

## The effect of organochlorine insecticides on honey bee

### (*Apis mellifera*) deaths seen in Adana region

#### ABSTRACT

Our society has attached great importance to beekeeping and honey since the past, and the popularity of other bee products in recent years has made beekeeping and bee health even more important. There are many reasons that can cause colony loss, which is seen together with intense deaths in bees, one of them is death due to toxicity caused by pesticides. In the study, 188 bee samples received between January and April 2015-2018 in Adana region were examined. These samples were examined qualitatively in terms of organochlorine pesticides by scanning the pesticides with gas chromatography device and scanning analysis method with GC-MS technique in materials with suspected poisoning. In the examination of the bee samples, there was no detectable level of any substance in terms of organochlorine pesticides. This situation showed that organochlorine pesticides did not play a role in bee colony losses in the samples received in Adana province and its surroundings between 2015-2018 due to the suspicion of toxicity due to heavy bee deaths. It should be kept in mind that deaths with massive bee colony losses may be due to different pesticide types and / or subacute and acute diseases.

**Keywords:** *Apis mellifera*, honey bee, organochlorine, insecticide, poisoning

## INTRODUCTION

Pesticides are used to protect economically from the negative effects of diseases, pests and weeds, which are a problem in agricultural production in our country as well as all over the world (Durmuşoğlu et al., 2010). Pesticides, living or found on or around human and animal bodies, plants and inanimate objects; and also, substances used to kill pests that reduce or damage their nutritional value during the production, preparation, storage and consumption of nutrients (Kaya and Bilgili, 1998). Pesticides are toxic chemicals with a specific mechanism of action; that is, they are designed to specifically control a target group of organisms by interfering with certain metabolic pathways (Sanchez-Bayo and Gako, 2016). Pesticides pose a danger to humans and animals, including wildlife, as they carry the risk of acute, subacute and chronic poisoning and mutagenic, carcinogenic and teratogenic effects. And also, they can cause food and environmental pollution. (Daş ve Kaya, 2004). Pesticides can be classified in various ways according to their mode of action, rate of action, chemical structure, types of parasites they affect, formulations and toxicity (Kaya and Bilgili, 1998). Pesticides are named according to the organism they affect and the pesticide function; insecticide (against insects and pests), fungicide (against fungi), herbicide (against weeds), molluscicide (against mollusks), rodenticide (against rodents), nematicide (against nematodes), acaricide (against mites).

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#### Research Article

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Classification of pesticides by their chemical structure is the most common and useful method of pesticide classification. Insecticides are also divided into various subgroups such as organochlorines (OC), organophosphorus (OF), pyrethroids, carbamates, neonicotinoids, spinosyns (Akashe et al. 2018). Pesticides contaminate non-target areas of blooming plants, wildflowers, beehives, pollen and nectar, so bees can be exposed to insecticides that are harmful to them. In cases where exposure is suspected, it is necessary to examine dead bee samples and identify the cause of death and possible pesticides (Lozowicka, 2013).

In this study, our aim is to investigate whether organic chlorine insecticides have an effect on intense bee deaths between January and April, which is the wintering period in Adana region.

### MATERIAL and METHOD

Due to the intense bee deaths that occurred between January and April in the Adana region, a total of 188 jar bee samples sent to Adana Veterinary Control Institute and collected from the field by experts were examined for OC insecticide (89 jar in 2015, 10 jar in 2016, 57 jar in 2017, 32 jar in 2018). Each jar sample contains 100 g of newly dead bees or dying bees. Incoming bee samples were analyzed within 3-4 days and kept in the refrigerator at  $\pm 4^{\circ}\text{C}$  until analysis. "Scan analysis method of pesticides with GC-MS technique in suspected poisoning materials" was used as the analysis method (GTHB, 2013). Scanning was performed with gas chromatography (GC) device and detectors connected to the device (Agilent 5975C Series GC/MSD System with triple-axis detector; MSD: mass spectrophotometer detector, ECD: electron capture detector, D-FPD: flame photometric detector x2).

According to the working method, each bee sample was thoroughly shredded in the shredder

in the extraction of the samples, and then the disintegrated sample was weighed as 25 g in the erlenmeyer, and then 75 ml of acetonitrile was added to it, and then mixed in the mixer and left for 4 hours. After 4 hours, it was filtered into a separatory funnel using filter paper and a funnel. 125 ml of 2% saline and 50 ml of n-hexane were added to the filtrate, respectively, and waited until phase separation, and then the upper phase was passed through a funnel in which some powdered sodium sulfate was placed and taken into a 120 ml porcelain capsule, and then the lower phase was taken to a separate erlenmeyer, and then 50 ml of chloroform was added to the lower phase in erlenmeyer and it was taken back to the separating funnel and a phase difference was expected. Depending on the phase difference, the lower phase was passed through a funnel with powdered sodium sulfate and added to the same capsule, and then the liquid in the capsule was expected to evaporate in the fume hood. The dried extract in the capsule was dissolved by adding 2 ml of methanol, and then the liquid was passed at high pressure through a glass pasteur pipette providing filtration with powdered sodium sulfate, and then the filtered liquid was given to the GC device in a vial. Analysis was performed by injecting 2  $\mu\text{l}$  of each sample into the GC device system. Device defined "pp'DDD, pp'DDE, op'DDT, pp'DDT, hexachlorobenzene, alpha/beta/gamma-hexachlorocyclohexane, alpha-endosulfan, beta-endosulfan, endosulfan sulfate, cis-chlordane, beta-chlordane, aldrin, dieldrin, diclofop-methyl, heptachlor, bromopropylate, tetradifon, acetochlor, phenoxoprop-P-ethyl" OC pesticides were analyzed and also other OC pesticides in the device library were examined.

### RESULTS

Three detectors are connected to the GC device in the laboratory, and with these three detectors, the mass weight, electron and light intensity

measurement of the analyte, if present in the sample, are defined simultaneously. As seen in Table 1, no detectable residue was found in

terms of OC insecticides that could cause toxication in the examination of a total of 188 jar bee samples.

**Table 1.** The number of samples examined in terms of OC insecticide by years

Year	Pesticide Group	Number of Samples Analyzed	Positive Sample	Detected Analytes
2015	Organochlorine	89	None	No analyte
2016	Organochlorine	10	None	No analyte
2017	Organochlorine	57	None	No analyte
2018	Organochlorine	32	None	No analyte

OC analytes: pp'DDD, pp'DDE, op'DDT, pp'DDT, hexachlorobenzene, alpha/beta/gamma-hexachlorocyclohexane, alpha-endosulfan, beta-nedosulfan, endosulfan sulfate, cis-chlordane, beta-chlordane, aldrin, dieldrin, diclofop-methyl, heptachlor, bromopropylate, tetradifon, acetochlor, phenoxoprop-P-ethyl

## DISCUSSION

Honey bees play an important role in the environmental ecosystem, as pollinating species with contribute to the production of honey, beeswax and other bee products (Panseri et al., 2020). The European or western honey bee, *Apis mellifera* L., is used for pollination in growing agricultural and horticultural plants and its contribution to agricultural production in the world is around 170-200 billion dollars (Cloyd, 2019). Pesticides are used to protect agricultural products, but some can affect bee health with their toxicity, depending on the active ingredient used and the formulation of different pesticide products (Lozowicka, 2013).

In a survey study conducted in the United States in the 2000s, one hundred and twenty-one different pesticides and metabolites were found in 887 samples, including beeswax, pollen, bee and hive samples. Among them are 6 OC insecticides. Only 16 samples could not detect pesticides (Johnson et al., 2010). Although it has been stated that the use of OC such as DDT and lindane has been prohibited in Poland for more than 10 years, Lozowicka (2013) found traces of these OC insecticides in bee samples in southeastern Poland. Since their detected concentrations were very low, these substances were not considered to be responsible for bee deaths, but these determinations revealed that the environment is

still polluted by these toxins (Lozowicka, 2013).

In 2019 and 2020, 98 honey samples were collected from hives in 4 different areas (dense orchards, agricultural land, areas close to the city without agriculture, and arable land with orchards) in the Apulia Region in southern Italy. Hexachlorobenzene (approximately 83% of samples), 4,4'-DDE (61% of samples) and Aldrin (19% of samples) insecticides were detected throughout the all-region. They also detected different OC insecticides in some, but not all, of the field (Panseri et al., 2020).

OC insecticides were not found in our study between 2015-2018, however, Johnson et al. (2010), Lozowicka (2013), and Panseri et al. (2020) as seen in the articles of, OC insecticides are always widely used in agriculture in the world and it is seen that this situation affects bee products as well as bee health.

The risk of affecting bee health increases when legal requirements or good practices for pesticide application are not followed, among other potential factors (Lozowicka, 2013). Beekeeping and bee health activities in Turkey have been put on the agenda with the "Regulation on Beekeeping" (TKB, 2003). In Turkey, honey and other beekeeping products are protected from pesticides according to the

'Turkish Food Codex Regulation on Maximum Residue Limits of Pesticides'. The maximum residue limit (MRL) values for pesticides allowed for bees and other bee products are available in the table in Annex-5 of this regulation. For pesticides not included in Annex-5, an evaluation is made on the basis of 0.01 mg/kg as MRL (GTHB, 2016).

### CONCLUSION

In the Adana region, there were intense bee deaths in the 2015-2018 periods, but it was determined that there were no deaths caused by OC insecticides in line with the analyzes made. It should be noted that while poisoning and death may be caused by different insecticide groups, bee deaths may also be caused by subacute and/or acute infectious diseases, sudden weather changes and other environmental factors.

To prevent possible poisoning; pesticides with low toxicity should be preferred, the beekeeper should be notified a few days in advance of the application of pesticides, the beekeeper should also take into account the warnings, pesticides should be applied in the afternoon or in the evening, dosage adjustment of the drugs used should be done according to the prospectus/instructions for use, pesticides should not be combined with different substances other than the leaflet information.

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