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Residues and dissipation of kresoxim methyl in apple under field condition

Farag Malhat [a,*], Essam Kamel [b], Ayman Saber [a], Ehab Hassan [a], Ahmed Youssef [c], Monir Almaz [a], Ayman Hassan [a], Abd El-Salam Fayz [a]

[a] Pesticide Residues and Environmental Pollution Department, Central Agricultural Pesticide Laboratory, Agricultural Research Center, Dokki, Giza 12618, Egypt
[b] Toxicology Unite, Animal Health Research Institute, Dokii, Giza, Egypt
[c] Packing and Packaging Materials Department, National Research Center, Dokki, Cairo, Egypt

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ABSTRACT

The dissipation and residual levels of kresoxim methyl in apple under field condition were determined by using HPLC-DAD with QuEChERS method. At fortification levels of 0.05, 0.1, 0.5 and 1.0 mg kg^{-1} in apple, it was shown that recoveries were ranged from 91.1% to 96.9% with coefficient variation of the method (CV%) for repeatability ranged from 1.27% to 4.77%. The limit of quantification (LOQ) of the method was 0.05 mg kg^{-1}. The dissipation rates of kresoxim methyl were described by using first-order kinetics and its half-life, as they are ranged from 4.58 to 4.77 days in apple. The terminal residues of kresoxim methyl were below the FAO/WHO maximum residue limit (MRL, 0.2 mg kg^{-1}) in apple when measured 14 days after the final application, which suggested that the use of this fungicide was safe for humans. This study would help in providing the basic information for developing regulation to guard a safe use of kresoxim methyl in apple orchard and to prevent health problem from consumers.

1. Introduction

Apple is a fleshy sweet fruit with many health benefits. It is one of the most important commercial fruit crops which grow on a large scale on Egypt; it is cultivated in 24.55 thousand hectare with an annual production, that reaches to 550,743 thousand tones and most of these areas in modern reclaimed land. This crop is attack by many fungi, which require frequent use of pesticides. Kresoxim-methyl(methyl(E)-methoxyimino[2-(o-tolyloxymethyl)phenyl] acetate (Fig. 1), is strobilurin group fungicide with protective activity, which belongs to a new class of substances which are synthetic active ingredients (β-methoxyacrylate derivative group) with similar action to the natural strobilurin A (obtained from different wood rotting fungi) (Bartett, Clough, Godfrey, & Godwin, 2001). Kresoxim-methyl is mainly used for the control of *Oidium* spp. on different vegetable crops, including cucurbits (Tomlin, 2009). Kresoxim-methyl is considered a reduced risk fungicide with low mammalian toxicity and a benign profile for avians as compared to that of conventional fungicide, except some aquatic (Wang & Pan, 2005). Because of the excellent properties, the use of kresoxim-methyl is continuously increasing. It is important to study the dissipation and the residue of kresoxim-methyl in crop.

Many methods for kresoxim-methyl analysis have been reported in recent years, including headspace solid-phase microextraction and gas chromatography–mass spectrometry in groceries (Navalon, Prieto, & Araujo, 2002), acetone extraction followed by liquid–liquid partition in cucumber and soil prior to chromatographic analysis (Li, Wu, & Ji-Ye, 2006). Sannino, Bolzoni, and Bandini (2004) employed liquid chromatography with electrospray tandem mass spectrometry to determine kresoxim-methyl in the processed food and vegetables. Abreua, Cabonib, Cabrasb, Garaub, and Alvesa (2006) investigate liquid chromatographic with diode array detection method for kresoxim-methyl analysis in grapes and wine. Christensen and Granby (2001) investigate a GC-multimethod with electron capture, nitrogen/phosphorus and mass spectrometry detection of kresoxim-methyl in cereals and fruits.

The QuEchERS "Quick Easy Cheap Effective Rugged and Safe" method, which developed between 2000 and 2002 and first reported in 2003, had been widely accepted by the international community of pesticide residue analysts (Payá et al., 2007). The QuEchERS method covers a very wide analyte scope such as highly polar pesticides, and highly acidic and basic ones. This method involves extraction with acetonitrile and partitioning after the addition of a salt mixture. The final extract in acetonitrile could be directly amenable to determinative analysis that based on LC/MS. The LC–MS instruments are costly and not commonly available in Egypt. With the focus on food safety issue, there are many grass-roots institutions were founded for food examination. The residue dynamics of kresoxim-methyl have been studied in different matrixes, such as Zucchini, grape, cucumber and soil (Aguilera, Antonio, Francisco, Mourad, & Luis, 2012; Cabras, Angioni, & Vincenzo, 1998; Li, Wu, & Ji-Ye, 2006). The use of pesticides for combating pests in agricultural production has no doubt enhanced

* Corresponding author.
E-mail address: farag_malhat@yahoo.com (F. Malhat).

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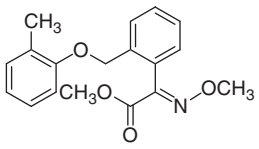

Fig. 1. Structure of kresoxim methyl.

food production and quality of the product, but their indiscriminate use has led to undesirable side effects on environmental quality and human health. Consequently, analysis of residual quantities of pesticides in raw agricultural crops is in forefront among preventive measure of public health safety. In addition, the maximum residue limits (MRL) regulations require a pre-harvest interval (PHI) to ensure the dissipation of a pesticide below the proposed MRL at harvest time (Karmakar & Kulshrestha, 2009). Therefore, to ensure food safety and protecting the environment, field dissipation studies on pesticide persistence in foodstuffs and pesticide residue behaviour in agricultural fields are needed.

To the best of our knowledge, no paper has reported the residue dissipation behaviour of kresoxim-methyl on apple under field condition. The present study deals with QuEChERS procedures followed by HPLC-DAD to establish a simple and reliable analytical method of kresoxim methyl residue in apple and to evaluate its dissipation rate and residue level under field condition. This work would help the government to provide guidance on the proper and safe use of this fungicide.

2. Materials and methods

2.1. Materials

The certified reference standard of kresoxim-methyl was provided from central agricultural pesticide laboratory, Egypt, and was of >99% purity. All organic solvents used in the study were pesticide grade or HPLC grade and purchased from Merck (Germany). Primary secondary amine (PSA, 40 μm Bondesil) and graphite carbon black (GCB) sorbents were purchased from Supelco (Supelco, Bellefonte, USA). Anhydrous magnesium sulphate was of analytical grade, purchased from Merck and was activated by heating at 400 °C for 4 h in the muffle furnace (for phthalates and moisture removal), cooled and kept in desiccators before use. Sodium chloride was of analytical grade and purchased from El Naser Pharmaceutical Chemical Com. (Egypt).

Kresoxim-methyl standard solutions of 100 mg/L were prepared in methanol and stored at −20 °C. Standard solutions prepared in acetonitrile were used for spiking apple samples at 0.05, 0.1, 0.5, and 1 mg/L levels and stored at 4 °C.

Calibration standard solutions were prepared in solvent and in apple extract from untreated samples at 0.01–1.0 mg/L concentration range. Matrix matched standard solution were prepared by evaporating to dryness portion of the final extract solutions obtained from untreated apple sample and taking up the remained residue with the calibration solution.

2.2. Field experiment design

The field trails including the dissipation study and final residue study were conducted in 10 year old apple orchard. The supervised field trials were carried out in Meit-Gamer and Menof, Egypt, in the year 2011. Every experiment plot was three apple trees. Each treatment was designed with three replicated plots; 20 m distance was maintained as a buffer area to separate each plot in the same field. During the whole trial, the average minimum/maximum daily air temperatures was 27/38 °C, the average relative humidity was 80.5%. There was no rainfall at any time during the experimental period.

2.3. Residue dynamic experiment

To investigate the dissipation of kresoxim methyl in plant, apple were sprayed with kresoxim methyl formulation (50% SC) in the experiment plots each with three replicates, the dosage was 100 g a.i. ha^{-1} (two time the recommended dosage) with one time spray. A plot with the same size but no kresoxim methyl application was compared simultaneously. Samples (apple fruit) were collected at random from sampling plots at 0 (2 h after spraying), 1, 3, 7, 10, 14, and 21 days after the treatment. Immediately after picking, the samples were put into polyethylene bags and transported to the laboratory, where they were chopped and thoroughly mixed. The sample was kept deep-frozen (−20 °C) until analysis. Control samples were obtained from the control plots.

2.4. Final residue experiments

To investigate the final residue of kresoxim methyl in apple, the plants were sprayed, in three replication, with kresoxim methyl formulation (50% SC) at two dosage of 50 g a.i. ha (recommended dosage) and 75 g a.i. ha (1.5 time recommended dosage). Each dosage level was designed to spray two and three times. Representative apple samples were collected at days 7 and 14 before harvest. Collected apple samples were chopped immediately after harvesting, mixed with the same plot, packed separately in polyethylene bags, labelled, and stored at −20 °C until analysis.

2.5. Analytical methods

2.5.1. Sample preparation

The apple samples were homogenised in a food processor (Thermomix; Vorwerk) and 10 g of the homogenate of each sample was placed into 50-ml centrifuge tube.

2.5.2. Sample extraction and clean up

As for the apple sample (10 g), 10 ml of acetonitrile (1% acetic acid) was added, the screw cap was closed and the tube vigorously shaken for 1 min using a vortex mixer at a maximum speed. Next 1 g sodium chloride and 4 g anhydrous magnesium sulphate was added, the sample was vortexed for 30 s. The extracts were centrifuged for 5 min at 3800 rpm and 4 °C. An aliquot of 4 ml was transferred from the supernatant to new clean 15 ml centrifuge tube and cleaned up by dispersive solid-phase extraction with 100 mg PSA, 20 mg GCB and 600 mg $MgSO_4$. The sample was again vortexed for 1 min and then centrifugation was carried out as mention above. Then, 2 ml of the supernatant was taken, and evaporate to dryness at 35–40 °C under a gentle stream of nitrogen. The residues were redissolved in 2 ml of mobile phase (methanol/water = 80 + 20, v/v), filtered through 0.22 μm PTFE filter (Millipore, USA) and transferred into a 1.5 ml glass vial for HPLC-DAD analysis.

2.5.3. Instrumental determination

The HPLC analysis was performed with an Agilent 1260 HPLC system (USA), with quaternary pump, autosampler injector, thermostat compartment for the column and photodiode array detector. The chromatographic column was Zorbax C_{18} XDB (250 mm × 4.6 mm, 5 μm film thicknesses). The column was kept at room temperature. Flow rate of mobile phase (methanol/water = 80 + 20, v/v) was 0.8 ml/min., and injection volume was 20 μl. Detection wavelength for detection of kresoxim methyl was set at 210 nm. The residues in the real samples were