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Evaluation of potential Residue of *Imidacloprid* and *Abamectin* in Tomato, Cucumber and Pepper Plants after Sprayingusing High Performance Liquid Chromatography (HPLC)

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Abstract
Recently,one of the most indispensable methods has beenthe usage ofimidacloprid(Konfedor®) and Abamectin (Vertimk®) in crops, which made great improvements towards crops such as tomatoes, cucumbers and peppers. On the other hand there are disadvantages demerits of using such pesticides toward the human health. In this study, two types of pesticides were chosen:imidacloprid and abamectin, for many reasons.First of all they are the most commonlyused among farmers in Palestine, second, these pesticides are the most effective in destroying spiders and many insects. Imidaclopridand abamectin(Vertimk®) where sprayed on tomato, cucumber and pepper crops in three greenhouses using the same concentration that farmers used to spray their crops, with a concentration of 14.5mg /L for imidaclopridand a concentration of 29 mg /L for abamectin.The residues showed that bothimidacloprid and abamectin were higher in quantity than those of residues determined by previous researchers. Also, results obtained from this study showed that the quantities of residues were higher than the maximum residue levels (MRLs) in the samples that were collected on the first, the fifth and the tenth day of spraying. Furthermore, this study showed that the residues of abamectin are higher than imidacloprid, and residues of both abamectin and imidacloprid are higher in soil than in plant parts.The rate of degradation for both pesticides followed the first order with more than 0.97 fitting correlation.

Keywords: abamectin, imidacloprid, pesticides, plants, soil, adsorption, residue.

Introduction
Pesticide residue refers to the pesticides that remain on or in food after they are applied to food crops [1]. Pesticide residues also include any breakdown products from the pesticide itself [2].
The Environmental Protection Agency and European Food Safety Authority (EFSA) set maximum residue limits (MRLs), or tolerances, for pesticides that can be used on various food and feed commodities. Imidacloprid is a nicotine-based systemic insecticide, which acts as a neurotoxin and belongs to a class of

chemicals called the neonicotinoids. As a systemic pesticide, imidaclopridtranslocates or moves readily from the soil into the leaves, flowers, fruiting bodies, pollen, nectar, and guttation fluid of plants. Imidacloprid is also mobile and persistent in the environment and has the potential to accumulate in soil [3-4].

Abamectinis metabolised in the plant into the avermectin B1a when degraded rapidly. The only residues of toxicological significance areabamectin, a mixture of two components: avermectins B1a and B1b the first being the major component. Two of the derivatives from abamectin include emamectin benzoate and ivermectine, and these chemicals are used as acaricides or parasiticides on animals or plants [5].

Pesticide residues have an effect on the health of humans and animals (wild life), ranging betweenslightly to very dangerous.Sometimes, some of pesticide residues affect hormones in human and cause disturbances to natural hormones in our bodies [6]. These hormone disruptors are said to risk affecting brain development, behavior, and the development of reproductive organs. They have also been associated with manifestations such as falling sperm counts and girls entering puberty earlier. The greatest risk from hormone disruptors is in their ability to cause problems at very low doses. Some disrupts the body's natural hormones from working, while others mimic the action of natural hormones [7-8].

Most of the food produced for human consumption is grown using pesticides. Chemical control of weeds, insects, fungi, and rodents has enabled agricultural productivity and intensity to increase. However, these economic benefits are not without their risks to human and environmental health. Small amounts of some pesticides may remain as residues on fruits, vegetables, grains, and other foods. If exposureis high, many pesticides may cause harmful health effects, including delayed or altered development, cancer, acute and chronic injury to the nervous systems, lung damage, reproductive dysfunction, and possible dysfunction of the endocrine (hormone) and immune systems. For some pesticides, residues at levels below detection limits may pose important risks, while for other pesticides detectable levels of residues may not pose a significant health concern [9].

In this study the pesticides residues (*imidacloprid andabamectin)* on some vegetables include tomatoes, cucumbers, and peppers have been studiedusing the extraction from plant including roots, foliage and fruits for analysis using high performance liquid chromatography (HPLC).The residues of the previous pesticides and their fate in soil as a function of time were studied. In the past, the adsorption and kinetics of abamectin and imidacloprid in greenhouse soil in Palestine have been studied [10].

2. Materials and Methods

*2.1 Chemicals***.**

All chemicals like methanol, acetone acetonitrile and hexane were HPLC grade and purchased from Merck in Germany**.** Pesticide standards of abamectinand imidacloprid were analytical grade and were purchased from Wako in Japan. Water was purified witha Milli-Q SP TOC system (Nippon Millipore, Tokyo, Japan). Each standard was dissolved in methanol to make a stock solution of 100 mg/L. Stock solutions were equally mixed and diluted with methanol to make for mixture spiking and working standard solutions. Standard solutions were stored at 4 C in a refrigerator for further use.Calibration curves for both abamectin and imidacloprid were made using different concentrations (0 - 10 mg/L).

2.2 Instrumentation

HPLC analysis was carried out with a Hewlett - Packard 1050, controlled by Chem Station and equiped with UV detector in the range of 200-300 nm. HPLC separation was conducted using a Lichoro CART®, C18 Column (150x4.6mm,20µm) (Imtakt, Kyoto, Japan). The Mobile phase was a gradient elution of methanol/0.05 M KH_2PO_4 solution with the following methanol content: 0-10 min, 10%,;10-20 min, 20-25 min 100%; 25-30 min, 30-35 min, 5%, at the flow rate of 0.8 mL/min. The column temperature was maintained at 50 C.An aliquot of 20 µL was injected with an auto sampler.

*2.3 Extraction and Cleanup***.**

About 20 samples of all vegetables were cut into small pieces and 50 g of the samples were put in a stainless steel blender cup. Then 200 mL of acetonitrile were added. After blending for two minutes, the sample was extracted and filtered through a shark skin filter paper into a 250 mL bottle with 10 g of sodium chloride [11].
The acetonitrile extract (50 mL), in a round-bottom flask, was evaporated to neardryness, and the residue was dissolved in acetone (2 mL). The flask was washed with another 2 mL of acetone, which was combined with hexane (4 mL) to make an acetone concentration of 50%. The mixture was added to a C_{18} Bond-Elute cartridge which was connected with a glass syringe (10 mL) as a reservoir. A 5-mL mixture of 1:1 acetone/hexane was further used to elute the pesticides from the C_{18}column. The cartridge elution was evaporated and dissolved in about 2 mL of 2:8 acetone/hexane. An aliquot of 10 mL of 4:6 acetone/hexane was further eluted for anacetamiprid and imidacloprid collection, followed by acetone (20 mL) for nitenpyram elution. Each elution was evaporated separately and dissolved in methanol to make up to 2 mL.

2.4 Location of Experiments

A greenhouse consisting of tomatoes, cucumbers, and peppers located at Burqeen village near Jenin city was used in this study. The sample collection was conducted with the help of the farmers, who were given questionnaires. After the farmers answered the questionnaire, we judged how to collect best the sample. This greenhouse had moderate climates during the period of growing. The average seasonal temperature was 21C°, average relative humidity was 60% and there was good water holding capacity for the soil in which the vegetables were planted.

2.5 Sampling Procedure

Tomatoes (2030) that were characterized as medium-sized fruits with highly roots and suitable for consumption and processing were used in the presented work.
Cucumbers (Jana) that were characterized as small to medium-sized fruits, dark green color and suitable for consumption. Peppers (Sharabee) that were characterized as small to medium-sized fruit, light green color and suitable for marketing and consumption.
During the collection of fruits and samples from greenhouse we noticed that most farmers were notcommitted to the recommended amount of pesticides spraying on vegetables, which is 1.0 mL of imidacloprid per liter of water and 2.0 mL of abamactine per liter of water. The farmers used imidacloprid to control a broad spectrum of insects such as leaf hoppers, white flies and other insects. These insects attack the roots, leaves, stems and fruits. Each green house was treated every 3 weeks with imidacloprid pesticide (0.0145%) of spray solution, starting at the beginning of the growing season. Abamactinewas treated every 2 weeks (0.029% mg/L). Samples were collected from the first days of spraying, until the end of the 2 week period.
Fruits, leaves and root samples were picked up at 1, 5, 10, and 20 days after the spraying of the two pesticides. The samples were stored in the refrigerator at 2 – 4^{o}C in order to be analyzed for the residues of both pesticides by the HPLC. In addition, each sample was represented by 4 replicates, used for calculation of the mean value of the pesticide residue level for both pesticides.

3. Results and Discussion

Leaves, fruits, roots and soil of tomatoes, cucumbers and green peppers were collected and analyzed for pesticide residues, using the method of extraction and analysis by UV-Visible spectrophotometer. From Fig.1 it was noticed that the concentration of abamectin was decreasingwith time, due to abamectin degredation by sunlight to the many derivatives: BHT, avermactinB1a and avermactin B1b [12]. Degredation of abamectin was faster than imidacloprid with time. At the tenth day it was noticed that the concentration of imidacloprid was higher than abamectin; however at the first day it was noticed that the concentration of abamectin was higher than imidacloprid, due to more drops of spraying solution falling on the soil during the experiment.At the fifth day,the concentration of abamectin was 10.17 mg/L, while the concentration of imidaclprid was 8.52 mg/L.It was noticed that the concentration of abamectin was decreasing between the first and the fifth day of spraying, while imidacloprid concentrationswere also decreasing between the first and the fifth day. The concentrations of abamectin were decreasing at a rate faster than in imidacloprid. The concentrations of abamectin and imidaclopridon the first and the fifth day of spraying, were higher than those oftheirmaximum residue levels (MRLs). At the tenth day abamectin concentration was less than maximum residue levels (MRLs), while the concentration of imidacloprid at the tenth day was 2.32 mg/L more than themaximum residue levels (MRLs). The concentrations of both abamectin and imidacloprid at twentieth day was less than maximum residue levels (MRLs).

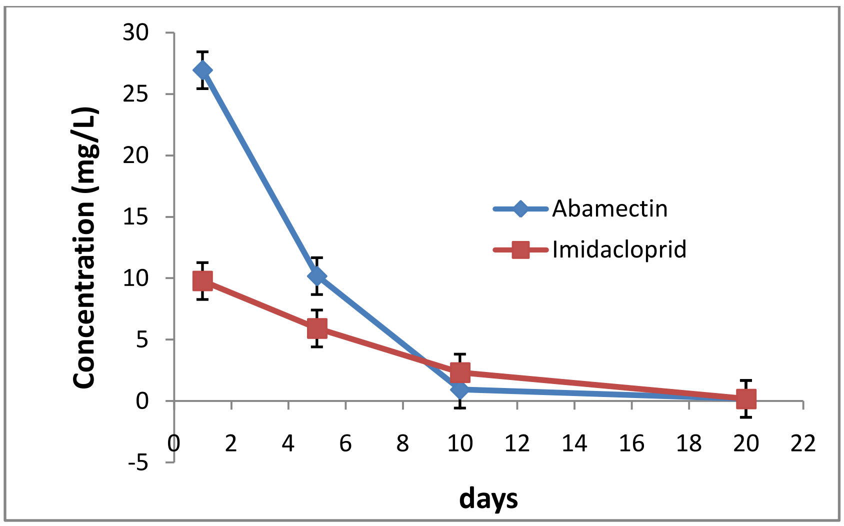

Figure 1. Concentration of Abamectin and Imidacloprid Residues in Soil Cultivated with Pepper(DT50 for abamectin = 2.57 s and for imidacloprid = 3.30 s).

From Fig. 2a it was noticed that the concentration reading between the fifth to the tenth daywas higher for imidacloprid than for abamectin,and it was noticed in general that the pesticide residuesin the different fruits were lower than the residues in soil due to the soil adsorption of the residues[13]. At the first day the concentration of abamectin was higher than the concentration of imidacloprid; concentration of both abamectin and imidacloprid on the first day was more than maximum residue levels (MRLs). At the fifth day the concentration of abamectin was 1.54 mg/L; a high drop of the abamectin concentration was noticed in pepper fruits and this was due to photodegradation and the action of enzymes that degrade abamectin [14]. At the fifth day the concentration of imidacloprid was 4.03 mg/L; it was noticed that the degredation of imidacloprid was slower than the degredation of abamectin, and it was noticed that the concentration of both abamectin and imidacloprid was higher than maximum residue levels (MRLs). It was noticed at the tenth day that the concentrations of both abamectin and imidacloprid was less than themaximum residue levels (MRLs). It was noticed at the twentieth day that the concentration of abamectin was not detectable and less than maximum residue levels (MRLs), while imidacloprid was detectable, but the concentration of imidacloprid was also less than maximum residue levels (MRLs).

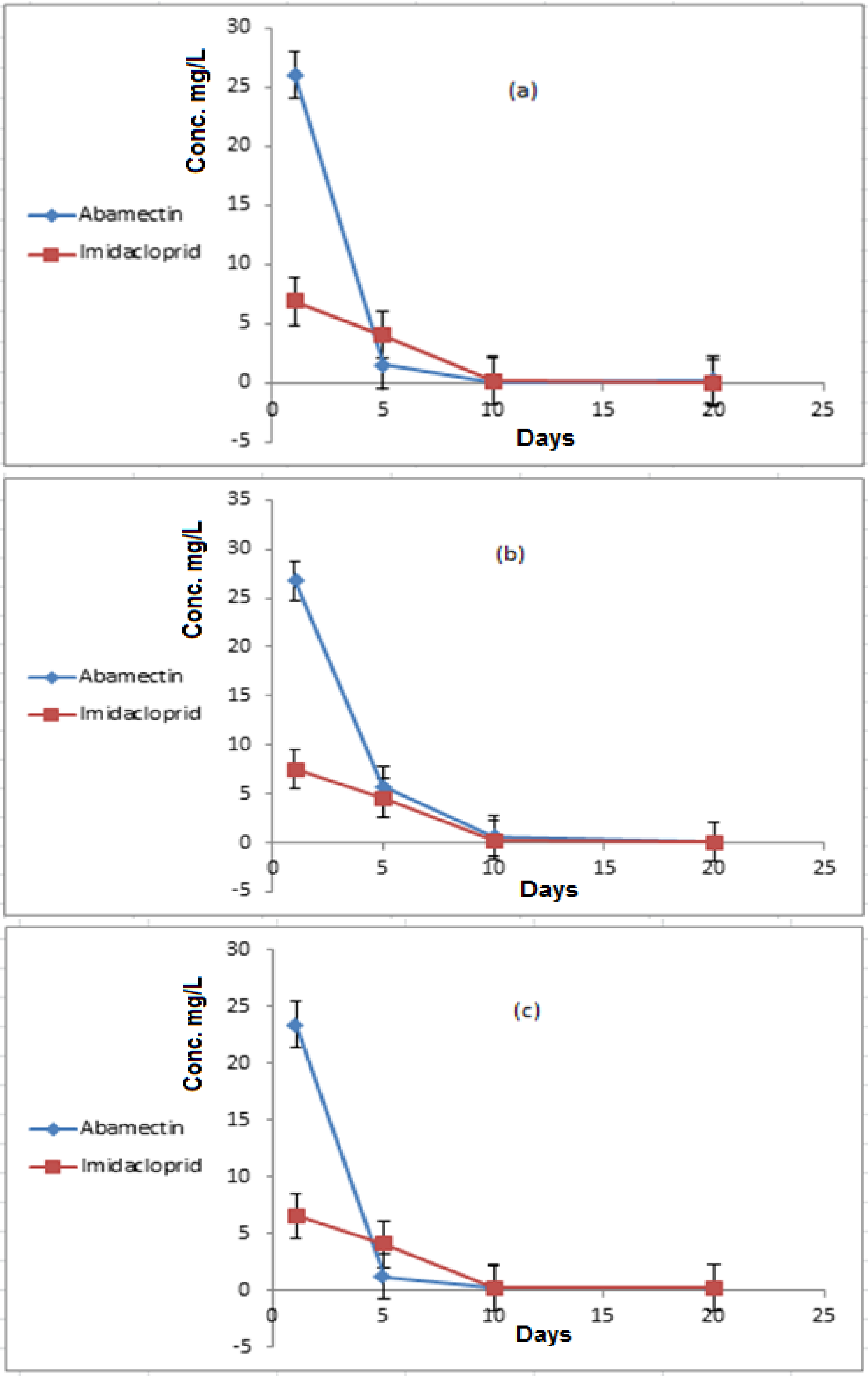

Figure. 2: Concentration of abamectin and imidacloprid residues from pepper (a) fruits (b) leaves (c) roots(n = 3 runs)[a) DT50 = 2.78 abamictin; 3.26 imidacloprid; b) DT50 = 2.88 abamictin; 3.35 imidacloprid; c) DT50 = 2.91s abamictin; 3.42 s imidacloprid]

From Fig. 2b it was noticed that the concentration of abamectin was higher than the concentration of imidacloprid, except on the twentieth day. The concentration of imidacloprid was higher than abamectin due to abamectin having more volatization than imidacloprid [15, 10]It was noticed that the concentration of abamectin and imidacloprid was detectable on the twentieth day. At the first day, the concentration of abamectin was higher than the concentration of imidacloprid; concentration of both abamectin and imidacloprid on the first day was more than maximum residue levels (MRLs). At the fifth day the concentration of abamectin was 5.77 mg/L; very quick decreasing of abamectin concentrations in pepper leaves was noticed and this was due to photodegredation and the action of enzymes that degrade abamectin.It was noticed that the concentration of abamectin in pepper leaves was more than the concentration of abamectin in pepper fruits. At the fifth day the concentration of imidacloprid was 4.53 mg/L; it was noticed that degredation was slower than the degredation of abamectin, and it was noticed that the concentration of both abamectin and imidacloprid was higher than maximum residue levels (MRLs). It was noticed that the concentration of imidacloprid in pepper leaves was more than the concentration of imidacloprid in pepper fruits.

From Fig. 2c it was noticed that the concentration of abamectin in pepper roots was higher on the first day than imidacloprid, but the concentration of abamectin in pepper roots on the fifth day was less than the concentration of imidacloprid of spraying. The abamectin and imidacloprid were not detectable on the twentieth day, at the fifth day the concentration of abamectin was 1.17mg/L; very quick decreasing of abamectin concentration in pepper roots was noticed and this was due to the action of enzymes that degrade abamectin and the effect of fertilizers that were used on the peppers [16]. At the fifth day the concentration of imidacloprid was 4.02 mg/L; it was noticed that the degredation of imidacloprid was slower than the degredation of abamectin, and it was noticed that the concentration of both abamectin and imidacloprid was higher than maximum residue levels (MRLs). It was noticed at the tenth day that the concentration of both imidacloprid and abamectin was less than the maximum residue levels (MRLs). It was noticed at the twentieth day that the concentrations of both abamectin and imidacloprid were less than maximum residue levels (MRLs).

From Fig. 3a it was noticed that the concentration readings of imidacloprid and abamectin after the tenth day were approximetly equal, and the low concentration of abamectin and imidacloprid refer to tomato fruit decompostion of pesticides residues [17]. At the first day, the concentration of abamectin was higher than the concentration of imidacloprid; concentration of both abamectin and imidacloprid on the first day was more than maximum residue levels (MRLs). At the fifth day the concentration of imidacloprid was 4.08 mg/L; it was noticed that degredation was slower than the degredation of abamectin, and it was noticed that the concentration of both abamectin and imidacloprid was higher than maximum residue levels (MRLs). It was noticed at the tenth day the concentration of both abamectin and imidacloprid was less than maximum residue levels (MRLs). It was noticed at the twentieth day the concentration of both abamectin and imidacloprid was not detectable, also the concentration of both abamectin and imidacloprid less than maximum residue levels (MRLs).

From Fig. 3b it was noticed the concetration of abamectin in tomato leaves was higher than the imidacloprid on the first day and the fifth and the tenth day of spraying, but the concentration of imidacloprid on the twentieth day was higher than that of abamectin. At the fifth day the concentration of abamectin was 4.61 mg/L; very quick decreasing of abamectin concentration in tomato leaves was noticed and this is possible due to photodegredation and the action of enzymes that degrade abamectin [18]. At the fifth day, the concentration of imidacloprid was 4.39 mg/L; it was noticed that degredation was slower than the degredation of abamectin, and it was noticed that the concentrations of both abamectin and imidacloprid was higher than maximum residue levels (MRLs). It was noticed at the tenth day that the concentration of both imidacloprid and abamectin was less than the maximum residue levels (MRLs). It was noticed at the twentith day that the concentrations of both abamectin and imidacloprid was less than the maximum residue levels (MRLs).

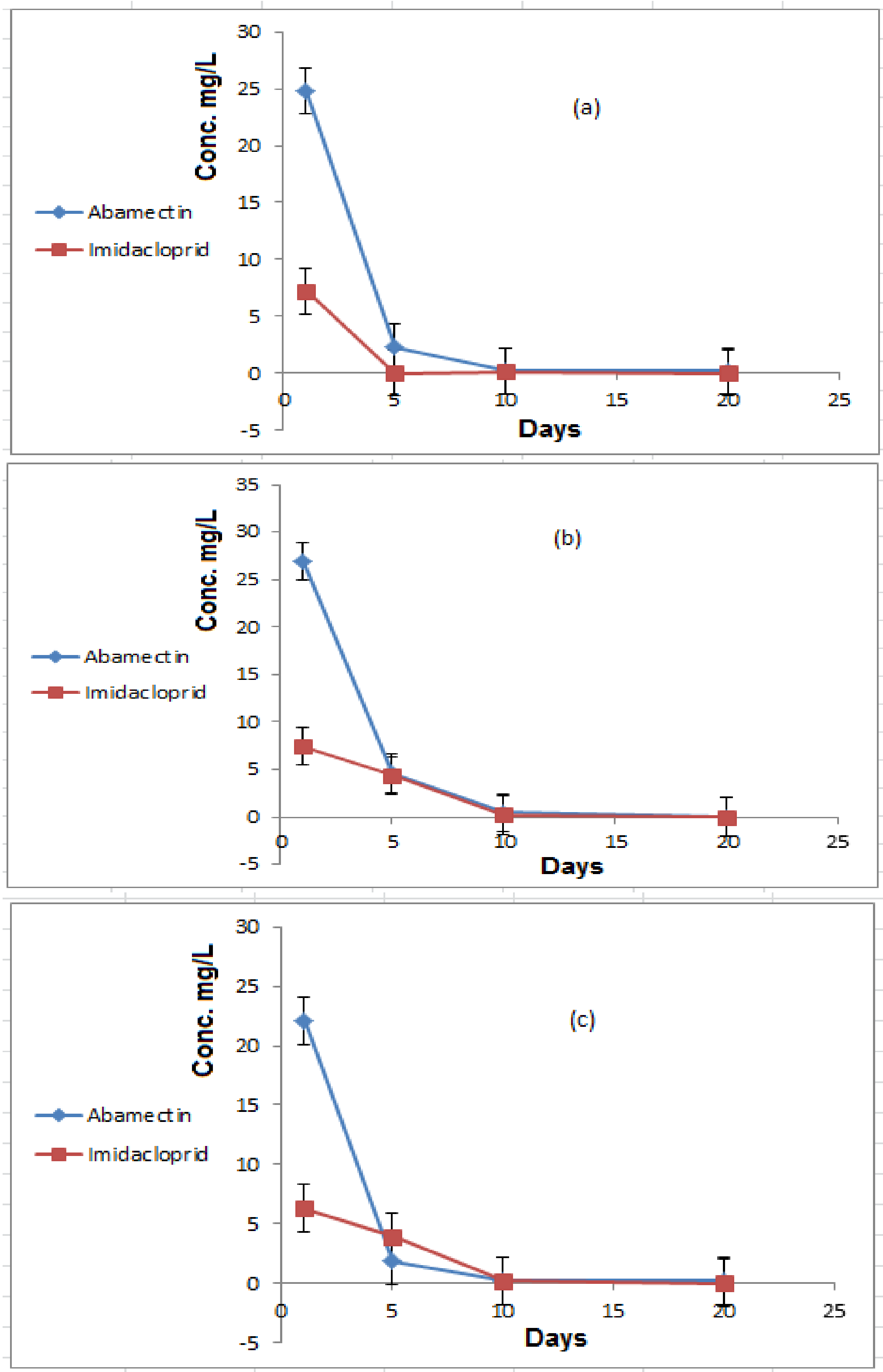

Figure. 3: Concentration of abamectin and imidacloprid residues from tomato (a) fruits (b) leaves (c) roots (n = 3 runs)[a) DT50 = 2.76 s abamictin; 3.93 s imidacloprid; b) DT50 = 2.62 s abamictin; 3.26 s imidacloprid; c) DT50 = 2.84 s abamictin; 3.46 s imidacloprid]

From Fig. 3c it was noticed that the concentration of abamectin in tomato roots on the first day was higher than that of imidacloprid, but the concentration of abamectin on the fifth day in tomato roots was less than the concentration of imidacloprid on the fifth day. The concentration of abamectin on the twentieth day in tomato roots was not detectable but the imidacloprid was detectable due to high concentractions used by farmers [19]. At the fifth day the concentration of abamectin was found 1.84 mg/L; a drop in the abamectin concentration in tomato roots was noticed, and this was due to the action of enzymes that degrade abamectin and the effect of fertilizers on tomato. At the fifth day the concentration of imidacloprid was 3.95 mg/L; it was noticed that dissipation was slower than degredation of abamectin, and it was noticed that the concentration of both abamectin and imidacloprid was higher than maximum residue levels (MRLs). It was noticed at the twentieth day the concentration of both abamectin and imidacloprid was less than maximum residue levels (MRLs).

From Fig. 4a it was noticed at the first day that the concentration of abamectin was higher than the concentration of imidacloprid; concentrations of both abamectin and imidacloprid on the first day was more than maximum residue levels (MRLs). At the fifth day the concentration of abamectin was 0.99 mg/L; it was noticed that very quick decreasing of abamectin concentration in cucumber fruits occured and this was due to photodegredation and the action of enzymes that degrade abamectin [19]. At the fifth day the concentration of imidacloprid was 4.08 mg/L; it was noticed that degredation was slower than the degredation of abamectin, and it was noticed that the concentration of both abamectin and imidacloprid was higher than maximum residue levels (MRLs). It was noticed at the tenth day that the concentration of both abamectin and imidacloprid was less than maximum residue levels (MRLs). It was noticed at the twentieth day that the concentration of both abamectin and imidacloprid was not detectable, and that the concentrations of both abamectin and imidacloprid were less than maximum residue levels (MRLs).

From Fig. 4b it was noticed that the concentration of abamectin in cucumber leaves was higher than the imidacloprid on the first, fifth and the tenth day of spraying, but the concentration of imidacloprid on the twentieth day was higher than abamectin. At the fifth day the concentration of abamectin was 6.82 mg/L; it was noticed that very quick decreasing of abamectin concentration in cucumber leaves occured and this was due to photodegredation and the action of enzymes that degrade abamectin [18]. At the fifth day the concentration of imidacloprid was 4.89 mg/L; it was noticed that degredation was slower than the degredation of abamectin, and it was noticed that the concentration of both abamectin and imidacloprid was higher than maximum residue levels (MRLs). It was noticed at the tenth day that the concentrations of both imidacloprid and abamectin were less than maximum residue levels (MRLs). It was noticed at the twentith day that the concentrations of both abamectin and imidacloprid were less than maximum residue levels (MRLs).

From Fig. 4c it was noticed the concentration of abamectin in cucumber roots on the first day was higher than in imidacloprid, but the concentration of abamectin in cucumber roots on the fifth day was less than in imidacloprid due to abamectin being more volatilie [20]. The concentration of abamectin and imidacloprid residues on the twentieth day was not detectable. The adsorption constant related to soil organic carbon content for imidacloprid is high (Koc = 210) [20] At the fifth day, the concentration of abamectin was 1.63 mg/L; it was noticed that very quick decreasing of abamectin concentration in cucumber roots occurred and this was due to the action of enzymes that degrade abamectin, and the use of fertilizers on the cucumber [21]. At the fifth day the concentration of imidacloprid was 4.28 mg/L; it was noticed that degradation was slower than the degradation of abamectin, and it was noticed that the concentration of both abamectin and imidacloprid was higher than maximum residue levels (MRLs) [22]. Finally, the rate of degradation in both pesticides followed the rate of second order with correlation of fitting being more than 0.97 and the results are shown in Fig 5.

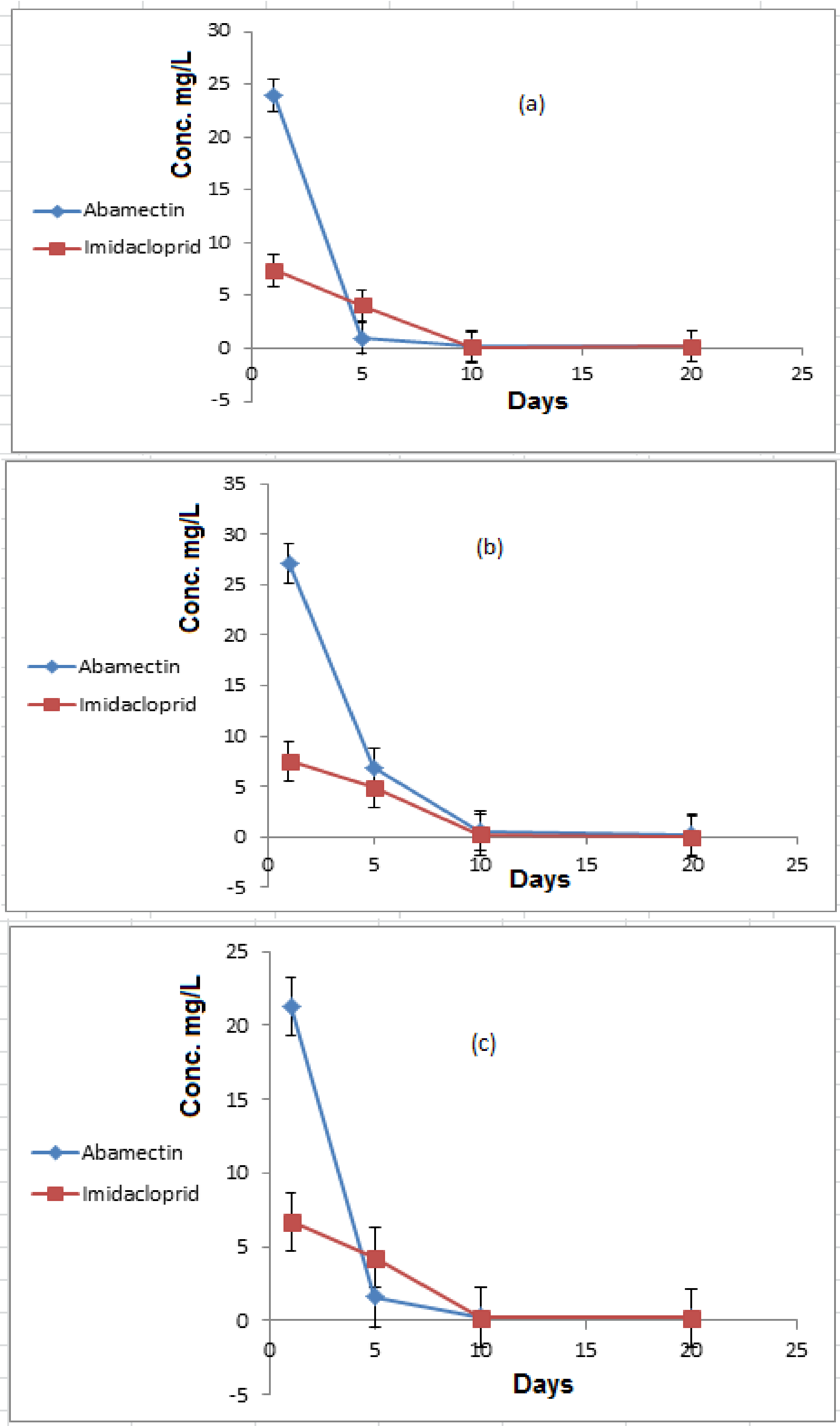

Figure: 4. Concentration of abamectin and midacloprid residues from cucumber (a) fruits (b) leaves (c) roots(n = 3 runs)[a) DT50 = 3.28 s abamictin; 3.56 s imidacloprid; b) DT50 = 2.52 s abamictin; 3.76 imidacloprid; c) DT50 = 2.88 s abamictin; 3.46 s imidacloprid]

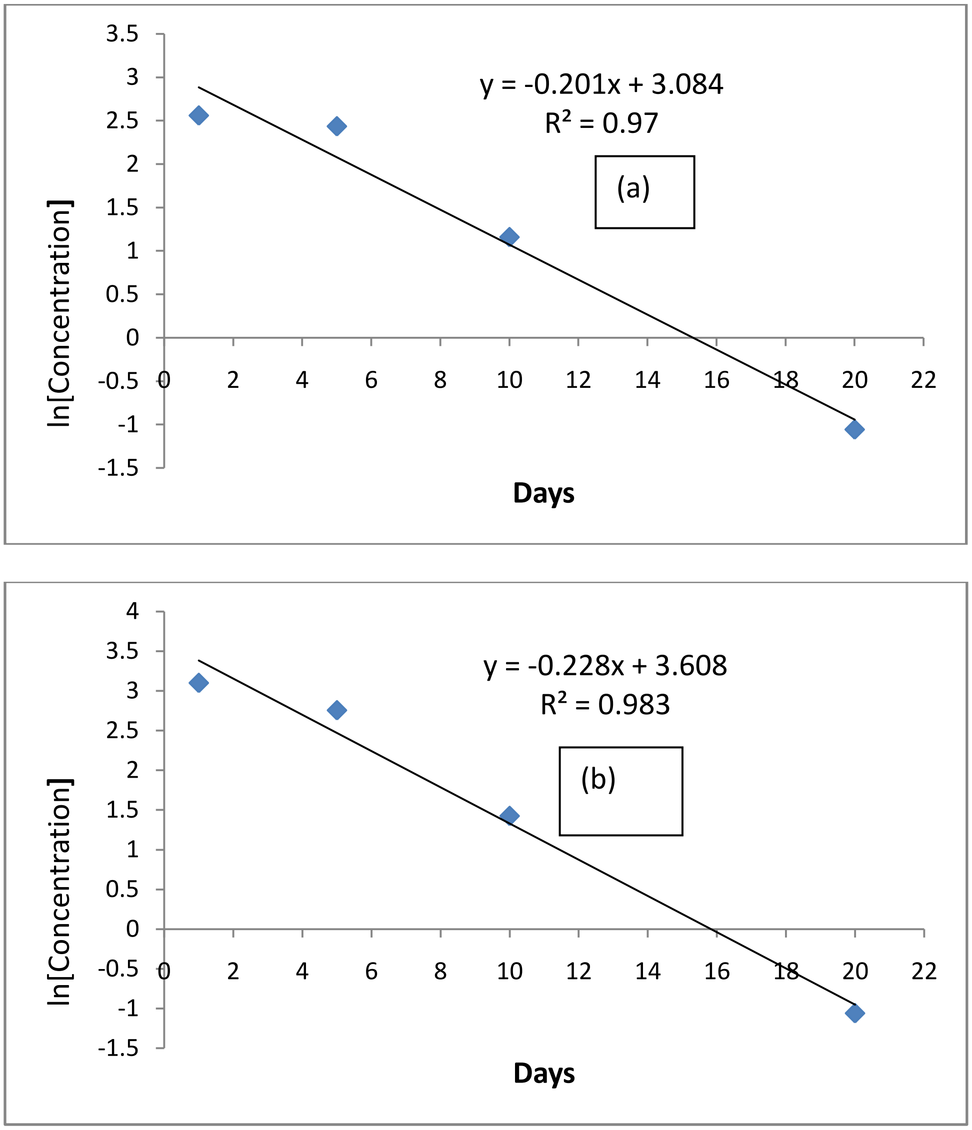

Figure 5: Dissipation Rate of (a)Abamectin and (b)Imidacloprid for fruit versus Time.

Conclusion

Results obtained from this study showed that residues of imidacloprid and abamactine remain in vegetables: tomatoes, cucumbers and peppers, especially after a few days of spraying of pesticides.

During the course of the study, we noticed that the amounts of imidacloprid and abamactine residues were very high after the first day of spraying. Until the fifth day of spraying, the amounts of residues of the two pesticides remained high (due to high concentrations used by farmers) and this may pose a serious risk to human health via food consumption. However, photodegradation caused a decrease of the pesticide residues in the plant parts.

The ministries of Health and Agriculture should continue to control the harvesting of the products and prevent farmers from selling them before the tenth day of spraying, due to the existence of pesticide residue. This is in accordance to the law followed by the ministries of Health and Agriculture.

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