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Dissipation and Residue of Myclobutanil in Lychee

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Abstract The dissipation and residue of myclobutanil in lychee under field conditions were studied. To determine myclobutanil residue in samples, an analytical method with a florisil column clean-up and detected by gas chromatography-electron capture detector (GC-ECD) was developed. Recoveries were found in the range of 83.24 %–89.00 % with relative standard deviations of 2.67 %–9.88 %. This method was successfully applied to analyze the dissipation and residue of myclobutanil in lychee in Guangdong and Guangxi Province, China. The half lives in lychee were from 2.2 to 3.4 days. The residues of myclobutanil in lychee flesh were all below the limit of quantification (LOQ) value (0.01 mg/kg), and most of the residues were concentrated in the peel. The terminal residues of myclobutanil were all bellow the maximum residue limit (MRL) value set by European Union (EU) (0.02 mg/kg). Hence it was safe for the use of this pesticide and the results also could give a reference for MRL setting of myclobutanil in lychee in China.

Keywords Myclobutanil residue · Dissipation · Lychee

Lychee is a specialty fruit in China, and originated from Lingnan area. It is rich in sugar, vitamin C and protein, which helps to enhance human's immune function and the capability of resistance to disease. However, lychee suffers a series of disease during growth, such as anthracnose, grey mould, etc. Some fungicides (such as myclobutanil, mancozeb, metalaxyl and so on) are used to protect the lychee from disease (Li et al. 2009).

Myclobutanil (2-*p*-chlorophenyl-2-(1H-1,2,4-triazol-1-ylmethyl) hexanenitrile, Fig. 1) is a kind of triazole fungicide, and a sterol demethylation inhibitor, with protective and curative action. It was developed by Dow AgroSciences and first marketed in 1989 (the e-pesticide manual, Version, 3.0) and could be used to protect lychee from the disease. Gas chromatography (GC) with electron capture detector (ECD) and GC-mass spectrometry (MS) were used to determine the residue of myclobutanil in different matrices (Tan 2010; Chen and Lin 2006; Cui et al. 2011; Deng et al. 2010; Tian et al. 2010). And previous studies also investigated the dissipation of myclobutanil in onion, pear, wheat and banana (Cui et al. 2011; Shi et al. 2008; Liu et al. 2009; Han et al. 2007). However, to our knowledge, there was no research on analytical method and the dissipation of myclobutanil in lychee.

The MRL (maximum residue limit) set by EU was 0.02 mg/kg. There was no MRL of myclobutanil in lychee in China, so it is very necessary to study the dissipation and residue of myclobutanil in lychee to give a reasonable use of this pesticide and provide a reference for MRL setting in lychee.

Materials and Methods

Myclobutanil standard (purity 99.2 %) was purchased from National Research Center for Certified Reference Materials (Beijing, China); analytical reagent (AR), methanol, ethyl acetate, and hexane were purchased from Beijing Chemical Reagents Company, China; anhydrous magnesium sulfate

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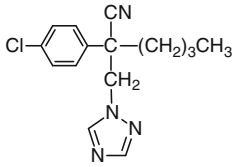
Fig. 1 Chemical structure of myclobutanil



and ganister sand (analytical grade) were purchased from Beijing Chemical Reagents Company, China. The shaker (HY-5) was from Jiamei Chemical Instrument Company of Jiangsu Jiantan, China; vacuum rotary evaporator (SHZ-III) was from Xinghaiwang Biochemical Co., Ltd, China; homogenizer (IKA T18) was from IKA Laboratory Equipment Company, Germany; GC (Agilent 6890) with ECD was from Agilent Company, USA.

The field trial was conducted in Guangdong and Guangxi Province in two consecutive years. The experiment was designed according to NY/T 788-2004 (Guideline on Pesticide Residue Trials) issued by Ministry of Agriculture, People's Republic of China. Four field plots (recommended dosage application with 3 and 4 times, dissipation, blank control of lychee) each with two trees were prepared with a buffer area to separate each plot, and each experiment plot with three replicates.

In the dissipation study of myclobutanil in lychee, the formulation 40 % WP (Wettable powder) was sprayed with a dosage of 150 mg/kg (1.5 times of the recommended dosage). A plot with the same size with no myclobutanil application was as control simultaneously. The lychee samples were collected on day 0, 1, 3, 7, 14, 21 and 28 after spraying. All the samples were stored at −20°C before analysis.

In terminal residue plot, 40 % WP was sprayed with a dosage of 100 mg/kg (the recommended dosage), each with two treatments: sprayed 3 times and 4 times. The spraying interval was 7 days. The lychee samples were collected at 7 days after spraying. All the samples were stored at −20°C before analysis.

The whole lychee sample was blended after removing of the rotten fruit, and the flesh of lychee was blended after removing of fruit stone.

Twenty gram of the blended sample (whole lychee sample or flesh sample) was weighed into a conical flask and 80 mL methanol was added. The samples were minced with a high pressure homogenization and shaken for 30 min. The extracts were filtered with a filter paper and ganister sand and washed with 60 mL methanol. The filtrate was transferred to a conical flask.

The extract was added with 100 mL distilled water and 20 mL saturated solution of sodium chloride, and then was extracted three times by liquid–liquid extraction with 60 mL ethyl acetate each time. The organic layers were combined and filtered through anhydrous sodium sulfate. It was concentrated to dryness on a rotary evaporator under vacuum and redissolved with hexane. A glass column packed with 3 g florisil in between two layers of 1.5 cm anhydrous sodium sulfate was used for further cleanup. It was preconditioned with 30 mL hexane and the concentrated extract was loaded to the column. The rinsing solvent with 70 mL acetone/hexane mixture (V/V = 1:9) to remove some interferences, and the elution solvent was 80 mL acetone/hexane mixture (V/V = 3:7). The eluent was collected and evaporated under a gentle nitrogen stream and redissolved in 2 mL hexane for GC analysis.

The myclobutanil was determined by an Agilent 6890 GC-ECD with a DB-1701 capillary column (30 m × 0.53 mm × 0.25 μm). The injector was operated at 260°C with an injection volume of 2 μL. Oven temperature was programmed as follows: the initial temperature was 120°C, and rising to 200°C at a rate of 10°C/min, hold for 8 min, then rising to 240°C at a rate of 3°C/min and hold for 10 min. Nitrogen was used as the carrier gas at a flow rate of 1.0 mL/min. The detector was operated at 300°C. The retention time of myclobutanil was 20.0 min.

Residue concentration and half life of myclobutanil were calculated by the first order kinetics equations: $C_t = C_0 e^{-kt}$ and $t_{1/2} = (\ln 2)/k$, where C_0 means the initial concentration residue of myclobutanil, C_t means the concentration residue of myclobutanil at time t, $t_{1/2}$ is the dissipation half life and k is the rate constant.

Results and Discussion

To determine the linearity of myclobutanil, different concentrations of working solutions (0.01, 0.1, 0.2, 0.5, 1.0 mg/kg) were prepared by diluting the stock solution with hexane. The results showed a good linearity with a correlation coefficient of 0.9999. Recoveries were determined at three levels of 0.01, 0.10 and 1.00 mg/kg for whole lychee and lychee flesh with 5 replicates. The average recoveries were ranging from 83.24 % to 89.00 % with relative standard deviations (RSDs) of 2.67 %–9.88 %. The limit of quantification (LOQ) was defined as the lowest fortified level of recovery, which was 0.01 mg/kg in whole lychee and lychee flesh. The limit of detection (LOD) was 0.005 mg/kg in whole lychee and lychee flesh which set at a signal to noise ratio of 3:1. The results are shown in Table 1.

Figure 2 and Table 2 show the dissipation of myclobutanil in lychee. From the results, it can be seen that the half lives of myclobutanil in lychee were from 2.2 to 3.4 days in Guangdong and Guangxi, which means the dissipation rate of myclobutanil was very fast. Previous research showed that the half lives in onion were 3.4 days (Cui et al. 2011), in pear were 2.9–4.8 days (Shi et al. 2008), and in banana were 7.7–10.4 days (Han et al. 2007). There had a

Table 1 The average recoveries, LODs and LOQs of myclobutanil in lychee (n = 5)

Sample	Spiking level (mg/kg)	Mean recovery (%)	RSD (%)	LOD (mg/kg)	LOQ (mg/kg)
Whole lychee	0.01	89.00	9.88	0.005	0.01
	0.10	83.24	2.67		
	1.00	86.24	4.37		
Lychee flesh	0.01	84.18	5.47	0.005	0.01
	0.10	83.44	3.39		
	1.00	85.06	4.16		

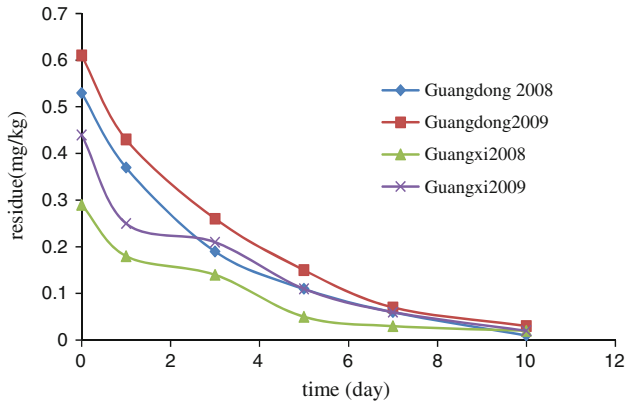


Fig. 2 Dissipation of myclobutanil in lychee of Guangdong and Guangxi in the year 2008 and 2009

different dissipation rate in those crops. That is to say, the dissipation rate of myclobutanil may be affected by the kinds of crop, for example, the plant growth, the water content and the acid–base character of the crops. On the other hand, the dissipation rate of myclobutanil in Guangdong and Guangxi had the similar result, which is mostly because of the similar geographical location and weather conditions.

The terminal residue results showed that, when the myclobutanil (40 % EC) was applied 3–4 times with the recommend and 1.5 times of recommended dosage in lychee, the residue of myclobutanil in lychee flesh were all below the LOQ value (0.01 mg/kg). However, the residue in whole lychee was much more than in flesh, and the maximum value was 0.06 mg/kg 14 days after the application, respectively, which means that most of the residues were concentrated in the peel of lychee.

The terminal residue was investigated with the flesh basis and whole fruit basis, respectively (Table 3). The residue in lychee flesh were all bellow LOQ value (0.01 mg/kg) 7 days after application either in Guangxi or Guangdong Province in the year 2008 and 2009. The residues in peel were ranged from 0.15 to 0.86 mg/g, and the residues of myclobutanil in whole lychee were ranged from 0.03 to 0.15 mg/kg at the recommended dosage. It can be seen from Table 3 that most of the myclobutanil residues were concentrated in the peel of lychee.

The EU MRL value was set at 0.02 mg/kg. It can be seen from the terminal results that when the myclobutanil was applied at recommended dosage after 7 days, the residues in lychee flesh were all below 0.01 mg/kg. However, the residue in whole lychee and lychee peel were all higher than 0.02 mg/kg. It means that most of the myclobutanil residues were concentrated in the peel of lychee. The concentration in peel was 5–6 times of residue in whole lychee (Table 3). The results shows that although it is safe for the human to consume the lychee flesh, the high residue in the peel of lychee should attract much attention, for the high active ingredients such as flavonoids, phenolic acids, and multi-phenols which have good oxidation and free radical scavenging capacity in lychee peel (Wen et al. 2008). As there was no MRL value of myclobutanil in lychee in China, those results could give a reference to establish the MRL of myclobutanil in lychee in China.

To conclude, A GC-ECD method for analysis of myclobutanil in lychee was developed. Based on it, the dissipation and residue of myclobutanil in lychee of Guangdong and Guangxi Province were investigated. It could be seen that the half lives of myclobutanil in lychee were 2.2–3.4 days. The residues of myclobutanil in lychee

Table 2 The half lives for myclobutanil dissipation in lychee

Matrix	Experiment plot	Regression equation	Correlation coefficient (γ)	Half life (days)
Lychee	Guangdong, 2008	$C = 0.486e^{-0.312t}$	0.973	2.2
	Guangdong, 2009	$C = 0.427e^{-0.204t}$	0.967	3.4
	Guangxi, 2008	$C = 0.260e^{-0.278t}$	0.980	2.5
	Guangxi, 2009	$C = 0.406e^{-0.275t}$	0.992	2.5

Table 3 The distribution of myclobutanil in lychee flesh and peel

Experiment spot	Spraying time	Residue in flesh (mg/kg)	Residue in whole lychee (mg/kg)	Residue in peel (mg/kg)	C_{peel}/C_{whole}
Guangdong, 2008	3	<0.01	0.03–0.15	0.17–0.86	5.7
	4	<0.01	0.04–0.11	0.23–0.63	5.7
Guangdong, 2009	3	<0.01	0.07–0.14	0.41–0.83	5.9
	4	<0.01	0.05–0.13	0.29–0.77	5.9
Guangxi, 2008	3	<0.01	0.05–0.11	0.25–0.54	5.0
	4	<0.01	0.03–0.09	0.15–0.45	5.0
Guangx,i 2009	3	<0.01	0.05–0.14	0.27–0.77	5.5
	4	<0.01	0.03–0.09	0.16–0.49	5.4

flesh were all below the LOQ value (0.01 mg/kg), and most of the residues were concentrated in the peel of lychee. The terminal residue of myclobutanil when applied at the recommend dosage 14 days were all bellow the MRL value set by EU (0.02 mg/kg). Hence it was safe for the use of this pesticide and the results also could give a reference to MRL setting of myclobutanil in lychee in China.

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