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ORIGINAL ARTICLE

Persistence of metalaxyl residues on tomato fruit using high performance liquid chromatography and QuEChERS methodology

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Abstract Metalaxyl is a widely used fungicide around the world. Very limited data have been reported concerning the dissipation and residue of metalaxyl in agricultural products. Residues and dissipation rate of metalaxyl were estimated in tomato fruit by HPLC following single application of the fungicide at 262.5 g a.i. ha^{-1}. The average initial deposit of metalaxyl on tomato fruits was found to be 2.39 mg kg^{-1}. Residues of metalaxyl dissipated below the maximum residue limit (MRL) of 0.5 mg kg^{-1} in 7 days. Half-life ($t_{1/2}$) for degradation of metalaxyl on tomato fruit was observed to be 1.81 days. A waiting period of 7 days is suggested for safe consumption of tomato. The results would be useful for the safe use of metalaxyl and to prevent any health problem to customers.

1. Introduction

Metalaxyl [*N*-(2,6-dimethylphenyl)-*N*-(methoxy-acetyl)-DL-alanine ester] (Fig. 1) is an acylalanine compound used as a systemic fungicide. It can be used to control disease caused by air- and soil-borne *Peronosporales* on a wide range of temperate, subtropical and tropical crops such as apple, cucumber, grape, tobacco, millet, potato, tomato, betel, sunflower, etc. It is used extensively in Egypt both as foliar spray as well as soil drench for control of various diseases of crop plants such as root and lower stem rot, downy mildew and damping off. The fungicide is available in different formulations such as Apron 35 WS (for seed treatment), Ridomil 5 G (for soil treatment), Ridomil 25% WP, Vacomil 35% WP (for foliar sprays) and in a mixture with mancozeb; captan; folpet, carbendazim and copper oxychloride. Metalaxyl is registered for use in many countries worldwide including USA, European nations, Australia, India and Egypt.

It is well recognized that there are risks attached to the consumption of pesticide-treated crops because of the presence of residues on them. To prevent the presence of pesticide residue above the MRL, the time between pesticide application and harvest must be determined, since the pesticide decay time depends on crop type, pesticide and environmental conditions. Although metalaxyl has relatively low toxicity to people (maximum allowable intake, 0.03 mg/kg body weight/day), it

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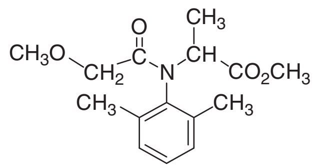

Figure 1 Molecular structure of metalaxyl.

is necessary to measure the persistence and distribution of metalaxyl residues in crops after application. However, there are few references available on these topics (Spack and Dirr, 1980; Businlli et al., 1984; Reddy et al., 1990; Mohapatra et al., 1998; Yirong et al., 2003). This study was therefore conducted to evaluate the residue persistence of metalaxyl in tomato fruit after plant treatment at the recommended dosage for pest control.

2. Materials and methods

2.1. Equipment

The HPLC system was an Agilent 1100 series (Agilent Technologies, Wilmington, DE) attached to a photodiode array detector. The food processor was a Thermomix, Vorwerk. For water purification, a Millipore-Q system was used. Vortex shaker and high speed centrifuge were made in Germany. Rotary evaporator was a Buchi.

2.2. Reagents

All organic solvents employed throughout the study were of HPLC grade and were purchased from Merck (Darmstadt, Germany). Primary secondary amine (PSA, 40 μm Bondesil) and graphite carbon black (GCB) sorbents were purchased from Supelco (Supelco, Bellefonte, USA). Anhydrous magnesium sulfate was of analytical grade and purchased from Merck Ltd. Sodium chloride is of analytical grade and was purchased from El-Naser Pharmaceutical Chemical Com., (Egypt). Anhydrous magnesium sulfate and sodium chloride were activated by heating at 400 °C for 4 h in the oven before use and kept in desiccators. Ultra pure water was prepared by the Millipore system. The neat standard of metalaxyl (purity $\geqslant$ 98%) was obtained from central agricultural pesticide laboratory (Egypt). A stock standard solution (100 mg L^{-1}) was prepared in acetonitrile and stored at −18 °C.

2.3. Field experiment

Tomato plants were raised in plots consisting of eight rows. Plots were arranged in complete randomized block design at El-Hakimayia village, Miet-Gamer Province, El-Dkahlyia Governorate, Egypt, on 2 August 2011. Common agricultural and fertilization practices were used. Metalaxyl (Vacomil 35% WP) was applied at the recommended dosage of 262.5 g a.i. ha^{-1}. The pesticide was applied as high volume spray using knapsack sprayer (capacity 20 L).

2.4. Sampling

Samples of tomato were randomly taken from each treatment plot after 0 (2 h after spraying), 1, 3, 7, 10, and 15 days after the application of fungicide. About eight to ten marketable size fruits of tomato were collected at random from each plot. The samples were collected in polyethylene bags, labeled and brought to the laboratory for analysis.

2.5. Extraction and clean-up

Each fruit sample was comminuted using the laboratory blender and the representative of homogenized (15 g) of each was then placed into a 50 mL polyethylene tube. Samples were extracted and cleaned up immediately after sampling using the QuEChERS method. Fifteen milliliters of acetonitrile was added into each tube. The samples were well shaken using a vortex mixer at maximum speed. Afterward, 6 g of anhydrous magnesium sulfate and 1.5 g of sodium chloride were added, then extracted by shaking vigorously on a vortex for 5 min and centrifuged for 10 min at 4000 rpm. An aliquot of 4 mL was transferred from the supernatant to a new clean 15-mL centrifuge tube containing 100 mg PSA, 20 mg GCB and 600 mg anhydrous magnesium sulfate. The samples were again vortexed for 3 min and then centrifuged for 10 min at 4000 rpm. An aliquot of 2 mL was filtered through a 0.2 μm PTFE filter (Millipore, USA). The sample was then ready for the final analysis in LC system.

2.6. Liquid chromatographic analysis

HPLC analysis was performed with an Agilent 1100 HPLC system (USA), with quaternary pump, manual injector (Rheodyne), thermostat compartment for the column and photodiode array detector. The chromatographic column was ODS H optimal (150 mm × 4.6 mm, 5 μm film thicknesses). The column was kept at room temperature. Flow rate of mobile phase (acetonitrile/methanol/water = 40/20/40 v/v/v) was 0.8 mL/min., and injection volume was 20 μL. Detection wavelength for detection of metalaxyl was set at 205 nm. The retention time of metalaxyl was about 8.01 min. Residues were estimated by the comparison of peak area of standards with that of the unknown or spiked samples run under identical conditions.

2.7. Recovery assays

Untreated tomato samples were homogenized before being spiked with a standard solution of metalaxyl. Recovery assays were performed in the 0.05–0.5 mg kg^{-1} range. The quantification of recovery was carried out with the standard dissolved in pure solvent. The samples were processed according to the above procedure. At each fortification level, five replicates were analyzed. Results of recovery study are shown in Table 1. Results were corrected according to recovery rate. Blank analyses were performed in order to check interference from the matrix.

Table 1 Recoveries and relative standard deviations for metalaxyl in tomato at various fortification levels.

Fortified level (mg kg^{-1}) (n^{a} = 5)	Recovery	RSD
0.05	93	10
0.10	95	12
0.50	98	5.3

[a] Number of replicate.

Table 2 Dissipation of metalaxyl in/on tomato fruit.

Time (days)	Residue level (mg kg^{-1}) mean[a] ± SD	Dissipation%
Zero	2.39 ± 0.12	0.00
1	0.996 ± 0.09	58.32
3	0.504 ± 0.05	78.91
7	0.105 ± 0.01	95.60
10	0.046 ± 0.01	98.07
15	ND[b]	–
$t_{1/2}$ (days)	1.81	

[a] $N = 3$.
[b] Not detectable.

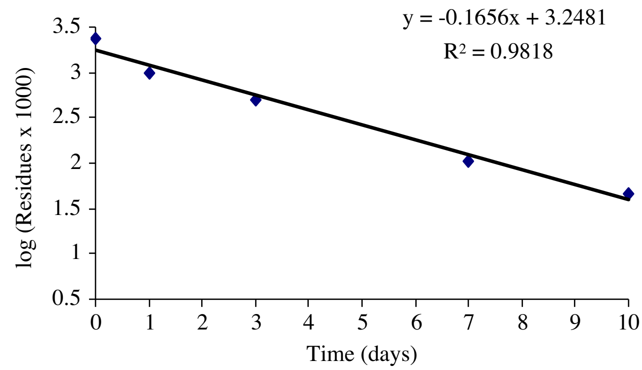

Figure 2 Linear plot for first order kinetics of metalaxyl residues in tomato fruits.

2.8. Statistical analysis

All statistical analyses were done using the Statistical Package for Social Sciences (SPSS 16.0) program.

3. Results and discussion

3.1. Analytical method efficiency

Linear range, limit of detection (LOD), limit of quantification (LOQ), and repeatability were determined for metalaxyl. The linear range was from 0.01 to 2 μg mL^{-1}. Good linearity was obtained for metalaxyl and for the entire range of studied concentration with correlation coefficients better than 0.9999. The LOD was found to be 0.01 mg kg^{-1} and resulted by considering a signal-to-noise ratio of 3 with reference to the background noise obtained for the blank sample. The limit of quantification of the method was 0.04 mg kg^{-1}, the S/N of which was generally >10. The matrix effect of the present method was investigated by comparing standards in solvent with matrix-matched standards for 5 replicates at 0.5 mg kg $^{-1}$. The results showed that, no interfering endogenous peak appeared, and the retention times of the tested analytes at the spiked sample completely matched those of the standard samples. The analytes were eluted as separate symmetric peaks. Repeatability expressed as RSD was below 12.3%. Recovery experiment was carried out by spiking tomato at different levels to establish the reliability and validity of analytical method adopted. Tomato fruits from control plots were spiked with metalaxyl at levels of 0.05, 0.1 and 0.5 mg kg^{-1} five times. These were extracted, cleaned up and analyzed by following the method as described. The mean recoveries of metalaxyl fortified at these levels were found to be consistent and more than 90% (Table 1).

3.2. Dissipation of metalaxyl in/on tomato fruits

The mean initial deposit of metalaxyl in/on tomato fruits was found to be 2.39 mg kg^{-1} following application at the recommended dosage. More than 90% of these metalaxyl residues dissipated after 10 days after application (Table 2). The rapid degradation/dissipation of fungicide in subsequent sampling after spraying may be due to dilution of the toxicant due to plant growth coupled with favorable climatic conditions, i.e., clear sunshine, temperature, and relative humidity prevailing during the application period. Milgroom and Fry (1988) observed that metalaxyl residues decreased rapidly in the first two days after application and thereafter the decrease was quite slow.

Half-life value ($t_{1/2}$) for degradation of metalaxyl on tomato fruits was calculated as per Hoskin (1961) and observed to be 1.81 days, at the recommended dosage (See Fig. 2). Persistence of metalaxyl has been studied earlier by Hanumantharaju et al. (2002) on the tomato fruits. It was found that metalaxyl residues dissipated on tomato fruits with a half-life of 5.23–6.95 days, which was higher as compared to those as obtained in the present study. Singh et al. (1998) studied the persistence of metalaxyl in potato tubers. It was found that with double conc. of Ridomil MZ, metalaxyl accumulates in tubers but the level was within prescribed limits. Maximum residue limit (MRL) of metalaxyl on tomato has been prescribed as 0.50 mg kg^{-1} (Codex, 2011). Residues of metalaxyl on tomato fruits were less than its MRL value after 7 days of its application at the recommended dosage. So it is suggested that a period of 7 days should be observed before consumption of tomato fruits, as it will be safe for the consumer's health.

4. Conclusion

In this study, the dissipation rate of metalaxyl after single application at the recommended dose on tomato was evaluated. The results of this study indicated that metalaxyl disappears rapidly in tomato fruit and field under natural conditions and exhibited first-order kinetic dissipation. Usually, the degradation of pesticides in the plant besides the effect of some physical and chemical factors like light, heat, pH and moisture, growth dilution factor might have played a significant role. These data could provide guidance for the proper and safe use of this pesticide on tomato in Egypt.

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