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RESEARCH PAPER

The Effects of Sugar Syrup Enriched with Amino Acid Mixtures at Different Concentrations on Colony Population Dynamics and Development Characteristics of Bumble bee (*Bombus terrestris*)

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Abstract

In this study, we tested the effects of amino acid-supplemented diets on colony development performances in *Bombus terrestris*. A total of 75 queens artificially hibernated were randomly separated into three groups. These groups were fed with different diets: normal sugar syrups and normal pollen (Control), sugar syrup which contains recommended dose (for honey bees: 10 mL/L, Sucrose syrup) of amino acid supplement and normal pollen (10 mL/L), sugar syrup which contains twice higher recommended dose of amino acid supplement and normal pollen (20 mL/L). Some developmental traits of queens and their colonies were determined. According to our findings, there were no significant differences in any of the traits of colony development except the number of total individuals among the groups ($P<0.05$). Results showed that feeding with an amino acid-supplemented diet is not influencial on colony development traits in *B. terrestris.*

Introduction

Bumble bees are vital pollinators of wild flora and agricultural crops. *Bombus terrestris* is polylectic and the most commercially reared bumble bee species (Velthuis & van Doorn, 2006). In mass rearing of the *B. terrestris*, all life stages such as founding colonies from queens, rearing queens and males from colonies, enabling the virgin queens to mate, controlling the diapause period of mated queens, and ensuring that queens emerging from diapause are carried out under controlled conditions (Beekman & Stratum, 2000; Gosterit et al., 2009; Amin et al., 2010). Nevertheless, there are some losses in each of these stages in the rearing process, and these losses affect the colony founding success in suitable quality for pollination (Gosterit, 2011). Besides, even if all these stages are processed under the same conditions and in the same laboratory, there are many variations in colony development traits including the number of workers, queens, and males, colony initiation, colony production ratio, and competition and switch points. Genetic structure, environmental conditions, diseases and parasites, and food quality and food availability affect variations of these traits in mass rearing of *B. terrestris* (Riberio et al., 1996; Cnaani et al., 2000).

Food quality and availability are crucial for eusocial bees in terms of egg-laying of queens, improving brood rearing, obtaining more yield, and preventing diseases and stress, etc. (Herbert & Shimanuki, 1978; Brodschneider & Crailsheim, 2010; Gosterit & Cicek, 2017). In some harsh conditions in nature, honey bee colonies are fed artificially by using food supplements containing some ingredients such as vitamins and amino acids (Kumova, 2000). *B. terrestris* sometimes may face short-term food shortfalls just as honey bees. When harsh conditions occur, reduction of brood temperature and mobility in workers, increased brood developmental time, less and/or smaller individual production, and less sexual production may occur in bumble bees (Plowright & Pendrel 1977, Heinrich 1979, Sutcliffe & Plowright 1990, Schmid-Hempel & Schmid-Hempel 1998). Moreover, larvae ejection from the nest occurs in long-term food shortfalls (Plowright and Plowright 1999). To avoid this situation, queens and colonies of *B. terrestris* are fed *ad-libitum* with 50 Brix sugar syrup and honey bees-collected pollen in year-round rearing (Riberio et al., 1996; Gosterit, 2016).

Honey bee colonies are fed with different supplementary food to improve brood rearing, protect against disease, and increase yield. Investigation of the effects of this supplement on bumble bee colonies is also valuable for the sustainability of their rearing activities. This study aimed to determine the effects of amino acid-supplemented diets on colony development performances in *B. terrestris.*

Materials and Methods

In this study, 75 hibernated *Bombus terrestris* queens, purchased from commercial bumble bee supplier (Bio Group Antalya, Türkiye) were used. Queens and their colonies were reared under standard laboratory conditions (28 ± 1°C, 50 ± 5% R.HAs a supplemental food material, BeeTonic was added to sugar syrup according to its recommended dose (for honey bees: 10 ml/L Sucrose syrup). BeeTonic is a food supplement used for honey bee feeding and contains 20 different amino acids (choline chloride 2500 mg, glycine 34900 mg, methionine 2400 mg, histidine 1340 mg, lysine 5650 mg, hydroxylysine 2250 mg, inositol 2500 mg, hydroxyproline 18300 mg, leucine 5000 mg, phenylalanine 2390 mg, isoleucine 3740 mg, proline 20530 mg, alanine 12600 mg, serine 5090 mg, arginine 11500 mg, threonine 2830 mg, aspartic acid 6700 mg, tyrosine 890 mg, glutamic acid 15000 mg, and valine 3740 mg). These queens were randomly assigned into three groups containing 25 queens feeding with standard sugar syrup and pollen (Control), feeding with sugar syrup containing recommended dose of the amino acid supplement and normal pollen (10 mL/L), feeding with sugar syrup containing twice higher recommended dose of amino acid supplement and normal pollen (20 mL/L). Supplemental food material was mixed with 50 Brix sucrose syrup, and queens and their colonies were fed with their assigned diets *ad-libitum*. Queens were transferred to starting boxes (8x8x6 cm) allowed to begin the colony founding process. After the first workers emerged, colonies were moved into larger rearing boxes (26x23x14 cm). Colony developmental characteristics were observed twice a week periodically. Egg-laying ratio, colony production ratio and marketable colony production ratio of queens, colony initiation (time of the first egg-lay) time, the timing of first worker emergence, the timing of the young male and queen production, the timing of switch point and competition point, and the total number of individuals were recorded. Queens that produced more than 10 workers were considered to produce colonies, and colonies that had 50 or more workers were deemed marketable (Gosterit & Cicek, 2017).

Descriptive statistics of parameters were analyzed in Minitab Statistical Software. Parameters were tested for normality. One-Way ANOVA analyses were run to determine the effects of amino acid-supplemented diets on development characteristics. Two proportion z-tests were used to compare the percentages of the queens that laid eggs and produced 10 and 50 workers.

Results and Discussion

The ratio of egg-laying, colony founding, and marketable colony have a wide range of variations reported in previous studies in *Bombus terrestris* (Velthuis & van Doorn, 2006; Baloglu & Gurel, 2015; Gosterit & Gurel, 2016). The effects of feeding with amino acid-supplemented diets on egg-laying ratio, colony founding ratio, and marketable colony ratio were given in Table 1. According to our results, there were no significant differences among experiment groups for each characteristic. The highest egg-laying ratio and colony founding ratio were determined in the control group (96% and 88%, respectively), while the highest marketable colony production ratio was in 20 mL/L (76%).

Table 1. Ratios of egg-laying, colony founding, and marketable colony production (%)

Groups	N	Egg-laying	Colony founding	Marketable colony
Control	25	96.00	88.00	64.00
10 mL/L	25	84.00	72.00	64.00
20 mL/L	25	92.00	84.00	76.00

There are three crucial stages for colonies of *B. terrestris*: colony initiation, switch point, and competition point. In the first stage, the queen (founder queen) lays eggs, and the first workers emerge from these eggs (beginning of social phase). Switch point, the second stage, is that the queen changes her reproductive strategies and starts laying haploid eggs (males) instead of diploid eggs (females). Egg-robbing and conflict between workers and the founder queen are seen in the competition point, the last stage (Duchateau & Velthuis, 1988; Gurel et al., 2008). Switch and competition points indicate that the end of colony life is approaching. When these stages are observed in detail, the production time of individuals and marketable colonies are also important for sustainable mass rearing success. In this study, the findings belonging to mentioned developmental characteristics were given in Table 2. The results showed that no significant differences were found among the groups in terms of colony developmental characteristics.

Table 2. Some developmental characteristics of queens and colonies fed with amino acids supplements

Characteristics (days)	Groups	N	$\bar{x} \pm s.d$	*P* value
Colony initiation time	**Control**	24	10.54 ± 4.12	0.650
	10 mL/L	21	10.38 ± 4.78	
	20 mL/L	23	9.48 ± 0.76	
Timing of first workers emergence	**Control**	22	31.86 ± 4.29	0.870
	10 mL/L	18	31.89 ± 5.04	
	20 mL/L	22	31.23 ± 4.60	
Timing of first male emergence	**Control**	17	72.06 ± 4.70	0.769
	10 mL/L	16	70.76 ± 6.58	
	20 mL/L	18	72.00 ± 6.07	
Timing of young queen emergence	**Control**	16	43.29 ± 5.98	0.549
	10 mL/L	17	45.00 ± 5.83	
	20 mL/L	19	45.21 ± 5.07	
Competition point	**Control**	17	29.53 ± 4.96	0.725
	10 mL/L	16	28.94 ± 4.80	
	20 mL/L	18	30.22 ± 4.22	
Switch point	**Control**	16	16.06 ± 4.28	0.537
	10 mL/L	17	13.88 ± 7.46	
	20 mL/L	16	15.31 ± 4.63	
Timing of marketable colony production	**Control**	16	58.19 ± 3.25	0.587
	10 mL/L	16	59.56 ± 9.00	
	20 mL/L	19	57.42 ± 4.83	

According to the findings of previous studies, founder queens are affected by various factors such as food quality and quantity, worker/larva ratios, etc. (Duchateau et al., 2004; Holland et al., 2013). Gosterit and Cicek (2017) investigated the effects of pollen and syrup which include vitamin supplements on colony developmental characteristics in bumble bees. According to their reporting, it was determined that feeding with a diet supplemented with vitamins did not have a positive effect on the colony developmental characteristics of *B. terrestris*, but there was a significant difference in the number of young queens. In this study, the number of egg cells in the first brood, the number of workers in the first brood, and the total number of workers, males, young queens, and the total number of individuals were determined (Table 3). Our results showed that there were no significant differences among the experimental groups except in the total number of individuals ($P<0.05$).

Table 3. Some characteristics in the colonies fed with amino acids supplements

Characteristics	Groups	N	$\bar{x} \pm s.d$	*P* value
Numbers of egg cells in first brood	**Control**	24	3.625 ± 0.275	0.377
	10 mL/L	21	3.429 ± 1.207	
	20 mL/L	23	3.957 ± 1.224	
Number of workers in first brood	**Control**	22	8.500 ± 2.988	0.158
	10 mL/L	18	7.778 ± 3.209	
	20 mL/L	22	6.727 ± 2.914	
Total number of workers	**Control**	22	156.40 ± 73.50	0.192
	10 mL/L	18	183.60 ± 49.40	
	20 mL/L	21	189.50 ± 60.30	
Total number of males	**Control**	16	89.80 ± 44.90	0.050
	10 mL/L	17	128.50 ± 50.80	
	20 mL/L	18	125.00 ± 48.80	
Total number of young queens	**Control**	17	90.41 ± 35.42	0.089
	10 mL/L	16	100.10 ± 48.80	
	20 mL/L	19	128.30 ± 66.50	
Total number of individuals	**Control**	22	291.60 ± 154.60 b	0.020
	10 mL/L	18	393.80 ± 125.30 ab	
	20 mL/L	21	412.80 ± 155.50 a	

For each characteristic, means followed by different letters (a, b) in the same column are different for each characteristic ($P<0.05$)

Data on sex (queen and male) production strategies in colonies produced in the study groups were given in Figure 1. Gosterit (2011) and Gosterit and Baskar (2016) categorized the reproductive strategies of bumble bees into four groups: colonies that produce only males, produce only queens, produce both queens and males, and produce neither queen nor male bees in their studies. According to our results, all reproductive strategies belonging to bumble bees were observed in this study.

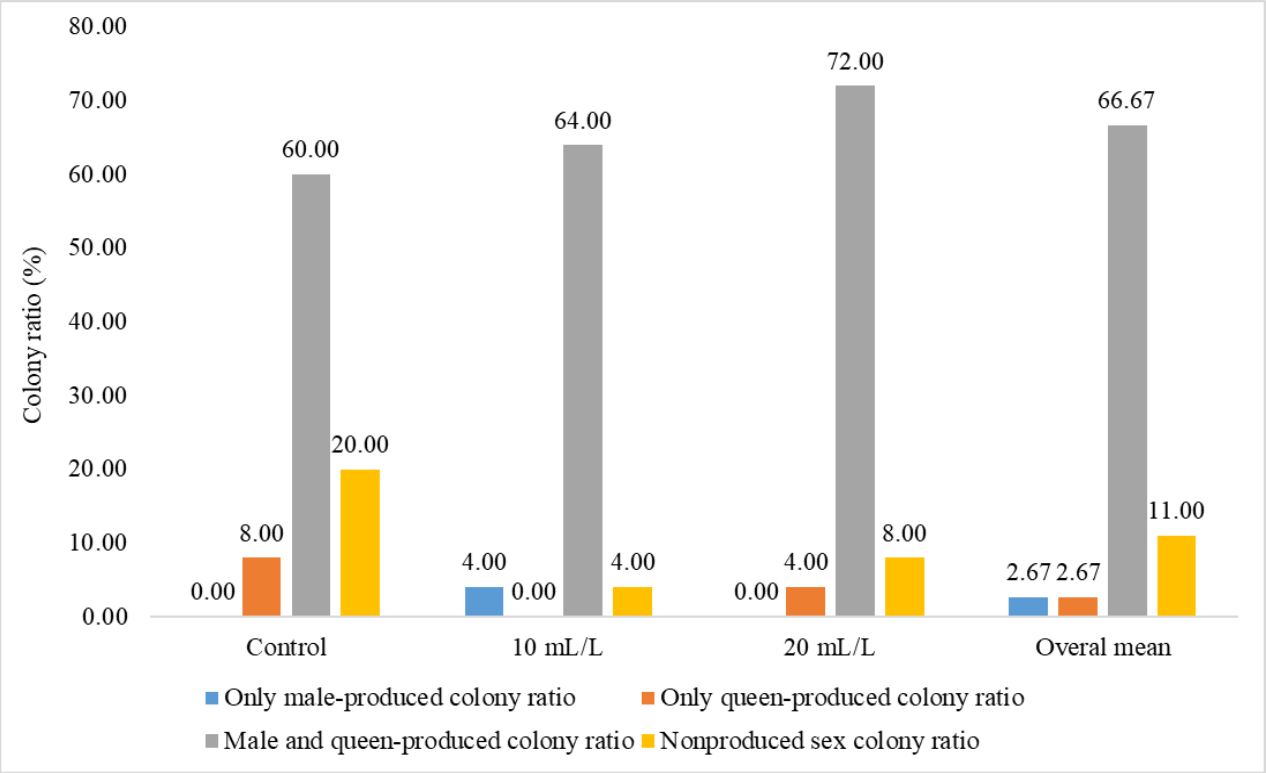

Figure 1. Sex production strategies in colonies

Conclusion

Our results have shown that feeding with the amino acid mix is not effective for bumble bees. When twice the recommended dose of amino acid supplement (BeeTonic) was added to the sugar syrup and pollen, a margin of 20% was observed in the total number of males and queens in comparison with the control groups. While this margin is not statistically significant, it should not be overlooked considering it affects the colony productivity ratio and efficient use of resources for sustainable Bombus terrestris mass rearing. For this reason, it becomes important to investigate even the smallest factor that may affect the success of the mass rearing process of bumble bees.

Ethical Statement

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Author 1: Investigation, Writing – review & editing,
Author 2: Investigation, Writing – review & editing; Supervision, Formal Analysis
Author 3: Methodology, Writing – review & editing; Supervision, Formal Analysis

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REVIEW PAPER

An Overview on the Effects of Propolis Administration in Different Branches of Livestock Production

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Abstract

Recently, the increase in the awareness of nutrition with natural products has gradually increased the popularity of honey bee products. Popularity of honey bee products like honey pollen, propolis, bee bread, royal jelly, bee venom and apilarnil are beekeeping products. Propolis is one of the most popular bee product and has anticancer, anti-inflammatory, antibiotic, antioxidative, antibacterial, antiviral, antifungal, anesthetic, immunostimulant and cytotoxic effects. Approximately 300 compounds have been identified in the content of propolis, including polyphenols, phenolic aldehydes, sequiterpene quinines, coumarins, amino acids, steroids, and inorganic compounds. These properties have been used in folk medicine for centuries. The use of propolis in the livestock sector has become popular with the restriction on the use of antibiotics and synthetic drugs in this sector. Various studies were carried out to determine the effetcs of propolis administration in different branches of livestock sector. This study aimed to reveal the effects of propolis administration in animal husbandry by analyzing the current studies and provide brief information on the subject.

Introduction

Honey is the most widely produced bee product in Türkiye. However, honey bees produce variety of other products with high economic value such as royal jelly, pollen, bee bread, beeswax, apilarnil and propolis. These products are both highly nutritious and are used extensively as a preventive and supplementary product in the field of alternative medicine, which is quite popular all over the world (Silici, 2015). Honey bees prepare propolis by mixing the bark, leaves and plant secretions with their salivary enzymes and beeswax. Bees use this resinous product for many purposes in the hive such as narrowing the hive entrance hole, closing the cracks in the hive, fixing the frames, disinfection of the honeycomb cells and mummification of the pests that are killed inside the hive. Around 35℃ and 40-70% humidity are required inside the hive for a bee colony to survive and grow. On the other hand, these requirements are significant risk factors since the temperature is extremely suitable for the growth of various viruses, bacteria, and fungi in the hive. The propolis produced by the bees protects the hive against such harmful microorganisms and regulates the humidity and temperature of the hive (Ndımballan, 2021; Yücel et al., 2015). The color of propolis can vary from light yellow to dark brown, depending on the source of the resin. Propolis is a soft, flexible, and very sticky substance at temperatures of 25-45 ºC. When the temperature drops to 15 ºC, it is partially frozen or near freezing, and is in a hard and brittle state. Its stickiness increases above 45 ºC, it becomes liquid at 60-70 ºC. However, in some samples, the melting point can reach 100 ºC (Krell, 1996). The chemical structure of propolis was revealed by the studies carried out at the beginning of the 20th century. The content of propolis may vary depending on the plant source collected, bee species, bee breed, and ecological conditions. The composition of propolis varies according to its source, it generally consists of 50% resin, 30% wax, 10% essential and aromatic oils, 5% pollen, 5% other organic compounds and mineral substances, and flavonoids, which are the active substances of many drugs, antioxidants, substances with biological activity, antibiotics, antimycotics, antiviral effective substances are some of the compounds propolis possesses (Kılıç Karabaş et al., 2020; Doğan and Hayaoğlu, 2012; Kumova et al., 2002). The amount and distribution of these substances in

propolis and their pharmacological properties have been demonstrated by various studies.

Propolis has been used for different purposes in public health throughout human history. About 300 compounds have been identified in the content of propolis, polyphenols, phenolic aldehydes, sequiterpene kinins, coumarins, amino acids, steroids and inorganic compounds in propolis samples are some of them (Khan, 2017). Propolis collected from nature by bees is an extremely important substance for human health and life. People have benefited from propolis collected from nature in the treatment of various infections from ancient times to the present. It was reported that propolis was used as an ointment in surgical interventions mixed with petroleum jelly to heal wounds and tissues in wars instead of medical wax (Kumova et al., 2002). With all the above-mentioned features, propolis has been widely used in traditional and complementary medicine for many years.

The European Union banned the use of antibiotics in animal production as growth and development enhancers in 2006 with the discovery of residues in animal products caused by antibiotic use (Saeed et al., 2017). This decision has driven scientists and manufacturers to look for new natural alternative additives that can be used instead of antibiotics to prevent economic losses due to the ban on the use of antibiotics. Antibacterial (Silici & Kutluca, 2005), antiviral (Vynograd et al., 2000), anti-inflammatory, analgesic, and tissue regenerative (De Castro, 2001), antioxidant (Banskota et al., 2000), and cytostatic and hepatoprotective (Banskota et al., 2000; Khan, 2017) effects of an ethanolic extract of propolis have been reported in many studies. With all its stated properties, propolis stands out as a strong alternative substance that can be used in the livestock sector instead of antibiotics. This study discusses the usability and effects of this product as an alternative natural substance in the animal production sector.

Use of Propoolis in Poultry Farming

The positive effects of propolis in poultry farming were reported in various studies. In a study conducted on Ross breed broilers, researchers reported that with propolis administration, daily weight gain and feed efficiency ratio increased, while death rates decreased significantly (Shalmany & Shivazad, 2006). Similarly, Zhi-Jiang et al. (2004), reported that 0.1% propolis supplementation to the ration increased the body weight in boilers by 2.03% compared to the control group. In a similar study conducted in ducks from egg hatching to 60 days of age, it was reported that the live weight increased by 10.5% and 13.5% as a result of the addition of 20 and 40 mg propolis to kg/diet (Bonomi et al., 2002). Similar results in terms of body weight gain and feed efficiency were also reported in quails by Denli et al. (2005).

In another study conducted on laying hens, it was reported that the addition of propolis to the diet decreased egg cholesterol and triglyceride levels and increased serum HDL (high-density lipoprotein) levels (Silici & Güçlü, 2018). In addition, propolis administration was reported to increase eggshell quality when used on laying hen rations (Tatli Seven, 2008). Mahmoud et al. (2015), reported that propolis causes an increase in calcium digestibility and absorption with different acids such as benzoic, 4-hydroxy-benzoic acids, and this increase may be the reason for this increase in eggshell quality. In a similar study, different doses of propolis were added to laying hen rations and performance parameters were monitored. Researchers reported that the live weights of laying hens increased with the addition of propolis, but there was no difference in other parameters (Özkök et al., 2013). In another study examining the effects of 250, 500, and 1000 mg/kg propolis supplementation in layer hen rations on performance, egg production, and quality of laying hens, 250 mg/kg dietary propolis supplementation was reported to improve feed efficiency, egg production, and egg mass significantly (Abdel-Kareem & El Sheikh, 2015).

Propolis contains bioactive substances with high antioxidant activity. Tatli Seven (2008), reported that the use of propolis was significantly effective in reducing oxidative damage caused by heat stress, increasing growth performance, and increasing egg shell thickness and egg weight in layer hens. Comparable results were reported for Ross breed broilers exposed to heat stress by Mahmoud et al. (2015). Propolis was reported to improve the performance of turkeys by increasing immunity, when exposed to an herbicide called Paraquat, which is used in agriculture and causes inflammation and oxidative stress (Abass et al., 2017).

It has been reported that propolis used in chicken rations also increases blood total protein, albumin and globulin levels (El-Neney & Awadien, 2014; Abdel-Kareem & El Sheikh, 2015). Moreover, Çetin et al. (2010), determined that the application of propolis resulted in an increase in serum IgG and IgM levels and red blood cell counts in White Leghorn chickens, and they reported that the addition of propolis to the 3 g/kg diet was effective in enhancing immunity in chickens. Similar results were also reported for layer hens by Freitas et al. (2011). Eyng et al. (2014), used 0, 1000, 2000, 3000, 4000 and 5000 ppm doses in 1-21 day old male chicks to investigate the effect of ethanolic extract of propolis added to the rations of broiler chickens on small intestine morphology and digestive enzymes activity. Propolis with ethanolic extract at a dose of 1000-5000 ppm added to the starter feeds of broilers decreased the performance due to the decreased sucrase activity at their stage. However, 3000 ppm propolis extract improved the small intestine morphophysiology of 21-day-old chickens, but did not affect the performance or carcass yield of 42-day-old chickens.

Tekeli (2007), investigated the possibility of using natural herbal extracts and propolis as growth factors as an alternative to antibiotics due to the ban on the use of antibiotics in broiler feeds. In the third experiment of the study 0, 500, 1000, 2000 ppm doses of propolis were used, and it was observed that especially 1000 ppm propolis increased feed consumption, live weight gain, feed conversion ratio and intestinal villi length, and was important in terms of being an alternative to antibiotics. In the fourth experiment, doses of Zingiber officinale and propolis were used separately and in combination. It was observed that 240 ppm of Zingiber officinale and 1000 ppm of propolis showed similar effects with antibiotics in terms of body weight gain, feed consumption and feed conversion ratio.

It is evident by above-mentioned studies that propolis is a promising natural alternative product that can be used in the poultry sector to enhance immunity, reduce oxidative stress, improve feed efficiency since live weight gain, and improve egg quality. In addition, propolis administration shows comparable effects on poultry when compared to antibiotics which created residue in animal products and endanger consumer health.

Use of propolis in Ruminants

There have been limited studies carried out to investigate the effects of propolis administration on ruminant nutrition. In a current study, Slanzon et al. (2019), revealed that the incidence of diarrhea in calves decreased significantly with the supplementation of 4 mL of propolis daily to newborn calves' diets. Similarly, propolis was reported to be effective for both preventive and therapeutic purposes in neonatal calf diarrhea (Kwon et al., 1999). These findings were also confirmed by Yücel et al. (2015), who fed newborn calves by 2 cc of propolis / per day and reported that propolis was effective in the treatment of diarrhea in newborn calves and significantly increased growth and development. Kupczyński et al. (2012), also reported a significant decrease in diarrhea cases in calves with a daily application of 4 mL of propolis. Furthermore, it was reported by Manav and Yılmaz (2021), that the incidence of diarrhea in goat kids decreased significantly with propolis administration. The effectiveness of propolis in diarrhea treatment in young ruminants may be explained by its strong antimicrobial activity.

Propolis improves the general health status of animals and increases feed efficiency, thus increasing animal productivity. A significant increase in the daily live weight gain of newborn calves was revealed by Tolon et al. (2002), with the administration of propolis. Similar results were also reported by Yücel et al. (2015). Moreover, Kupczyński et al. (2012), investigated some health and performance parameters of calves with propolis supplementation and reported that calves fed with 4 mL of 10% propolis extract/day had a significantly superior 21st day body weight compared to the control and 2 mL propolis groups. A significant increase was observed in calf starter feed consumption and in 5th week body weight with the addition of flavonoids extracted from propolis to calf diets (Yaghoubi et al., 2008). Furthermore, Slanzon et al. (2019), observed that the feed efficiency of the calves in the treatment group (694.2 g/d) was superior to that of the control group (654.5 g/d) after the supplementation of a daily 4 mL ethanolic extract of propolis.

Abd-Allah and Daghash (2019), examined the efficiency of flavomycin and propolis in dairy buffaloes and calves and reported that they both had similar positive effects on the performance of water buffalo calves. In a similar study conducted on sheep, it was reported that with the daily supplementation of 3 g of propolis in pregnant sheep's diets, significant increases were observed in milk yield and lamb live weight gains (Morsy et al., 2016). On the other hand, Zawadzki et al. (2011), reported that propolis administration significantly increased feed efficiency and daily live weight gain in feedlot-finished bulls. In addition, it was also reported that propolis application (5 g/kg diet) in pregnant Barki sheep significantly increased milk yield and lamb performance (Shedeed et al., 2019). These effects of propolis in enhancing the performance of young ruminants may be due to its strong antimicrobial effect, which preventing the development of pathogenic microorganisms that suppress growth by creating disease factors in calves, and reduces oxidative stress with its antioxidant effect (Abd-Allah & Daghash, 2019).

The effects of propolis on immunity, oxidative stress and certain blood parameters were also examined in many studies and propolis was found to be effective on these parameters. It was reported that antioxidant enzyme levels in blood samples decreased significantly and immune system function increased with propolis administration in pregnant sheep (Shedeed et al., 2019). Similarly, Yaghoubi et al. (2008), reported that propolis application reduces the effectiveness of bacteria and viruses that cause damage in newborn calves and decreases the IgM and IgG levels. On the other hand, Morsy et al. (2016), reported that an increase in total leukocyte, total protein, albumin and glucose levels were observed in the Santaine sheep that were fed propolis supplemented to their diets.

All these studies provide clear evidence that propolis is highly effective in increasing general health, enhancing immunity, preventing oxidative damage, increasing feed efficiency and weight gain and most importantly decreasing diarrhea which is responsible for the considerable amount of young ruminant losses in the livestock industry.

Use of Propolis in The Fishery Industry

In aquaculture, it is extremely difficult to treat diseases and the eradication of diseases requires hard work. Various chemotherapeutic agents such as antibiotics, nitrofurans and sulfonamides have been

used for a long time for both preventive and therapeutic purposes against infections that occur for any reason and cause significant economic losses in the fishing industry (Arda et al., 2005). Recently, natural treatment methods became popular in order to prevent financial losses in treatments, to minimize fish losses and prevent the negative effects of chemotherapeutics. In recent years, studying the applicability of natural products that have been widely used by people for centuries in the treatment of various diseases, in the field of aquaculture and their pharmacological use has become an important field of study (Yonar, 2012; Yonar, 2010). Propolis is among the most prominent substances used for natural treatment (Silici, 2015), and the effects of propolis administration on the fish have been studied by many researchers.

Yonar et al. (2018), investigated the effect of propolis on some immunological parameters in rainbow trout. For this purpose, propolis was injected intraperitoneally into fish 4 times at a dose of 2.5, 5 and 10 mg/kg fish weight. Blood samples were collected from the experimental and control groups on the 3rd, 9th, 15th, and 21st days, and oxidative radical production [nitrobluetetrazolium (NBT) activity], total protein, and total immunoglobulin levels were measured. At the end of the experiment, it was reported that there was a statistically significant increase in the oxidative radical production, total protein and immunoglobulin levels of the groups treated with propolis compared to the control group.

Segvic-Bubic et al. (2013), investigated the effect of low temperature stress in European sea bass fish, and reported that the group that fed with 2.5 g/kg propolis showed higher growth performance, and propolis administration was found to be highly effective against low temperature stress in fish. In a similar study 10 g/kg of diet propolis was supplemented to catfish diets and propolis was found to be significantly effective in increasing the growth performance and feed efficiency ratio (Nur et al., 2017). Comparably, Abbass et al. (2012), studied the effects of propolis and bee pollen supplementation on the growth and feed efficiency of Nil tilapia fishes and revealed that supplementation of propolis to these fish's diets increased their feed efficiency ratio and growth performance. In a 10-week feeding study conducted by Deng et al. (2011), in trout, it was determined that the growth rate, feed efficiency rate and protein efficiency rate did not change in the groups fed 1 g propolis/kg diet, but increased significantly in the groups offered 2 and 4 g propolis/kg diet. Moreover, Bae et al. (2011), supplemented 0.25%, 0.5%, 1%, 2% and 4% propolis to the diets of juvenile eels (*Anguilla japonica*) and determined that weight gain, growth rate, feed rate, feed rate at the end of 12 weeks of feeding, and protein efficiency rates were the highest in the diets supplemented with 0.5% propolis.

In a study conducted by Yonar et al. (2012), the effect of propolis on malondialdehyde (MDA), glutathione (GSH) and glutathione-S-transferase (GST) enzyme activity in common carp (*Cyprinus carpio carpio*) under different water temperature conditions was investigated. Fish were kept at 20, 24 and 28 °C and propolis (10 mg kg^{-1} bait) was applied to these fish. It was determined that the MDA level of the fish at 20 °C and 28 °C increased significantly, while the GSH level and GST activity decreased. Furthermore, Keleştemur et al. (2012), determined that the blood total protein and BAUN values of the fish fed with the diet supplemented with propolis were significantly lower than the control group, and the blood cholesterol and VLDL triglyceride values were higher compared to the control group. While the difference between the creatinine values of the groups was not statistically significant. Another study was conducted to investigate the effect of propolis on some hematological parameters in rainbow trout (Orkinos mykiss) by Yonar and Silici (2010). Researchers reported that the differences were not significant in terms of hematocrit value (P>0.05), hemoglobin amount (P>0.01) and erythrocyte indices (P>0.001) of the fish treated with propolis. However, the differences among groups for leukocrit and leukocyte values were reported to be statistically significant.

Result

Türkiye has a significant potential for beekeeping and the production of bee products. However, aside from the use of other bee products other than honey in "Apitherapy" studies, the targeted level for their production has not been reached yet. The increasing awareness of the side effects of synthetic drugs which are widely used today and the resistance of disease agents to these drugs have led people to demand natural medicine products and food produced naturally and safely. Despite being used in folk medicine for centuries by people use of propolis in animal farming is a new field of study. Propolis is proven to be effective in increasing the feed efficiency and growth performance. Moreover, it also shows activity in the protection against disease agents, strengthening and building immunity as well as serving as an antioxidant agent in animal farming. All the above mentioned studies provide evidence that propolis can be a promising alternative to antibiotics and other synthetic drugs in different branches of animal production. Furthermore, propolis stands out as a significant substance in ecological agriculture where the use of antibiotics and synthetic drugs are restricted.

Ethical Statement

Not applicable.

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Conflict of Interest

The author declare no conflict of interest.

Author Contributions

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

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RESEARCH PAPER

Molecular Characterization of the Honeybee (*Apis mellifera*) from two Vegetational Zones in Nigeria

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Abstract

The genetic structure and diversity of *Apis mellifera* from eight populations across two vegetational zones of Nigeria was investigated in order to provide base line information that will enhance its conservation, genetic improvement and sustainable yield. Genomic DNA was extracted from 40 specimens randomly selected from eight colonies in derived savannah and tropical rainforest regions of Nigeria. The specimens were amplified using five Random Polymorphic DNA (RAPD) primers and the amplicons generated were assessed using appropriate statistical indices. Results generated revealed significant genetic polymorphisms among the eight populations of *A. mellifera* ranging from 38.89 to 68.52% with a total of 281 bands amplified from 54 loci in all samples. The derived savannah population (Offa) had a greater amount of genetic diversity than the rest seven (7) populations as revealed by the percentage of polymorphic loci, expected heterozygosity and Shannon information index (I). The genetic structure as revealed by the Neighbour-Joining dendrogram showed that the eight populations studied represent two major genotypic groups with intra-group relationships. Eleyoka and Offa populations were the genetically closest in the first group while Ayetoro-Gbede and Ayegunle-Gbede populations were the genetically closest in the second group. The study suggests that the two major genotypic groups represent two distinct genetic stocks and can thus be managed accordingly.

Introduction

Apis mellifera, also referred to as the western honey bee is a beneficial and economically important insect. The usefulness of honey bees cannot be overemphasized in terms of the role it plays in the pollination of many native plants as well as in the production of essential seed crops, fiber and food crops (Ratcliffe et al., 2011; Awodiran et al. 2021). 80% of all insect pollination are done by honey bees (Sung et al., 2006). The honey bee is also appreciated for its hive products which has both medicinal and economic benefits. The hive products include honey, bee wax, royal jelly, bee venom, propolis, bee bread etc. Some of these products are known for their anti-oxidant properties, wound healing properties, immunity boosting potential and treatment of infection among others. Bees and other pollinators have been known to contribute greatly to the agricultural sector; with an annual estimate of between $235 - $577 billion worldwide (FAO, 2018). *A. mellifera* has suffered a recent population decline due to overexploitation, habitat degradation, poor beekeeping practices, the threat of pests and diseases as well as exposure to non-target or residual pesticide toxicity in the farm (Shaibi & Moritz, 2010; Awodiran et al., 2021). Conservation of genetic diversity is a major way to ensure the sustainable yield of economically important species. The need to preserve the genetic diversity of domesticated species cannot be overstressed. High genetic diversity helps in the adaptability of a population to its environment and promotes the long term health and persistence of that species (Awodiran et al., 2016). The fitness of honey bee colonies is being enhanced by high genetic diversity (Mattila et al., 2008). The honeybee (*Apis mellifera*) has been extensively studied from different perspectives such as ecological, morphometric and genetic studies (Ruttner et al., 2000; Whitfield et al., 2006; Szalanski & McKern, 2007; Tunca & Kence, 2011). Yogesh and Khan (2014) investigated the genetic

diversity and structure of *Apis mellifera* sampled from nine colonies in different regions of India using five Random Amplified Polymorphic DNA primers. Awodiran et al. (2021), studied the genetic characterization of *Apis mellifera* populations from the rain forest and derived savannah zones of Nigeria using morphological and microsatellite markers. Munoz and De La Rúa (2021), reported the genetic structure of honey bee, *A. mellifera* ecotypes and subspecies whose evolutionary lineage is of east European origin using mitochondrial and microsatellite markers. In spite of the widespread knowledge of the numerous benefits of conserving the genetic diversity of domesticated species, there are very few reports on the genetic diversity of arthropod species that have beneficial values (Yogesh & Khan, 2014). Moreover, considering the commercial and economic importance of *Apis mellifera,* there is need for more studies that will provide information on their genetic characterization in Nigeria. In this study, the genetic diversity and structure of *Apis mellifera* from eight populations across two vegetational zones of Nigeria was investigated in order to provide baseline information that will enhance its conservation, genetic improvement and sustainable yield.

Materials and Methods

Sample collection and study areas

Live samples of *Apis mellifera* workers were collected randomly from eight colonies in two vegetational zones of Nigeria namely: the tropical rainforest and derived savannah zone as shown in Table 1 and described in Figure 1. The tropical rainforest samples were collected from two towns in two different states respectively; Osun (Ipetumodu town and Modakeke town), Oyo (Ibadan town and Eruwa town) while the derived savannah zone samples were also collected from two towns in two different states respectively; Kwara (Offa town and Eleyoka town), Kogi (Ayegunle-gbede town and Ayetoro-gbede town). Five specimens were obtained from each colony in eight different apiaries which do not practice migratory beekeeping (i.e. one apiary was sampled in each location). The specimens were sacrificed in ether vapour and preserved in 80% ethanol for genomic DNA extraction. Identification was done with the aid of a dissecting binocular microscope, using identification keys prepared by Michener (2007).

Table 1. Geographical parameters *of the studied Apis mellifera* Populations

Population	Region	Altitude (m)	Latitude (N)	Longitude (E)	Annual Rainfall (mm)	Annual Temperature (°C)
Ayetoro-Gbede	**Derived Savannah**	508	7°59′	6°0′	1200	30
Ayegunle-Gbede	**Derived savannah**	515	7°21′	5°58′	1300	31
Eleyoka	**Derived savannah**	349	8°13′	4°49′	1150	29
Offa	**Derived savannah**	419	8°9′	4°43′	1250	25.7
Ipetumodu	**Rainforest**	252	7°22′	4°30′	1347	26.2
Modakeke	**Rainforest**	264	7°18′	4°16′	1350	25.8
Ibadan	**Rainforest**	230	7°23′	3°55′	1420	23.94
Eruwa	**Rainforest**	252	7°32′	3°27′	1320	26

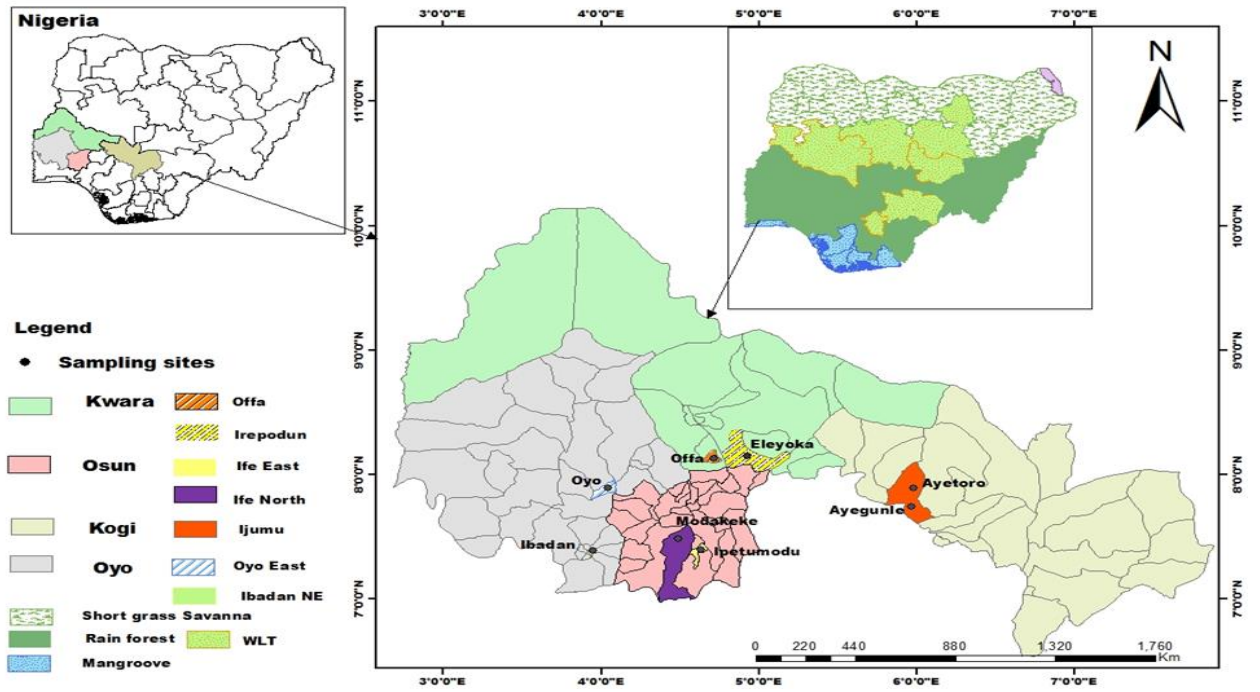

Figure 1. Map showing the vegetation zones and sampling locations of *Apis mellifera*

RAPD/PCR

Whole Genomic DNA of honey bees was extracted from the preserved thorax using CTAB method (Saghai et al., 1984). The concentration of the DNA samples was measured at 260 nm and 280 nm using Nanodrop spectrophotometer. The integrity of the DNA samples was further detected by agarose gel electrophoresis. The genomic DNA was amplified using Random Amplified Polymorphic DNA (RAPD) primers. A total of 10 primers (OPA02, OPA05, OPA07, OPA08, OPAB11, OPAB02, OPA13, OPAB14, OPA15, OPAB08) with different combinations of annealing temperature (37°C - 40°C) were tested, out of which five primers which showed good amplification and exhibited the highest variability were selected for population analysis (Table 2).

The amplification was done according to standard PCR protocols as follows: first denaturation at 95°C for 3 mins, another denaturation at 94°C for 30 secs, annealing for 30 secs, and primer extension at 72°C for 30 secs followed by another extension for 10 mins. Fragment analysis was accomplished by agarose gel electrophoresis. The gel was viewed under UV light, using ethidium bromide as the fluorescent dye. DNA sizing was done using 100 bp DNA ladder (Norgen PCR Sizer). The DNA ladder was loaded along with the gels. The amplified bands were scored as numerical values using GelQuest (GelQuest, 2008).

Table 2. List of RAPD primers used, their sequence and annealing temperature

PRIMERS	SEQUENCE (5'→3')	TM (°C)
OPA 02	TGCCGAGCTG	37.0
OPA 05	AGGGGTCTTG	37.0
OPA 08	GTGACGTAGG	37.6
OPA 13	CAGCACCCAC	37.3
OPAB 11	GTGCGCAATG	36.9

Data and statistical analysis

The data generated was analysed using the Genalex 6.502 (Peakall & Smouse, 2006; 2012). The indices of genetic diversity that were assessed for each population include number of effective alleles (Ne), number of different alleles (Na), analysis of molecular variance (AMOVA), fixation indices, percentage polymorphism, expected heterozygosity, Shannon's Information Index, genetic identity and distance (Nei, 1978). Neighbour-Joining dendrogram was constructed using Evolview (He et al., 2016) and Phylip-3.695 (Felsentein, 2014)

Result

A total of 281 bands were amplified from 54 loci in all samples for the five RAPD primers studied. The derived savannah populations had a total of 145 bands, while the tropical rainforest populations had 136 bands. The amount of genetic diversity, as measured by expected heterozygosity, number of bands, number of private bands, band frequency, and number of locally common bands among the eight populations studied is shown in Figure 2. The Analysis of molecular Variance (AMOVA) (ϕPT =0.001, ϕRT = 0.084, ϕPR = -0.091) indicated that the honey bee populations are significantly different from one another at p = 0.001, but not at greater probability levels (ϕPT is the fixation index). AMOVA also revealed that 92% of the total variation existed within the population, 0% among populations, and only 8% among regions. The number of different alleles (Na), number of effective alleles (Ne), Shannon's information diversity index (I), expected heterozygosity (He), and unbiased diversity values are shown in Table 3. The expected heterozygosity estimate for the honeybee populations ranged between 0.151 (Ibadan) and 0.249 (Offa). Shannon's Information diversity index values for the populations ranged between 0.222 (Ibadan) and 0.371 (Offa). Eleyoka and Eruwa populations had the highest number of bands (39), while the lowest number (27 bands) was recorded in the Ibadan population (Figure 2). Ayetoro, Eleyoka, Offa, and Modakeke populations have one different private band each; Ibadan and Eruwa each have two different private bands while Ayegunle and Ipetumodu have no private bands (Figure 2). Nei's genetic identity, which measures the similarities across all the eight populations, revealed 95% similarity, while the genetic distance, a reciprocal of the genetic identity is 0.05 (Tables 4 & 5). The percentage of polymorphic loci ranged between 38.89 and 68.52%. The lowest and highest percentages were recorded in Ibadan and Offa populations respectively (Table 6). The Neighbour-Joining dendrogram revealed two major genotypic groups with intra-group relationships. The first genotypic group further separates into three distinct clades. The first clade comprises of *A. mellifera* population from Eleyoka, Offa, and Ipetumodu; with Eleyoka and Offa being the closest in the clade. The second clade consists of Ibadan and Eruwa populations while *A. mellifera* population from Modakeke singly represents the third clade. The second major genotypic group comprises of Ayetoro-Gbede and Ayegunle-Gbede populations (Figure 3).

Table 3. Basic indicators of genetic variation across populations for the RAPD data

Population		N	Na	Ne	I	He	uHe	
Ayetoro	Mean	4.000	1.241	1.378	0.332	0.222	0.254	0.031
	SE	0.000	0.132	0.051	0.039	0.027		
Ayegunle	Mean	4.648	1.241	1.378	0.326	0.219	0.246	0.031
	SE	0.066	0.129	0.053	0.040	0.028		
Eleyoka	Mean	4.000	1.352	1.309	0.305	0.196	0.224	0.027
	SE	0.000	0.122	0.043	0.035	0.024		
Offa	Mean	4.815	1.370	1.425	0.371	0.249	0.278	0.030
	SE	0.053	0.128	0.051	0.038	0.027		
Ipetumodu	Mean	3.278	1.278	1.360	0.330	0.219	0.261	0.031
	SE	0.104	0.128	0.046	0.038	0.026		
Modakeke	Mean	4.259	1.204	1.358	0.310	0.208	0.239	0.032
	SE	0.157	0.131	0.053	0.039	0.028		
Ibadan	Mean	3.630	0.889	1.259	0.222	0.151	0.176	0.032
	SE	0.107	0.129	0.049	0.039	0.027		
Eruwa	Mean	3.889	1.389	1.391	0.354	0.235	0.275	0.031
	SE	0.158	0.122	0.048	0.037	0.026		

Na = No. of Different Alleles
Ne = No. of Effective Alleles
I = Shannon's Information Index
He = Diversity' Expected Heterozygosity
uHe = Unbiased Diversity
SE = Standard Error

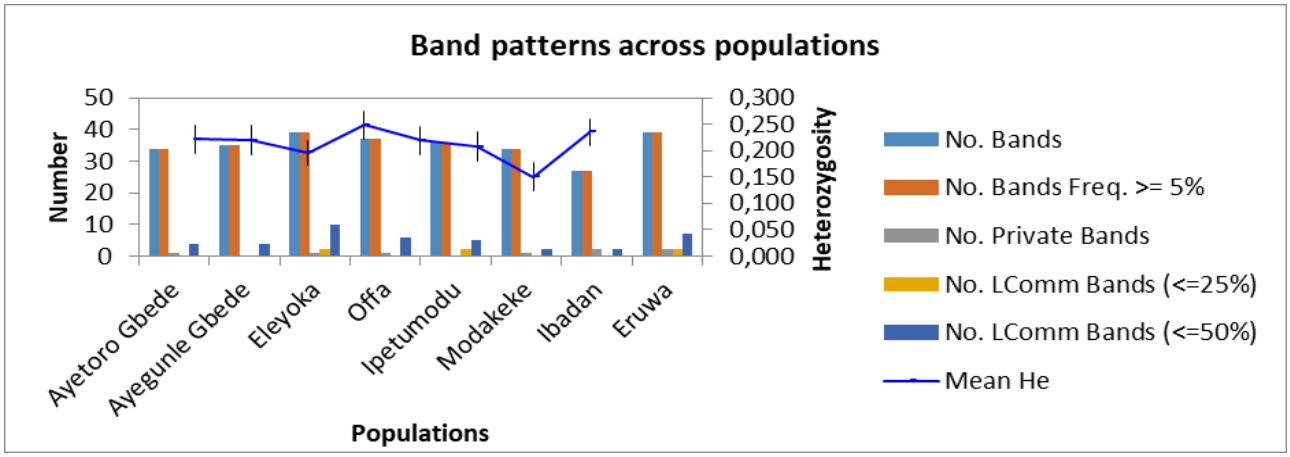

Figure 2. Band patterns and mean diversity of *Apis mellifera* across populations

Table 4: Nei's Unbiased Genetic distance among the eight populations of *Apis mellifera* studied.

Ayetoro	Ayegunle	Eleyoka	Offa	Ipetumodu	Modakeke	Ibadan	Eruwa	
0.000								**Ayetoro**
0.029	0.000							**Ayegunle**
0.076	0.092	0.000						**Eleyoka**
0.005	0.047	0.011	0.000					**Offa**
0.010	0.024	0.067	0.019	0.000				**Ipetumodu**
0.023	0.032	0.113	0.044	0.002	0.000			**Modakeke**
0.037	0.080	0.123	0.065	0.074	0.091	0.000		**Ibadan**
0.010	0.029	0.113	0.044	0.039	0.040	0.063	0.000	**Eruwa**

Table 5: Nei's Unbiased Genetic Identity among the eight populations of *Apis mellifera* studied

Ayetoro	Ayegunle	Eleyoka	Offa	Ipetumodu	Modakeke	Ibadan	Eruwa	
1.000								**Ayetoro**
0.971	1.000							**Ayegunle**
0.927	0.912	1.000						**Eleyoka**
0.995	0.954	0.989	1.000					**Offa**
0.990	0.976	0.935	0.981	1.000				**Ipetumodu**
0.977	0.969	0.894	0.957	0.998	1.000			**Modakeke**
0.964	0.924	0.884	0.937	0.928	0.913	1.000		**Ibadan**
0.990	0.972	0.894	0.957	0.962	0.961	0.939	1.000	**Eruwa**

Table 6: Percentage Polymorphism across the Populations

Population	Polymorphism (%)
Ayetoro Gbede	61.11%
Ayegunle Gbede	59.26%
Eleyoka	62.96%
Offa	68.52%
Ipetumodu	61.11%
Modakeke	57.41%
Ibadan	38.89%
Eruwa	66.67%
Total Mean	59.49%
SE	3.22%

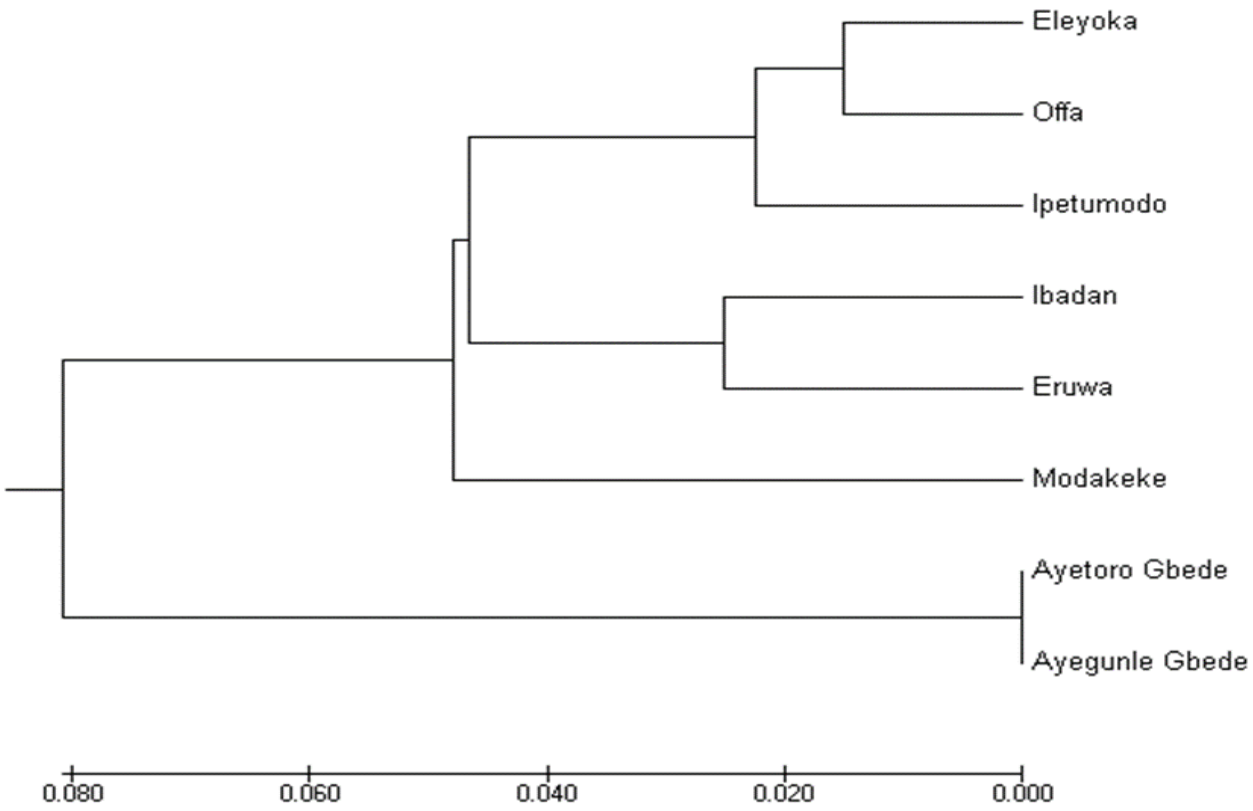

Figure 3. Neighbour-joining dendogram based on Nei's (1978) unbiased genetic distance showing the genetic relationship among the eight populations of *Apis mellifera* from two vegetation zones in Nigeria

Discussion

Amplification of five RAPD-PCR primers was used in this study to comparatively estimate genetic diversity and structure in eight populations of *Apis mellifera.* The result of which revealed significant genetic polymorphisms among the eight populations of *A. mellifera* from derived savannah (Ayegunle-Gbede, Ayetoro-Gbede, Offa, and Eleyoka) and rainforest (Ipetumodu, Modakeke, Eruwa, Ibadan) zones of Nigeria. The derived savannah population (Offa) had a greater amount of genetic diversity and allelic richness than the rest seven (7) populations as shown by indicators such as the number of alleles (Na), the number of effective alleles (Ne), percentage of polymorphic loci (P%), expected heterozygosity and Shannon information diversity index (I). Gene heterozygosity which is a suitable parameter for investigating genetic diversity had values that ranged from 0.151 to 0.249 with an average value of 0.212. This implies that the studied populations of *A. mellifera* can be said to be moderately diverse. The expected heterozygosity values observed in this study are comparable to those reported in other studies on *A. mellifera*. Tunca and Kence (2011), reported expected heterozygosity levels ranging between 0.035 and 0.175 estimated from *A. mellifera* L. populations in Turkey using RAPD markers. Qamer et al. (2021), also reported heterozygosity values ranging from 0.254 to 0.320 in honey bees, *Apis dorsata* collected from two districts in Pakistan using the RAPD marker. Awodiran et al. (2021), however, reported unbiased expected heterozygosity (uHe) values ranging from 0.830 to 0.997 with an average value of 0.902±0.118 in *A. mellifera* L. populations from 28 colonies in the tropical rainforest and the derived savanna zones of Nigeria. The values were estimated from five microsatellite DNA primers. High heterozygosity estimates in a population of a species may be due to long-term selection for adaptation, and the introduction of different strains from several viable populations constituting the parent stock. Expected heterozygosity estimate from a RAPD marker refers to the loci that show evidence of more than one allele (i.e. polymorphic loci). A high level of average heterozygosity in a population is presumed to correlate with high levels of polymorphisms at loci with consequential significance for adaptive response to environmental changes (Kotzé & Muller, 1994). The mean unbiased expected heterozygosity (uHe), a better expression of gene diversity reported in this study was 0.244. The overall fixation index value (ϕPT), which is a good estimate of the genetic differentiation of populations, was very low (0.001). This is an indication that the eight honey bee populations (colonies) studied are genetically similar. This is corroborated by the high value estimated from the genetic similarity matrix. The highest genetic similarity estimate (0.998) was recorded between the pair of Ipetumodu and Modakeke populations (both of Rainforest zones). This is expected because the two populations are the geographically closest, followed by Ayetoro-Gbede and Offa (both of derived savannah zones) with a similarity value of 0.995. The lowest genetic similarity estimate (0.884) was recorded between Eleyoka and Ibadan populations. Genetic distances (GD) according to Nei (1978) were calculated for each pair of populations. The lowest GD (0.002) was found between Ipetumodu (rainforest) populations and Modakeke (rainforest) populations, while the highest GD (0.123) was found between Eleyoka (derived savannah) populations and Ibadan (rainforest) populations. Nei's genetic identity, which measures the similarities across all eight populations, revealed 95% similarity, while the genetic distance, a reciprocal of the genetic identity is 0.05. This is consistent with the findings of Thorpe and Sole-Cava (1994), who reported that most same-species populations have a genetic similarity index that is above 0.85. The genetic structure as revealed by the Neighbour-Joining dendrogram showed that the eight populations studied represent two major genotypic groups with intra-group relationships. Eleyoka and Offa populations were the genetically closest in the first major group while Ayetoro-Gbede and Ayegunle-Gbede populations were the genetically closest in the second group. The study suggests that the two major genotypic groups represent two distinct genetic stocks and can thus be managed accordingly.

Conclusion

The study therefore revealed that RAPD markers can be used as a simple and cost-effective alternative relative to other advanced markers in providing base-line information on the genetic characterization of *A. mellifera* populations. Meanwhile, conservation efforts should be geared towards increasing the inherent genetic diversity in the studied *A. mellifera* populations in order to enhance its sustainable yield.

Ethical Statement

Not applicable.

Funding Information

No external funding was received for this study.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Author 1: Conducted field and laboratory work, acquisition of data and analysis,

Author 2: Conceptualized and supervised the study; critically reviewed the manuscript

Author 3: Led manuscript writing/wrote original draft

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RESEARCH PAPER

Analysis of Honey Export Potential and Competitiveness of Türkiye

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Abstract

The rich vegetation of Türkiye, different climatic zones and honey bee gene resources make the country's beekeeping activities advantageous. Türkiye has an important potential with the presence of colonies and honey production. In 2021, 96344 tons of honey was produced with 8733394 colonies, and 9994 tons of honey was exported to 66 countries for 31148000 dollars. This study was designed to reveal the honey export structure and competitiveness of Türkiye in the 2002-2021 period. In Türkiye's honey trade, the USA (29.64%), Germany (22.32%) and Spain (7.22%) are the main importing countries. It has been determined that Türkiye is a net exporter country in the 2002–2021 periods. Türkiye is predominantly a country with medium and weak comperative advantage. The Revealed Comparative Advantages (RCA), Revealed Symmetric Comparative Advantages (RSCA), Trade Balance (TBI) values calculated with Türkiye's 2021 data was found 1.13, 0.06 and 0.98 respectively. These values show that Türkiye is a net exporter and a highly competitive country with a declared comparative advantage. Providing Türkiye's access to rapidly growing markets in direct proportion to its beekeeping capacity and potential will make Türkiye a more competitive country

Introduction

Honey; it is a natural product that plant the nectars, secretions from living parts of plants, or secretions of plant – sucking insects living on living parts of plants after being collected by the honey bee *Apis mellifera*, modify it by combining with its unique substances, reduce its water content, and nature it by storing it in the honeycomb (Anonymous, 2022a).

1 million 770 thousand 119 tons of honey were produced with 93999656 colonies in the world. In the last 10 years, an increase of 16.9% in the number of colonies and an increase of 8.81% in honey production has been achieved. In terms of the number of colonies, India comes first with 2204850 colonies. India is followed by China with 9337361 colonies, Türkiye with 8179085 colonies, Iran with 7140561 colonies, Etiopia with 6986100 colonies, Tanzania with 3003012 colonies, Argentina with 2983247 colonies, Russia with 2982452 colonies, USA with 2706000 colonies and Korea with 2162250 colonies respectively. In terms of honey production, China is followed by Türkiye with 104403 tons, Ukraine with 68028 tons, USA with 66948 tons, Russia with 66368 tons, India with 62123 tons, Mexico with 54165 tons and Brazil with 51508 tons respectively (Anonymous, 2022b).

Compared to sugar and other sweeteners, the higher nutritional properties of honey and the positive health effects of honey are the most important factors that trigger the global honey supply. Honey trade in the world is increasing its importance day by day. The world honey market is undergoing a major transformation as a result of fluctuating exchange rates and commodity prices, increasing raw material costs, global farming, colony losses and globalization of cultures. This transformation brings the competitiveness between countries to the fore.

Although there are many studies on the concept of competitiveness, a general theory on international competitiveness has not been established (Mitschke, 2008). International competitiveness can be examined at different levels such as product, company, sector, region, country, trade block or a part of world trade, as the reasons for the lack of an accepted definition of international competitiveness. Because there are different forms of competitiveness, different ways of

measuring the competitiveness of companies, sectors and economies are required, depending on the level of micro or macro discussion. There is no consensus on the concept among academics dealing with international competition issues. Existing literature reviews reveal a lack of clear consensus on the exact meaning of international competitiveness stems from the concept of competition. Others argue that the roots of international trade theories. For this reason, many definitions describing the concept of international competitiveness make it a variable concept (Olczyk, 2016a; Olczyk, 2016b).

It is seen that the competitiveness studies on the international honey trade of the countries are quite limited. The main studies on the subject are as follows; China's honey export competitiveness (Ma, 2009; Song & Jensen, 2014); Serbia's honey export competitiveness (Ignjatijević et al., 2015; Ignjatijević et al., 2018; Cvijanović & Ignjatijević, 2020); Italy's honey export competitiveness (Pippinato et al., 2019); Mexico's honey export competitiveness (Magana Magana et al., 2017; Avila et al., 2019); honey export competitiveness of Brazil (Paula et al., 2016a; Paula et al., 2016b; Paula et al., 2016c; Paula et al., 2017; Campos et al., 2018); honey export competitiveness of selected European Countries (Pocol et al., 2017; Covaci, 2020). Comparison of honey export competitiveness of Türkiye with Balkan countries (Terin et al., 2018).

In Türkiye, 89631 beekeeping enterprises produced 96344 tons of honey with 8733394 colonies in 2021. Honey production in 2021 decreased by 7.4% compared to the previous year due to adverse climatic conditions and forest fires. In the last 20 years in Türkiye, the beekeeping business has increased by 297% (Anonymous, 2022c). The rich honey bee gene resources and advantageous flora and climatic conditions, which spread over a wide area in the Anatolian geography, have caused Türkiye to become one of the important actors of the World beekeeping sector.

In this study; It is aimed to explain Türkiye's honey export performance an international competitiveness between the years 2002-2021 with the Revealed Comparative Advantages (RCA), Revealed Symmetric Comparative Advantages (RSCA) and Trade Balance (TBI) indexes.

Material and Methods

In this study; it is aimed of the study consists of secondary data at the macro level. Honey foreign trade data used in the study and covering the period 2002 – 2021 were taken from the "Trade statistics for international business development (Trade Map)" database. In the study, foreign trade data of 0409 coded '' Natural Honey', which is a subgroup of 04 coded ''Dairy produce; egs; natural honey; edible products of animal origin, not elsewhere specied or inclued'' product group and included in Harmonized product Classification (HC), was used.

The first criterion used to determine the competitiveness of Türkiye's honey foreign trade is the Revealed Comparative Advantages index. Liesner (1958), was the first to introduce the Revealed Comparative Advantages (RCA) index to the literature. Later, it was redefined and developed by Balassa (1965). Balassa's RCA approach assumes that the true form of comparative advantage can be observed from post – trade data. With this approach, Balassa tries to determine whether a country has an 'explained' comparative advantage in the relevant commodity or sector.

Balassa's RCA index is formulated as:

$$RCAij = [(Xij Xi) / (Xwj Xw)]$$

RCAij, the comparative advantage index announced for the 'j' sector of country 'i',

Xij exports of 'j' sector of country 'i',

Xi 'i' country's total exports,

Xwj 'j' sector's exports to the world,

Xw represents the world's total exports.

Where the index value is greater than 1, the country has a revealed comparative advantage in the relevant good; in cases where the index value is less than 1, it is concluded that the country does not have a comparative advantage in the relevant product (Mushanyuri & Mzumara, 2013).

The RCA index value was categorized as follows by Hinloopen and Marrewijk (2001).

$0 < RCA \leq 1$: No comparative advantage
$1 < RCA \leq 2$: Weak comparative advantage
$2 < RCA \leq 4$: Medium comparative advantage
$4 < RCA$: Strong comparative advantage

The second criterion used in the measurement of competitiveness is the "Revealed Symmetric Comparative Advantages (RSCA)" index. This index is formulated as follows:

$$RSCAij = (RCAij - 1) / (RCAij + 1)$$

The RSCA index takes a value between -1 and +1. If the index value is positive, the country has competitiveness in that product; if it is negative, it indicates that the country has a comparative disadvantage in the trade of that product (Dalum et al., 1998).

The last criterion used to measure competitiveness is the Trade Balance Index (TBI). This index developed by Lafay is used to determine whether a country is a net exporter or a net importer of the relevant product (Ishchukova & Smutka, 2013a; Ishchukova & Smutka, 2013b).

The index is formulated as:

TBIij = (Xij − Mij)/(Xij + Mij)

TBIij, the country's trade balance of goods j;
Xij, the exports of product "j"
Mij, the imports of product "j"

TBI index takes a value between -1 and +1 (ShariatUllah & Kazuo, 2012).

If TBIij > 0, the country is a net exporter.
If TBIij < 0, the country is a net importer.

Results and Discussion

World honey exports increased by 378% in the 2002-2021 period. In the analyzed period, the total honey export value in the world was 33239558000 dollars, and the total honey export value in Türkiye was 326429000 dollars. Türkiye's average share in world honey exports over the years has been 1.121%. In the category of all goods subject to export, Türkiye's total exports constitute an average of 0.814% of the world's total exports (Table 1).

Table 1. Export data for Türkiye and the World for 2002-2021($ 1000), (Anonymous, 2022d)

Years	Türkiye's Honey Exports	World's Honey Exports	Share (%)	Türkiye's Total Goods Exports	World's Total Goods Exports	Share (%)
2002	32335	719019	4.497	35761981	6432105964	0.556
2003	37090	960880	3.860	47252836	7498530918	0.630
2004	16329	852304	1.916	63120949	9110737596	0.693
2005	6564	708660	0.926	73476408	10360495753	0.709
2006	5499	829836	0.663	85534676	11979108568	0.714
2007	1759	897138	0.196	107271750	13809800618	0.777
2008	2286	1329366	0.172	132027196	16007659828	0.825
2009	4495	1293905	0.347	102142613	12384813282	0.825
2010	5811	1497393	0.388	113883219	15098981170	0.754
2011	5206	1724579	0.302	134906869	18141372916	0.744
2012	6007	1779265	0.338	152461737	18399990900	0.829
2013	13020	2081259	0.626	161480915	18858726557	0.856
2014	18934	2338421	0.810	166504862	18862399126	0.883
2015	25072	2324938	1.078	143844066	16416895796	0.876
2016	14926	2228806	0.670	142606247	15923096945	0.896
2017	23385	2391744	0.978	156992940	17561440015	0.894
2018	25669	2264578	1.134	167923862	19327897410	0.869
2019	24763	1988243	1.245	180870841	18750885146	0.965
2020	26161	2320105	1.128	169657940	17488466269	0.970
2021	31148	2717309	1.146	225264314	22112533133	1.019

In 2021, Türkiye exported 9994 tons of honey to 66 countries for 31148000 dollars. In Türkiye's honey trade, the USA (29.64%), Germany (22.32%) and Spain (7.22%) are the main importing countries (Table 2).

Table 2. Countries importing honey from Türkiye in 2021, (Anonymous, 2022d)

Importers	Value exported in 2021 (USD thousand)	Share (%)	Quantity exported in 2021 (ton)	Share (%)
World	31148	100	9994	100
United States of America	9231	29.6	2916	29.2
Germany	6951	22.3	1544	15.4
Spain	2250	7.2	1101	11.0
Bulgaria	1125	3.6	799	8.0
Israel	975	3.1	492	4.9
Belgium	907	2.9	217	2.2
United Arab Emirates	831	2.7	176	1.8
United Kingdom	738	2.4	229	2.3
Slovakia	721	2.3	497	5.0
Netherlands	623	2.0	136	1.4
Kuwait	603	1.9	103	1.0
Qatar	532	1.7	116	1.2
Italy	505	1.6	324	3.2
Canada	410	1.3	75	0.8
Japan	397	1.3	57	0.6
Poland	393	1.3	259	2.6
Oman	346	1.1	73	0.7
Australia	301	1.0	85	0.9
Hong Kong, China	277	0.9	59	0.6
Slovenia	273	0.9	43	0.4
Other	2759	8.9	693	7.0

The countries importing honey from Türkiye and their distribution by value are given in Figure 1 and Table 2. Comb honey constitutes 23% of honey exports in terms of quantity. Türkiye provided 12427000 dollars income from honeycomb export. When we look at the countries where honeycomb honey is exported, Germany takes the first place with 933635 kg. Germany is followed by the USA with 652082 kg, United Arab Emirates with 105621 kg, Spain with 92186 kg, Belgium with 88976 kg, and Japan with 50097 kg, respectively. Türkiye's income from the export of filtered honey covers 59.1% of the total honey export. Türkiye earned 18427000 dollars in exchange for 7701743 kg of filtered honey. The USA comes first with 2263000 kg of filtered honey from Türkiye. The USA is followed by Spain with 1008550 kg, Germany with 609979 kg, Bulgaria with 798536 kg, Slovakia with 496825 kg, Israel with 491625 kg (Anonymous, 2022d).

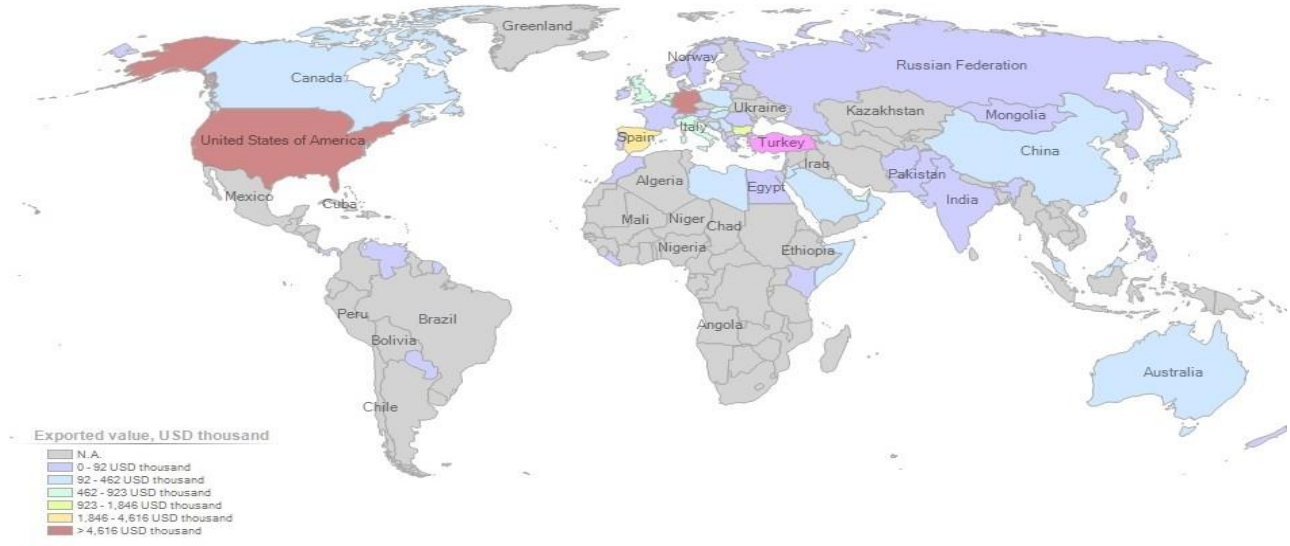

Figure 1. Countries to which Türkiye exports honey in 2021, (Anonymous, 2022d)

While countries such as Kuwait, Poland, Spain, Japan, Qatar, Oman, Canada, Italy, the United Arab Emirates, Belgium, Slovakia, the Netherlands and Bulgaria, which have a limited share in Türkiye's honey export, are rapidly growing markets; England, Israel, Australia and Hong Kong appear as shrinking markets. Germany, which has a large share in Türkiye's honey export, represents the shrinking market, while the USA represents the rapidly growing market. The USA and Germany, whose shares in Türkiye's honey export are 29.64% and 22.32%, respectively, and whose shares of global imports are 24.8% and 11.61%, respectively.

Average annual growth rates in the export value of countries in Türkiye's honey trade between 2017-2021 were 3% in the USA, 22% in Spain, 31% in Israel, 15% in Belgium, 39% in the United Arab Emirates, and 28% in Netherlands 39% in Kuwait, 14% in Qatar, 6% in Italy, 23% in Canada, 185% in Japan, 58% in Poland, 103% in Oman, 77% in Australia, 93% in Hong Kong and 28% in Slovenia, respectively (Figure 2).

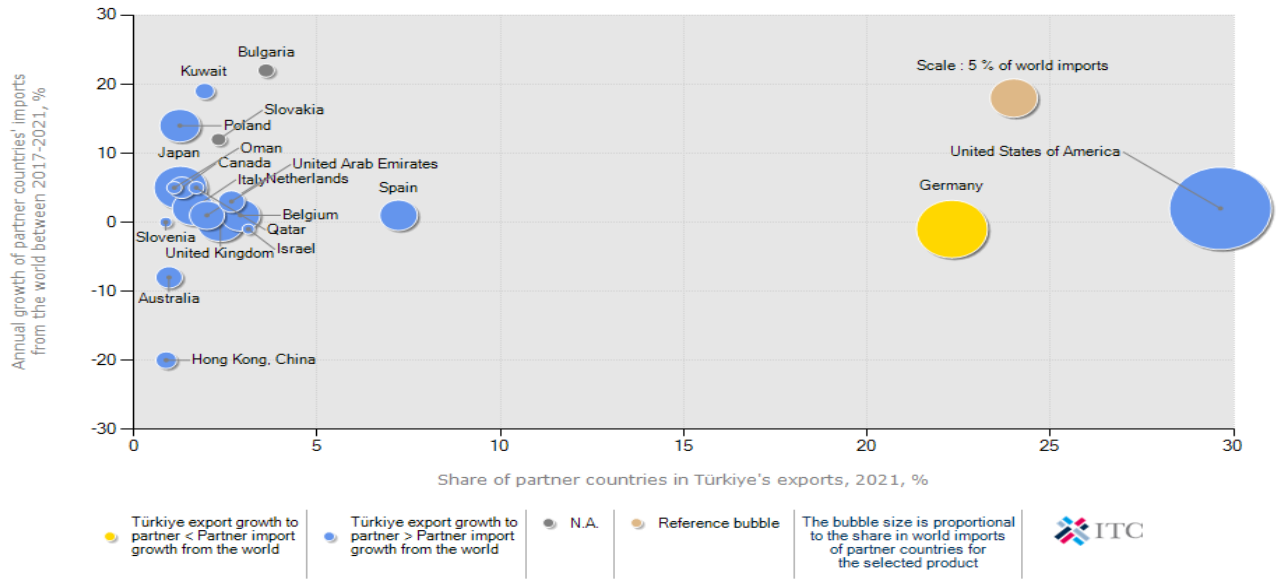

Figure 2. Potential graph for market diversification of honey exported by Türkiye, (Anonymous, 2022d)

Table 3. Türkiye's competitiveness indexes by years

Years	RCA	RSCA	TBI
2002	8.09	0.78	0.96
2003	6.13	0.72	0.94
2004	2.77	0.47	0.92
2005	1.31	0.13	0.85
2006	0.93	-0.04	0.96
2007	0.25	-0.60	0.78
2008	0.21	-0.65	-0.27
2009	0.42	-0.41	0.95
2010	0.51	-0.32	1.00
2011	0.41	-0.42	1.00
2012	0.41	-0.42	1.00
2013	0.73	-0.16	0.97
2014	0.92	-0.04	0.98
2015	1.23	0.10	1.00
2016	0.75	-0.14	1.00
2017	1.09	0.04	1.00
2018	1.30	0.13	0.99
2019	1.29	0.13	0.98
2020	1.16	0.08	0.98
2021	1.13	0.06	0.98
Average	1.55	-0.03	0.90

The Revealed Comparative Advantage Index calculated for Türkiye's honey export is given in Table 3. The RCA average for the 2002-2021 period was found to be 1.55. The average value obtained shows that Türkiye has a comparative advantage. Between the years 2006-2014 and in 2016, it is seen that the RCA values remained below 1 and did not have a comparative competitiveness. When the RCA indices are evaluated according to the Hinloopen and Van Marrewijk (2001), classification, it is seen that Türkiye had a strong comparative advantage in 2002 and 2003, moderate comparative advantage in 2004, did not have a comparative advantage in 2006-2014 and 2016, and appeared to have a weak competitiveness for other years. Türkiye's Revealed Symmetric Comparative Advantages (RSCA) index results are similar to the RCA index results. In the analyzed period, Türkiye's RSCA average was -0.03. Although Türkiye seems to have had a competitive advantage in the last 5 years according to the RSCA index; the values obtained show a tendency towards a comparative disadvantage in honey trade. When the values of Turkey's trade index are examined, it is clear that the country is a net exporter in all years except 2008. The mean TBI in the analyzed period was found to be 0.90. (Table 3). Türkiye,which became a net importer only in 2008; it imported 4 million dollars of honey from Argentina, Mexico and Uruguay, and also exported 2286000 dollars to mainly Germany and Hungary, as well as Cyprus, Saudi Arabia, Denmark, Belgium, Iraq, the United Arab Emirates and Albania. Terin et al. (2018), in the study that put forward the competitiveness of Türkiye and the Balkan Countries in honey exports in the period 2001-2015, stated that although Türkiye is a net honey exporter country, its competitiveness in honey trade is weak. In the honey trade competition, Türkiye is less competitive than Moldova, Bulgaria, Romania, Serbia, Croatia and Greece; it was determined to be more competitive than Macedonia, Albania, Bosnia and Herzegovina, Montenegro and Slovenia. In the RCA index study conducted by Ma (2009), with export values for 2006, it was found that China, Argentina and Mexico have a competitive advantage in honey exports, and Argentina has significantly higher index values than Mexico and China, it was reported that China's competitive advantage, affected by international trade barriers, is relatively weak. It is stated by Pippinato et al. (2019), that European Union countries are not very competitive in honey exports and are strongly inclined towards imports. It is noticed that Italy shows a significant comparative disadvantage when compared to Romania, which produces larger quantities in the trade of this product, Spain, and Germany highly specialized in trade. Chinese honey is less competitive than Argentine honey in the US and EU markets (Song & Jensen, 2014). It was reported by Paula et al. (2017), that Brazil was competitive in exporting natural honey products from 2002 to 2015.

Conclusion

As a result, it has been determined that Türkiye is a net exporter country in the 2002-2021 period. Türkiye is predominantly a country with medium and weak comparative advantage. The average RSCA value of the examined period shows that the country has a comparative disadvantage in honey trade. Considering the last 5 years of RSCA values for Türkiye, it is seen that it is a country with competitiveness in line with its potential. With its colony presence and honey and beeswax production potential, Türkiye is one of the most important players in the global beekeeping sector. Türkiye is a country with a high potential in terms of different geographical features, different climatic zones, honey bee gene resources, and plant genetic richness and diversity as it progresses from north to south and west to east. Beekeeping in Türkiye is an agricultural activity that has a conventional and organic production types has gained a professional status, and is carried out in every region of Türkiye. In the Mediterranean region of Türkiye, the nectar flow that starts with citrus continues with monofloral and polyfloral honeys in the Black Sea, Marmara and Anatolian regions and ends with pine honey produced in the Aegean and Mediterranean regions. 24 honeys including thyme, chestnut, geven, pine and oak monofloral honeys and flower honeys produced in different locations between 2017 and 2022 by the Turkish Patent and Trademark Office have been given geographical indications. When the distribution of geographical indications in Türkiye by product groups is evaluated, honey comprises 2% of all product groups. This rate is expected to increase steadily in the coming years. Many monofloral and polyfloral honeys such as oak, chestnut, sunflower, cotton, acacia, geven, thyme,lavender, chasteberry, etc., which have high yields, especially pine honey, deserve to be registered on an international scale. Considering the current potential of Türkiye's place in the world honey trade, it is seen that it is not at the desired level. Bringing the high capacity in the beekeeping sector to the fore and providing access to rapidly growing markets will make Türkiye a more competitive country.

Ethical Statement

Ethics certificate is not required.

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Conflict of Interest

The author declare no conflict of interest.

Author Contributions

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

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