Chapter 8

Paclobutrazol Residues in Mango Fruits 'Chok Anan' cultivar

8.1 Abstract

A block of four or five-year-old 'Chok Anan' mango trees was treated with a single soil application of paclobutrazol by soil drench technique at the rate of 0.5 grams active ingredient per square meter. The mango fruits were collected and PBZ was extracted by conventional method associating with SPME technique, and analyzed by GC-MS. It was found that paclobutrazol residues in the mango fruit was less than the detection limit (0.005 milligrams per kilogram) and less than the internationally accepted amount (0.05 milligrams per kilogram in stone fruits) and also lower than the accepted values of New Zealand and Japan at 0.01 mg/kg.

8.2 Introduction

Mango (*Mangifera indica* L.) is one of the important economic fruits of Thailand. The big markets are Singapore, Malaysia, Hong Kong and some European countries. Presently, we can conclude that the use of paclobutrazol is a popular method for growers in Thailand to produce off-season mango (Nartvaranant *et al*., 2000). When it is applied as soil drench it is more effective than foliar spray (Tongumpai *et al.,* 1997). The paclobutrazol was taken up through the root and was transported primarily in xylem through stem and accumulated in the leave and fruit (Wang *et al.*, 1986). By analogy, studies on paclobutrazol mobility in castor oil plant (*Ricinus communis*) and *Pistachia chinesnsis* (Anacardiaceae) suggested that paclobutrazol is not exclusively xylem mobile and that phloem pathway exists as secondary transport mechanism for paclobutrazol (Witchard, 1997a,b). The residues level of paclobutrazol depends on the method of application, doses and crop species. As it persisted 2-5 years in apple, 1-3 years in peach (Singh and Ram, 2000), and 2 years in *Rhododenron* and *Kalmia* (Gent, 1997). The maximum residue limit (MRLs) of paclobutrazol accepted by Food and Agriculture Organization (FAO) in apple and stone fruits are 0.5 mg/kg (previously, 0.2 mg/kg) and 0.05 mg/kg (FAO, 2002), respectively. On the other hand, New Zealand Food and Safety Authority's regulation (2005, 2007) indicates MRL of paclobutrazol in avocados and stone fruit at 0.01 mg/kg and banana in Japan (FAO and WHO, 2006). Singh and Ram (2000) reported the paclobutrazol residues in mango mesocarp after soil application of paclobutrazol for off-season fruit production are not excepted. Consistently, semi-quantitative paper chromatography indicated paclobutrazol residues a below MRL with less than 0.004 mg/kg fruit weight. Moreover, Bicchi *et al*. (2001) detected triazol pesticide residues in apple and pear pulps by liquid chromatography with ultraviolet detection including the paclobutrazol residue to be same as 0.006 mg/kg in there. The objective of this study is to apply the valid method for analysis of paclobutrazol residue to mango cv. Chok Anan as it is one of the potential produced and exported fruits of Thai market.

8.3 Materials and Methods

GC-MS Conditions

The GC-MS conditions was followed in the chapter 5

SPME procedure

The SPME conditions and processes were followed in the chapter 5

The extraction procedure of paclobutrazol from mango samples

The extraction procedure of paclobutrazol residue was followed in the chapter 6

Paclobutrazol application and collection of mango samples

A field experiment was conducted at a mango orchard consisting of 4-5 year old mango trees (cultivar Chok Anan) located at Mae Jo University field, San Sai district, Chiang Mai province, Thailand. Paclobutrazol used was Paclobutrazol-10 (WP), China produced, imported by Inter Crop Ltd, and sold by WESCO Ltd, Bangkok which was available at Chiang Mai city market. Paclobutrazol was applied to the tree basin soil (under the canopy), called ‘soil drench technique’. Trees in 5 replicates (1 tree represented 1 replicate) were maintained for the treatments, namely with concentration 0.5 grams active ingredient per square meter. The addition of control was not treated with paclobutrazol. The trees were irrigated 3 times per week or more (35.87 L/tree/time) after application which depended on soil moisture until harvesting. However the irrigation was stopped only short time during flowering period.

The mature green mango fruits were collected and brought to laboratory at Faculty of Agriculture, Chiang Mai University. Peeled and chopped mango in cube,

samples were kept in the pouches and sealed with a 100 % vacuum. Samples were frozen in deep freezer (-80 ˚C) and sent by airplane for further analysis of paclobutrazol residues in University of Hohenheim, Stuttgart, Germany. Prior to the analysis, the samples were thrown at room temperature. The analysis was followed as the same as validate method.

8.4 Results

The analysis of paclobutrazol residues in mango fruits could not be continued because the GC-MS system lost its signal sensitivity and could not be repaired. In addition, it took such a long time to setup the new machine. For example, it would have required spending the time for rerunning tests such as limit of detection, recovery, etc. Therefore, the data shown are for a limited number of fruit lots that were able to be analyzed. Two lots were observed mainly control and treatment (PBZ) which were harvested on April, 28-29, 2005 (Table 8.1). The samples were extracted twice and diluted 1:10 and 1:5 prior to injection. All of data exhibited were below the detection limit of 0.005 milligrams per kilogram sample. The average of paclobutrazol residue was 0.00028 milligrams per kilogram. Hence, it was assumed that paclobutrazol is not transported into the mango fruits. The optimum dilution of samples solution prior to injection was important for giving good results. The coefficient variance (CV) and the standard deviation (S.D.) were achieved in range of 0.86 %-20.87 % and 0.06-1.62, respectively.

Table 8.1 Paclobutrazol residues in mango pulp harvested from Mae Jo University field

Sample	Reference	Analysis	Paclobutrazol (mg/kg mesocarp)				
			Dilution ratio	Mean detected	S.D.	CV (%)	n
Mango							
Chok Anan (Control)	**In this work**	SPME + GCMS	1:10	0.00131	0.06	0.86	2
			1:10 & 1:5	0.00060	0.98	13.24	2
Chok Anan (PBZ 0.5 g.*a.i.*/m^2)	**In this work**		1:5	0.00014	0.36	4.05	2
	Adapted from Reintjes (2005)		1:10 & 1:5	0.00043	1.62	20.87	2
Dashehari and Langra (PBZ 2.3 g *a.i.*/canopy)	Sing and Ram (2000)	Bioassay by corn root	-	< 0.004	-	-	-
Nam Dok Mai (PBZ 8 g/tree)	Subhadrabandhu *et al.* (1999)	HPLC + UV	-	Non detected	-	-	-
Alphonso (PBZ 5, 10 g *a.i.*/tree)	Sharma and Awasthi (2005)	GLC + ECD	-	Below permissible level and detection limit at 0.001	-	-	-
Tommy Atkins	Osuna-García *et al.* (2001)	-	-	Very low levels	-	-	-
Apples, Pears, Lemon and Tomato							
Apple (puree) lemon juice and tomato (puree)	Sannino *et al*. (2004)	LC-ESI- MS-MS	-	Non detected higher than 0.010	-	-	-
Apple and pear	Bicchi *et al*. (2001)	LC + UV	-	0.006	-	-	-
48 commercial pears	Sancho *et al*. (2003)	LC-ESI-MS-MS	-	Non detected in any of the commercial samples analyzed	-	-	-

8.5 Discussion

Paclobutrazol residue in mango fruits occurred under the acceptable daily intake (ADI) for paclobutrazol established by CODEX for apple which is 0.1 milligram per kilogram body weight and the maximum residue limits (MRL) is 0.05 milligrams per kilogram (FAO, 2002). In comparison with a study by Sing and Ram (2000), they found that 'Dashehari' and 'Langra' mango with an application of paclobutrazol at 2.3 grams active ingredient per meter canopy had only 0.004 milligrams per kilogram fruit weight residue in the fruit, which was much lower than the internationally accepted values (0.05 milligrams per kilogram fruit weight). In general, seeds contained higher level of paclobutrazol than the mesocarp. Sannino *et al*. (2004) determined that 24 new pesticide residues (i.e. paclobutrazol, etc) in apple puree, concentrated lemon juice and tomato puree. The extracts were analyzed by LC-ESI-MS-MS. None of samples contained residues higher than 0.010 milligrams per kilogram. Sharma and Awasthi (2005) studied the application of paclobutrazol at 5 and 10 grams active ingredient per tree for three consecutive years with mango cv. 'Alphonso'. Residues of paclobutrazol were found in unripe mango fruits at below permissible levels (also below detectable limit at 0.001 microgram per gram). Meanwhile, the same amount was generally not detected in fully mature mango fruits ready for harvest. Paclobutrazol residues were also detected in 'Tommy Atkins' mango at very low levels after two consecutive years of application (Osuna-García *et al*., 2001). Accordingly, Subhadrabandhu *et al*. (1999) used 8 grams per tree on 'Nam Dok Mai' mango and chemical residues were not detected in the mature fruit. They suggested that the dose of paclobutrazol that they applied was applicable for mango production in terms of food safety. Similarly, Sancho *et al*. (2003) reported that the validated method was applied to 48 pear samples: eight commercial samples taken from the market and 40 samples from field residue trials. Paclobutrazol was not detected in any of the commercial samples analyzed, while treated samples presented different concentration levels according to the collection date after field application. Bicchi *et al*. (2001) detected triazole pesticide residues in apple and pear pulps by liquid chromatography with ultraviolet detection including paclobutrazol residue at a comparable concentration of 0.006 mg/kg. Chuanfan and Jiuxue (1998) found that

paclobutrazol residue can be extracted by 80 % methanol-water, and then transferred to methylene chloride. After the evaporation of methylene chloride, methanol is added and cleaned up by a florisil-active carbon column. Paclobutrazol is then detected by HPLC-UV. After the application of 15 grams paclobutrazol (10 % WP) the residue in matured mango was not detectable.

According to previously documented evidence, residues of paclobutrazol remained in mango much less than MRL, and were not detectable in the fruits. Thus, when compared to this experiment, the data observed was also at the lowest level. It could be assumed that the residues of paclobutrazol would not be detected or only discovered in very small amounts below the internationally accepted values of 0.05 milligrams per kilogram (FAO, 2002) or 0.01 milligram per kilogram in the EU for pesticide residues in baby food (Commission Directive 1999/39/CE). Moreover, there was concurrence with the translocation of paclobutrazol from root uptake, which was assumed to occur primary though xylem and was not via phloem (Wang *et al*., 1986; Quinlan and Richardson, 1986; Hamid and Williams, 1997). In addition, the rates of paclobutrazol used in the current experiment were lower than the rate used by Subhadrabandhu *et al*. (1999).

According to Osuna-Garcia *et al*. (2001), the United States has established a set of restrictions to use this substance due to its high persistence and it has been identified as a possible cause of irrigation water pollution, which could also damage fruits. These facts may reduce export of mango to the United States, which produces an income of 40 million dollars per year for Mexico. In addition, Thailand is also one of mango cultivating countries and nowadays, the mangoes are one of the most exported fruits. The established big markets are Singapore, Malaysia, Hong Kong and some European countries. If paclobutrazol residues are determined before exporting, it will establish a highly valuable cost of production.

8.6 Conclusion

It can be concluded that the mango ‘Chok Anan’ with application of paclobutrazol at 0.5 grams active ingredient per square meter will correspond with current food safety levels.