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Investigation of Pesticide Residues in Natural Comb Honey from Tokat Province

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Abstract − Pesticides used in agricultural production pose a threat to bee health and may lead to residue issues in honey. While foraging for food, bees can carry chemicals to the hive from the environment, which may result in pesticide residues in bee products. In this study, 24 samples of natural comb honey collected from beekeepers in various districts of Tokat province, including Almus, Artova, Erbaa, Merkez, Niksar, Reşadiye, Pazar, Turhal, and Zile, were analyzed for 261 different pesticide residues. Natural comb honey samples were preferred to avoid potential pesticide contamination from the foundation comb or beeswax. Pesticide analyses were conducted at the Central Laboratory of Gaziosmanpaşa University in Tokat. In a sample from the Reşadiye district (Re-15), tebuconazole fungicide was detected at 0.025 mg/kg and pirimicarb insecticide at 0.469 mg/kg, both exceeding Maximum Residue Limit (MRL) values. However, all other samples tested had pesticide residues below detectable levels. The results suggest that the natural comb honey produced in Tokat province is safe, and the region is conducive to pesticide-free honey production.

Keywords − *Tokat, natural comb honey, pesticide, residue*

1. Introduction

Pesticides are commonly used in agriculture to control harmful organisms and minimize their impact on crop production. However, these chemicals not only affect the target pests but also have detrimental effects on non-target organisms over a wide area. Pesticides can contaminate groundwater and spread into the atmosphere, posing risks to bees and other pollinators, which are crucial for ecosystem health [1]. Bees and their products are indicators of environmental pollution, reflecting the presence of pesticides in the environment [2, 3, 4]. Pesticides can enter bee colonies through various routes, including contaminated pollen and nectar consumption, contact with treated plants and soil, inhalation during flight, drinking contaminated water, and direct exposure to sprayed pesticides [5,6].

The foraging behavior of honey bees, which involves collecting nectar and pollen from various sources, exposes them to environmental pollutants that can be transported back to the hive [7]. Their long-distance flights in search of food further increase the risk of pesticide exposure. The social structure of bee colonies, with food sharing and close contact among individuals, facilitates the rapid spread of contamination within the colony. Pesticides can harm bees by affecting their behavior, immune system, and ability to reproduce, leading to weakened or collapsed colonies, mainly due to worker bee deaths. Contaminated be products can also pose health risks to humans, causing short-term symptoms like skin and eye irritation, headaches, vomiting, and

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long-term health issues such as cancer, diabetes, asthma, Parkinson's disease, and genetic mutations [7,8]. Furthermore, decreasing pollinator populations, including bees, can reduce pollination and lower crop yields.

Various pesticides, including insecticides, fungicides, herbicides, bactericides, and acaricides, have been detected in apiaries [9-11]. The levels of pesticide residues tend to increase from honey to pollen and beeswax [4,12]. Regularly monitoring pesticide levels in honey is crucial for consumer health and guides producers [6]. The maximum allowable pesticide residue limit in honey is 0.01 mg/kg according to the "Turkish Food Codex Honey Communiqué" [13]. Exceeding these limits in bee products can also result in export issues, financial losses, and health problems.

According to the Turkish Food Codex Honey Communiqué, honey produced by bees with their combs without using a foundation comb is defined as “natural comb honey” [13]. Due to its lipophilic nature, Beeswax can accumulate pesticides and absorb volatile chemicals [8]. During the maturation process, honey is stored in beeswax cells, and the combs are reused in honey production in subsequent years, accumulating pesticide residues in old combs [19]. As a result, natural comb honey is considered less risky in terms of pesticide residues compared to other types of honey.

As far as we know, no pesticide analysis has been conducted on natural comb honey yet. The aim of this study is to determine the pesticide contents of natural comb honey produced in Tokat province. The paper is structured as follows: Section 2 provides a brief overview of sample collection and analyses. Section 3 discusses the results and implications, highlighting the issue of pesticide residues in honey in Turkey and the production of pesticide-free honey. Finally, Section 4 concludes the study by summarizing key findings, proposing future research directions, and offering recommendations to beekeepers.

2. Materials

A total of 24 natural comb honey samples were gathered from various districts in Tokat province, including Almus, Artova, Erbaa, Merkez, Niksar, Reşadiye, Pazar, Turhal, and Zile, once from each locality between August 2023 and September 2023 (Table 1). Prior to the honey season, beekeepers in these districts were provided with frames known as 'hoop bee frame' without basic combs. The bees were allowed to fill these frames with honey by constructing beeswax combs within them. Subsequently, the natural comb honey samples from the frames were collected, each weighing approximately 250 g, and stored in sealed jars using the leakage method at room temperature until analysis. The samples were properly labeled and sent to the Tokat Gaziosmanpaşa University Central Laboratory for further testing.

Table 1. Honey sample collection locations and dates in Tokat

Sample district	Sample locality	Proximity to	Sample number	Date
Almus	Almus	5km	Al-01	September 2023
Artova	Artova	11km	Ar-02	September 2023
Artova	Artova	200m	Ar-03	September 2023
Artova	Artova	100m	Ar-04	September 2023
Erbaa	Evciler village	12km	Er-05	September 2023
Erbaa	Karayaka village	8km	Er-06	September 2023
Erbaa	Erbaa	100m	Er-07	September 2023
Niksar	Başçiftlik	50m	Ni-08	September 2023
Niksar	Çamiçi plateau	20km	Ni-09	September 2023
Niksar	Hanyeri village	100m	Ni-10	September 2023
Niksar	Şıhlar village	7km	Ni-11	September 2023
Pazar	Kalaycık village	500m	Pa-12	September 2023
Pazar	Pazar	100m	Pa-13	September 2023
Reşadiye	Karaağaç village	20km	Re-14	September 2023
Reşadiye	Karlıyayla village	2km	Re-15	September 2023
Reşadiye	Özlüce village	25km	Re-16	September 2023

Table 1. (Continue) Honey sample collection locations and dates in Tokat

Sample district	Sample locality	Proximity to	Sample number	Date
Tokat Merkez	Aydoğdu village	8km	To-17	September 2023
Tokat	Tokat	2km	To-18	August 2023
Turhal	Yazıtepe village	10km	Tu-19	August 2023
Turhal	Çivril village	12km	Tu-20	August 2023
Turhal	Turhal	250m	Tu-21	August 2023
Zile	Karakuzu village	100m	Zi-22	August 2023
Zile	Kervansaray village	500m	Zi-23	August 2023
Zile	Ağılcık village	50m	Zi-24	August 2023

3. Methods

In the present study, pesticide residue analyses of honey samples were conducted using the method described for dried fig samples by Yeladı et. al. [15] at Tokat Gaziosmanpaşa University Central Laboratory. A total of 24 honey samples were tested for 261 different pesticide residues. The names of the pesticides screened are listed in Table 2.

Table 2. Pesticides analyzed in the study

2,4-D	Diafenthiuran	Hexaconazole	Pirimicarb
Abamectin	Diazinon	Hexaflumuron	Primiphos -ethyl
Acephate	Dichlofluanid	Hexythiazox	Primiphos -methyl
Acequinocyl	Dichlorfos	Imazalil sulfate	Prochloraz
Acetamiprid	Diclofop -methyl	Imazapyr	Profenefos
Acetochlor	Dicrotophos	Imidacloprid	Profoxydim-lithium
Acrinathrin	Diethofencarb	Indoxacarb	Promecarb
Alachlor	Difenacozole	Iodosulfuron-methyl-sodium	Prometryn
Aldicarb	Diflubenzuran	Ioxynil	Propaquizafob
Aldicarb-sulfone	Dimethenamid	Isocarbofos	Propargite
Aldicarb-sulfoxide	Dimethoate	Kresoxim Methyl	Propazine
Ametoctradin	Dimethomorph	Lenacil	Propiconazole
Amitraz	Diniconazole	Linuron	Propoxur
Atrazine	Dinocap	Lufenuron	Propyzamide
Azinphos-ethyl	Dioxacarb	Malaoxon	Prothiophos
Azinphos-methyl	Diphenamid	Malathion	Pymetrozine
Azoxystrobin	Diphenylamine	Mandipropamid	Pyraclostrobin
Benalaxyl	Diuron	MCPA	Pyrazophos
Benfuracarb	DMF	Mecarbam	Pyridaben
Benomyl	Dodine	Mepanipyrim	Pyridaphenthion
Bensulfuron-methyl	Emamectin benzoate	Mepanipyrim-hyroxypropyl	Pyridate
Bentazone	EPN	Metaflumizone	Pyrimethani
Bifenazate	Epoxiconazole	Metalaxyl M	Quinalphos
Bitertanol	EPTC	Metamitron	Quizalofop-ethyl
Boscalid	Ethiofencarb	Methacrifos-poz	Rimsulfuron
Bromoxynil	Ethion	Methamidophos	Sethoxydim
Bromuconazole	Ethirimol	Methidathion	Simazine
Buprimate	Etofenprox	Methiocarb	Spinosyn A
Buprofezin	Etoxazole	Methiocarb-sulfone	Spinosyn D
Butralin	Famaxadone	Methiocarb-sulfoxide	Spirodiclofen
Butylate	Fenamidone	Methomyl	Spiromesifen
Cadusafos	Fenamiphos	Methoxyfenozide	Spiroxamine
Carbaryl	Fenamiphos-sulfone	Metolachlor-S	Sulfoxaflor
Carbendazim	Fenamiphos-sulfoxide	Metosulam	Tebuconazole
Carbofuran	Fenarimol	Metrafenone	Tebufenozide
Carbofuran-3-hydroxy	Fenazaquin	Metribuzin	Tebufenpyrad

Table 2. (Continue) Pesticides analyzed in the study

Carbosulfan	Fenbuconazole	Mevinphos	Teflubenzuron
Carboxin	Fenbutatin oxide	Molinate	Tepraloxydim
Carfentrazone-ethyl	Fenhexamide	Monocrotophos	Terbutryn
Chlorantraniliprole	Fenoxycarb	Monolinuron	Terbutylazine
Chlorbufam	Fenoxyprob -ethyl	Myclobutanil	Tetraconazole
Chlorfenvinhos	Fenpropathrin	Novaluron	Tetramethrin
Chlorfluazuron	Fenproxymate	Nuarimol	Thiabendazole
Chloridazon	Fenthion	Omethoate	Thiacloprid
Chlorpyrifos	Fenthion-sulfone	Oxadixyl	Thiamethoxam
Chlorsulfuron	Fenthion-sulfoxide	Oxamyl	Thifensulfuron-
Clethodim	Fipronil	Oxycarboxin	Thiobencarb
Clodinofop-propargyl	Fipronil-sulfone	Oxydemeton-methyl	Thiodicarb
Clofentezine	Fluazifop-p-butyl	Paclobutrazol	Thiophanate-methyl
Clothianidine	Fluazinam	Paraoxon-ethyl	Tolclofos-methyl
Cyantraniliprole	Flubendiamide	Paraoxon-methyl	Tolfenpyrad
Cyazofamid	Fludioxinil	Parathion-ethyl	Tolyfluanid
Cycloate	Flufenoxuron	Penconazole	Tralkoxydim
Cycloxydim	Fluopicolide	Pencycuron	Triadimefon
Cyflufenamid	Fluopyram	Pendimenthalin	Triadimenol
Cyhalothrin	Fluquinconazole	Permethrin	Triasulfuron
Cymoxanil	Fluroxypyr	Phenmedipham	Triazophos
Cypermethrin	Flusilazole	Phenthoate	Tribenuron methyl
Cyproconazole	Flutriafol	Phorate	Trichlorfon
Cyprodinil	Forchlorfenuron	Phorate-sulfone	Trifloxystrobin
Dazomet	Formetanete hydrochloride	Phorate-sulfoxide	Triflumizole
Deltamethrin	Fosthiazate	Phosalone	Triflumuron
Demeton-s-methyl	Furathiocarb	Phosmet	Triticonazole
Demeton-S-methyl-sulfone	Haloxyfop-R-methyl	Phosphamidon	
Desmedipham	Heptenophos	Pirimicarb-Desmethyl	

4. Results and Discussion

In this study, natural comb honey samples taken from beekeepers in nine districts of Tokat province in 2023 were examined for pesticide residues. Natural comb honey samples were chosen to prevent contamination from honey produced in frames with foundation combs or repeatedly used frames, guaranteeing that any pesticide residues detected in the honey samples came from nectar or pollen sources in the environment.

In this study, 24 natural comb honey samples were analyzed for 261 different pesticide residues. According to results, only one sample (Re-15) exceeded the Maximum Residue Limit (MRL) for Pirimicarb and Tebuconazole pesticides (0.469 mg/kg and 0.025 mg/kg, respectively) (Figure 1). The remaining samples had pesticide levels below detectable limits. Despite heavy pesticide use in Tokat province, most of our samples (23 out of 24) were found free of pesticide residues. The detected pesticides, Tebuconazole and Pirimicarb, are moderately harmful to human health according to the World Health Organization [16]. The presence of these chemicals in the honey sample suggests contamination from agricultural pesticides used in the vicinity of the apiary. Our findings are consistent with previous studies that detected pirimicarb in honey samples from Egypt [17] and tebuconazole in honey samples from Italy [18]. These two pesticides have not been detected in the studies conducted so far in Türkiye.

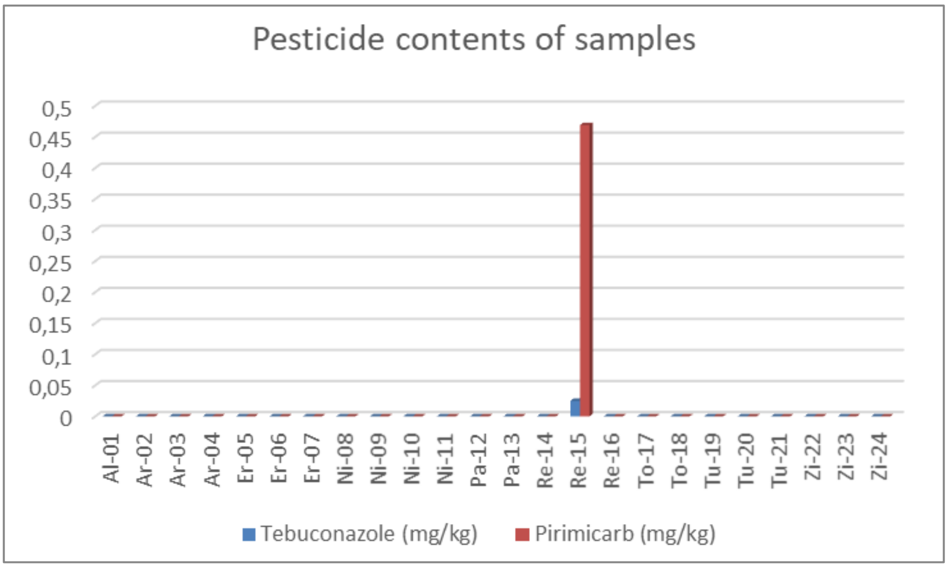

Figure 1. Pesticide contents of honey samples (mg/kg)

The distances of sample collection locations to industrial and agricultural areas are given in Table 1. Out of the samples analyzed, eleven were located in areas at least 5 km away from industrial or agricultural zones where pesticides were not used, while thirteen of them were close to pesticide-use areas. Beekeepers applied pesticides to control hive parasites either before the honey flow season or after harvesting honey. This practice likely explains why no chemical residues from hive treatments were detected in the honey samples analyzed. Furthermore, the natural comb honey samples were free from any contamination of pesticides by beeswax, as they were not produced in frames containing previously used beeswax or foundation comb.

In a study in Tokat province [19], pesticide residues were found in three out of 24 flower honey samples, likely due to in-hive spraying, pesticides, or environmental pollutants. Natural comb honeys have lower pesticide residue risk compared to extracted honeys, with old combs more likely to be contaminated. Nevertheless, additional research is required to investigate the potential transfer of pesticides through beeswax. To make a more relevant comparison, it would be beneficial to analyze natural comb honey and basic/old comb honey from the same hives.

Pesticides carried by bees to hives mainly accumulate in beeswax, with residue levels increasing from honey to pollen and beeswax [4,12]. A study found that honey had lower contamination levels compared to propolis from the same region [20]. Although pesticides in honey were mainly detected at low levels and not considered a health risk, honey serves as a biological indicator of environmental pollution [17, 21]. The study revealed that most honey samples from Tokat province, near agricultural areas, were free of pesticide residues, indicating their suitability for natural honey production. Possible reasons for the absence of residues include pesticide application outside the honey production season, lack of residues on visited plants, or minimal contamination of honey.

Recent studies on pesticide residues in honey in Türkiye have shown that some honeys contain pesticide residues, while others do not. The findings of these studies are briefly discussed below. Pesticides like malathion, ethion, cypermethrin, and deltamethrin were found in seven out of 20 flower honeys tested in Isparta. Diazinon and chlorpyrifos were also detected above the Maximum Residue Limit (MRL). These chemicals are commonly used insecticides in the region for apple and cherry cultivation [22].

Carbendazim, chlorpyrifos, imazalil, metalaxyl, and thiabendazole were found in 20 citrus honeys from various regions in Türkiye, including Antalya, Alanya, Aydın, Bozdoğan, Adana, and Kozan. The levels of imazalil and thiabendazole exceeded the Maximum Residue Limits (MRL) in these samples [23]. Specifically, imazalil was detected at 10.96 ng/g and thiabendazole at 12.11 ng/g, slightly above the MRL values.

In a study conducted in Gümüşhane province, Cyfluthrin, Cypermethrin, Malathion, and Deltamethrin were detected in various flower honeys. Cyfluthrin, Cypermethrin, and Malathion were found in two honey samples, while Deltamethrin was found in four honey samples [24].

As mentioned above, some studies have reported pesticide residues in honeys from Türkiye. On the other hand, no pesticide residues were detected in honey samples collected from the provinces of Adana, Hatay, Mersin, [25], Çanakkale [26], Antalya [27], Muş [28], and Tekirdağ [29].

Health risk assessments indicate that the levels of pesticides in honey are below the acceptable daily intake and pose minimal risk to consumers [17]. However, exposure to multiple chemicals simultaneously can lead to synergistic toxic effects in the body [30]. Therefore, it is crucial for honey production to be pesticide-free and for continuous monitoring of honey quality to ensure food safety.

5. Conclusion

The findings show that Tokat province has suitable conditions for producing pure natural honey. Beekeepers in Tokat province are well-organized through the Tokat Beekeeping Association, following seasonal maintenance schedules, hive treatments, and production practices. The low presence of pesticide residues in the honey samples, except for one, reflects the beekeepers' awareness.

The production of natural comb honey is limited among beekeepers, with many using basic or old combs, which may contain pesticide residues. Encouraging beekeepers to produce natural comb honey or "log hive" honey can reduce the risk of pesticide contamination. Beekeepers should source basic combs from reliable sources, avoid areas with pesticide use, and maintain hygiene in beekeeping practices. Education on producing additive-free honey, especially natural comb honey, is crucial for consumer health and environmental sustainability. Promoting the production of safe honey products is essential. It is recommended to produce safe products and time pesticide applications when bees are less active to protect their health.

Future studies should include the analysis of pollen, bee bread, and beeswax samples for pesticide residues in beehives. Comparing these bee products, particularly those from the same hives, can provide more precise information on the source of pesticide residues.

Author Contributions

The first author wrote and edited the manuscript and interpreted the results. The second author conducted the literature search, collected samples, and wrote the results. This article is based on the second author's master's thesis. Both authors reviewed and approved the final version of the paper.

Conflict of Interest

All the authors declare no conflict of interest.

Ethical Review and Approval

No approval from the Board of Ethics is required.

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