RESEARCH ARTICLE (Open Access)

The degradation of the insecticide Imidacloprid in greenhouse tomatoes and an estimation of the level of residues.

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Abstract

A liquid chromatographic (LC) method using UV detection was used to study the degradation of imidacloprid in tomatoes grown in greenhouses. A liquid-liquid extraction with acetonitrile/methanol (60/40, v/v) and a cleanup step with Florisil were combined with LC to isolate, recover, and quantities the pesticide. Average recoveries obtained at spike levels of 0.03 and 0.40 mg/kg were 93.2-94.7%. Determination limits were 0.012 mg/kg. The experiment was conducted in the greenhouses located in Durres. Treatment was performed using Confidor20 EC (Imidacloprid), an insecticide with a systemic action. The aim was to confirm the residue of Imidacloprid in tomatoes and to find the decline curve after the last application in minimal and maximal concentration, 0.25% and 0.5% respectively. Samples of tomato fruits were taken in an interval of 1, 3, 5, 7 days after the application. The degradation of Imidacloprid, in maximal concentration exceeds the allowed limit of 7 days, this is day of PHI, thus influencing harvest and marketing.

Key words: Imidacloprid, Greenhouse Tomato, Pesticide Residues .

1. Introduction

Greenhouse tomato is considered as the main major vegetable crop grown on a large scale in Albania but, the plant is attacked by many insects, making frequent use of neonicotinoide insecticides necessary.

Imidacloprid has high molecular mobility in the xylem of treated plants because of its high water solubility [1]. This insecticide is extensively used during vegetable and fruit production, to control pests. It is directly applied to the crops which lead to its persistence in the form of residues in vegetables and fruits at the time of harvest. The environmental and health problems and the risk involved in the use of chemicals, especially pesticides, in agriculture are very high [5], which not only leads to the chemical build up of pesticide residues in crops but also disrupts the biochemical parameters of plants. Imidacloprid causes a blockage in a type of neuronal pathways (nicotinergic) that is more abundant in insects than in warm blooded animals. This blockage leads to accumulation of acetylcholine, an important neurotransmitter, resulting in the insect's paralysis and eventually death [2,3]. A number of researchers have reported residues of imidacloprid in different agriculture crops substrate[4,6,7,8]. This study was undertaken with a view to analyze residues of imidacloprid, in some of the most popular crops such tomatoes.Various analytical methods used in neonicotinoid pesticides residues analysis have been reported by different researchers [9,10].

2. Material and methods

2.1 Field trial design

In order to estimate pesticide residue levels, a field trial was carried out in a 230 m^2 greenhouse located in Rada - Durres, Albania. Tomato plants, were cultivated in a spring-summer cycle with a density of 2.2 plants $m^2$and a total yield of 20 kg/$m^2$of tomato fruits.

The treatment was carried out using a portable motor sprayer equipped with a gun nozzle using the following commercial formulation: Confidor(20% of Imidacloprid). Spraying was carried out at the minimal recommendeddose 0.025 %.and maximal recommended dose of 0.05 %.Sampling were made after the treatment and repeated after 1, 3, 5, 7 and 9 days. Only mature fruits were used for analysis.

Experimental plan for designing the evaluation of the level of residues of imidacloprid in tomatoes:

Experimental design inside the tomatoes greenhouse:

V1 – first line. It is not treated.

V2 –Second line, it is treated with confidor , with minimal dose of 0.025 %.

V3 – third line, it is treated with confidor, with maximal dose of 0.05 %.

For every line are treated 30 plants and within the lineare left some buffer areas.

Crops planted: Tomato

Crops are treated with confidor (Imidacloprid) which is a systemic insecticide.

Treatments are done by professional sprayer from Plant Protection Laboratory in Durres.Albania

The treatment date: 01.06.2012.

2.3 Standards and chemicals

Pesticide standard;Pesticide standard reference material was purchased from Fluka analytical. The purity of imidaclopridwas more than 95 % by liquid chromatography (LC).

Pesticide working solutions;Pesticide standard solutions (1000 µg/ml) were prepared by dissolving the pesticide standard in methanol HPLCgradeand diluting to suitable concentrations with the same solvent.

Organic solvents and reagents; Acetone, ethyl acetate, acetonitrile, methanol were of special grade for pesticide residue analysis..Anhydrous sodiumsulfate (Na_2S04) was of analytical grade. These reagents were used without prewashing. Florisil (60-100 mesh) was obtained from fluka. Florisil and anhydrous Na_2S04 were heated overnight at 130°C and desiccated before use.

Samples;Tomatoes were obtained from greenhouses, treated with commercial insecticideformulation – Confidor 20 EC.

ApparatusLiquidchromatograph;Shimadzuequipped with a UV detector was used for determination of imidacloprid.Shim-pack VP-ODS (250mmL.×4.6mmI.D.) columns were used for pesticide content determination.

Chromatographic tube for column chromatography;A glass column of 40 cm x 22 mm I.D. wasused in Florisil column chromatography for purification of sample solution.

Rotary evaporator; Equipped withwater bath and vacuum pumpwere used to concentrate the organic solvents. A water bath was set at 35-40 ^{0}C.

2.4 Extraction and Clean-up

Approximately 1000 g ofwhole tomatoes were mechanically minced to provide a homogeneous tomato mix, from which sub-samples were taken.A subsample of 10 g oftomatoes was transferred to a blender cup, to which 60 ml of Acetonitrile/methanolhad been added. The contents were then blended for 30 minutes. The mixture was filtered through No. 5A filter paper, and the residue was re homogenized with the same amountof Acetonitrile /methanoland then filtered again. The filtrate was combined, than was transferred completely to a Florisil column along with 10 goffflorisil. and 20 g of anhydrous Na_2S04 Imidaclopridwas eluted with 100 ml of this mixture. The eluate was concentrated using a rotary evaporator. The residue was re dissolved in acetonitrileand made up to final volume of 3 ml (the sample solution).

2.5. Determination of pesticide.

Test solution prepared was subjected to HPLC for imidacloprid content determination under the following conditions: apparatus HPLC Shimadzu:

Determination of imidacloprid residue was done on HPLC-UV (Shimadzu) equipped with an analytical column Shim-pack VP-ODS (250mmL. × 4.6 mm i.d.).The mobile phase was acetonitrile: water (90:10 v/v)at a flow rate was 0.8 mL/minute. Detection was done by UV detector at 252nm wavelength.Identification of imidacloprid residues were accomplished by retention time (Rt) and compared with a known standard at the same conditions. The quantities were calculated on peak area basis. The injection volume of 10 µl was used in all experiments.

3. Results and discussion

The analytical method was developed to provide a rapid, accurate, and efficient means of determining imidacloprid residues in tomatoes. For recovery experiments homogenized, untreated tomato samples were spiked with imidacloprid at 0.05 and 1.0 mg/kg. With a graduated microsyringe of 20 µL and 50 µL of the 50 µg/mL pesticide stock solution was added to 10 g blank matrix in a blender tube. Samples were mixed and allowed to stand for 1 h before extraction. For each fortification level, 10 replicates were analyzed. Mean recovery value obtained for imidacloprid was 94.03% with relative standard deviation (RSD) values below

with relative standard deviation (RSD) values below 15% in the fortification range from 0.05 to 0.5 mg/kg.

Calibration for quantification was carried out by use of external standard calibration curves; calibration curves were linear with correlation coefficients beingbetter than 0.998 . Retention time of imidacloprid under these conditions was observed to be 1.92 minutes.

The LOD and LOQ in the tomato fruits were 0.0089 and 0.0178 mg/kg, respectively, ensuring LOQ values significantly lower than the MRLs established by the EU.

3.1 Imidacloprid Residue Levels in Fresh Harvested tomatoes

The results of imidacloprid residue analyses and the percent dissipation at different intervals at single and double dosages are presented in Table 1 and Figure2.

Below it is presented thelinear calibration curve of Imidaclopridstandard.(Figure.1).

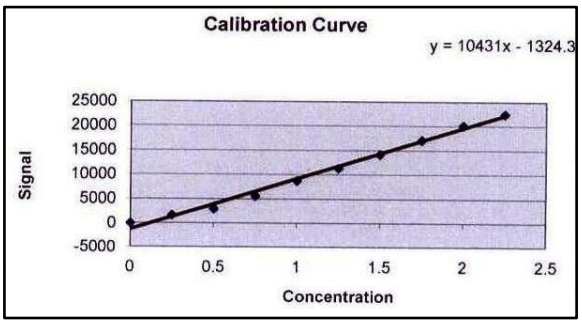

Figure. 1 Linear calibration curve of Imidacloprid standard(Concentration rangeµg ml^{-1} 0.01 - 2.25 µg ml^{-1}) in methanol.

The extractable residue of imidacloprid in tomatoes samples was found to be recpectively 0.42and 0.93 mg/kg at the time of harvest. It was under the MRL value (dose level 0.025%) and higher MRL value (dose level 0.025%).The EU MRL value for imidacloprid is 0.5 mg/kg for tomato.

Table 1. Imidacloprid residue in mg/kg according the sampling days after the application

Sampling	***Sampling days after treatment***	***Residues in mg/kg***	
		Dose level 0.025 %	Dose level 0.05 %
02.06.2012	1	1.1	2.11
03.06.2012	3	0.81	1.56
05.06.2012	5	0.63	1.13
07.06.2012	7	0.42	0.93
09.06.2012	9	0.22	0.65

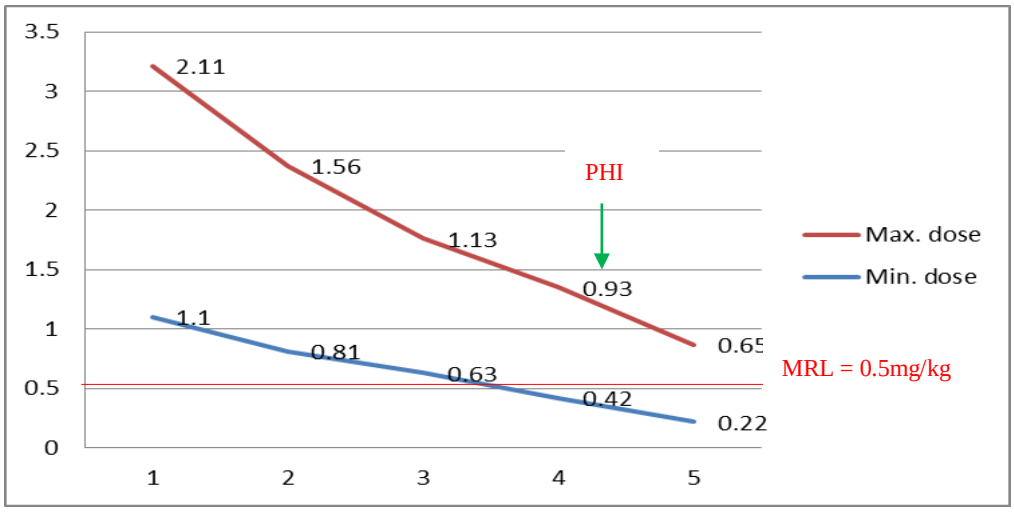

Figure2. Degradation of Imidacloprid in tomatoes for both dosing level and comparison with MRL values. MRL for imidacloprid in tomatoes is 0.5 mg/kg.

The Decline rate of imidacloprid according toboth dosesin mg/kg according the sampling days after the application, are presented in Figure 2.

Linearity and Limits of Detection (LODs)

Calibration graphs for tomato samples were constructed for imidacloprid in the range of 0.05–1.0mg/kg absolute amounts injected.Correlation coefficients were > 0.998 in all cases. LODs were calculated by using asignal-to-noise ratio of 3. The LOD for imidacloprid were 0.0089ppm.

The recovery experiments for were carried out and calculated. Table 2 shows the recoveries and precision for the pesticide added at 0.05 and 1.0 mg/kg to untreated tomato samples. Fortification levels were representative of the tolerance limits of the European Union (EU). Recoveries were calculated by using an external standard.

Table 2. Validation parameters for the method of determination of Imidacloprid in tomatoes.

Validation parameter	***Value***
Linearity, correlation coefficient	0.9982
LOD	0.0089ppm
LOQ	0.0178 ppm
Reproducibility (S)	4.64 %
Repeatability(S)	3.48 %
Range	0-4 ppm
Bias	13.86 %
Recovery	94.03%

4. Conclusions

In the tomato samples collected in the greenhouse, imidacloprid residues higher than their MRL values were observed in initial days. When the pre-harvest intervals between pesticide applications and crop harvest are not observed by the farmers, the risk of having higher pesticide levels is likely to increase [11].

From the above study it can be concluded that the injudicious application of pesticides on vegetable and fruit crops results in persistence of high levels of pesticide residues (some cases above MRL) in the crops at the time of harvest. These residues produce disastrous effects on the crop quality by lowering or enhancing the biochemical parameters which make the crop unfit for consumption.

In this case, the higher levels of pesticide residues can cause considerable consumer health risks and environmental pollution.

Therefore, it is suggested that a waiting period of 9days (for max dose) should be observed before consumption of tomato fruits, as it will be safe for the consumers' health.

5. References

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