Method Validation and Investigation of Paclobutrazol in Soil Using SPME-GC-MS Technique

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ABSTRACT

Solid-phase microextraction (SPME) is a solvent-free sample preparation technique. 85 µm polyacrylate fiber was used to extract the analytes directly from aqueous samples, associating with the conventional extraction, and then thermal desorption was carried out in the hot injector of GC. The application of them to soil sample in paclobutrazol residue analysis was validated. This technique was coupled mainly with gas chromatography and mass spectrometry (GC-MS). The preparative soil procedures for extraction of paclobutrazol, extraction time and analysis were obtained. 67% of the recovery from spiked soil samples was an average. Limit of detection was found at the concentration of 0.01 mg/kg sample, an appropriate extraction time was at 30 min. However, the simple dilution of the extraction to avoid negative matrix effects is more important. This technique was applied to determine paclobutrazol residues in soil corresponding with soil depth at mango orchard which was treated by soil drench method. The results showed high volume of paclobutrazol residue in upper soil layer (0-5 cm) after application, while low quantity was obtained in lower soil layer (10-20 cm). Moreover, the persistence of paclobutrazol residue was evaluated to be about 3-5 months.

Key words: Paclobutrazol, Soil, GC-MS, SPME-GC-MS, Residue, Method Validation

INTRODUCTION

The residue of paclobutrazol depends on the methods of application, doses and crop species. It was found to persist 2-5 years in apple and 1-3 years in peach (Singh and Ram, 2000). Soil application of paclobutrazol has been found to be more effective as regard to suppressing the vegetative growth and enhancing the reproductive growth in mango than foliar application (Burondkar and Gunjate, 1991; Tongumpai et al., 1996; Singh, 2002). It may remain active for many years

and can severely affect the growth and development of subsequent crops (Jackson et al., 1996; Silva et al., 2003). Persistence of paclobutrazol in soil further com-plicates agricultural environment by affecting non-target succeeding crops (Bhattacherjee and Singh, 2002). Paclobutrazol residue in soil may result in contamination of nearby water bodies which in turn may also be a hazard to human and animal health and may influence the soil microbial activity (Sharma and Awasthi, 2005). Soil microbial count of a mango orchard soil where paclobutrazol was frequently applied was shown to be reduced by up to 58% and it may be recommended for use in short-rotation cropping systems (Jackson et al., 1996; Silva et al., 2003). Bhattacherjee and Singh (2002) used gas chromatographic technique for the estimation of paclobutrazol residues in soil. Methanol was used for extraction of paclobutrazol from soil samples. Electron capture detector (ECD) was chosen for gas chromatographic analysis. Singh and Bhattacherjee (2005) studied the chemical residue in soil for analyzing the persistence of paclobutrazol by GLC method. Methanol was used to extract paclobutrazol residues in soil samples. Bolygo and Atreya (1991) developed a multi-residue analytical method for the analysis of paclobutrazol in groundwater by GLC, using nitrogen–phosphorus detector (NPD). HPLC with UV detector was used to analyze paclobutrazol in soil (Subhadrabandhu et al., 1999). In this work, we aim at finding out to develop and validate method, proposed for analysis of paclobutrazol residues in soil using a sample preparation step by conventional extraction coupling with SPME extraction and analysis by GC-MS.

MATERIALS AND METHODS

1. GC-MS and SPME condition

1.1 Gas chromatographic (GC) and mass spectrometry (MS) detection condition *(Alteration from Crook, 1999)*

The GC-MS analysis was performed by a Varian model Star 3400 gas chromatograph equipped with Electronic Flow Control (EFC) and fitted with a Saturn II ion trap mass spectrometer (Varian Instruments, Walnut Creek, CA, USA). The GC chromatographic column consisted of a BPX5 capillary column (SGE GmbH, Darmstadt, Germany), length 30 m, internal diameter (I.D.) 0.32 mm and containing 5% phenyl-polysilphenylen-siloxane with a phase thickness of 0.5 μm connected to the splitless injector (The I.D. and thickness were changed from the previous test (I.D.=0.25 mm, thickness = 0.25 μm). The carrier gas was helium (99.999%).

The oven temperature program of GC was to hold the temperature initially at 60°C for 6 min to a final temperature of 280°C at a rate of 20°C per minute and then held at this temperature (280°C) for 8 min. A column head pressure of 11 p.s.i. and an injector temperature of 300°C were used. The injector was operated by manual holder into splitless mode (SPI/1077) for 6 min, the lapse of time for SPME fiber desorption was set at a fixed constant temperature of 300°C. The GC transfer line was maintained at continual 300°C. The mass spectrometer was operated in the electron-impact ionization (EI) scan mode with a source temperature of 300°C.

Ionization mode was obtained at fixed mode. The electron energy was 70 eV and the filament current 10 μA. The manifold temperature was set at 180°C. The electron multiplier voltage was established at 1800 volts. The amplitude voltage (A/M) was 4.0 volts. The external event 1 was turned on.

Chromatograms were acquired in 'scan' mode, scanning the mass range from *m/z* 50 to *m/z* 300 (with scan rate 1000 milliseconds), with a background mass of m/z 45 segments acquire time 25 min. In order to improve the peak identification, three fragment ions were monitored from the spectrum of each compound to quantify the response in the selected-ion monitoring (SIM) mode. The mass spectrum of *m/z* 125, 236 and 294 for paclobutrazol (Retention time 17.038 ± 0.2 min) and *m/z* 159, 270 and 272 for Diclobutrazol (Internal standard, Retention time 17.518 ± 0.2 min) were ion monitored as references. In this way, it could be easily identified.

1.2 Solid-phase microextraction (SPME) analytical procedure

The method was partly adapted from Crook, 1999. The SPME holder and fiber assembly for manual sampling were provided by Supelco (Bellefonte, PA, USA) and used without modification. The silica fiber was coated with an 85 μm thick polyacrylate (PA) film. Prior to the measurements, the fiber was preconditioned in the injector for 3 hours at 300°C, with the split vent opened, to fully remove any contaminant or impurities present in the coating or introduced during the manufacture of the fiber which might have caused very high baseline noise and injected into the GC system until interfering peaks disappeared. This was performed according to instruction provided by the supplier. All SPME was made directly from an aqueous sample. The vials were filled with 1.2 mL aqueous sample or standard solution with the addition of 0.4 g sodium chloride (NaCl, Merck). The vials were then sealed with the hole caps and Teflon-faced silicone septa (Supelco, USA). The samples were stirred for dissolving the salt for 15 min. Then, the vials were placed in water bath with circulating water associated with magnetic stirrer (IKA-Combimag RCO) that controls the temperature at 35°C, stirred again to adjust the temperature of aqueous sample to 35°C at 15 min. The speed of stirring was set at level 3.5. Next, the fiber was then exposed to aqueous phase for an appropriate time period of 60 min with stirred sample at the control temperature 35°C (in order to improve mass transfer from the aqueous sample onto the fiber coating). After extraction, the fiber was removed from the sample and directly introduced into the hot injector of the GC system for analysis (desorption) where the thermal desorption of the analysis at 280°C for 6 min was carried out. After the desorption, the fibers were reused but they should be inserted into the split port for 10 min at 300°C and turned on the helium (He) gas flowing at 11 p.s.i. for cleaning proposes. Then, the next sample could be continually operated.

2. Methods

2.1 Preparation of standard solutions

To prepare individual stock standard solutions (paclobutrazol, PBZ and diclobutrazol, DBZ) at the concentration of approximately 2.8 mg/100 g, all standards were prepared in acetonitrile (99.8%) with distilled water (final ratio 3.5%:

96.5% (w/w)). Into a 100 mL volumetric flask, approximately 2.8 mg (± 0.00001) of PBZ or DBZ standard was weighed and dissolved with 3.5 (± 0.00001) g of acetonitrile. Distilled water was then added to the flask but not reaching the mark. Then, the flask was stored at 20°C for about 20 min and readjusted the volume to 100 mL by distilled water with pasture pipette and thoroughly shaken. A stock solution was used by spiking (paclobutrazol) and internal standard (diclobutrazol). The concentration of PBZ or DBZ solution should be maintained at 0.7 mg/kg sample after spiking into the samples.

2.2 Preparation of spiking soil sample

The soil samples were collected at three depth levels from University of Hohenheim and Mae Jo University field for spiking and analytic experiment. Soils were frozen (- 20°C), cut into 3 segments according to the corresponding depth (0-5, 5-10 and 10-20 cm). Samples were dried at 105°C for 1 hour and cooled down. Stones were removed 2 times, 10 min at a time by sieving machine (5 sieves mesh size: 3.15mm- 1mm- 0.5mm- 0.315mm- 0.25mm, Retsch, Haan, Germany) at 70% of speeds. Then, soils were weighed exactly 100 g (± 0.001) into a glass bottle, the paclobutrazol standard 0.7 mg/kg sample and distilled water with a ratio of 1:1 (w/w, water: dry soil) were added. The mixer was continuously shaken for 24 hours. Next, these were taken arid again at 100°C for 3 hours and milled by Mixer-Grinder (diameter of metal beads 3 centimetres, 5 beads Retsch, Haan, Germany) for 5 min at 60% of speeds. The fine soil samples were kept in the pouches for sterilization and spiking analysis as shown in Figure 1.

2.3 The extraction procedure of paclobutrazol from soil sample

A soil sample after milling of 100 g was exactly weighed (± 0.001) into a 500 mL of glass bottle, the internal standard (diclobutrazol [DBZ] 0.7 mg/kg sample) and magnetic stir bar were added and homogenized for 5 min. The distilled water was added in the ratio of 1:1 (w/w) and homogenized as well by stirring for 5 min. Acetonitrile and water (Crook, 1999) in ratio of 70.0%:30.0% (w/w) were added and the mixture was homogenized for 30 min, using magnetic stirrer at high speed. The 20 g of Celite 503 was added to trap mango pulp during the filtration and stirred for 2 min. The extraction bottle was weighed and tared. The mixture was filtered through a 110 mm Büchner funnel with paper filter (No.589/3, Blue ribbon, Microscience GmbH, Dassel, Germany) associated with vacuum to the flask, and the mixture used was weighed for calculation. The bottle was washed 4 times with 5 g solution (ACN/H_2O, 70.0%:30.0%, w/w), rinsing of filter cake 2 times with 5 g solution (ACN/H_2O, 70.0%:30.0%, w/w) and weighed again. The mixture solution was evaporated under the vacuum (30°C, <150 millibars) but not until dryness, only until ACN has been removed (66.5% weight loss). The addition of acetonitrile was used to adjust the ACN/H_2O ratio (3.5%:96.5%, w/w). Finally, the supernatant liquid 1 mL was collected and diluted to 10 mL (ACN/H_20, 3.5%:96.5%, w/w) in volumetric flask with additional weight control. It was filtered through a 70 mm glass funnel with paper filter (No.595½, Microscience GmbH, Dassel, Germany) to glass bottle. Every step just referred to the MS-excel® sheet form for the calculation (FB). The scheme of the extraction is shown in Figure 2.

Table 1. Rocovery of paclobutrazol from fortified processes in different soil depths.

Soil depth (cm)	Dilute Ratio	Mean Recovery (%)	S.D. Recovery	PBZ (mg/kg soil)		
				Expected	Mean detected	CV (%)
0-5	1:10	64.56	7.05	0.7020	0.4532	3.94
5-10	1:10	77.75	3.07	0.7054	0.5485	8.18
10-20	1:10	50.78	4.15	0.7014	0.3562	10.92

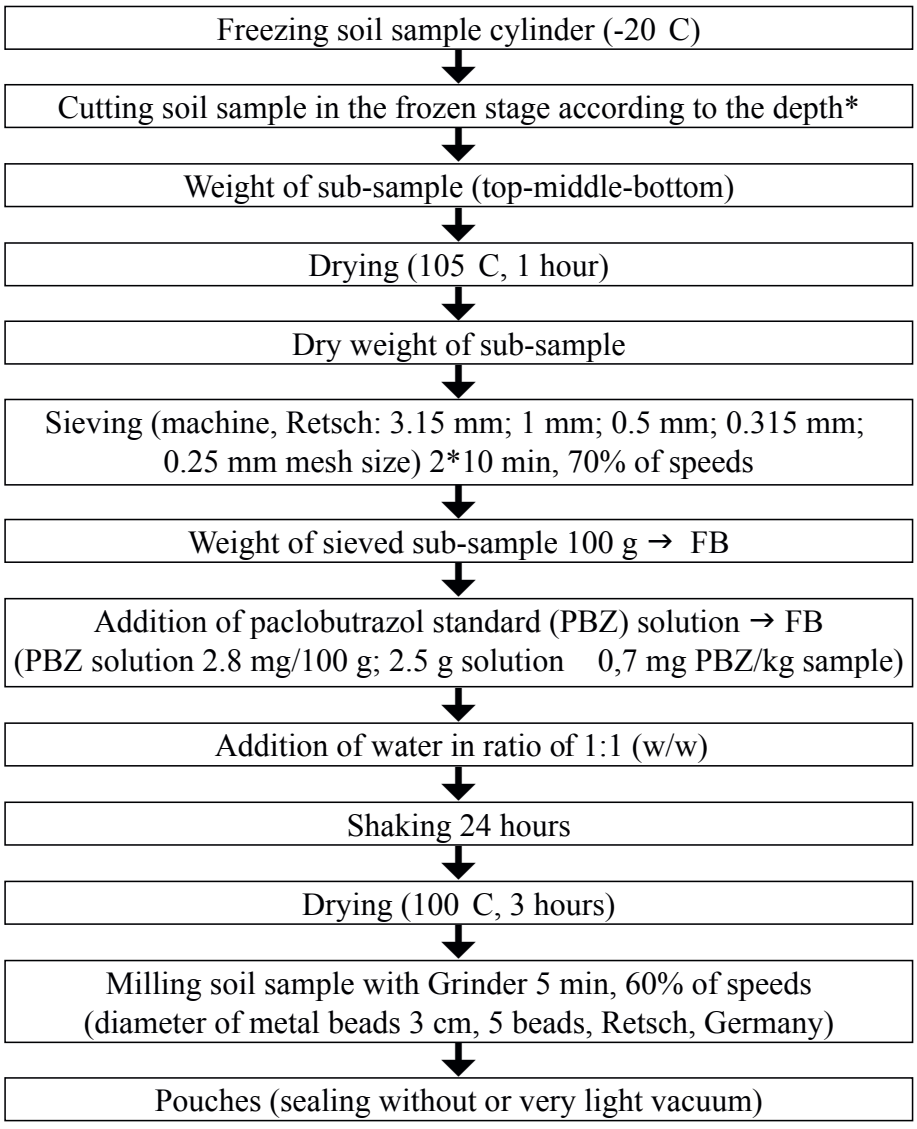

Figure 1. Scheme of spiking paclobutrazol for recovery and detection limit analysis.

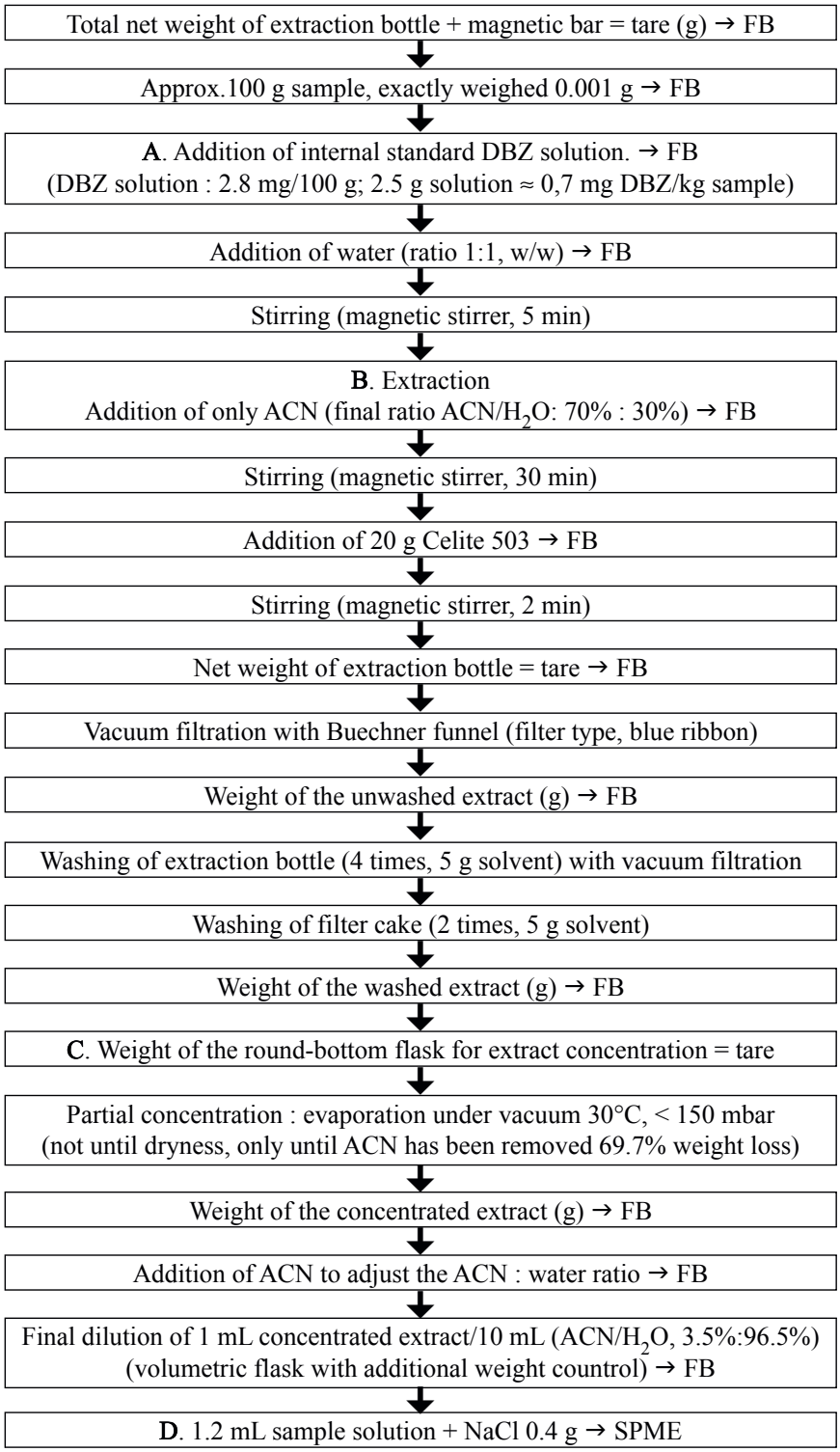

Figure 2. Scheme of soil sample extraction procedure. A. addition of internal standard, B. sample extraction, c. concentration step, D. SPME extraction.

Table 2. Limit of detection and recovery in different concentrations of paclobutrazol in pooled soil samples (Mean data, n=4; ND=not detected).

Spiked (mg/kg)	Dilute Ratio	PBZ (mg/kg soil)				Mean Recovery (%)
		Expected Results	Mean Detected	S.D. Detected	CV (%)	
0.5	1:10	0.4909	0.3446	0.027	7.77	70.19
0.1	1:10	0.1025	0.0852	0.010	11.37	83.17
0.05	1:10	0.0524	0.0357	0.006	16.11	69.76
0.01	1:10	0.0137	0.0076	0.000	5.69	55.80
0.005	1:10	0.0052	ND	ND	ND	ND

3. Validations

3.1 Stability of paclobutrazol in soil under the sterile condition

The stability of paclobutrazol under sterilization condition, 121°C at 20 min by Autoclave (Type FVS3, Fedegari, Italian) was studied at the Institute of Microbiology (150a), University of Hohenheim, by weighing 100 g (± 0.001) of soil samples which were prepared as previously described (Figure 1). Then it was followed as the same as the scheme of soil extraction procedure (Figure 2). The data were compared with the control (not sterilized).

3.2 Optimizations of paclobutrazol extract time in extraction procedure

A 100 g of each sample were taken (previously described, PBZ 0.7 mg/kg sample added) and then the internal standard (diclobutrazol [DBZ] 0.7 mg/kg sample) was added and homogenized. Samples were extracted by acetonitrile (ACN) and water (70.0%:30.0%, w/w). The homogenization was done by stirring for various times of 10, 30 and 60 min with the addition of Celite 503 and filtrating. The mixture solution was followed as shown in Figure 2.

3.3 Recovery study of paclobutrazol in the soil

The soil samples were collected at three depth levels from Mae Jo University field for spiking and recovery study of paclobutrazol in soil. Soils were prepared as shown in Figure 1. Then the extraction was followed according to Figure 2.

3.4 Detection limit and recovery study of paclobutrazol in the soil

The pooled soil samples were used for this experiment. Samples were prepared as described in Figure 1; the only difference was the concentration of paclobutrazol which was added at 0.005, 0.01, 0.05, 0.1 and 0.5 mg/kg. The extraction of paclobutrazol was processed as in Figure 2. The samples were then analyzed, using GC-MS in the electron-impact ionization (EI) mode.

4. Analysis of paclobutrazol on-site evaluation at mango orchard

The application of paclobutrazol use was soil drench technique. The plot at Mae Jo University orchard was divided into two groups (blue and yellow) and 5 trees were studied in each group. The blue-labeled trees were treated in April when it was hot and dry; the yellow ones were done 4 months later in July at the beginning of the rainy season. Two litres of paclobutrazol solution, concentration calculated

depending on canopy diameter but in this case, was treated with paclobutrazol at 1.0 g active ingredient per canopy diameter. It was equally spread within the application ring around each tree at a distance of 20 cm from the trunk. The ring was divided into 12 equal segments for each tree. The application of the volume per segment was 2/12 litres. The drilling machine was used to collect soil samples. The cylinders of soil samples were frozen. It was cut into 3 segments according to the corresponding depth of the soil layer in the field. Stones of the dried soil were removed by sieving at a mesh size of 3.35 mm. For the transportation to University of Hohenheim, the sieved soil was sterilised at 121°C for 20 min. Within each group (blue and yellow), samples were pooled for each soil layer and sampling date from the soil of 2 or 3 trees by grinding the dry soil in a ball mill for 5 min. The pooled samples were extracted and analysed as the same process used with GC-MS (Figure 2).

RESULTS AND DISCUSSION

1. Stability of paclobutrazol under the sterilization condition

Stability of paclobutrazol under the sterilized condition of 121°C for 20 min was observed. The treatments in comparison to control sample (not sterilized) were not significantly different in term of volume of the concentrations and recovery percentage (Figure 3). The recovery of paclobutrazol in S2 treatment was below the theoretical line but the mean of total was raised. In other words, paclobutrazol was highly stable, although it was subjected to high pressure and temperature at the same time. Furthermore, it was confirmed that PBZ was very strongly persistent in the environment. Thus, the preparation of soil for transportation had to meet the restrictions of European's laws. Soil had to be eliminated for contamination of microorganism in the sample before shipment. Consistently, Bromilow et al., (1999) reported that several triazole fungicides are very persistent, though no deleterious effects on soil microbial processes have been reported. Triadimenol, flutriafol and epoxiconazole were all very persistent with DT_{50}>400 days whilst propiconazole had DT_{50} *c* = 200 days.

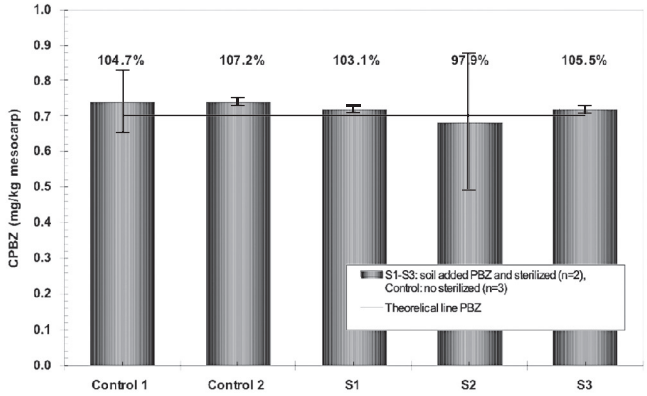

Figure 3. Stability of PBZ in pooled soil samples under sterilized condition.

2. Optimization of paclobutrazol extract time in soil extraction procedure

The various extract times of 10, 30 and 60 min were evaluated in soil extraction procedure. The recovery percentage of paclobutrazol at 10 and 60 min were below the theoretical concentration line of paclobutrazol, they were: 72.9% and 71.5%, respectively. However, 30 min extract time in which the recovery was 100.9% was better than the others as shown in Figure 4. It may be due to numerous organic matters of soil components which were bound to paclobutrazol, rather moderately to strongly, were sorbed to soil (Bromilow et al., 1999). The extractable fraction may also be sorbed to the soil solid phases (Gevao et al., 2000) which increased time or intensity of extraction.

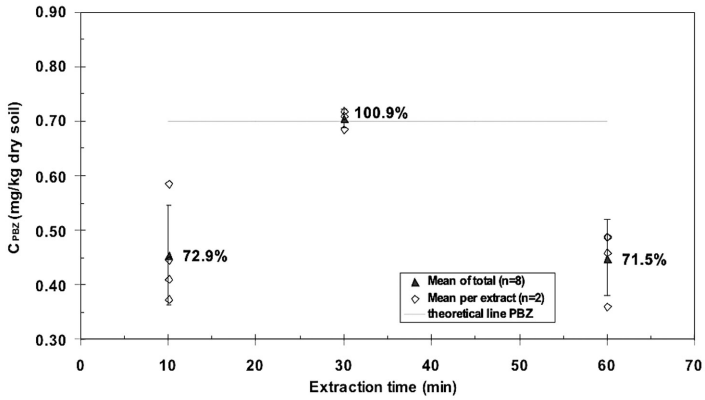

Figure 4. Recovery percentage of optimizing extract time in soil extraction procedure.

3. Recovery of paclobutrazol in the soil

The results of the recovery studies are given in Table 1. The percent mean recovery was below 78.0% from all depth levels. The recoveries from each level at the top (0-5 cm), middle (5-10 cm) and bottom (10-20 cm) layers were observed to be 64.56%, 77.75% and 50.78%, respectively. However, the standard deviation (S.D.) and coefficient of variant (CV) in all cases were achieved in range of 3-11%. When the recovery in Tables 1 and 2 was inclusively calculated, the mean was about 67.0%. It was in medium level and thus could be accepted. On the other hand, Sharma and Awasthi (2005) reported that the percent recovery of paclobutrazol residues was 83±3.4% for soil samples with the syringe injection and gas liquid chromatography. In addition, Bhattacherjee and Singh (2002) reported that a simple gas chromatographic method for the estimation of paclobutrazol residues in soil was standardized, using electron capture detector and megabore column. No paclobutrazol-TMSi (trimethylsilyl ether) was required for GLC-ECD determination of paclobutrazol. Soils were simply extracted in methanol, filtered twice, methanol was evaporated completely and residues were redissolved in 1 mL before analysis. An average recovery of 86.82% of paclobutrazol was obtained from soils fortified with 0.1, 1.0 and 2.0 ppm solutions with a minimum detection limit 0.0001 ppm. Also

Shalini and Sharma (2006) found that the percent mean recovery of paclobutrazol residues from soil at fortification levels of 0.01 and 0.1µg/g was 85.4% and 89.2%. Beltran et al., (2000) mentioned that the organic matter in the soil sample greatly influenced the recovery of compounds from the soil. The resultant paclobutrazol recovery in different soil depths were not excellent, it may be influenced by various factors such as degree of its adsorption on soil particles, rate of its release from soil particles to soil solution (Jacyna and Dodds, 1995), including the reason of bound chemical residues in soils as previously described. Moreover, the quantitative application of SPME to soil samples does not allow the direct use of external standard calibration curves, being necessary to use internal standard quantitation or the standard addition procedure (Beltran et al., 2000).

4. Detection limit of paclobutrazol in the soil

The limit of detection was obtained at 0.01 mg/kg sample, although the recovery was beneath 55.80%. The peak of paclobutrazol at the concentration of 0.005 mg/kg was not found in the present case. The detectable means of paclobutrazol were also lower than the expectation. The standard deviation (S.D.) and coefficient of variant (CV) were satisfactory. All of mean recovery values were at an acceptable level at percentage coefficient of variant (CV) values of ≤ 20%. In the experiment at the concentration of 0.05 mg/kg, it gave approximately 16%. The quantitative analysis of the linearity of response of the concentration is displayed in Figure 5. Linearity plots obtained indicated a high correlation coefficient values ($R^2 > 0.99$) of the linear regression analysis, showing for fitting the paclobutrazol concentration rate in spiking soil. However, if the supernatant was diluted in grater dilutions such as 1:100, 1:1000 or 1:5000, etc., it could probably help a higher recovery (Yang et al., 2006). Moreover, to reduce organic solvent content when a previous solvent extraction is required, it is usually achieved by diluting sample extracts prior to SPME application (Beltran et al., 2000). In addition, Simpl?cio and Boas (1999) showed that the pesticide recoveries could be much improved by diluting the samples up to a 100-fold dilution in the determination of organophosphorus pesticides in pear fruit and juice. Nevertheless, in this case it was not necessary to know the limit detection at lowest concentration of paclobutrazol in soil as the same to mango pulp.

According to the work of Prosen and Zupancic-Kralj (1998), it indicated that for isolation of residue pesticides and their degradation products, solid-phase microextraction (SPME) could be used in combination with conventional extraction method. This modern separation method was optimized for extraction of organochlorine and triazine pesticides from soil samples. Analytes were desorbed from the fiber in the injector of gas chromatograph and determined by either electron capture or mass spectrometric detection. Linearity and limit of detection were tested in the 0.1 – 20.0 ng/g range for organochlorines and 10 – 100 ng/g range for triazines. The method presented could be used for screening of pesticides in contaminated soil samples and offers a simple alternative to established methods of pesticide analysis in soil. Hence, it could be used in similar work and modified for other pesticide analysis in the future.

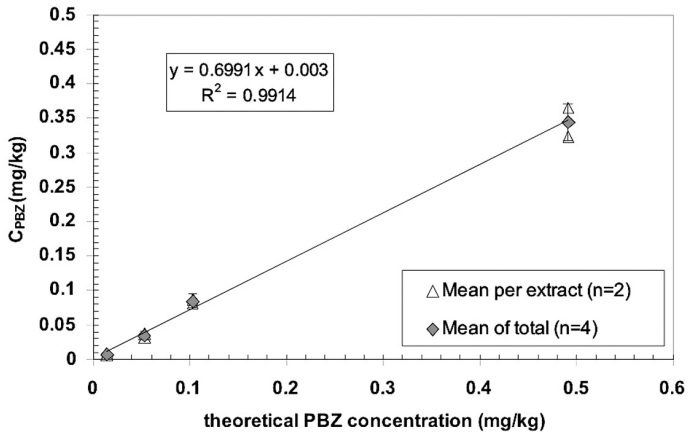

Figure 5. Linearity plot by means of the paclobutrazol standard addition method.

5. Analysis of PBZ in soil for mango orchard

The results obtained showed that the residues of paclobutrazol in top layer (0-5 cm) more present in high amount after application in both groups. The middle layer (5-10 cm) and bottom layer (10-20 cm) also decreased in both cases. Then they decreased gradually with time for both groups and in each layer. The paclobutrazol at an initial study was observed from 0.013- 0.380 mg/kg of dry soil. Paclobutrazol in only the top layer decreased after first application from 1,030–1300 mg/kg of dry soil to 18–25 mg/kg of dry soil. This fall was caused by leaching to lower layers with water. In the bottom layer in both groups, the paclobutrazol content remained nearly constant at 10 – 40 mg/kg of dry soil. As shown in Figure 6, the result showed the dislocation of paclobutrazol for blue group from top to bottom layer (Figure 6(A)), also the same in yellow group (Figure 6(B)). All of data obtained gave the coefficient of variance (CV) below 18%, also with the lowest of standard deviation (S.D.). In addition, the persistence of paclobutrazol residue in soil for first application was evaluated to be about 90-150 days (~3-5 months).

The paclobutrazol residue in soil was observed after the first application. The persistence was also still detected in soil at 10-50 mg/kg of dry soil. In comparison to Sharma and Awasthi (2005), it has been reported that paclobutrazol was applied at the soil at tree basins in the month of September every year. The persistence of paclobutrazol residues in the soil was found to be a little over 8 months after application. It was observed that paclobutrazol residues accumulated in the surface soil (0-15 cm) at the tree basins and that the soil residues at season-1 were 0.065 and 0.302 µg/g from the lower (5 g a.i.) and higher (10 g a.i.) treatments, respectively. At the end of each season, there was a small increase in the amount of residues corresponding to the number of a yearly application. Similarly, Mcarthur and Eaton (1989) found that paclobutrazol could still be detected in the soil 50 weeks after application at cherry orchard. Moreover, the persistence of paclobutrazol residues at different soil depths which was treated at 5 and 10 g active ingredient per tree was found but only in laboratory condition. The present study was therefore carried out

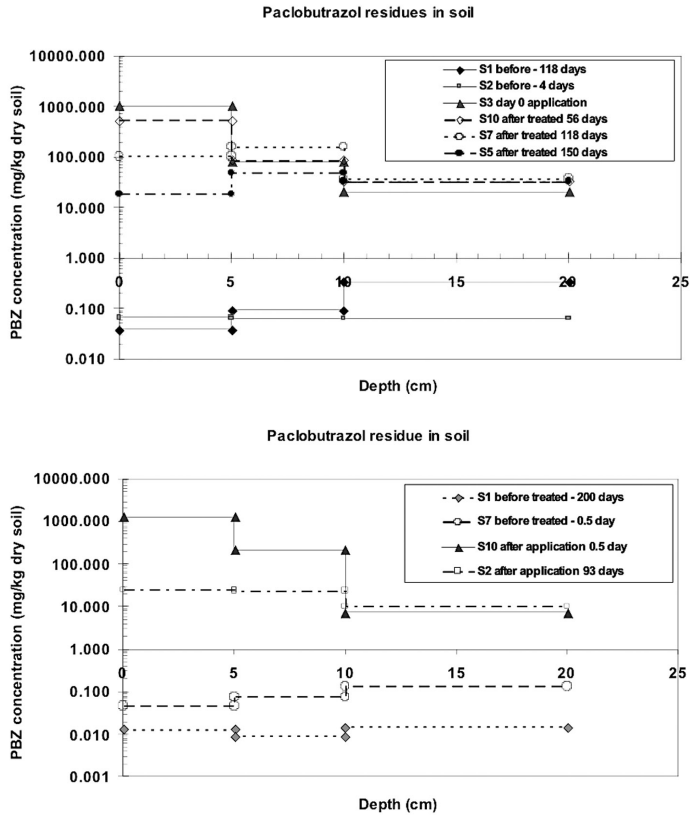

Figure 6. The dislocation of paclobutrazol within the soil layer in Blue (*A*) and Yellow (*B*) group (adapted from Reintjes, 2005; Niedhart et al., 2006).

to determine the persistence and mobility of paclobutrazol residues in soil following its soil application in mango orchard from the point of environment significance of such residues. The residues of paclobutrazol in surface soil (0-15 cm) decreased gradually with time from 1.77 and 4.87 µg/g at 0 day after treatment to 0.37 and 2.19 µg/g at 60 days and just above the detectable level (0.01 µg/g at 210 days at both the treatment concentrations (Shalini and Sharma, 2006). Subhadrabandhu et al., (1999) also studied the residues of paclobutrazol and reported to remain upto 11 months. In addition, a rate of 2 g paclobutrazol per tree effectively controlled vegetative growth for 3 years in apricot. Bioassay with broad bean (*Vicia faba* L.) confirmed that paclobutrazol was still present in the treated soil for almost 3 years after application (Jacyna and Dodds, 1995).

The previous documents showed that the trunk-drench application contributed to deeper penetration of paclobutrazol into the soil than other applications, such as foliar spray or trunk injection. On the other hand, the movement of paclobutrazol from upper to lower soil layer occurred by leaching due to regular rainfalls during the beginning of rainy season, as revealed by the comparison of both groups and the climate data obtained for this period at Mae Jo University field.

The irrigation was also another reason. Accumulation of high paclobutrazol levels in the top soil may be also ascribed to the limited water solubility of paclobutrazol (26-35 mg/L). The persistence of paclobutrazol in the soil may result in the contamination of nearby water bodies, thus presenting a possible hazard to human and animal health, and could also influence soil microbial activity with further effects on biodiversity. When paclobutrazol was applied to soil drench several times, an accumulation of residue could be increased.

CONCLUSION

In conclusion, this method is veritably suitable for residue analysis of paclobutrazol in soils. However, the dilution is more important or necessary for soil analysis which the technique of SPME has been applied to analyze soil extracts.

Even the results are based on a one-season experiment, it can be seen from the current trial that there was still paclobutrazol residues in soil. The persistency of paclobutrazol was 3-5 months.

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