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Determination of insecticide imidacloprid residues in Tokat city water by using enzyme-linked immunosorbant assay (ELISA)

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Abstract

Imidacloprid is a common neonicotinoid insecticide applied in agricultural fields worlwide. Imidacloprid residues can be detected in water resources as it can accumulate in the surface and ground water after insecticide applications through many years. Because of this, a fast and efficient method, enzyme-linked immunosorbant assay (ELISA), is used to determine the imidacloprid levels in water sample collected from Tokat city water system and compared with a commercially avaliable bottled water sample. Imidacloprid residue was not detected in bottled water sample. However, imidalcoprid residues was detected in Tokay city water close to the maximum residue limit of imidacloprid. The results indicate that the Tokat city water system could be at the risk of imidacloprid contamination. This study also shows that ELISA technique can be effectively used for imidacloprid determination since it is fast and reliable and well correlated with other time consuming and cost-effective analytical methods.

Keywords: *Imidacloprid, ELISA, water, pesticide residue.*

Tokat ili su örneklerinde insektisit imidacloprid kalıntılarının enzim-bağımlı immunosorbent analizi (ELİSA) ile belirlenmesi

Özet

İmidacloprid, dünya çapında tarım alanlarında uygulanan yaygın bir neonikotinoid insektisittir. Uzun yıllar boyunca insektisit uygulamaları, yüzey ve yeraltı sularında imidacloprid kalıntılarının birikebileceğini göstermektedir. Bu çalışmada, hızlı ve etkili bir yöntem olan ELİSA tekniği kullanarak Tokat ili su şebekesinden alınan örnekte

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imidacloprid seviyesine bakılmış ve ticari olarak satılan şişe suyu ile karşılaştırılması gerçekleştirilmiştir. Şişelenmiş su örneğinde imidacloprid kalıntısı saptanmamıştır. Fakat Tokat ili şebeke suyunda maksimum kalıntı limit alt sınır miktarı kadar imidacloprid tespit edilmiştir. Bu sonuç, Tokat ili suyunun imidacloprid ile kontamine riskini göstermektedir. Ayrıca bu çalışma, ELİSA tekniğinin hızlı, güvenilir ve diğer pahallı ve uzun analiz süresi gerektiren analitik metodlarla korelasyon göstermesi bakımından etkili bir şekilde kullanılabileceğini göstermektedir.

Anahtar kelimeler: *İmidakloprid, ELİSA, su, pestisit kalıntısı.*

1. Introduction

Pesticides are heterogenous chemicals that are used to increase crop productivity and fight with insect-borne diseases. An insecticide is a pesticide that targets insects to control their destruction. Imidacloprid, 1-(6-chloro-3-pyridylmethyl)-2-nitroimino-imidazolidine, is a new class of neonicotinoid insecticide [1-3]. It has been effectively used to cope with hempiteran (sucking) insects in agriculture worldwide due to its low toxicity in mammals and high effectiveness against insects [4-7]. It can be used directly on seeds and plants or can be applied to soil. Despite its benefits, it is a cause of environmental risk to other organisms and human being [8-11].

Several methods have been developed for the analysis of various insecticides. Detection of imidacloprid by analytical methods include gas chromatography-mass spectrometry (GC-MS), high-performance liquid hromatography (HPLC) and liquid chromatography coupled with tandem mass spectrometry (LC MS/MS) [12-17]. These methods are very sensitive but require complex sample extraction procedure, large scale expensive instruments and trained personelle. Alternatively, enzyme-linked immunosorbent assays (ELISAs) have been widely used for the determination of imidacloprid residues in various samples [18-29]. ELISA is a faster, more sensitive, more effective, and high-throughput method which requires low budget to run various samples at the same time.

Pesticide consumption is gradually increasing worlwide, including Turkey, to increase agricultural production. To overcome problems associated with its misusage, application regulations were made by Government agencies and European Union Commission and maximum residue limits (MRLs) have been established for many consumable products [30-31]. For drinking water, the MRL has been determined as 0.1 µg/l for individual pesticides [32-33]. Despite these regulations, imidacloprid residue contaminations have been detected in soil and in water sources after many years of application [34]. In California (USA), it has been reported that 19 % of the water samples from three different agricultural fields has been found to be contaminated with imidacloprid, exceeding the limit of 1.05 µg/l [35]. A few study in Turkey have also indicated that the surface and groundwater were contaminated with pesticides in regions related with agricultural activities [36-38].

In this study, imidacloprid contamination was investigated in drinking water sample collected in the city of Tokat by ELISA technique. A bottled water sample is used as a control since we assume that the bottled water should be tested for many chemicals and pesticides. Tokat province is one of the largest agricultural areas located in Middle

Black Sea region and is a major contributor to the national agricultural food stock [39]. Despite the fact that 37% of the landscape in Tokat is occupied with agriculture, there is no evaluation of imidacloprid insecticide residues in agricultural samples and water resources in this region. This work provides the first preliminary study that determines imidacloprid residues in the Tokat city water sample. ELISA method can be effectively used in measurement of imidacloprid residues in water samples and a variety of agricultural products for proper assessment of human exposure to pesticides.

2. Material and methods

2.1. Reagent, materials and samples

The analytical grade imidacloprid was purchased from Sigma-Aldrich (Germany). The stock standard solution (50 ppm) was prepared by dissolving imidacloprid in methanol (Merck, Germany). Seven different standard levels (6, 4, 2, 1, 0.5, 0.2, 0.1 ppb) were prepared by serial dilutions of the stock solution and stored at 4 ºC until use.

The EnviroLogix QuantiPlate Kit for Imidacloprid (Model EP-006, Portland, ME), with an assay range from 0.2 to 6 parts per billion (ppb) was used for ELISA. Absorbances were measured at 450 nm with a microplate reader (Multiskan GO, Thermo Scientific).

The water samples tested in this study is a tap water collected in Tasliciftlik Campus which is about 10 km apart from Tokat city center. This region is mainly surrounded by agricultural fields. A bottled drinking water is also used as a control for comparison.

2.2. ELISA analysis

The ELISA kit consists of 12 strips of 8 anti-imidacloprid coated antibody wells each and three calibrators (0.2, 1, and 6 ppb). Briefly, 100 µl of negative control, each calibrator, serial dilutions of the standards, and water samples were added to individual wells on the microtiter plate in duplicate. 100 µl of imidacloprid-enzyme conjugate was added immediately to each well and mixed gently. The plate was covered with parafilm to prevent evaporation and placed on an orbital shaker at 200 rpm for 1 hr at room temperature. After incubation, the contents of the wells were discarded and the wells were washed four times with cold water and tapped dry. 100 µl substrate was added to each well, mixed gently and covered with parafilm and shaken at 200 rpm on the orbital shaker. After 30 min of incubation, 100 µl stop solution was added to each well and yellow color development was checked. Quantitation of the assay was based on the optical density reading on each well at 450 nm.

Measured absorbances were normalized using the formula B/B_0, where B_0 is the absorbance measured at zero concentration of imidacloprid and B refers to the absorbance of the standard (or calibrator) point [32]. A B_0 of 1.0 (or % B_0 of 100%) indicates the maximum amount of imidacloprid enzyme-conjugate bound to the antibody in the absence of imidacloprid (such as negative control). The data were fitted with the four-parameter logistic equation using SigmaPlot Version 14.0 (Systat Software, San Jose, CA, USA).

3. Results and discussion

The competitive ELISA assay results were determined as absorbance measurements that are inversely proportional to the imidacloprid determination. Figure 1 shows the competitive standard curves as a plot of % B_0 values versus concentration of imidacloprid with self-made standard solutions (0.1, 0.2, 0.5, 1, 2, 4, 6 ppb) and the kit-provided calibrators (0.2, 1, and 6 ppb).

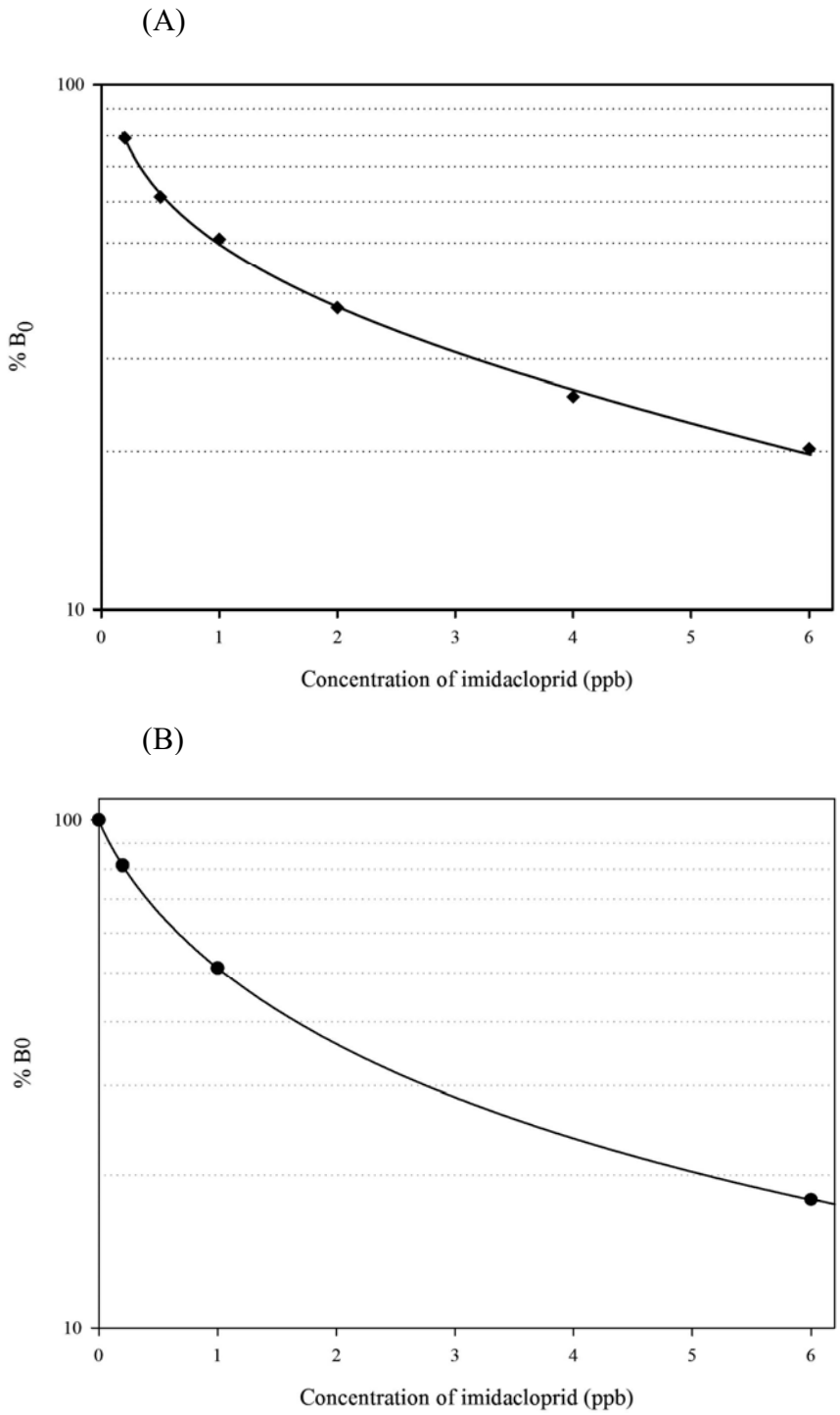

Figure 1. ELISA calibration curve for imidacloprid produced with self-made standard solutions (A) and kit-provided calibrators (B). Data are means of two replicates.

The linearity of each curve was determined by the slope calculated as -0.21 and -0.89 for self-made standard solutions and the kit-provided calibrators, respectively. The sensitivity of the ELISA test was determined by the IC_{50} value (50 % inhibition of control), which is interpreted as the smaller the IC_{50} value, the higher the sensitivity of the ELISA for imidacloprid detection. Accordingly, the IC_{50} values were 1.04 ppb and 0.11 ppb for the calibrators and standards, respectively. The sensitivity of the ELISA assay in this study agreed with previoulsy reported work that had IC_{50} values in this range [24]. The limit of detection (LOD) of the kit was 0.07 ppb and the experimental LOD was calculated as 0.09 ppb which also indicates higher sensitivity of the assay. The coefficient of variation (CV) for absorbances for the standards, calibrators and the samples were as predicted (lower than 15 %). These results indicate that the ELISA method is sensitive for imidacloprid detection in water samples used in this study. While the bottled drinking water had no detectable imidacloprid residue, drinking water collected from Tokat province had imidacloprid residue level at the acceptable lower limit of MRL accepted by the European Union Commission.

4. Conclusion

A rapid and sensitive ELISA method was conducted to determine imidacloprid residue level in city water and bottles water samples. Due to the large scale usage of imidacloprid in agricultural fields and lack of studies on imidacloprid contamination in water resources in Tokat province, it is important to assess a preliminary work to determine imidacloprid contamination in Tokat. According to our results, although the imidacloprid residue level does not exceed the MRL accepted in Europe, it is highly possible that it can be gradually increased in the long term if the usage of insecticide is not controlled in the future. This work clearly indicates that there could be a potential risk of imidacloprid contamination in Tokat. As an alternative to analytical methods, ELISA provides a simple and cost-effective screening method to efficiently quantify imidacloprid residues in various samples including water samples, agricultural products and food consumables. In the future work, agricultural products will be checked for imidacloprid contamination to understand the risk of insecticide contamination in the same location.

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