroldi*	Comment
How similar are the climatic conditions that would affect pest establishment the the PRA area and in the area of origin?	3	2	Comparison hampered by lack of detailed temperature, relative humidity, total rainfall and rainfall patterns data in Africa. However, comparing the avaredge temperatures suggest that countries of the Medditerenean basin, esspecialy south Spain, Italy, Sicily and Greece are similar to most of South Africa.
How similar are other abiotic factors in the PRA area and in the area of origin?	3	2	Little data available but likely to be similar at least in the Mediterraninan basin.
How likely is the pest to have competition from existing species in the PRA area for its ecological niche?	1	1	Little native species reported to feed on bee brood-competition unlikely.
How likey is estabishment to be prevented by natural enemies alredy present in the PRA?	2	2	No published records on the species natural enemies reported form Africa. It is unlikely the species would have a natural enenmy, at least early during the establishment in the PRA area.
If there are differences in apicultural practices in the PRA area and the area of origin, are they likely to aid establishment?	7	7	Greater extent of migratory beekeeping may aid dispersal through the EU.
Are the control measures which are aleredy used against other apicultural pests likely to prevent establishment of the pest?	2	2	In Europe, no beetles are controled within the hives. It is possible that medication used against *Varroa destructor* may be effective agaisnt the hive beetles, but seems unlikely.
Is the reproductive strategy and the duration of the life cycle likely to aid establishment?	3	3	No, the life cycle is long which will not aid the establishment.
How likely are relatively low populations of the pest to become established?	7	7	Likely, a number of bee pests in the history became established on a new territory following the introduction of a few individuals (*Vespa velutina* in France, Varroa destructor in the Czech Republic)
How probable is that the pest could be eradicated form the PRA area?	4	4	Eradication of the species was not attempted before, but our experience with eradicating *Aethina tumida* in Italy suggests the process may be complicated.
How genetically adaptable is the pest?	X	X	Little knowlede on the species genetic adaptability hampers objective decison.
How often has the species been intoroduced outside its native range?	3	2	Only spread regionaly, or to neigbhouring countries. However, this may be as a result of lack of migratory beekeeping in the area of origin.
How important is the economic loss caused by the pest within its existimg geographical range?	3	3	No published estimates available, the beetles may act in syngerny with other factors to elevate bee colony losses.
How important is the environmental damage caused by the pest within its existing geographical range?	5	5	Weakening and killing bee colonies has massive impact on pollination availibility for agricultural and horticultural crops including wild plants.
How important is the social damage caused by the pest within its existing geographical range?	4	4	Lowering beekeepers and farmers profit.

Entry and Establishent Potential	*O. fuligineus*	*O. haroldi*	Comment
How extensive is the part of the PRA area likely to suffer from the pest?	2	2	Meditarenan basin, but it remains unknown if the species could spread further.
How rapidly is the pest liable to spread in the PRA area by natural means?	3	3	Does not spread very quickly on their own, which may explain their small distribution range in Africa.
How rapidly is the pest liable to spread in the PRA area by human assistance?	6	6	Apicultural pests spread rapidly with human activity (*Varroa destructor, Aethina tumida*)
How likely is it that the spread of the pest could be contained within the PRA area?	6	6	Likely, mainly due to climatic differences.
Considering the ecological conditions in the PRA area, how serious is the direct effect of the pest on bee products quantity and/or quality likely to be?	3	3	The yield of honey would decrease during the early years of introduction, possibly elevating the price. However, due to climatic conditions, only a part of the EU may be prone to the damage.
How likely is the pest to have a significant effect on producer profits due to changes in the production cost, yields etc. in the PRA area?	X	X	Impacts in Africa unpublished, potential impacts in EU uncertain.
How likely is the pest to have a significant effect on consumer demand in the PRA area?	3	3	Consumers in the affected regions would need to look for foraign supplies as an alternative to the growing prices.
How likely is the presence of the pest in the PRA area to affect export markets?	4	4	As has been the case with the introduction of *A. tumida* in Italy in 2014, the EU is likely to impose a stop to bee product export from the affected area to avoid further pest spread.
How important would other costs resulting from the intrdiuction be?	6	5	More research would be required on the control of the pests.
How important is the environmental damage likely to be in the PRA area?	4	4	Decrease in pollination in the invaded areas resulting into lowered plant yields, loss of plant biodiversity and increased likelyhood of soil erosion and flood risk.
How important is the social damage likely to be in the PRA area?	4	4	Beekeeping may become unprofitable to many hobby beekeeprs who may leave the industry.
How probable is it that the natural enemies, aleready present in the PRA area, will affect the populations of the pest if introduced?	3	3	No records of natural enemies are knwon from Africa, it seems that the situation may be similar inn Europe.
How easily can the pest be controlled?	6	5	No published data on the pest control are available, research needed.
How likely are control measures to disrupt existing biological or integrated systems for control of other pests?	3	3	Medication of the bees against the ectoparasitic mite Varroa destructor is rutine in the EU.
How likely are control measures to have other undesirable side-effects?	5	5	If control measures will include the use of pesticides, biocides or baited traps, side-effects may be expected.
Is the pest likely to develop resistance to control products?	X	X	No resisatnce reported.

Distribution Modeling (SDM) to predict the high-risk areas for pest establishment.

The gaps in our knowledge of the biology of these two hive beetles are still considerable. This study especially stressed the importance of detailed knowledge of the beetles alternative hosts and control. Also, the mechanisms involved in the hive beetles orientation warrant further study. So far it seems that food selection depends on olfactory and contact cues [11,18].

4. Conclusion

The honey bee (*Apis mellifera*) provides essential pollination services, but sustainable beekeeping is hampered by a wide range of pests. Many of these pests are now globally distributed, but in their native range cause little damage. For the future sustainability of beekeeping it is important that potentially important pests are identified in advance and their introduction is prevented. This study focused on two African hive beetles, *Oplostomus fuligineus* and *Oplostomus haroldi*. A Pest Risk Assessment (PRA) scheme was adopted for this purpose. The known biology of the two beetles was revised. The PRA scheme revealed that the beetles may be introduced to Europe with soil via horticultural trade, with bees, bee products or on alternative hosts that include certain fruits or in paper wasp nests. The Mediterranean basin may be particularly susceptible to the hive beetle´s establishment, due to climate similarities. Although the impact of the hive beetles on European beekeeping is debatable, it is advised that high risk commodities which may contain the hive beetles are inspected thoroughly for *O. fuligineus* and *O. haroldi* so their introduction is prevented. In addition, it will be crucial to gain more knowledge on the species biology, especially control and alternative hosts.

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Yuva Paraziti Böceklerin, *Oplostomus fuligineus* ve *Oplostomus haroldi* (Coleoptera: Scarabaeidae), Avrupa Arıcılığı Üzerine Potansiyel Riskleri

ÖZ

Yuva paraziti böcekler *Oplostomus fuligineus* Olivier, 1789 ve *Oplostomus haroldi* Witte, 1880 (Coleoptera: Scarabaeidae) Apis mellifera Linnaeus, 1758 yavruları üzerinde predatör olup, sırasıyla güney ve doğu Afrika'da zararlı olarak kabul edilmektedir. Buna rağmen, biyolojileri ve diğer coğrafik bölgelerde oluşabilecek potansiyel riskler ile ilgili bir çalışma bulunmamaktadır. Bu çalışmada iki Scarabid böceğinin Avrupa arıcılığı için oluşturulmuş zararlı risk değerlendirmesi şeması kullanılmıştır. Avrupa Birliği'ne girişi ve bu bölgede yayılış göstermeye başlamasıyla oluşabilecek potansiyel ekonomik etkiler değerlendirilmiştir. Bu kapsamda, türlerin ekolojileri, şu anki dağılışları ve Apis mellifera zararlı statüleri revize edilmiştir. Bu zararlıların, bugün itibariyle yayılış alanlarının Afrika ile sınırlı olduğu tespit edilmişse de, gelecekte Avrupa üzerinde de yayılış gösterebileceği düşünülmektedir. *Oplostomus fuligineus* ve *Oplostomus haroldi* böceklerinin biyolojilerine ilişkin bilgimiz kısıtlıdır. Bu böceklerin ekolojileri, özellikle de konakları ve bunların kontrolü hakkında bilgi sahibi olmak Avrupa'ya olabilecek olası girişlerin kontrolü açısından çok önemlidir.

Anahtar Kelimeler: *Oplostomus fuligineus; Oplostomus haroldi*; zararlı risk değerlendirilmesi; Avrupa Birliği; bal arısı; Apis mellifera

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Losses of honey bee colonies over the 2015/16 winter

Published November 17, 2016

Preliminary results from an internationalstudy

The honey bee research association COLOSS1 has today announced the preliminary result soft heir international study of colony losses over the 2015-16 winter. Data were collected from[2] 9 countries in this initiative, which is the large stand longest running international study of honeybee colonylosses. Intotal 18, 693 respondents provided over wintering mortality and other data of their colonies. Collectively, all responding beekeepers managed 399, 602 honeybee colonies. The over all proportion of colonies lost was estimated as 11.9%.

Co-Chairs of the COLOSS Core project for colony losses monitoring Dr Alison Gray and DrRobertBrodschneider say: "These loss rates vary considerably between countries. In this year's survey thehighestlosses were found in Ireland and Northern Ireland, followed by Wales and Spain. The pattern of lossratesdiffers from last year, when higher mortality and loss rates were found in central Europe and countries totheeast. This year the higher loss rates tend to be in the west and northern countries, although Spain hadhighrates of loss in both years. All the loss rates quoted here include losses due to unresolvable queen problem safter winter, as well as colonies that died over winter for various reasons. Losses due to queen problems were unexpectedly high in some countries and this will be a matter of further investigation."

The protocol used to collect this COLOSS data has been internationally standardized 2,3 to allow comparisonsand joint analysis of the data. A more detailed analysis of risk factors calculated from the whole dataset ,aswell as further colony loss data from other countries, will be published later in theyear.

Romée van der Zee of the COLOSS Core project for colony losses monitoring says: "Spring and early summer (March-July) were cold in Norway, Scotland, Sweden, Denmark and Ireland, with mean temperatures ranging from 12.8 - 14.4 °C. This may have had negative effects on colony development, resulting in both relatively high numbers of dead colonies and unsolvable queen problems after winter. A more detailed analysis may reveal the effects of other important factors, such as the role of the honey bee parasite Varroadestructor."

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[1] COLOSS is a honey bee research association formerly funded by the European UnionCOSTProgramme (Action FA0803) and currently by the Ricola Foundation – Nature & Culture, whichaimsto explain and prevent massive honey bee colony losses. COLOSS does not directly supportscience,but aims to coordinate international research activities across Europe and worldwide,promotingcooperative approaches and a research programme with a strong focus on the transfer ofscienceinto beekeeping practice. COLOSS has 781 members drawn from 91 countries worldwide. ItsPresident is Prof. Peter Neumann of the University of Bern,Switzerland. Websitehttp://www.coloss.org/

[2] The standard protcols are available in The COLOSS BEEBOOK . Volumes 1 and 2 are availableonlineat: http://ibrabee.org.uk/index.php/component/content/article?layout=edit&id=3664

[3] The COLOSS BEEBOOK Volume 2 is available in hard copyfrom:- http://ibrabee.org.uk/index.php/component/k2/item/3028

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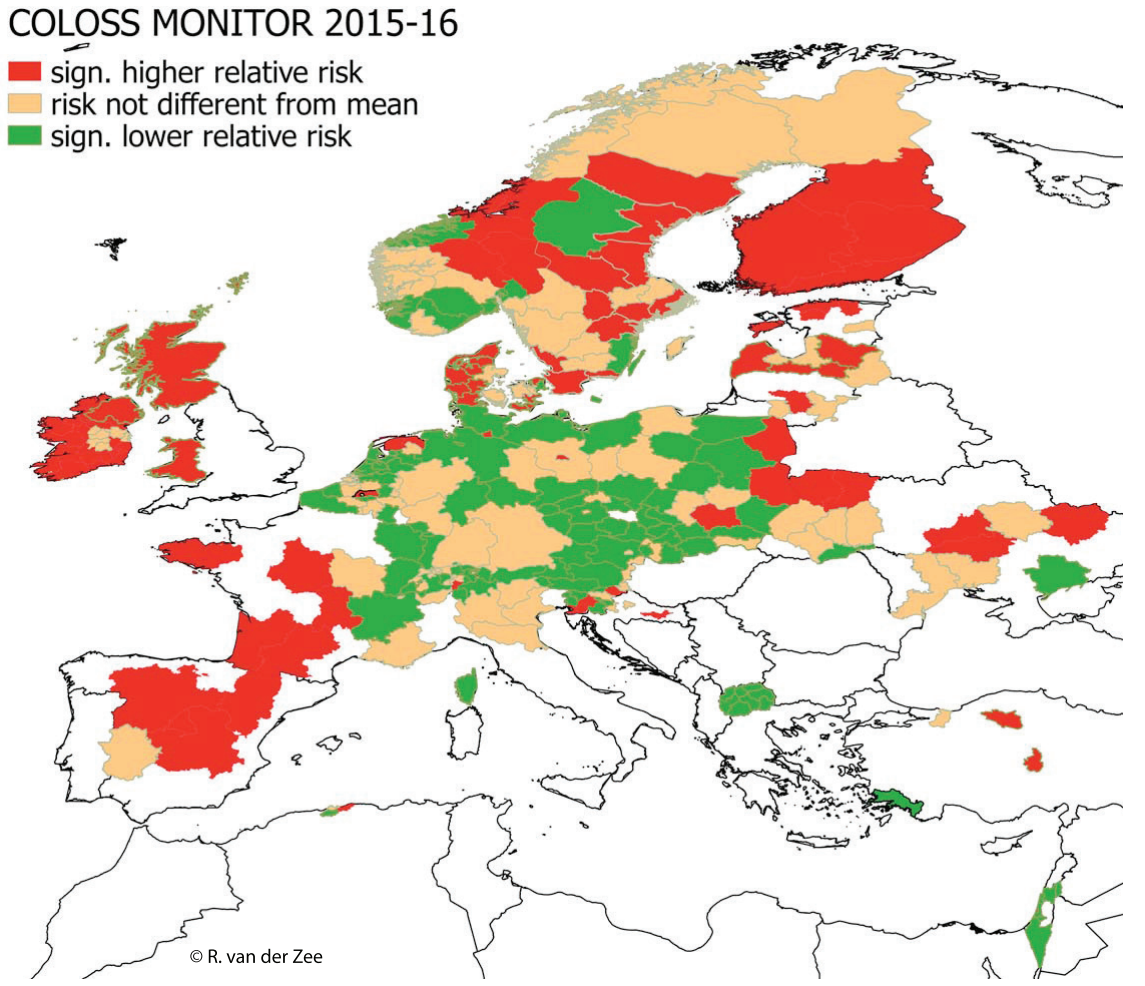
2015-2016 Yılı Kış Sonu Bal Arısı Koloni Kayıpları Uluslar Arası Bir Çalışmanın Ön Sonuçları

Bal Arısı Araştırmaları Derneği COLOSS'un, uluslar arası bir çalışması olan 2015-2016 yılı kış sonu bal arısı koloni kayıplarının ön sonuçları sunulmuştur. Bal arısı koloni kayıplarının uluslar arası seviyede yürütülen en uzun soluklu ve en büyük çalışması olan sözkonusu çalışmanın dataları 29 ülkeden toplanmıştır. Kolonileri hakkında kış sonu kayıpları ve diğer bilgileri toplamda 18.693 katılımcı kaydetmiştir. Bilgi sağlayan tüm arıcıların yönettiği arı kolonisi sayısı 399.602'dir. Tüm değerlendirmeler sonucunda bu yıla ait koloni kaybı oranı %11,9 olarak hesaplanmıştır.
Koloni kayıplarının ülkeler arasında büyük oransal farklılıklar gösterdiği rapor edilmektedir. Bu yıla ait araştırmada en yüksek koloni kayıpları İrlanda Kuzey İrlanda'da meydana gelmiştir. Söz konusu rakamları Galler ve İspanya'daki kayıplar izlemektedir.Kayıplara ait bulgular geçen yıldan büyük farklılık göstermektedir. Geçtiğimiz yıl en büyük ölüm ve kayıp oranı Orta Avrupa ve Doğu ülkelerinde görülmüşken, bu yıl yüksek kayıplar Batı ve Kuzey ülkelerinde rapor edilmiştir (İspanya verileri her iki yılda da yüksek görünmektedir).


COLOSS MONITOR 2015-16
sign. higher relative risk
risk not different from mean
sign. lower relative risk
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Preliminary results for COLOSS monitoring group for press release on bee losses spring 2016
Note: All results should be regarded as preliminary; a more detailed final report is being prepared for later publication.

Country	No. of respondents	No. of colonies going into winter	Overall no. of colonies lost (dead colonies and colonies lost due to queen problems)	Overall winter loss rate (%)	95% confidence interval for overall winter loss rate Lower limit	95% confidence interval for overall winter loss rate Upper limit	Estimated no. of beekeepers in country in 2015
Austria	1289	23418	1897	8.1	7.4	8.8	25000
Belgium	451	4064	494	12.2	10.5	14.0	9490
Czech Republic	968	17350	1107	6.4	5.8	7.1	57000
Denmark	1186	12359	1913	15.5	14.4	16.7	6200
Estonia	71	5115	792	15.5	12.2	19.5	5969
Finland	299	6072	1043	17.2	15.4	19.1	3000
France	488	36734	4926	13.4	12.2	14.7	41850
Germany	4772	57165	6118	10.7	10.3	11.2	110000
Ireland	427	4059	1199	29.5	27.4	31.7	3000
Israel	49	32165	3362	10.5	8.2	13.2	500
Latvia	472	16367	2462	15.0	13.1	17.2	4300
Macedonia	296	17288	1378	8.0	7.1	8.9	3000
N.Ireland	93	574	162	28.2	22.6	34.6	1000
Netherlands	1425	11815	1276	10.8	9.9	11.7	7000
Norway	743	13249	1604	12.1	11.0	13.3	3500
Poland	492	17822	2012	11.3	10.2	12.5	57550
Scotland	154	701	126	18.0	14.6	21.9	1400
Slovakia	276	6783	553	8.2	6.8	9.7	17170
Slovenia	267	7910	1127	14.2	11.8	17.1	9000
Sweden	2092	25403	4039	15.9	15.1	16.8	13500
Switzerland	1259	17813	1769	9.9	9.2	10.7	17500
Ukraine	399	13850	1368	9.9	8.5	11.4	254010
Countries with a data set mostly for a limited number of regions							
Algeria	59	5729	759	13.2	11.0	15.9	20000
Italy	309	6815	855	12.5	10.9	14.5	55000
Spain	113	10786	2388	22.1	18.7	26.0	25535
Turkey	139	22160	1702	7.7	5.7	10.2	83467
Countries with limited data at this time							
Croatia	62	4303	706	16.4	11.6	22.7	12500
Lithuania	43	1733	324	18.7	14.4	24.0	not available
Wales	39	232	52	22.4	16.0	30.4	4110
Totals	18693	399602	47461	11.9			847441

MELLIFERA
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Contents / *İçindekiler*