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RESEARCH PAPER

Antioxidant Variability of Propolis Collected from Different Zones in Hives

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Abstract

Propolis is biologically highly active honey bee product. The popularity of propolis is increasingly growing because of its contribution to human health. Propolis composition is highly variable depending on its sources. Different honey bees subspecies can collect propolis having different anti-bacterial effect. Honey bees, collect propolis for a couple of different purposes such as narrowing the entrance of their own hives and airflow isolation. In this study, propolis collected from entrances (EC) and top of deep supper (FC) of six different hives, and the antioxidant properties of these propolis samples were compared. Average values of total phenolic content were 68.5 and 62.6 mg GAE/g propolis extract, IC_{50} value of DPPH were 0.14 and 0.16 mg/mL, and FRAP value were 43.5 and 38.4 mg TE/g propolis extract samples collected from EC and FC, respectively. Statistically significant differences have not been found in terms of antioxidant activity analysis between EC and FC collected propolis ($P>0.05$).

Introduction

Propolis (bee glue) is collected from variable plant sources and the name came from the Greek, pro-for or in defence, and polis- the city, and means is defence of the city (or the hive) (Ghisalberti, 1979). Propolis is used by bees for versatile purposes such as covering holes and cracks, repairing combs, sticking the border combs, narrowing the entrance of the hive for easy defending, and embalming the aliens (Ghisalberti, 1979). The composition of propolis is highly variable depending on collected sources, and the most important botanical propolis sources are poplars (*Populus* spp.), briches (*Betula* spp.), willows (*Salix* spp.), chestnut tree (*Aesculus hippocastanum* L.), elms (Ulmus spp.), pine trees (*Pinus* spp.), oaks (*Quercus* spp.), spruces (*Picea* spp.) and ashes (*Fraxinus* spp.) (Bonvehí & Coll, 1994; Greenaway, Scaysbrook, & Whatley, 1990). Marcucci (1995) and Bankova, Christov, Kujumgiev, Marcucci, and Popov (1995) identified that propolis have more than 300 constitutes. It is reported that-variable biological activities of the ethanolic extract of propolis such as hepatoprotective effect (González et al., 1994), antitumor activity (Mitamura et al., 1996), antioxidative activity (Matsushige, Kusumoto, Yamamoto, Kadota, & Namba, 1995), antimicrobial activity (Bankova et al., 1995), and anti-inflammatory effect (Krol et al., 1996).

Honey bees accumulate propolis to entrances of their hives for narrowing, to top of deep supper the hives for air isolation, between the combs for sticking the combs, and some more hive region. There are various research indicate that the composition of propolis not only be affected from environmental and botanical origin factors and this can be affected from the race of bees and bees diseases as well (Popova, Antonova, & Bankova, 2017; Silici & Kutluca, 2005).

Honey bees can consciously prefer to choose the different resource of propolis in nature. Bees accumulate propolis to the entrance of hive for narrowing the entrance and maybe grooming themselves with propolis,

but bees accumulate propolis to top of deep supper for airflow isolation. Hypothesis of this study is that the antioxidant effect of propolis collected from the entrance of the hive and top of deep supper the hives can be different due to bees collect this propolis for different purposes.

Materials and Methods

Propolis collected from 6 colonies in Apicultural Research Institute apiary. The raw propolis were collected from two different zones in hives; the entrance (EC) of the hives and the top of deep supper the hives (FC). Collected raw propolis extracted by using 70% ethanol solution. Samples placed to erlenmeyer flasks and ethanol solutions were added. Propolis/solvent ratio was 3:10 (m/V). Maceration lasted 7 days at room temperature. Samples were filtered through the filter page after maceration and were held at 4ºC for one day. Samples were filtered again with filter page, then ethanol vaporized at the rotary evaporator. Thus, extracted propolis obtained.

Extraction of propolis samples

0.1 g from each propolis sample weighed and ethanol added up to 10 mL in falcon tubes. Samples left at 4°C for one day, then all samples centrifuged 10 min at 5000 rpm. The supernatant was used for antioxidant analysis (Elmastas, Isildak, Turkekul, & Temur, 2006; Lachman, Orsak, Hejtmankova, & Kovarova, 2010).

Determination free radical scavenging activity (DPPH)

Trolox was used as standard for free radical scavenging activity analysis and activity was determined by reading absorbances of compounds at 517 nm which reacted with 1,1-diphenyl-2-picryl-hydrazil (DPPH). 3 mL ethanol and 1 mL DPPH were added to 80 µL sample solution. All absorbances obtained after holding of all samples at room temperature for 30 minutes (Shimada, Fujikawa, Yahara, & Nakamura, 1992).

Determination Total Phenolic Content (TPC)

200 µL from supernatants of each sample were transferred to tubes. 0.1 mL Folin-Cicoalteu reactive and 0.3 mL 2% Na_2CO_3 solution added and all tubes filled up to 5 mL with distilled water. A standard curve obtained by reaction of gallic acid with Folin-Cicoalteu. Absorbances of samples were read at 760 nm and results were calculated as mg GAE/g propolis extract (Gülçin, Şat, Beydemir, Elmastaş, & Küfrevioğlu, 2004; Slinkard & Singleton, 1977).

Determination ferric reducing ability of plasma (FRAP)

80 µL samples were transferred to tubes for determining FRAP value of each sample and 1.25 mL sodium phosphate buffer (0.2 M, pH 6.6) and 1.25 mL 1% potassium ferro cyanide solution were added, and solutions left for incubation at 50°C for 30 minutes. 1.25 mL 10% 3-chloro acetic acid solution and 0.25 mL 1% $FeCl_3$ solution were added after incubation. Trolox was used to obtain standard curve and all samples read at 700 nm at spectrophotometer (Oyaizu, 1986).

Statistical Analysis

IBM SPSS Statistics 20 program was used for statistical analysis. Kruskal Wallis h test was used to compare mean ranks of all values of 2 groups.

Results

The value of total phenolic content of propolis extract between 47.9 and 88.1 mg GAE/g propolis extract showed in Table 1. Even though the propolis were collected from different zones of the hive, there are no statistical differences between the groups (P>0.05). The result of DPPH was expresed IC_{50} values that ranged between 0.12-0.19 mg/mL and while average value for propolis EC collected was 0.14 mg/mL, average value for propolis FC collected was 0.16 mg/mL. IC_{50} values of all samples can be seen in Table 1. DPPH values of propolis samples collected from different areas of hives were not different statistically (P>0.05). FRAP values ranged between 32.9-58.9 mg TE/g propolis extract and average values propolis samples EC and FC collected were 43.5 and 38.4 mg TE/g propolis extract respectively. FRAP values of samples were also indifferent statistically (P>0.05).

Discussion

Free Radical Scavenging Activity Analysis (DPPH)

IC_{50} value of DPPH analysis expresses the concentration of antioxidant compound which required for scavenging 50% of free radicals found in medium. Marghitas, Dezmirean, Moise, Mihai, and Laslo (2009) obtained IC_{50} values between 0.3-5.6 mg/mL in the study which propolis samples collected from Romanian. Mercan et al. (2006) indicate that IC_{50} value range from 3.4 to 4.6 mg/mL. IC_{50} values calculated in this study were lower in contrast with values of these works. Talla et al. (2017) compare DPPH value of Cameroonian propolis and vitamin C. The IC_{50} value of Cameroonian is found 0.30 mg/mL. That value is higher than our findings. The possible explanation of this non-overlapping is that our propolis sample were collected Black See region of Turkey, but Talla et al. (2017)'s study propolis samples were collected from Cameroon.

Table 1. The TPC, DPPH and FRAP analysis results of propolis collected from FC and EC.

Samples	TPC	DPPH (IC_{50})	FRAP
FC1	57.0±3.76	0.17±0.03	33.3±2.45
FC2	60.0±2.60	0.13±0.04	37.9±2.52
FC3	54.0±3.21	0.13±0.03	36.2±1.60
FC4	68.2±4.05	0.19±0.04	34.0±1.04
FC5	64.8±3.33	0.17±0.02	43.6±3.23
FC6	71.4±5.49	0.14±0.04	45.5±3.21
Avg.	62.6±2.51	0.16±0.01	38.4±1.24
Min.	54.0±2.21	0.13±0.03	33.3±2.45
Max.	71.4±5.49	0.19±0.04	45.5±3.21
EC1	76.8±3.18	0.17±0.03	39.0±3.53
EC2	47.9±2.64	0.13±0.03	37.0±2.89
EC3	75.3±2.89	0.13±0.25	44.8±3.09
EC4	49.6±3.23	0.18±0.03	32.9±2.71
EC5	73.3±3.09	0.17±0.26	48.4±2.43
EC6	71.4±3.04	0.14±0.03	58.9±2.37
Avg.	68.5±5.29	0.14±0.06	43.5±3.78
Min.	47.9±2.64	0.12±0.03	32.9±2.71
Max.	88.1±4.01	0.18±0.03	58.9±2.37

FC: Propolis collected from top of deep supper the hives.
EC: Propolis collected from entrance of the hives.
All analysis were performed three times.
TPC (mg GAE/g propolis extract).
DPPH (IC_{50} mg/mL) and FRAP (mg TE/ g propolis extract) of propolis collected from FC and EC. ±standart error of mean.

Total Phenolic Content (TPC)

Total phenolic content was illustrated with gallic acid equivalency (GAE). The average of the total phenolic content of propolis EC and FC collected are 68.5 and 62.6 mg GAE/g propolis extract respectively (Table 1). There appears to be no previous research exploring about comparison propolis collected from EC and FC. Socha, Galkowska, Bugaj, and Juszczak, (2015) indicate that the total phenolic content of propolis range from 150.05 to 197.14 mg GAE/g propolis extract which are higher than our total phenolic content. The possible explanation is that Socha et al. (2015) collect propolis from Polland, but we collected our propolis from Turkey Black Sea region. Aliyazıcıoglu, Sahin, Erturk, Ulusoy, and Kolayli, (2013) study show that the phenolic content of the propolis collected from different part of Turkey is between 115 and 210 mg GAE/g. The potential explanation of these differences is that propolis collected region. Propolis collected from Black Sea region in this study, but in Aliyazıcıoglu et al. (2013) study propolis collected from different parts of Turkey. Also, different honey bees subspecies can be caused by this difference because it is well known that different honey bee species collect propolis from different propolis sources (Silici & Kutluca, 2005).

Ferric Reducing Ability of Plasma Analysis (FRAP)

Trolox equivalent (TE) value of FRAP analysis show the concentration of antioxidant compounds. Average FRAP value was 156.59 mg TE in Ozdal, Sari-Kaplan, Mutlu-Altundag, Boyacioglu, and Capanoglu (2018) study which samples collected from different regions of Turkey. Barlak, Değer, Ucar, and Çakıroğlu (2015) calculated average FRAP value as 59.5 mg TE in a similar work. Regional differences and use of different honey bee species may be caused these FRAP value differences. Ahmed et al. (2017) research indicate that FRAP value of propolis is calculated 62.5 TE/g that is compatible with our findings.

Conclusion

In this study, it is hypothesized that honey bees can behave selectively at propolis collection for different purposes in hives. Antioxidant activity of propolis collected from different zones of hives could be different due to this selective behaviour. However, there are no differences at antioxidant values of propolis collected from different zones. Similar future works from different areas which are rich for plant sources and contain additional analysis such as volatile compounds composition and phenolic composition have potential for exploring differences at propolis samples collected for particular purposes.

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REVIEW PAPER

The Use of Medicinal Aromatic Plants Against Bee Diseases and Pests

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Abstract

Nowadays, aromatic plants and oils have significant contributions in growth performance, regulation of the digestive system and struggle with disease factors in farm animals. In beekeeping, many synthetic drugs are used against bee diseases and pests. Medicinal aromatic plants and oils, whose beneficial effects are known, are used in beekeeping in order to overcome the mentioned problems by natural means. In multiple researches it has been reported that herbs and essential oils of thyme, clove, mint, lemongrass, cinnamon, grapefruit, rosemary, marigold, laurel, eucalyptus and tea tree have lethal effects against mites, bacteria and fungi. When used appropriate doses, aromatic oils do not cause any toxic effects on the bee colony and have no negative effects on colony development. In addition, the use of medicinal aromatic herbs and oils in beekeeping supports organic production, as well as preventing residue problems in bee products (honey, beeswax and others).

Introduction

In order to combat/fight against diseases and improve the overall bee health, researchers are constantly developing medicines for honey bees by the use of microbiology, molecular biology and chemistry tools (Tauber et al., 2019). The effects of medicinal herbs have been known since ancient times, and in traditional healing, these herbs have been used to treat diseases from past to present. With the development of the scientific perspective, the compounds and active substances contained in these plants were identified. Thus, mechanisms of action against microorganisms causing diseases in humans, animals and plants have been discovered. The reason for this is the search for natural or environmental-friendly compounds that can be effectively and safely used as an alternative to pesticides (Altundağ & Aslım, 2005).

Generally, aromatic plant essential oils are obtained by steam and/or water distillation from the plants themselves or related parts. They contain volatile compounds (alcohols, esters, aldehydes, ketones etc.), non-volatile compounds (paraffin, wax etc.) and hydrocarbons (terpenes, sesquiterpenes etc.) (Losa, 2001). Nowadays, both aromatic plants and essential oils are used in poultry, rabbit and ruminant diets. A wide variety of essential oils have multiple positive effects on animals in regards to growth performance, digestive system, etc. They have also been reported to contain bioactive compounds that have the potential to act as multi-functional feed supplements (Bento et al., 2013; Cho et al., 2006; Hundal, Wadhwa & Bakshi, 2016; Küçükyılmaz et al., 2017; Simitzis, 2017; Ünal & Kocabağlı, 2014; Zeng, Zhang, Wang, & Piao, 2015).

The antimicrobial activity of essential oils in both human, animal nutrition and overall health depends on the chemical content of the active ingredients. There are differences in the content even within the same plant species. It is known that medicinal and aromatic plants may have adverse effects when taken in higher doses. For this reason, the effectiveness of the plant or oil used in this treatment system must be evaluated and demonstrated with more detailed scientific research (Kammon, 2017; Niculae et al., 2009; Novak, Zitterl-Eglseer, Deans, & Franz, 2001).

In later periods, plant-based insecticides have been used in bee colonies to fight parasites and pathogens (Blenau, Rademacher, & Baumann, 2011). Recent studies have demonstrated that thyme oil, blueberry oil, eucalyptus oil, walnut leaf oil, bay leaf oil, blackgrass oil, pine oil, guar gum, andiroba oil, citronella oil, garlic extract and other vegetable oils were used on bees. This and similar plants have been demonstrated to positively affect both colony development, along with health and bees behaviour (Bekret, Çankaya, & Silici, 2015; Higginson, Gilbert, & Reader, 2007).

In traditional beekeeping, many drugs and insecticides such as acaricides, fumigants, antibiotics are used for both bee pests and diseases such as *Varroa destructor* (Varroa mite), *Acarapis woodi* (tracheal mite), *Galleria mellonella* (large wax moth), American foulbrood, European foulbrood, Nosema etc. (Mert, Yücel, & Kösoğlu, 2007). Unconscious use of drugs leads to the resistance of disease factors, damage to beneficial microorganisms for bees and colonies, as well as residue problems in products obtained from beekeeping. For these reasons, alternative treatments or control methods are needed.

Recent studies have shown that essential oils of plants such as thyme, clove, peppermint, lemon grass, cinnamon, grapefruit, rosemary, marigold, eucalyptus, tea tree have positive effects against some mites, bacteria and fungi. In addition, active ingredients such as sanguinarin, thymoquinone, capsaicin, carvacrol, citral, eugenol, thymol isolated from these plants have been reported to have these effects on organisms (Demirel, Keskin, & Kumral, 2019; Khan et al., 2019; Tutun, Koç, & Kart, 2018). Recently, organic acids and essential oils are among the natural treatment methods for fighting against diseases in beekeeping. The most important point to note is that although the methods used are considered as natural ingredients, the usage doses should not be exceeded (Glavan, Novak, Božič, & Kokalj, 2020; Pinheiro et al., 2019; Porrini et al., 2017). In general, beekeepers use essential oils as an alternative to synthetic drugs for rotational purposes and also to prevent residues in bee products. To summarize, essential oils and essential oil compounds are used as an alternative treatment model against synthetic drugs.

In this review, the studies about some aromatic plants used in beekeeping and the effects of plants on bee health have been discussed.

Aromatic Plants Used in Beekeeping

Since the middle ages, citrus essential oils (lemon, citrus etc.) have been widely used in medicinal products such as bactericidal, virucidal, fungicidal, antiparasitic, insecticidal. Additionally, today, their active ingredients are used in the pharmaceutical, healthcare, cosmetic, agricultural, food and beekeeping industries. The best method of removing essential oils from citrus vegetable tissue is steam distillation, resulting in various volatile molecules extracted, such as terpenes and terpenoids, aromatic components derived from phenol and aliphatic components (Albo et al., 2003; Palazzolo, Laudicina, & Germana, 2013). Some compounds of lemon oil, the pheromone nasonov contains geranium, citral and geraniol. Lemon oil is used in different ways with honey bees, such as supplemental foods to increase the quality of the bees' food, encourage them to eat, soothe the bees and introduce the new queen. In addition, it has been claimed that the use of lemon balm (*Melissa officinalis* L.) in the hive is effective in attracting honeybee swarms (Allam & Zakaria, 2009; Burgett, 1980; Manning, Speijers, Harvey, & Black, 2010). Thyme (*Thymus vulgaris* L.) is a perennial herbaceous plant with a wide distribution area, having aromatic flowers (pink or white) and woody stems. Thyme oil, obtained by distillation from the flowering branches, is rich in thymol and carvacrol. Thymol is one of the most used essential oils in the treatment of varroa mites (*Varroa destructor*). Again, all the essential oils in the *Lamiaceae* (peppermint) family can be used to help control varroa. The mint plant, which has antifungal characteristics, is also used for the treatment of Chalkbrood or for the prevention of other diseases (Albo et al., 2003; Kouache et al., 2017; Tutkun, 2016). Rusenova and Parvanov (2009) demonstrated that the most powerful essential oils were reported in cinnamon, lemon grass and thyme plants according to the dilution method of agar. In general, mint is used as pheromone masking aroma due of its active compounds for bee pheromones. Curly peppermint (*Mentha x piperita* L.) is usually used with lemon oil during feeding to improve the health of the hive, is also used in the fight against varroa mites. Thymol and eucalyptus oils are known to be used to control varroa mites (Ariana, Ebadi, & Tahmasebi, 2002; Ismail, Ghoniemy, & Owayss, 2006). Bakar et al. (2017) evaluated the efficacy of eucalyptus, neem, lemon and orange oil at different doses against percent mortality of Varroa mites. Their results showed that the highest mortality percentage was observed in eucalyptus oil (76.13%) at 2.5 mL dose. It has also been reported that menthol is used for the control of tracheal mites. It has been established that all essential oils, with the exception of orange oil, are used to combat varroa mites. For example, in a recent study of Bakar et al. (2019) *in vitro* studies were conducted in order to assess the efficacy of multiple essential oils against percent mortality of *V. destructor* mites. Their experiments showed that after 72 hours at 400 ppm concentration, neem and eucalyptus oil showed the most promising results with a mortality percentage of 90% and 83.3%, respectively (Bakar et al., 2019). It is reported that the grey partridge (*Perdix perdix*) can be used to control the trachea mite and is useful against the small beetle when using the tea tree varroa (Amrine, Noel, Mallow, Stasny, & Skidmore, 1996a; Gashout & Guzmán-Nova, 2009; Imdorf, Bogdanov, Ochoa, & Calderone, 1999).

Studies on Bee Health of Medicinal Aromatic Plants and Oils

The studies of aromatic plants and oils which were used against diseases and pests of bees in recent years are summarized in Table 1.

The production potential of honey bee colonies depends on the colonies' health status. The use of aromatic plant extracts in late winter together with protein feed offered to bee colonies can be regarded as a method that has a positive effect on both improving bee health and colony's honey production. It has been reported that feeding bees with enriched garlic, echinacea, red reishi mushrooms and protein enriched sugar containing sage extracts increases the incubation of queen bee, compared to the vegetable extract used in the number of bacterial groups in the worker bees ((Pătruıcă, Moț, & Popovıcı, 2017).

The herbal products used in Varroa control are generally inexpensive and most of them pose little health risk. Terpenes (mainly monoterpenes) are the main compounds that make up 90% of the essential oils. In laboratory screening tests, over 150 essential oils and their compounds were evaluated. However, while very few were successful in field experiments, thymol and thymol mixtures proved to be a promising exception (Imdorf et al., 1999). The extract and leaves of many plants, such as tobacco, pine leaf, garlic, thyme, eucalyptus, juniper, peppermint, pear, walnut, citrus, teal and cumin are used in the fight against Varroa. These types of applications are 40-75% effective in reducing the varroa population, but these plants, which are kept in a very dense colony, can have negative effects on queen bees, due to their strong odour (Witherel & Bruce, 1990). Cinnamon oil, eucalyptus oil, pepper oil, green tea oil, neem oil and thyme oil are used in varroa control in multiple countries (Amrine, Noel, Mallow, Stasny, & Skidmore, 1996b). In another study, the success of 0.75 mg of menthol, cloves, origanum and thymol oil used to control mites was reported at 87%, 96%, and 100%, respectively (Gashout & Guzmán-Novoa 2009).

Table 1. Medicinal plants used against several bee diseases and pests.

Disease and/or harmful factor	Plant or oil used	References
Varroa destructor	*Thymus kotschyanus* Boiss. & Hohen. *Azadirachta indica* A.Juss. (neem or Indian lilac) *Foeniculum vulgare* Mill (fennel) oil *Syzygium aromaticum* (L.) Merr. & L.M.Perry (clove) oil	Emsen and Dodoloğlu, 2015; Ghasemi, Moharramipour, & Tahmasbi, 2011; González-Gómez et al., 2012; Gunes et al., 2017; Sabahi, Gashout, Kelly, & Guzman-Novoa, 2017; Li et al., 2017; Lin et al., 2020
Tropilaelaps spp	*Illicium verum* Hook. f. (True star Anise tree)	Su, Hua, Zhao, Fei & Zheng, 2016
Paenibacillus larvae	*Cinnamomum verum* J.Presl (cinnamon) oil; *Citrus limon* (L.) Burm. f. (lemon) oil	Alippi et al., 1996; Fuselli, de la Rosa, Eguaras, & Fritz, 2008
Chalkbrood disease (*Ascosphaera apis*)	*Andrographis paniculata* (Burm.f.) Nees (creat or green chireta)	Calderone et al., 1994; Davis and Ward, 2003
Nosema apis	Thymol extracted from *Thymus vulgaris* (thyme)	Chen et al., 2019; Costa, Lodesani, & Maistrello, 2010
Black queen cell virus (BQCV)	*Laurus nobilis* L. extract	Aurori et al., 2016

The most effective result in *Thymus kotschyanus* Boiss. & Hohen (thyme), *Ferula assa-foetida* L. (asafetida) and *Eucalyptus camaldulensis* Dehnh. (river red gum) essential oils, which evaluated the acaricidal activity against *Varroa destructor*, was obtained with *T. kotschyanus* (Ghasemi, Moharramipour & Tahmasbi, 2011). Ethanolic extracts of *Baccharis flabellate* Hook & Arn (baccharises) and *Minthostachys verticillata* (Griseb.) Epling (peperina) have been shown to be highly effective against mites, and their applications did not have a negative effect on bees (Damiani et al., 2011). As essential oils positively affect the caring behaviour of honey bee colonies against varroa (Abd El Wahab, Ebadah, & Zidan, 2012), it has been reported that the concentration of 21.1% of neem seed oil (*Azadirachta indica*) led to 100% mortality percentage of Varroa mites after 72 hours (González-Gómez, Otero-Colina, Villanueva-Jiménez, Peña-Valdivia, & Santizo-Rincón, 2012).

In the study conducted with *Lepidium latifolium* L. (perennial pepperweed), from *Brassicaceae* family and the *Zataria multiflora* Boiss. (Shirazi thyme) leaf extract against *V. destructor*, the methanolic extracts (100, 200, 400 and 500 ppm) were applied to hives in 4 different concentrations. Acaricide activity was determined to be the highest (100%) for *L. latifolium* extract at 500 ppm after 12 days and 86.26% for *Z. multiflora*. It has been reported that both extracts can be used for varroa on bees and that there are no acknowledged negative effects on honey bee colonies (Razavi, Asadpour, Jafari, & Malekpour, 2015). In another study performed in the same year, it was reported that 10 g of thyme added to bee cake had a mortality percentage of 92.85% in Varroa mite (Emsen & Dodoloğlu, 2015). Seven different treatment groups were created to combat varroa using mixtures of formic, oxalic acid and thymol-menthol in order to examine their impact on Heat Shock Proteins (HSP 70) in the brain tissues of bees. It was observed that HSP 70 was lower in the groups exposed to varroa treatment than in the untreated groups, and the thymol-menthol mixture was lowest in the exposed group among the treated groups (Güneș et al., 2017). In recent years, excessive use of traditional acaricides, such as tau fluvalinate and flumethrin against Varroa, has played an important role in colony losses; which has only increased its tolerance to them. Therefore, the application of essential oils on traditional pesticides has been reported as an alternative fighting option, with high yield, minimum residues and tolerance or resistance to mites (Li et al., 2017). In China, *Dalbergia odorifera* T. Chen (Odoriferous Rosewood) oil and *Foeniculum vulgare* (fennel) oil have been reported to be effective against over 65% of Varroa and have a high potential for varroacidal administration as a fumigant agent (Lin et al., 2020). To study the acaricidal activity of essential oils, clove oil (*Syzygium aromaticum* L.), a typical essential oil, with a wide range of field applications, was used to study its effects on enzyme activities of Ca^{2+}-Mg^{2+}-ATPase, glutathione-S-transferase (GST) and superoxide dismutase (SOD). Furthermore, its effects on the water-soluble protein content of *Varroa* body extracts after exposure to 0.1 µL and 1.0 µL of clove oil for 30 min was evaluated. The results showed that the water soluble protein content decreased significantly after the treatments, negatively affecting the mites' metabolism (Li et al., 2017).

Some essential oils were reported to be partially effective in determining the success of natural products in controlling the *in vitro* development of *Ascosphaera apis*. Oils that contain lemon extract as the main ingredient in its structure suppress fungi development at a concentration of 250 ppm. They found that *Corymbia citriodora* (Hook.) K.D.Hill & L.A.S.Johnson (lemon-scented gum), *Leptospermum petersonii* F.M.Bailey (lemon-scented teatree), *Leptospermum scoparium* J.R.Forst & G.Forst (mānuka) are more effective (Davis & Ward, 2003).

Paenibacillus larvae are one of the major bacterial pathogens that infect bee larvae and are a cause of American disease. Due to the increased resistance to commonly used antibiotics and the residual problem in the honey bee product, it is necessary to discover new agents to control this disease (Ansari et al., 2016). In the study conducted using eight plant extracts to determine their efficacy in *P. larvae*, *Ascosphaera apis* and *Paenibacillus alvei* larvae, 100 ppm cinnamon oil completely suppressed *A. apis* development for 7 days, whereas laurel, lemon, clove and thyme oil prevented their development at 1000 ppm for 7 days (Calderone, Shimanuki, & Allen-Wardell, 1994). In a different study, essential oils derived from aromatic plants such as thyme (*Satureja hortensis*, *Origanum vulgare*, *Thymus vulgaris*), lavender (*Lavandula* hybrids), eucalyptus, lemon grass (*Cymbopogon citratus* (DC.) Stapf), peppermint (*Mentha x piperita*), *Salvia rosmarinus* (rosemary). The antimicrobial activity against larvae was analyzed by *in vitro* study. It was found that among the tested essential oils lemon and thyme were the most effiecient in suppressing the development of *Paenibacillus larvae*. On the contrary, oils obtained from rosemary and eucalyptus plants showed a low antibacterial effect against *Paenibacillus larvae*. It was observed that instead of pure essences or their essential ingredients, mixtures of essential oils may show greater efficacy against *P. larvae* (Alippi, Ringuelet, Cerimete, Re, & Henning, 1996). The *in vitro* antibacterial activities of 28 essential oils of plants against *P. larva* ATCC 9545 were evaluated. Jamaican pepper oil, mountain pepper oil, ajwain oil (thymol), peppermint oil, star anise oil, oconut oil and camphor oil have been reported to be quite effective. Essential oils tested have shown significant antimicrobial activity against *P. larvae*. It was also reported that they may contain compounds that play an important role in the treatment or prevention of American foulbrood (Ansari et al., 2016).

The effect of 8 types of herbal extracts against *N. ceranae* infection was investigated under laboratory conditions to find alternative medicines to fight the nosema disease. It has been found that 1% of the source of *Andrographis paniculata* significantly reduces the number of *N. ceranae* spores at 7 days after infection (Chen et al., 2019).

Aurori, Bobis, Dezmirean, Mărghitas, and Erler (2016) evaluated the antiviral potential of *Laurus nobilis* leaf ethanolic extracts on forager honeybees naturally infected with BQCV. Their results showed that higher extract concentrations (≥5 mg/mL) significantly reduced virus replication, observing that viral loads decreased even at the lowest tested concentration (1 mg/mL). Researchers have revealed that secondary plant metabolites can reduce virus loads and virus replication in infected honey bees.

Conclusion

It is important to fight against/combat diseases and pests by using biological and organic methods because of suitable production models for healthy foods. As a result of the banned of some chemicals for animals breeding, medicinal and aromatic plants and oils have been commonly used. While some of the medicinal and aromatic plants are collected from nature, some of them are grown and produced commercially. It should be noted that carrying out studies with cultivated plants can facilitate the standardization of their chemical compunds. The compounds used against diseases and pests, as well as the dosage will reveal its positive effect on bee colony, as well as the environment. In many studies, it has been reported that alternative methods used in the organic control of both pest and diseases do not have toxic effects on larvae, pupae and adults and do not adversely affect colony development when they used at appropriate doses.

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REVIEW PAPER

Recent Studies in the Use of Propolis as a Traditional Medicine: A Review

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Abstract

Propolis is formed by honey bees through mixing a quantity of wax into the resinous substances, which they create by mixing the plant secretions in the pollen baskets on their hind legs with enzymes in their saliva. Depending on the source from which it is obtained, propolis is found in a wide variety of colours, such as yellow, green, dark brown, being solid-state at room temperature. For centuries, apicultural products (such as honey, pollen, propolis, bee venom, bee bread and royal jelly) have been used in traditional medicine for treatment purposes. In recent years, biochemical studies related to propolis have focused on the antimicrobial, anti-inflammatory, antitumoral, and antioxidant activities of propolis.

Introduction

Honey is the most known product among honey bee products. However, other products such as pollen, royal jelly, wax, propolis and bee venom, which are of great economic and ecological importance, also play an important role in human, is also used as food and they are used as food supplements in the treatment of many diseases (Kaftanoğlu, Kumova, & Yeninar, 1992; Kumova, 2001). Any use of these bee products for human health is called apitherapy, and this method forms an important part of traditional and complementary medicine (Stangaciu, 2006; Şirin, Çakırı, Can, Yıldız, & Kolaylı, 2016; Zumla & Lulat, 1989). Propolis is produced by honey bees using plant resins (Bankova, Castro, & Marcucci 2000; Silici, 2019). In addition to being considered as a substance produced by honeybees to protect their hives (filling the gaps, preventing the cracking of the hive, making the inner surface of the hive smooth), propolis has also been reported to have antiseptic properties that protect larvae and honeycombs against microbial infections. Covering the inside of the hive with propolis, the infection carried by honey bees, in close contact with each other, is prevented from spreading within the hive by Kuropatnicki, Szliszka, and Krol (2013). In short, propolis is used for many purposes such as protecting the honey bee colony from diseases and microbial infections, providing a hygienic habitat by covering hive walls and larval cells, and shrinking the hive entrance hole (Silici, 2019). Although the composition of raw propolis varies depending on its source, it is generally composed of 50% resin, 30% wax, 10% essential and aromatic oils, 5% pollen, and 5% other organic compounds and mineral substances (Doğan & Hayaoğlu, 2012). There are many studies on the content of propolis in the world and in our country. In a study conducted by (Silici, 2008), biological components of propolis samples belonging to poplar, *Castanea sativa*, *Eucalyptus globulus* and *Eucalyptus* with different botanical origins were analyzed with gas chromatography-mass spectrometry (GC-MS) and compared. The dominant compounds identified in the study were phenolic compounds, organic acids and fatty acids, and their esters, hydrocarbons, quinones, amines, alcohol, and terpenes. The difference between the analyzed chemical parameters was found statistically significant (P<0.05, 0.01). It was reported that the total amount of phenolic matter varied between 87.62-127.39 mg GAE (Gallic acid equivalents) propolis. Karlıdağ and Genç (2007) investigated the amount of resin containing biologically active agents in propolis samples produced by different methods from Caucasian, Carniolan, and Anatolian subspecies honeybees. It was determined that the resin content of propolis samples obtained in the study ranged between 11.40-67.79% and that the difference between the resin content of propolis samples in different treatment groups was statistically significant (P<0.05). Gençay and Salih (2009) compared the organic compound content of propolis samples obtained from different regions of Turkey, Brazil and Japan. As a result of the study, it was reported that the flavanone and flavonol content of Turkey propolis was higher compared to samples collected from Brazil and Japan. In the study in which they determined the chemical content of 64 propolis samples obtained from the districts of Hakkari province (Center, Çukurca, Yüksekova, and Şemdinli) by using GS-MS. Bayram, Sorkun, Cevahir Öz, Salih, and Topçu (2018) found that samples of Şemdinli contained a higher amount of flavonoids. 27 types of coumarin were identified in 28 of these 64 propolis samples, and it was reported that propolis samples belonging to Yüksekova were richer in coumarin ratio compared to others.

Propolis has been used in traditional medicine for treatment purposes. However, the interest in propolis has increased across the world recently, and by conducting studies related to the chemical composition and biological properties of propolis, it has been explicitly shown that propolis has antimicrobial, anti-inflammatory, antitumoral, antioxidant and immunomodulatory activities (Bogdanov, 2016; Cora, 2018). In this review, data regarding studies using propolis as a traditional medicine in the treatment of some diseases are discussed.

Studies Conducted Using Propolis

Since ancient times, the positive effects of propolis on human health has been known (Çelemli, & Özkırım, 2011). In the research conducted by El Adaouia Taleb, Djebli, Chenini, Şahin, and Kolaylı (2020), to determine the antidiabetic effect of propolis on rats, four groups of five rats each were formed. In the study, 0.5 mL/100 g doses of 30% and 15% propolis extacts were given to diabetic rats for 4 weeks. As a result of the study, it was reported that glucose values for propolis administered groups decreased from 393±192.7 to 154±28.0 mg/dL and from 386± 141.1 to 331.5±123.74 mg/dL. In the study, it was also demonstrated that the glucose value was significantly reduced in the 30% propolis administered group and that propolis was a remarkable product with clinical effect in the treatment of diabetes.

Hosseini et al. (2020) aimed to investigate the inhibition effects of propolis on the growth of the *Aspergillus parasiticus* fungal species, the production of aflatoxin classified as a group I carcinogenic compound by the International Agency for Research on Cancer, and the expression of aflatoxin biosynthesis pathway genes. In the study, the minimum inhibitory concentration (MIC) value was determined to be 100 µg/mL after the test was carried out. It was also stated that by 50 µg/mL of propolis treatment, the total level of aflatoxin decreased from 386.1 ppm to 3.01 ppm. After the treatment performed with propolis extract, a significant reduction in the expression level of the nor – 1, ver – 1, and omtA genes in the aflatoxin biosynthesis pathway was reported that propolis extract has inhibitory properties.

Afsharpourac, Javadiab, Hashemipourc, Koushand, and Haghighiana (2019) evaluated the effect of the propolis support on blood sugar, insulin resistance, and antioxidant status in Type 2 diabetes. In this study, sixty-two patients with Type 2 diabetes (aged 30-55) were randomly divided into two groups, propolis and placebo (n = 31). Patients were given propolis or placebo three times a day (500 mg each time and a total of 1500 mg). At the beginning and the end of the study, fasting blood glucose (FBG), two-hour postprandial glucose (2-hp), insulin, insulin resistance (IR), hemoglobin A1c (HbA1c), total antioxidant capacity (TAC), glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity values were measured. Measurements carried out after two weeks showed that the levels of FBG, 2-hp, insulin, IR, and HbA1c significantly decreased in the group of patients given propolis supplements compared to those given placebo. Additionally, it was found that propolis supplementation significantly increased TAC levels in the blood and GPx and SOD activity. As a result of this study, glycemic status, decreases insulin resistance and improves the antioxidant status and, therefore it may be useful as a dietary supplement in patients with Type 2 diabetes.

Karayel (2019) investigated the effects of propolis administration on oxidative stress parameters and acetylcholinesterase activity in brain tissue in rats whose epilepsy model had been generated. A total number of 35 rats were used in the study, divided into 5 groups (G1-G5) with 7 individuals each (G1-control; G2-Pentylenetetrazol (PTZ), G3-propolis, G4-PTZ + propolis, G5-PTZ + valproic acid). Seizure detection, seizure scores and total seizure duration were monitored in the study. After the administration, acetylcholinesterase enzyme activity in the brain tissue of rats was evaluated. In addition, as indicators of oxidative stress, total antioxidant capacity (TAC) and total oxidant status (TOS) levels were examined. As a result of the study, it was seen that compared to other groups, TAC levels in propolis groups increased significantly and TOS levels decreased, and it was found that the propolis extract reduced oxidative stress and behavioural symptoms of epilepsy.

In another study conducted on propolis samples obtained different regions of Turkey. It was revealed that propolis samples had inhibitory potentials on clinically important enzymes such as urease, xanthine oxidase and acetylcholinesterase. It is emphasized that these inhibition effects detected are due to the phenolic content of the propolis samples that vary depending on the floral origin (Baltaş, Yıldız, & Kolaylı, 2016).

Ebeid, Abd El Moneim, Benhawy, Hussain, and Hussain (2016) conducted a study to evaluate the radio-protective effect of propolis support in breast cancer patients undergoing radiotherapy. In this study, 135 subjects were divided into 3 groups the first group was control group consisting of healthy women (they were selected in a way to match with the women in the patient group in terms of age and menopausal status), the second group consisted of breast cancer patients taking chemotherapy, and then, undergoing only the radiotherapy and the third group consisted of patients taking radiotherapy and propolis supplements after the chemotherapy. In the patient group supplemented by propolis + radiotherapy, it was concluded that propolis had the ability to significantly reduce radiation-induced DNA damage. In relation to serum iron and hematologic parameters such as hemoglobin concentration, white blood cells, and platelet counts, it was observed that only in patients receiving radiotherapy treatment, these values significantly decreased. However, in patients who were given propolis supplements in addition to radiotherapy, these parameters increased significantly and reached normal control levels.

Because propolis-based preparations had a wide range of applications in various specialities of dentistry, AkhavanKarbassi, Yazdi, Ahadin, and Sadrabad (2016) conducted a study to test the effectiveness of propolis as a mouthwash in reducing oral mucositis that developed due to chemotherapy in a single center. For this purpose, 40 patients with oral mucositis who underwent chemotherapy were divided into 2 groups, and mouthwash with propolis was applied to the 1st group, while mouthwash with water was applied to the 2nd group. In the propolis group, significant differences were observed in oral mucositis, wound, and erythema compared to the placebo group; however, no significant difference was observed in the ability to eat and drink. Furthermore, on the 7th day of the study, 65% of the patients in the propolis group were reported to have fully recovered and no significant side effects were observed in the patients.

Ma et al. (2016) examined the advantages of the use of caffeic acid phenethyl ester (CAPE), which is an active antioxidant component derived from propolis, in the treatment of asthma and their role in the regulation of airway micro-environments. It was concluded that CAPE therapy reduced airway hypersensitivity and intense inflammatory cell infiltration in ovalbumin-sensitized rats, and inhibited goblet cell hyperplasia, collagen accumulation, and fibrosis. When all the findings obtained from the study were evaluated together, it was revealed that by balancing the airway micro-environment, which emphasizes a new CAPE profile as a powerful agent for asthma management, CAPE alleviates remodelling in the airway inflammation and chronic asthma.

Basma, Ghonome, Hodeib, and Elbadawy (2015), divided the 40 patients between the ages 13-14, who had facial acne, into two groups and applied propolis extract as a local solution to one group and ethanol local solution to the other group. As a result of the research, it has been reported that the local solution of propolis ethanol extract is clinically effective in the treatment of acne vulgaris.

Olczyk et al. (2014) investigated the effect of propolis on the healing of burn wounds. They reported that in burn wounds occurring on pig skins, by reducing the level of fibronectin, propolis reduced the keloid formation, one of the complications that can occur during the burn healing process.

In a research investigating the effect of propolis on the recovery of diabetic foot ulcers (DFU) in humans, Henshaw et al. (2014) administered propolis locally to 24 patients with diabetic foot ulcers at every clinical examination for 6 weeks. As a result of the research, it was reported that propolis had a curative effect and can improve diabetic foot ulcer recovery with weekly administrations.

In another study, Elshon, Muagam, Sadik, and Walaa (2012) examined the antimicrobial activity of propolis extract the upper respiratory tract infections (URTIs) infections in pediatric patients in Yemen. They tested the efficacy of propolis as a treatment for URTIs infections in 41 pediatric patients and observed that a mixture of propolis and goat's milk increased the antimicrobial effect. Complete remission of both streptococcal and candidal symptoms was achieved in all children within a time period less than the recovery time achieved by using each agent alone (2-5 days). Thus, propolis mixed with goat's milk was reported to be a very effective antimicrobial agent for the treatment and management of throat infections caused by bacterial and candidal species in children.

In the study of Çetin et al. (2011), the protection feature of propolis was investigated for liver damage occurring due to methotrexate (MTK) in rats. In the study, 48 rats were divided into four groups. Distilled water + MTK was given to the first group, propolis + MTK to the second group, propolis + isotonic water to the third group, and finally only distilled water + isotonic water to the control group. In liver homogenate samples, malondialdehyde (MDA) concentration, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) activity levels were investigated. In the study, MDA levels were increased and SOD, GSH-px and CAT levels were significantly decreased in the group given MTK. In contrast, it was observed that the MDA level decreased and GSH-Px levels increased in the group administered propolis and MTK, and oxidative stress caused by MTK decreased with propolis administration.

Conclusion

Propolis is a bee product that is collected from different plant sources by honey bees *(Apis mellifera L.),* containing densely resin and wax. Propolis has been used in many fields in traditional medicine since ancient times. Propolis, a proven natural bee product, is used in the form of oral intake, topical application (lozenge, mouthwash, cream) in many diseases such as oral mucositis, diabetes mellitus, herpes virus infection, candida infection, gastrointestinal cancers, upper respiratory tract infection, acute bronchitis, ulcer, gastritis, ulcerative coli. Today, studies on propolis are increasing day by day.

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REVIEW PAPER

Climate Change Prompts Monitoring and Systematic Utilization of Honey Bee Diversity in Turkey

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Abstract

Quantitative studies concerning the impact of climate change on pollinators are generally lacking. Relationship between honey bee diversity, present local adaptations and adaptive capacity of subspecies and ecotypes in the face of climate change is an urgent but rather poorly studied topic worldwide. Actually, such an effort lies at the crossroads of various fields of inquiry. Those include conservation of local honey bee diversity, breeding various local stocks for desirable traits, and enabling resilient ecosystem services. With the ever-increasing availability of genomic tools, now it is more probable than ever to simultaneously fill such gaps. Current knowledge and growing awareness on honey bee diversity in Turkey let us progress into a more systematic utilization of this resource through development of climate-conscious models. Here we provide a framework that takes genomic diversity into account for assessing and monitoring various aspects of species' response to climate change which can potentially lead to drastic impacts.

Introduction

As the global environment alters with an increasing pace, ecosystem resilience becomes more reliant on the readjustment of species to emerging conditions. For this reason, it is important to evaluate, monitor and manage genetic diversity and related adaptation capacity based on scientific results. Given the possible angular effects of climate change in the upcoming decades, it is necessary to expose how ecosystems can benefit from genetic diversity. In addition, it is essential to develop and test best practice protocols to monitor genetic diversity that varies in space and time.

In terms of honey bee (*Apis mellifera*) biodiversity, the current direction of anthropogenic impact is in line with the loss of native races and the adaptations they have accumulated over thousands of years (de la Rúa et al., 2013; Jensen, Palmer, Boomsma, & Pedersen, 2005; Soland-Reckeweg, Heckel, Neumann, Fluri, & Excoffier, 2009). The factors that cause colony losses in honey bees are very diverse. Possible loss or decline of pollinators are thought to be due to a combined result of destruction and degradation of habitats, pollution and pesticide related toxicity, pathogen and parasite related diseases, and invasive species many of which also effect honey bees (de la Rua, Jaffé, Dall'Olio, Muñoz, & Serrano, 2009; Goulson, Nicholls, Botías, & Rotheray, 2015; Potts et al., 2010; van der Zee, Gray, Pisa, & de Rijk, 2015).

Increasing hybridization of honey bee subspecies due to human activities like migratory beekeeping and queen and colony trade also threaten honey bees by potentially leading to loss of gene combinations that provide local success (Kükrer, Kence, & Kence, 2020). The absence of effective implementation of documentation and monitoring methods for uncovering the genetic basis of adaptive traits makes it difficult to understand and resist the trend of human induced loss of adaptive diversity. However, it is not possible to achieve success in long-term monitoring especially, without developing methods that are inexpensive and feasible but still able to provide meaningful data by deployment of technology-intensive procedures.

New risks and challenges are causing concern as global climate change potentially elevate temperatures and aridity in many parts of the world. We have very little information - not only in Turkey but in the world - about the overall impact of climate change on honey bees, even less on pollinators as a whole. However, most predictions suggest that climate change will worsen the situation by introducing new stressors (González-Varo et al., 2013; le Conte & Navajas, 2008).

A reduction in adaptive genetic diversity will not only be loss of a historic natural heritage that is intrinsically valuable but also of various economic and ecological benefits for the society (Espregueira et al., 2020). Urgently focusing on the genomic analysis of the relationship of honey bees with their environment in the era of global climate change will be to the benefit of both the society and the nature. There is now a strong incentive to consider and investigate pronounced influences of environmental conditions on honey bees through a perspective of ecosystem resilience.

This article aims to emphasize the need for developing a framework that takes genomic diversity into account for monitoring the adaptive capacities of honey bee subspecies and ecotypes present in Turkey in response to climate change.

It is Not Known in What Way the Global Climate Change Will Affect Honey Bee Populations

It is predicted that Turkey's climate will in general become hotter and more arid (Bilgin & Türkeş, 2008; Bilgin, 2013). However, the impact of this change on ecosystems and species still needs to be explored. It is of decisive importance whether the pollinators in general and honey bees in particular can adapt to a rapidly changing environment due to their role in nature and agricultural activities. However, our knowledge of the adaptation capacities in those species is limited. In addition to the identification of genes taking a role in adaption to hot and dry environments, documenting the existence and distribution of such genes in honey bee populations is important too.

Beyond single genes, the distribution of subspecies is determined under the influence of various climatic, geographical and biological factors. These complex factors can be combined to model the subspecies' ecological niches whose long-term characteristics will retain themselves under natural selection (Peterson, 2003). It is not always true that the combination of environmental conditions in which the species can survive is limited only by the current distribution of the species. Therefore, when it comes to modeling the distribution of a species, it is also necessary to refer to the basic niche, realized niche and potential niche concepts (Sillero, 2011).

Such models can be used not only to explain the current situation but also to model the distributions in the past - especially in the ice ages during which subspecies were drawn to refuges (Kozak, Graham, & Wiens, 2008). If a precise population genetic structure map can be generated based on genome surveys making use of high-density SNP data it might be possible to clarify how current distributions of the subspecies are affected by historical processes.

Similar models can be used to predict how species and sub-species would react under various climate change scenarios (Fordham, Akçakaya, Araújo, Keith, & Brook, 2013). Findings to be obtained in this way are good candidates as contributions to conservation planning, since they provide hints about how ecologically and economically important gene resources may change in the future.

There is no doubt of the various difficulties in terms of distribution modeling in species that interact with humans. However, these difficulties do not create insurmountable obstacles. For example, in the case of honey bee subspecies, the fact that these can be transported by people from one region to another would even be useful, as it will facilitate understanding of the potential niche (Jimenez-Valverde et al., 2011).

Of course, the purpose of creating models related to climate change cannot be to make definitive judgments about distributions, especially for species that humans utilize. The main purpose should be to reveal the stress factors and selection pressures that will occur in future ranges. Ecological niche models assist in determining relative weights of a wide variety of climatic and geographical factors that will require adaptation or species' adaptive capacities.

There is already evidence that the current climate might be playing a role in the distribution of honey bee subspecies. Separate studies in the Carpathians and on Africanized bees in South America indicate that borders of the subspecies might be determined by their capacity to adapt to vital factors such as temperature and precipitation (Coroian et al., 2014; Nelson, Wallberg, Simões, Lawson, & Webster, 2017). This is in contrast to artificial selection efforts by humans which are not mainly related to climate and geography, but rather agricultural characteristics such as yield and disease resistance.

Considering that honey bees have an intense interaction with the environment, it is almost impossible to think that they would not be affected by climate change. Therefore, the detection of genes that may prove to be useful in adapting climate change and investigating the effects of this change on the distribution of subspecies and ecotypes would fill an important gap.

Unique Adaptations of Honey Bees in Turkey Are Not Studied at the Genome Level

Migratory beekeeping and bee trade are shown to act like a hybrid zone mobile in space and time, facilitating the partial amalgamation of subspecies in Turkey (Kükrer, 2013; Kükrer et al., 2020; Oskay, Kükrer, & Kence, 2019). Despite that, high levels of geographically structured genetic diversity of honey bee subspecies in Turkey and the need to develop policies to maintain it, was also confirmed.

But how can the natural population genetic structure be preserved, when about 5 million of the 8 million hives in Turkey are taken from one region to another each year, and tens of thousands of queen bees change hands? Could environmental consequences play a certain role in the maintenance of distinct subspecies? In order to find answers, it should be examined whether there is a relationship between the distribution of various geographical and climatic factors such as temperature, humidity, altitude, precipitation regime, winter severity, insolation, flora, and the current distribution of subspecies. It can also be tested which particular genetic features obtained from whole genome sequencing change in a clinal fashion in line with environmental factors (Jones et al., 2013).

If honey bee populations are subject to natural selection due to their environment, then this selection force would emerge as a stabilizing factor for preserving locally adapted subspecies by acting against hybrids, and eventually restricting gene flow between populations (Feder & Nosil, 2010). In that case, natural selection would counterweigh the effect of gene flow between populations and random genetic drift. As a result, it is inevitable to observe different combinations of allele frequencies in various populations (Savolainen, Lascoux, & Merilä, 2013). Sudden changes are to be expected where selection is relatively strong while a smoother transition would be observed in regions where gene flow between populations is higher (Beekman, Allsopp, Wossler, & Oldroyd, 2008).

Since random genetic drift increases the differentiation between populations isolated from each other, the effects of geographical barriers also become measurable. In cases where a certain climatic factor or selection is not causative, it should be considered that the significant genetic distance between populations depends on geographical isolation (Manel, Schwartz, Luikart, & Taberlet, 2003).

The functions of the DNA regions candidates for selection can be easily inferred since honey bee genome was sequenced at an early stage and is studied relatively well (The Honey Bee Genome Sequencing Consortium, 2006). Therefore, it is possible to investigate the relationship between selected genes and environmental factors. At this stage, the goal is to make biologically meaningful inferences about the functions of any candidate genes selected in relation to climatic and geographical variables.

In a recent study on the relationship between environmental conditions and genome-wide selection, it has been observed that altitude-related adaptations are preserved in two African subspecies where gene flow between them is so intense that it prevents observation of a genetic structure (Wallberg, Schöning, Webster, & Hasselmann, 2017). It is normal to expect a similar process in Turkey where adaptations to environmental factors were preserved despite high levels of gene flow. In another study from Kenya, genes that could play an important role in adaptation to various climate types and geographies were investigated by comparing savanna, coastal, mountain and desert populations (Fuller et al., 2015). In a research conducted on a newly identified subspecies in China, researchers focused on the genetic effects created by transition from tropics to the temperate zone (Chen et al., 2016). In the Iberian Peninsula where genome-wide selection signals based on bioclimatic variables were investigated (Henriques et al., 2018) the findings demonstrate that genes involved in regulation of the biological clock by biosynthesis of macromolecules are associated with local adaptations.

Concerning honey bee subspecies in Turkey, various studies making use of SNP markers in honey bees have been carried out in the past. Whitfield et al. (2006) included samples from Turkey in their research, but this work was essentially in the domain of phylogeography. Although 11 genes were identified as candidates for selection, that comparison was carried out on Italian, Western European and African bees but bees from Turkey were excluded from that part of the study. Wallberg et al. (2014) focused on local adaptations but samples obtained from Turkey were only evaluated for extraction of global population structure. Here, the main comparison was made between A-C, A-M and C-M lineages leaving aside O-lineage bees which also includes subspecies in Turkey. Cridland, Tsutsui, and Ramírez (2017), did not themselves gather samples from Turkey but made use of data generated by Wallberg et al. (2014). Uncertainties caused by a sequencing method that is no longer available due to high error rates were revealed and the need for analysis of high-quality genome data belonging to samples from Turkey and South West Asia was emphasized.

Although different aspects of genetic diversity of honey bee subspecies in Turkey were examined, the way they are adapted to the local conditions were not studied at the genome level. In addition, despite extensive research, the exact distributional ranges of the subspecies and the core areas where they are found in "pure" forms are still not clear. This also holds for regions where subspecies' ranges overlap and they exchange genes with each other, as well as for critical regions where sudden changes in the subspecies composition occur.

It is possible that these deficiencies would be eliminated with a well-planned countrywide study which, in this way, would lead to a better understanding of genetic resources of native honey bee races and provide the most basic information that could be utilized in breeding efforts. Bearing in mind the global climate change, uncovering how climate and geography affect honey bees will be vital for the success of future breeding and conservation projects.

There is No Model Yet to Monitor Honey Bee Genetic Diversity in Turkey

In Turkey, within the last decade, awareness about the potential value of the honey bee diversity has radically improved due to intensive efforts of scientists, beekeepers' associations and civil society organizations. In parallel, there has been an increase in conservation implementations and rehabilitation in the field of honey bee ecotypes (Gül, 2020). Currently, breeding herds are either being created or have already been established in Ankara, Ardahan, Artvin, Çanakkale, Çorum, Düzce, Hatay, İzmir, Kırklareli, Kırşehir and Muğla provinces. Since these activities are aimed at local ecotypes, important genetic material is thus put under protection. In concordance with these efforts, a number of subspecies and ecotypes are in the process of being registered by The Ministry of Agriculture and Forestry as native genetic resources of Turkey. This action, too, can be expected to contribute to conservation and breeding efforts in Turkey.

Monitoring programs are implemented in order to detect changes in genetic variability or in the frequencies and the distribution of adaptive variants (Flanagan, Forester, Latch, Aitken, & Hoban, 2018). It is possible now, to further enhance the valuable steps taken till the moment and start monitoring of honey bee genetic diversity in Turkey and to consider making use of emerging technological tools in the field of genome sequencing as well as the decreasing costs.

However, till now, methods used for discrimination of subspecies in such efforts are mainly based on morphology, geometric morphometry and on mitochondrial as well as nuclear DNA markers like microsatellites. Resolution provided by such methods are far from precise discrimination of ecotypes, let alone allowing accurate reflection of diversity present in Turkey. Furthermore and more importantly, they do not let us to take into account a conscious incorporation of genomic elements that play role in adaptation of ecotypes to their natural environment. Today conservation and breeding efforts should focus more on genetic variation specifically improving the subspecies' capacity to adapt climate change. Constraints related to the adequate documentation of genetic diversity in Turkey do not enable yet, the development of functional and at the same time low-cost monitoring models.

An adaptive management context with an integrated monitoring step will enjoy the chances of both learning more about the local ecotypes and evaluating the effectiveness of management actions once they are initiated. After an initial genomic assessment by sequence capture methods or SNP arrays, it is possible to consistently genotype many individuals over time. This would certainly help to reach diverse objectives like diagnosing introgression and conservation efficacy, characterization of neutral and adaptive genetic variation especially related to climate change, as well as retrieving information about desirable traits (Aykanat, Lindqvist, Pritchard, & Primmer, 2016).

A Potent Long-Term Ecological Research Perspective and Scope

Basically, any research addressing the adaptive capacities of subspecies in Turkey against climate change should cover the following scope:

(i) Core regions in which 5 honey bee subspecies stay unmixed should be identified by an intense sampling effort across the country from stationary apiaries whose beekeepers reject to replace queens and colonies with non-native races. In order to achieve this, genome-wide data obtained with next generation sequencing techniques should be utilized.

(ii) Despite the anthropogenic impact in the form of migratory beekeeping and trade, subspecies are known to preserve their identities at certain places. Selection at the genomic level naturally relies on environmental conditions. Investigation of this phenomenon necessarily means comparing relative weights of natural selection, gene flow and genetic drift within populations.

(iii) Candidate genes located in genome regions under selection and playing a role in adaptation to local conditions should be identified. The functions of these genes and their relation with the environmental conditions should be examined. Genetic features that play a role in adaptation to elevated temperatures and aridity should be revealed through various comparisons between populations residing in such milieu. Existing conservation and breeding efforts like those supported and carried out by The Ministry of Agriculture and Forestry and Turkish Beekeepers' Association should better be reinforced by evolutionary knowledge. This will be achieved through purposeful introduction of locally adaptive genetic variants in addition to variants that provide adaptive potential under climate change within such stocks.

(iv) In order to preserve the genetic diversity and adaptation capacities documented in this way, a low-cost, feasible, but technology-intensive monitoring method should be developed. After an initial assessment, intensive sampling coupled with monitoring of conservation areas for these alleles by at least 5-year intervals should be guaranteed.

(v) Population structure obtained from genetic data should be used in models that will shed light on the evolutionary histories of subspecies and how their natural distribution would be affected under various global climate change scenarios.

Discussion

The most important needs of the actual period include the establishment of quantitative and regular implementations to appraise, monitor and manage the genetic resilience and adaptive capacity for species under human use or those not. This points to relevance for incorporation of genetic and evolutionary knowledge in policies concerning conservation planning and sustainability of ecosystem services, particularly under the severe impact of global climate change (COST Committee of Senior Officials, 2018).

The challenges faced in this area can be more easily overcome via piecing together of the following pursuit, akin but not limited to providing integration platforms in order to link together stakeholders and developing collaborations that combine experience in various areas of expertise to form the basis of a sustainable impact as well as integrating emerging technological tools into existing activities; explaining decision-makers how genetic diversity can benefit ecosystems; developing and testing best practice protocols for monitoring genetic diversity in space and time. As a key pollinator, honey bees (*Apis mellifera*) draw much attention among species aimed for determination and monitoring of the genetic adaptation capacities in response to climate change.

Although honey bees are intensively managed by humans, they cannot be regarded as fully domesticated. Apart from wild populations in the natural distribution range of the species or feral colonies that escaped from human hands, even colonies under human control act as part of wildlife due to nectar and pollen foraging activities. Their unique role in pollination makes bees a critical species for ecosystem resilience in addition to agricultural production and ecosystem services.

We need to put forward a monitoring model that can process honey bee diversity throughout the country. This also provides an opportunity to go beyond a general characterization of biodiversity. It can be aimed to monitor, in terms of presence and distribution, both specific alleles involved until now in local adaptation to native conditions and also genetic features that may contribute to adaptive potential under conditions of global climate change.

Long-term monitoring is a costly and labor-intensive process. This is also the most important reason for the fact that monitoring studies with a large spatial scale are not always possible. A technology-intensive monitoring model that combines the most cost-effective, feasible, state-of-the-art scientific methods developed and tested till now is likely to contribute to the goal of creating standard and routine tools.

Developing a model for monitoring and utilization of honey bee genomic diversity is not only useful for revealing the adaptive potential to climate change, but also with simple customizations, would provide new opportunities for implementation of marker assisted selection in breeding for disease resistance (varroosis, Nosema, foulbrood, etc.), obtaining desirable phenotypic characters (gentleness, wintering success, low swarming tendency, etc.) and increased yield (honey, royal jelly, pollen, propolis, bee venom and other bee products).

Genomic diversity and adaptive potentials are rapidly lost or undergoing serious changes under human influence. With such a model, decision-makers and field operators might have a chance to benefit from genomic and evolutionary information in the face of adverse human-induced effects.

This piece focuses on the limits of our knowledge on honey bee diversity in Turkey, its interaction with the environment, the consequences of this interaction for natural selection, and its implications for the future under global climate change. We recommend that further research in honey bee genetics would better seek previously unexplored phenomenon, structures and relationships. Such investigation would have the potential to innovatively apply to the situation the knowledge and techniques in the field of genomics and to contribute in the formation of an understanding that will be utilized in a way which may concern many stakeholders.

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