IZMIR DEMOCRACY UNIVERSITY HEALTH SCIENCES JOURNAL
IDUHES
e-ISNN:2651-4575

IDUHeS, 2022; 5(3): 805-813
Doi: 10.52538/iduhes.1191545

Araştırma Makalesi–Research Paper

DETERMINATION OF RESIDUES OF AMITRAZ AND FLUVALINATE IN HONEY SAMPLES COLLECTED FROM ÇUKUROVA DISTRICT

ÇUKUROVA BÖLGESİNDEN TOPLANAN BAL ÖRNEKLERİNDE AMİTRAZ VE FLUVALİNAT KALINTILARININ BELİRLENMESİ

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Özet

Bu çalışmada Çukurova bölgesinde bal örneklerinde amitraz ve fluvalinat kalıntılarının var olup olmadığı ve insan sağlığı için risk oluşturup oluşturmadıklarını araştırmak amaçlandı. Adana merkez, Kadirli, Kozan, Osmaniye, Ceyhan, Karataş, Yumurtalık, Tarsus ve Mersin merkezden 15'er adet süzme çiçek balı örnekleri olmak üzere toplam 135 adet bal örneği kullanıldı. Bal örnekleri Adana merkezden 1550, Kadirli'den 980, Kozan'dan 1050, Osmaniye'den 650, Ceyhan'dan 750, Karataş'tan 860, Yumurtalık'tan 740, Mersin merkezden 800 ve Tarsus'tan 950 adet kovanı temsil etti. Toplam 8330 kovandan bal örneği alındı. Bal örnekleri kapiller kolonlu gaz kromatografi kullanılarak Hammerling metoduna göre analiz edildi. Analizlerin sonucunda bal örneklerinde fluvalinat kalıntıları bulunmamasına rağmen, amitraz kalıntılarının 25 bal örneğinde 1.34 ile 33.48 ppm arasında değiştiği bulundu. Çukurova bölgesinden elde edilen balların halk sağlığı için risk oluşturacak düzeylerde amitraz kalıntılarıyla kontamine olduğu belirlendi. Balların üretilmesi ve tüketilmesi aşamasında tüketiciler için risk oluşturmayacak düzeylere kadar pestisit kalıntılarına karşı önlemlerin alınması gerektiği sonucuna varılmıştır.

Anahtar Kelimeler: Amitraz, Fluvalinat, Bal, Kalıntı

Abstract

In this study, it was aimed to investigate whether amitraz and fluvalinate residues were present in the honey samples in Çukurova district and whether they would pose a risk for human health. Total 135 honey samples were used with 15 extracted flower honey samples from each of Adana center, Kadirli, Kozan, Osmaniye, Ceyhan, Karataş, Yumurtalık, Tarsus and Mersin centre. Honey samples represented 1550 beehives from Adana centre, 980 from Kadirli, 1050 from Kozan, 650 from Osmaniye, 750 from Ceyhan, 860 from Karataş, 740 from Yumurtalık, 800 from Mersin center, and 950 from Tarsus. Honey samples were taken from total 8330 beehives. Honey samples were analyzed according to Hammerling method using gas chromatograhy with capillary column. In the result of analyses, although fluvalinate residues were not found in the honey samples, amitraz residues were found ranging from 1.34 to 33.48 ppm in 25 honey samples. The honeys collected from from Çukurova district were determined to be contaminated with amitraz residues at which levels they would pose a risk to public health. It has been concluded that in the stage of production and consuming of honeys, precautions are required to be taken against pesticide residues up to the levels not to pose a risk for consumers.

Keywords: Amitraz, Fluvalinate, Honey, Residue

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1. INTRODUCTION

Bee products can be contaminated by various chemicals due to environmental sources and veterinary drug treatments for beekeeping. Contamination with environment sources develops with carrying of nectar, pollen, propolis, and water by worker bees (Oldroyd 2007, p. e168; Sgolastra et al., 2019, pp. 22-35). The most important contamination way is by the use of acaricides and pesticides to beehives (Bogdanov 2006, pp. 1-18). Amitraz, cymiazole, bromopropylate, tau-fluvalinate, flumethrin, coumaphos with the purpose of control of varroa mites are commonly used by beekeepers in the world (Tette et al., 2016, pp. 124-141; Karazafiris et al., 2011, pp. 1-41).

Varroa mites are important parasites of bees and cause loss of them (Hernandez-Rodriguez et al., 2022, pp. 1179-1195). Loss of colonies occurs at significant rate in autumn and winter. Treatment of beehives with acaricides for the protection of bee colonies against varroa mites is performed (Tihelka, 2018, pp. 114-140). However, unconscious and excess drug use of beekeepers causes residues in the honey, royal jelly, and beeswax (Er, 1994; Kubik et al., 1995, pp. 13-22; Lozano et al., 2019, pp. 61-70).

Er (1994) has stated that honey samples from the district of Ceyhan do not contain fluvalinate residues but from Kazanlı the residue level is between 2.84-3.97 ppm. In addition, Hammerling et al. (1991, pp. 1047-1052) have revealed that of 330 honey samples, 8.5% samples contain amitraz residues over 0.05 ppm. Residue concerns in bee products in the result of acaricide use in beekeeping have been evaluated by other countries (Chauzat and Faucon, 2007, pp. 1100-1106; Wiest et al., 2011, pp. 5743-5736; Ravoet et al., 2015, pp. 543-548; Herrera Lopez et al., 2016, pp. 44-53; Ohba et al., 2018, pp. 2375-2386; Ohba et al., 2022, pp. 92-96).

Foundation of Development of Turkey has found that fluvalinate and amitraz use is the first choice against varroa mites (Anonim, 1987). In this study, it was aimed to investigate whether amitraz and fluvalinate residues were present in the honey samples in Çukurova district and whether they would pose a risk for human health.

2. MATERIALS AND METHODS

2.1. Chemical Substances

Methanol (Merck Cat no: 1.06008), ethyl acetate (Merck Cat No: 9.623), n-hexane (Merck Cat No: 1.04368), amitraz standard (Atabay Chemical Industry and Trade Company) and fluvalinate standard (Novartis, Turkey) are used.

2.2. Analysis Conditions of Capillary Gas Chromatography

Analysis conditions included capillary gas chromatography (Carlo Erba GC 6000 Vega Series-2), column (30 m in length, 0.25 mm in diameter, 0.25 µm in film thickness, stopping phase DB 17), nitrogen carrier gas (60 mL/min), oven temperature (200 °C), detector temperature (270 °C), detector (flame ionization detector), injector temperature (260 °C).

The recovery percentage of amitraz and fluvalinate was 75%. Limit of detection of amitraz and fluvalinate is 1.243 µg/g and 2.7 µg/g, respectively.

2.3. Analysis of Samples

In this study, total 135 honey samples were collected with 15 extracted flower honey samples from each Adana centre, Kadirli, Kozan, Osmaniye, Ceyhan, Karataş, Yumurtalık, Tarsus and Mersin centre. The honey samples represented beehives 1550 from Adana centre, 980 from Kadirli, 1050 from Kozan, 650 from Osmaniye, 750 from Ceyhan, 860 from Karataş, 740 from Yumurtalık, 800 from Mersin centre, 950 from Tarsus. Total beehives were 8330. Each honey sample of 60 g was taken to a glass jar and the glass jar was covered by aluminum foil. The honey samples were stored -20 °C until analysis. They were analyzed according to Hammerling (1987, p. 385) method using gas chromatography with capillary column (Carlo Erba GC 6000 Vega Series-2) within two weeks.

2.4. Amitraz and Fluvalinate Analyses

The extraction of samples was made according to the method of Hammerling (1987, p. 385). 15 honey samples obtained from each locations were labeled. The honey sample of 10 g was taken in an Erlenmeyer flask tared. Methanol-water (80:20) mixture of 10 mL was added to Erlenmeyer and mixed on magnetic stirrer until honey dissolved. Ethyl acetate of 10 mL was added to the dissolved honey, and mixed by stirring for 2 min. This mixture was taken to a separator funnel. Lower phase in the separator funnel was taken to the other Erlenmeyer. Lower phase was mixed with ethyl acetate of 10 mL and lower phase was again obtained from the separator funnel. Lower phase was washed twice with ethyl acetate, and obtained supernatant was filtered by the filter paper (Whatman No: 40). Filtrate was taken in evaporation flask with ground joints, and was evaporated at 40 °C until remained to 1 mL. n-hexane of 10 mL was added to obtained 1 mL solution and filtered with the filter paper (Whatman No: 40). This solution was evaporated under nitrogen stream until it remained to 1 mL, and 10 µL of this solution was given to gas chromatography with capillary column.

The standard chromatograms were prepared by injecting 10 µL of each standard amitraz and fluvalinate solutions between 0.2-0.8 µL/mL being a basis to detection of residues and quantifications.

Detection and quantifications were made by evaluating chromatograms regarding amitraz and fluvalinate residues in honey and amitraz and fluvalinate chromatograms of standard. Peak area calculations were made according to the triangulation method.

2.5. Statistical Analysis

In the results of laboratory analyses, the group means of the obtained data were calculated and standard deviations were calculated by variance analysis with SPSS programme.

3. RESULTS

Limit values regarding amitraz residue levels detected in honeys and percentages were given in Table 1.

Table 1: Amitraz residue levels in the honeys and percentages

Amitraz residue level (ppm)	Number of Samples	Percentage (%)
0.1-3	2	8
3.1-6	12	48
6.1-9	3	12
9.1-12	3	12
12.1-15	1	4
15.1-18	-	-
18.1-21	2	8
21.1-24	-	-
24.1-27	1	4
27.1-30	-	-
30.1-33	-	-
33.1-38	1	4

Fluvalinate residue was not detected in the honey samples collected from Adana centre, Kozan, Karataş, and Tarsus. However, amitraz residues were detected in 25 of total 135 honey samples ranging from 1.342 to 33.48 ppm (18.51%) at risk levels for public health considering that maximal residue level (MRL) is established 200 µg/kg by the Council Regulation (EEC) No. 2377/90 (CVMP, 1999, p. 4).

Amitraz levels in honey samples collected from locations and frequency of occurrence were given in Table 2 and a chromatogram of amitraz was given in Figure 1.

Table-2: Amitraz residue levels in the honeys collected from various locations and frequency of occurrence (ppm)

Location	Total Sample	Mean ± Standard Deviation (Minimum – Maximum)	Frequency of Occurrence
Adana	15	4.63 ± 3.19 (1.34-9.00)	4/15
Kozan	15	7.66 ± 3.83 (3.77 – 12.65)	5/15

Karataş	15	8.14 ± 7.64 (2.80 – 25.04)	10/15
Tarsus	15	12.59 ± 11.83 (4.46 – 33.48)	6/15
Total	60	8.51 ± 7.88 (1.34 – 33.48)	25/60

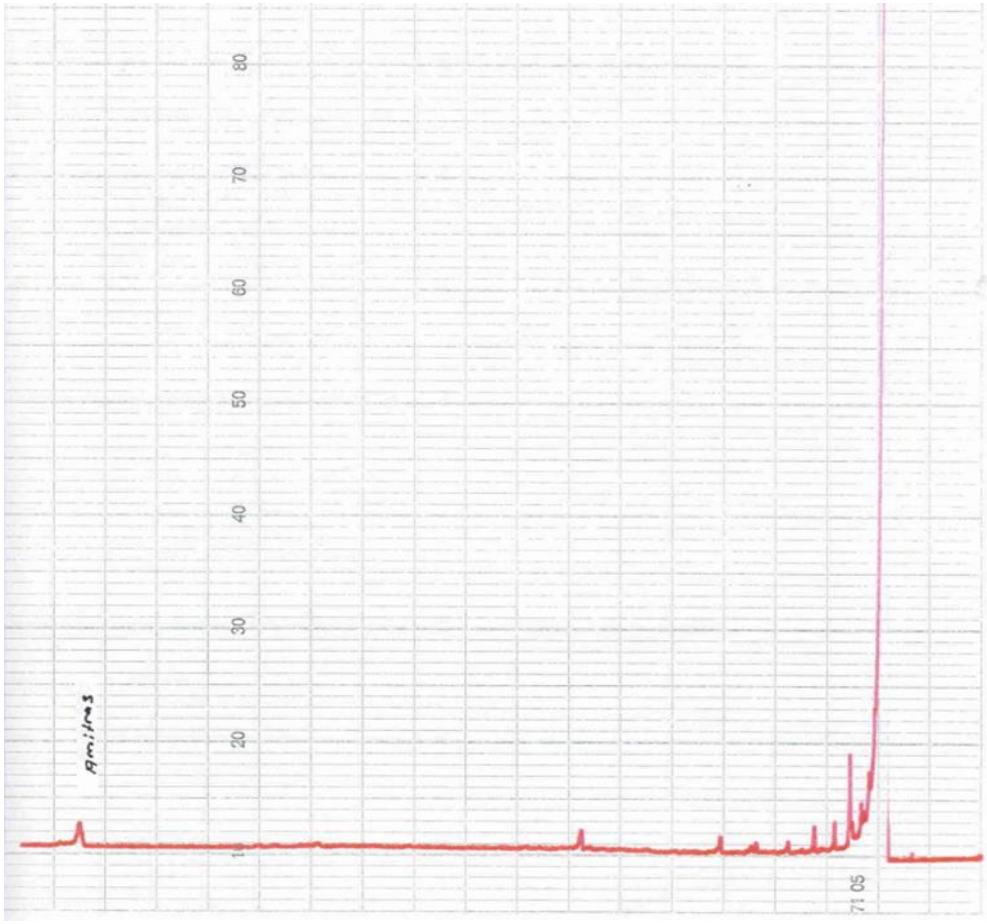

Figure 1. Chromatogram of amitraz in a honey sample collected from Adana center.

4. DISCUSSION

Acaricide treatment against varroa on bees is an inevitable application (Depaoli and Barbina, 1992, pp. 61-63). Major losses of colonies can occur if treatment is not made. Drugs against varroa mites are chosen from pesticides with minimum destructive to bee colonies and killing mites (Tsvetkova et al., 1981, pp. 93-98).

In beekeeping, amitraz, fluvalinate, bromopropylate, and malathion are used against varroa mites (Karazafiris et al., 2011, pp. 1-41; Tette et al., 2016, pp. 124-141).

In this study, amitraz and fluvalinate residues commonly used in Çukurova district were

investigated in 135 honey samples. Fluvalinate residues were not found in honey samples. Of 135 honey samples, 25 comprised amitraz residues. Amitraz residues were detected in the range of 3.1-12 ppm in the 18 (72%) of 25 honey samples. Similarly, Çobanoğlu et al. (2008, pp. 169-174) have found amitraz residues at high levels (5.35, 0.34, 0.23, 1.27, 0.92 and 0.40 ppm) in 6 of 32 honey samples in the districts of Ankara. However, Derebaşı et al. (2014, pp. 10-17) have found amitraz residues at acceptable levels (57.9-167.4 ppb) in the honey samples in Karadeniz region of Turkey. Studies from other countries have reported amitraz residues. For example, Hammerling et al. (1991, pp. 1047-1052) have reported that of 330 honey samples, amitraz residues are found to be at 8.5% more than 0.05 mg/kg in Germany. Herrera Lopez et al. (2016, pp. 44-53) have found amitraz residues at concentrations of 5-461 µg/kg in 20% of 60 beewax samples in Spain. Lozano et al. (2019, pp. 61-70) have found amitraz degradation product dimethylphenylformamide in honey at 117 µg/kg and this concentration is below the level of adverse effects. Mullin et al. (2010, p. e9754) have determined amitraz residues at levels of 3820, 1117 and 9040 ppb in bee wax, pollen and bees, respectively in North America.

In addition, this study found higher levels of amitraz residues in honey samples than those of other studies (Belda and Fernandez, 1989, pp. 58-59; Wiest et al., 2011). Er (1994) has found fluvalinate residues in honey samples in Kazanlı district of Mersin. In other countries, in the studies of honeys fluvalinate residues have been reported (Atienza et al., 1993, pp. 95-99; Faucon and Flamini 1990, pp. 57-58; Lambert et al., 2013, p. e67007). However, in this study, amitraz residue was found in the honeys of Çukurova district. The reason of amitraz residue in the honeys might be attributed to cheap and easy availability of amitraz compound in chemical struggle of varroa mites.

MRL of main amitraz compound and its metabolites [2,4-dimethylaniline, 2,4-dimethylformamide, N-(2,4 dimethylphenyl)-N-methylformamide] in honey is established 200 µg/kg by the Council Regulation (EEC) No. 2377/90 (CVMP, 1999, p. 4), European Communities Commission Regulation (EU) (2010, pp. 1-72), and EPA (2013, pp. 17123-17130). Amitraz is commonly used in the countries of EU and US for the effectiveness against varroa mites (Karazafiris et al., 2011, pp. 1-41). Thus, in this study, amitraz residues were found at high levels in honeys. In the result of accumulation of amitraz and its metabolites in beeswax from combs, again using combs affect honeys in terms of residues. In the honeys of Çukurova district, detection of amitraz presence revealed that the evaluation of amitraz residues was necessary in bee products such as beeswax, pollen, royal jelly.

A daily acceptable intake of amitraz is 0.03 mg/kg and one person can take 0.18 mg daily (CVMP, 1999, p. 4). In this study, the honeys produced in a specific area were revealed to pose a risk for public health. In the study, it was found that amitraz was a compound mainly used against varroa mites.

In the prevention of contamination of honeys for chemical control against varroa mites, there are legal regulations. Spraying is banned in the period of blooming of plants, and chemical applications are carried out after harvesting of honey (Ravoet et al., 2015, pp. 543-548). When acaricide strips are used in hives instead of powder, residues are at low levels (Wallner, 1999, pp. 235-248).

Honeys with chemical residues can cause public health concerns. In our country, beekeepers should be raised awareness about drug use for bee diseases. In addition, honey residue analyze should be made before offering to market.

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