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Preliminary Study of Difenoconazole Residues in Rice Paddy Watersheds.

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ABSTRACT Pesticide application to rice paddies may affect the quality of environmental resources such as groundwater and surface water. The distribution of residues of difenoconazole (an effective fungicide) used was monitored in the network of surface water bodies surrounding the main rice production area in Tajar, Kedah. The location monitoring sites was based on the potential risk for contamination of difenoconazole. The analysis was done by using liquid-liquid extraction (LLE) and detected by gas chromatography-mass spectrometer (GC-MS). Limit of detection (LOD) was 0.0618 ppm. Recoveries of difenoconazole at spiking level of 400 ppm, 8 ppm and 0.5 ppm were between 93% - 106%.

ABSTRAK Penggunaan racun pada tanaman padi boleh mempengaruhi kualiti sumber persekitaran seperti air galian dan air di permukaan. Taburan sisabaki difenokonazol (racun kulat yang efektif digunakan secara meluas) diuji pada rangkaian permukaan air yang mengelilingi kawasan pencemaran difenokonazol di Tajar, Kedah. Pengujian dilakukan menggunakan kaedah pengekstrakan cecair-cecair dan dikesan menggunakan kromatografi gas-spektrometer jisim (GC-MS). Had pengesanan adalah 0.0618 ppm. Peratusan mendapat kembali difenokonazol pada paras 400 ppm, 8 ppm dan 0.5 ppm adalah diantara 93% - 106%.

(**Keywords:** Difenoconazole, LLE, GC-MS, pesticide)

INTRODUCTION

Kedah is the main supplier of rice in Malaysia, producing one third of Malaysia's total production of the rice which fulfills 40 % of the national need. The crop is planted on 161,690 ha, including the 97,000 ha in Muda Agricultural Development Authority (MADA), 15,684 ha in the Drainage and Irrigation Department (DID) Scheme, 16,176 ha in the area outside the DID scheme and 32, 830 ha village land [1].

Water management is essential in the production of rice. Efficient water management will supply water from main canal, retain water at each plot and drain water from paddy fields. This is because the crops need a large amount of water to grow in paddy fields. Rice crop is grown mostly under flooded conditions on a range of soils, where organic matter content can vary from 0.5-20 %, and clay and sand contents can be greater than 50%. Due to these properties, agronomic practices can have impact on water quality in this rice growing area. For example, irrigation can increase the likelihood of pesticides leaching to groundwater or reaching surface water via drainage. In most cases, the surface water in flooded paddies saturates the soil. Consequently, this condition increases the potential for groundwater contamination. Moreover, the cultivation of rice throughout the river is very intensive, therefore, the potential loading of agrochemicals such as fertilizers, trace elements, and pesticides is quite high [2].

Rice pesticides are applied directly to surface water of the paddy field. The paddy field system is extremely susceptible to pesticides runoff upon a significant rainfall events or inappropriate water management. Irrigation drainage and runoff flow sequentially from the paddy fields to irrigation channels, small rivers and large rivers, whereby they are dispersed widely throughout the water systems. Simultaneously, the water flow carries along pesticide used in the paddy fields [3]. The runoff rate of pesticides varied depending on properties of pesticides and environmental conditions such as rainfall and temperature as well as amount of pesticide used per unit area and paddy water management methods. For example, high rainfall immediately after pesticide application as well as flow irrigation will increase the runoff rate into water systems [4]. Our concern is that runoff of pesticides applied to agricultural land may cause contamination of water bodies and give adverse effects on aquatic ecosystem.

There are several studies [5-11] have been reported on the runoff of pesticides from paddy fields to a river by monitoring their residues in river or sediments. From the pesticide-monitoring programme carried out in the paddy areas in Italy, the results showed that a diffuse pollution of both surface and groundwater bodies with pesticides at concentrations varying from 0.1 – 30 ppb [12]. Other study on DDE, DDT and heptachlor [14], these pesticide residues were found in the water of almost every river surveyed in Peninsular Malaysia. Another study conducted in a paddy field in Tanjung Karang indicated that a significant amount of endosulfan (4.17 – 5.18 ppb) persisted in water 21 days after application of the granular formulation, indicating that the toxic effects of the chemical may be quite prolonged.

Difenoconazole is an active ingredient found in Score 250 EC, a systematic triazole fungicide which commonly used by farmers during the rice plantation. This pesticide is applied directly to surface water of the paddy fields in order to combat related diseases. Difenoconazole is a fungicide effective against *Rhizoctonia solani,* a fungus that causes Sheath Blight (Hawar Seludang) and *Drechslera oryzae*, a fungus that causes brown spot [13]. These diseases can cause losses about 50 % of rice production. Sheath Blight occurs normally when the paddy age 50 days after sowing.

There are six ways these diseases can infect the crops which are water, wind, soil, touch, hay stubble and temporary host. The infection occurs when the following four conditions occur: high humidity, hot weather (higher than 25°C), urea fertilizer used exceedingly and critical time when paddy age 45 days after sowing. Normally, the infection occurs at stems and leaves of paddy. Meanwhile, there are three ways brown spot can infect the paddies; wind, seed and secondary host. The indication of brown spot is that there are brown color dots look like sesame at the side of the leaves and paddy fruits. Furthermore, there are white/grey dots at the centre of it.

Difenoconazole is typically sprayed two or three times a year, before or when there are early indications of the diseases with the rate of 180 L/ha for Sheath Blight and 300 L/ha for brown spot. Farmers normally sprayed difenoconazole fungicide at 40 days after planting.

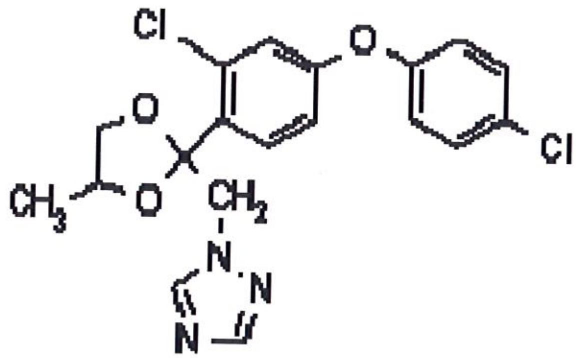

Figure 1: Molecular structure of difenoconazole

Figure 1: The molecular structure of difenoconazole (1-((2-(2-chloro-4-(4-chlorophenoxyl) phenyl)-4-methyl-1, 3-dioxolan-2-yl)methyl)-1H-1,2,4-triazole).

The aim of this study is to determine difenoconazole residues in the network of surface water bodies surrounding the rice production area in Tajar, Kedah after its application in the paddy fields. In this work, GC-MS was used to measure the pesticides content in the water sample.

MATERIALS AND METHODS

Sampling Location

The study was carried out in Tajar, one of the districts in Kedah. The selected paddy field area under studied was about 6.2 acres. Water samples were taken from four different locations (water irrigation, paddy field, water drainage (Alor Mengkuang River) and river basin (Pendang River). At each location, about 10-15 L of water samples were collected using a steel bucket, from which a composite sample (I L) was taken and kept in amber glass bottles. After that, the samples were filtered and stored at 4°C until analysis. In this study, all water samples were stored within 7 days before extraction and 40 days after extraction.

For monitoring study, the samples were collected three times (26 June 2006, 29 June 2006 and 6 July 2006). The first and second samplings were done before and after the application of difenoconazole to surface water of paddy field. Meanwhile, the third sampling was on 6 July 2006 when presumably most of the water used has been drained toward the main river. A control sample was obtained from Pedu Dam that supplied water for irrigation to this paddy field area.

For degradation study, water samples taken from paddy field were collected at one day before application and 1, 2, 3, 7, 8, and 9 days after application of difenoconazole. The collection of water samples for this degradation study was started on 13 Dec 2006.

Chemicals and Solvents

All chemicals used were of analytical reagent or chromatographic grade. Dichloromethane, and acetone and anhydrous sodium sulphate were obtained from Merck. The HPLC grade-water was obtained by purification of deionized water through a Milli-Q system. Pesticide standard of difenoconazole was obtained from Merck. A stock solution of 100 ppm was serially diluted in acetone ranging from 5 – 50 ppm.

Apparatus

Sampling bottles (1 L amber bottles), separation funnel: 250 ml, round bottom flask, glass stopcocks, glass filter funnels, filter paper, conical flask, beakers: 50 ml, 100 ml, 250 ml, 500 ml, measuring cylinders: 50 ml, 100 ml and 500 ml, pipettes, micropipettes, micro syringe, volumetric flasks: 5 ml, 10 ml, vials and parafilm (Laboratory film) were used throughout this work. An analytical balance from Mettler Toledo was used to prepare the working standard solution. An R-114 rotary evaporator (Buchi, Switzerland) was used for extract concentration.

Instrumentation Specification

GC-MS analysis was performed using a Hewlett-Packard system 6890 gas chromatograph coupled with a HP model 5972A quadrupole mass spectrometer (EI mode). An analytical column used was a cross-linked 5% phenyl methyl siloxane capillary column (HP-5MS, 30 m x 0.25 mm id x 0.25 mm film thickness). Data acquisition and processing were provided by a Vectra VL 5/90 Series 3 Computer equipped with HP G1030A Chemstation data system software. The parameters used for splitless as an injection mode and temperature program of column as in Table 1.

Table 1: The parameter used for splitless injection and temperature program

Parameters	Values
Injection mode	Splitless
Injection port temperature	240°C
Purge off time	0.75 min
Column flow rate	50 mL min^{-1}
Carrier gas	Helium
Interface temperature	280°C
Electron energy	70 eV
Oven temperature 1	100°C
Hold time	1 min
Rate 1	20°C min^{-1}
Oven temperature 2	290°C
Hold time	20 min
Run time	30.5 min
Injection volume	2 µl

The GC-MS system was set in the selective ion monitoring (SIM) mode and three ions were used for identification and quantification purposes. Difenoconazole compound was quantified based on one target ion (m/z 325) and two quantifier ions (m/z 323 and m/z 265). The retention time for the investigated pesticide is 15.17.

Sample Preparation

Water sample was filtered through glass fiber filter. A 200 ml aliquot of sample was transferred into a 250 ml separation funnel and extracted with five times of 20 ml aliquots of dichloromethane. The mixture was then shaken vigorously for about 2 minutes and the aqueous layer was remained in the separating funnel. The combined dichloromethane extracts were dried over anhydrous sodium sulfate. Therefore, the solvent layer was drained through a funnel containing solvent-moistened double phase separating filter paper in which approximately 2 g of anhydrous sodium sulfate was placed to adsorb extra water moisture. After that, the extract was concentrated in a Rotary Vacuum Evaporator (Buchi) until dryness and then 1 ml of acetone was added. After that, the extract was transferred quantitatively to a sample vial. Finally, a 2 µl aliquot of a sample extract was injected into a GC-MS system.

Method Validation

Linearity

A working standard of 100 ppm was obtained by dissolving an appropriate amount of standard in acetone. Then, calibration standards were prepared by diluting the working standard in acetone ranging from 5 – 50 ppm. A calibration curve was constructed using the ratio peak of pesticide standard against concentration of standard solutions.

Recovery Rate and Precision

The accuracy of the method was assessed by calculating the recovery rate of difenoconazole at three different fortification levels. The procedures were carried out four times for each blank sample for recovery study. For standard deviation calculation, injection to GC/MS was repeated four times for each selected water samples. Limit of detection (LOD) was calculated from standard deviation using the equation, LOD = 3 x standard deviation of the retention time ten blank samples spiked at a concentration level of five times the LOQ value.

RESULT AND DISCUSSION

For the study of difenoconazole in the water, samples were taken on 26/6/2006, 29/6/2006, and 6/7/2006. Meanwhile for the degradation study of difenoconazole, the sampling was carried out on 13/12/2006. The pH values of water obtained for each sampling location (irrigation, paddy field, Alor Mengkuang River (drainage) and Pendang River (river) are shown in Table 2. The results indicate that the water samples obtained from paddy field sites was quite acidic.

Table 2: pH values of water samples obtained from sampling sites

Type of water	Irrigation	Paddy field	Alor Mengkuang River	Pendang River	Dam
pH	6 – 8	6 – 8	6 – 8	6 – 8	6

Difenoconazole concentration in water

When the first sampling was carried out on 26 June 2006, this was the stage before application of difeneconazole in the paddy field. As shown in Table 3, there were difenoconazole residues detected in the paddy field and Alor Mengkuang River (as drainage) before the application of this pesticide. The presence might have originated from its application during previous season. Nevertheless, difenoconazole residues were not found in the irrigation and Pendang River.

The second sampling was on 29 June 2006, after one day application of difenoconazole. The concentration of difenoconazole in the irrigation water was 2.2164 ppm and after one week, it decreased to 1.7133 ppm. This indicates that difenoconazole residues could be detected in irrigation water because of the water used to supply into the paddy field was recycled back to the irrigation from the river by using pump. The recycling irrigation system was originally designed to save water. Meanwhile, the rainfall also considered as the reason why the residues can be detected in the irrigation.

The rainfall in June as shown in Table 4 was 401.16 mm. High rainfall after application of pesticides would increase the runoff rate systems and therefore it recycled back to the irrigation channel. According to MADA, the MADA surface water had been classified as a relatively fresh, slightly acidic and mesotrophic water source which was free from any serious toxic ion contamination [14].

Table 3: Mean concentration of difenoconazole residues in water on 26/6/2006

Sampling Location	Concentration (ppm) 26/6/2006	Concentration (ppm) 29/6/2006	Concentration (ppm) 6/7/2006
Irrigation	N. D	2.2164 $\pm$ 1.5672	1.7133 $\pm$ 1.2115
Paddy field	0.0118 $\pm$ 0.0083	8.7834 $\pm$ 6.2108	0.0082 $\pm$ 0.0058
Alor Mengkuang River	0.0068 $\pm$ 0.0048	0.9583 $\pm$ 0.6776	0.3007 $\pm$ 0.2127
Pendang River	N. D	0.1702 $\pm$ 0.1056	0.0082 $\pm$ 0.0058

Note: N. D – note detected

Table 4: The rainfall data in June, July and December 2006

Month	Amount of rainfall (mm)
June	401.16
July	132.5
December	23.83

As for paddy field water, from a pre-application level of 0.0118 ppm, the difenoconazole residue increased to 8.7834 ppm after application. After one week it decreased rapidly to 0.0082 ppm. After one day application, high concentration of difenoconazole was detected compared to one week later. This might be due to water flow to the drainage was quite fast. The results obtained from Alor Mengkuang River showed similar pattern as paddy field water.

The concentration of difenoconazole was increased from 0.0068 ppm before application to 0.9583 ppm after application. One week after that, it decreased to 0.3007 ppm. After application, about 0.1702 ppm of difenoconazole was present in Pendang River. About 95.2% from this value decline rapidly within one week. Therefore, an action to stop water flow of paddy field just after application of pesticides is a significant way to reduce the loading to aquatic systems and human health. The acceptable daily intake (ADI) for difenoconazole is about 0.01 ppm [15]. Furthermore, it is recommended that for at least 10 days after pesticide application, draining water from paddy field should be restricted.

Degradation of difenoconazole in water

The study of degradation of difenoconazole was begun in December 2006. At that time, the average rainfall at this area was 23.83 mm. Meanwhile, the temperature was 32.7°C and the air moisture was 5% only [16]. This condition is good for degradation study since the rainfall would not affected the water level that causes water flow into the river and also hot weather which affected the chemical degradation.

In this work, the degradation of difenoconazole was monitored within nine days after application (DAA). Before application there was no detection of difenoconazole in the water. After one day application (1 DAA), the highest mean concentration of difenoconazole detected in the paddy water was 235.16 ppm as shown in Table 5. The underlying cause is low rainfall occurred in December as shown in Table 4. On the second day after application (2 DAA), about 47.62% of difenoconazole degraded and the concentration of difenoconazole decreased to 33.6750 ppm at 3 DAA. After one week, about 96.42% of difenoconazole in water degraded and decreased rapidly to 3.1451 ppm at 9 DAA.

Table 5: Mean concentration of difenoconazole residues in water on 13/12/2006

DAA	-1	1	2	3	7	8	9
Concentration (ppm)	N. D	235.1594 ± 191.0901	123.1856 ± 17.4927	33.6750 ± 0.8908	8.4089 ± 0.4045	5.3399 ± 0.0250	3.1451 ± 0.0821

Note: DAA- days after application

Recovery The recovery experiments were done on four blank samples which were spiked with 400 ppm, 8 ppm and 0.5 ppm standard solutions of difenoconazole. The recovery for difenoconazole is within the acceptable range (93% - 106%).

CONCLUSION

Difenoconazole was present approximately in all water samples after its application in paddy field. The presence of difenoconazole did not persist in a length of time that maybe harmful to the environment. The farm paddies have no effective drainage control measures. This was reflected in the presence of a considerable amount of difenoconazole in the drainage channel, even though it would disappear rapidly after several days. Water management system should control the pesticide movement into the larger surface water bodies. The recommended holding period of ten days after application was not strictly maintain. Therefore, the difenoconazole ran off and/or overflowed through the drainage water and flow to the river. The presence of residues in drainage water indicates the potential of paddy water runoff to affect the surface water quality and the aquatic environment.

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