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Pesticide Residues on Sweet Basil, *Ocimum basilicum* L.(Labiatae) Under Different Production Systems From Central Thailand

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ABSTRACT

Pesticide residues on sweet basil under different production systems from Central Thailand from February 2010 to January 2011 were determined using gas chromatography. A total of 93 out of 360 samples had detectable levels of pesticide residues. However, 87 out of those 93 were sampled from conventional production systems; the other 6 samples were from a Good Agricultural Practice (GAP) farm while no pesticide residues were detected from samples of the organic production. Under the conventional systems, the residues of three organophosphorous pesticides were observed—namely, pirimiphos-methyl, chlorpyrifos and dimethoate. Moreover, the residue levels of dimethoate and pirimiphos-methyl in 1 and 10 samples, respectively, exceeded the Maximum Residue Limits of the European Union (EU MRLs). Although, two pyrethroid pesticides (cypermethrin and deltamethrin) were found more often, the residue levels were higher than those of the EU MRLs in only 1 and 4 samples, respectively. These samples were also collected from the conventional farms. Residues of organophosphates were more likely to be greater than the MRLs. The levels of pesticide residues depend on the persistence and application rates of pesticides as well as the pre-harvest interval. The results clearly indicated that the GAP certification system was effective in keeping pesticide residues to levels below the MRLs.

Keywords: sweet basil, pesticide residues, organic production system, Good Agricultural Practice, Maximum Residue Limit

INTRODUCTION

Sweet basil (*Ocimum basilicum* L.; Labiatae) is a popular spice because it is an important ingredient of Thai cuisine and is used for the preparation of various foods including pastas and other Italian foods (Joey, 2008). Sweet basil, however, has many serious insect pest problems in the field including thrips, cotton aphid, Ocimum leaf folder, lace bugs, spider mite, mealybug, tobacco whitefly, cutworms, Japanese beetles, grasshoppers and leaf miner fly (Paul and Bidlack, 1997; Michele, 2003; Cassandra and Bidlack, 2005; Leanne and Smith, 2006; Kristian, 2008; Sahaya *et al*., 2008; Srikacha *et al*., 2008; Dan, 2010; Mark, 2011). For the control of these insect pests, farmers normally use different types of insecticides. The major insecticides include: cypermethrin, chlorpyrifos, lambda-cyhalothrin, deltamethrin and dimethoate (Lee Fook Choy and Seeneevassen, 1998; Brian *et al*., 2001). Although dichlorodiphenyltrichloroethane (DDT), endosulfan, dieldrin and endrin, have been banned from agricultural use worldwide, their residues

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together with the residues of methomyl, malathion, dimethoate, profenofos, cypermethrin, chlorpyrifos and other pesticides have been documented on sweet basil in several reports (James, 1980; Abou-Arab and Abou Donia, 2001; Department of Agriculture Thailand, 2010; Farag *et al*., 2011; Selim *et al*., 2011; Loretta, 2012). In Thailand, no insecticides have been registered for use on sweet basil. However, due to problems with exporting to the European Union market, the Ministry of Agriculture and Cooperatives (2009) stipulated that sweet basil had to be regulated prior to export and must be inspected for microorganisms and other contaminants or both that could be a risk to human health. In turn, the government agencies were required to transfer their accumulated knowledge and information about the correct use, good agricultural practice and pest management, including post harvest technology, to the farmers and exporters.

Pesticide residues occur on sweet basil as a result of the direct application of pesticides. No comprehensive studies have been undertaken to determine the pesticide residues on sweet basil in the country with the exception of a few monitoring studies. The present study aimed to monitor and investigate pesticide residues on sweet basil produced under different production systems. The research is expected to result in preparing a list of pesticides found on sweet basil in Thailand. This will be important reference data for importing countries and represents a form of protection for sweet basil exports from Thailand to the global market.

MATERIALS AND METHODS

Study region and study sites

The experiments were conducted on different production systems in Latbualuang District (14°6'33"N, 100°14'53"E) Phra Nakhon Si Ayutthaya province, located in the central part of Thailand. The study sites were within an area of approximately 3 km^2. This region has an average rainfall of 34.46 mm per month (Anonymous, 2013a) and an average temperature of approximately 30 °C (Anonymous, 2013b).

Data collection

Field surveys of pesticide residues on sweet basil grown on conventional farm, farm certified by the Department of Agriculture as undertaking Good Agricultural Practice (GAP) and organic farm were performed using a systematic sampling technique. For each farm, the basil field was subdivided into 30 subplots of 1.15 × 1.90 m (that is, 15 plants per subplot). Plant samples from 10 randomly selected subplots were trimmed and approximately 1 kg was placed in plastic bags and transported for pesticide residue analysis by gas chromatography at the Laboratory of Department of Agriculture. The samples were placed in sterile, polythene bags and stored in a box maintained at approximately 4 °C with crushed ice in order to avoid any degradation between sampling and analysis. The field observation and sample collection procedures were repeated monthly for 12 consecutive months starting from February 2010 until the end of January 2011.

Sample preparation and extraction of pesticide residues

Samples brought into laboratory were registered and recorded in detail. About 200 g of each sample was then ground by robot coupe and a ground-up sample was placed into a Duran® round bottom flask for extraction of pesticide residues. The sample was thoroughly blended to obtain a homogenous representative sample for weighing. An aliquot of 25 g was then placed in a 250 mL Erlenmeyer flask, 50 mL acetone and 8 g NaCl were added to the flask and homogenized for 1 min at 10,000 cycles.min^{-1}. Dichloromethane (40 mL) was added to the flask and homogenized again for 1 min. Approximately 2 tablespoons of Na_2SO_4 were added into the flask and the sample was cleaned up with ethyl acetate 10 mL per time.

The organic solution of 50 mL was

transferred with a volumetric pipette into a round bottom flask and the pipette was washed twice with 10 mL ethyl acetate. The used ethyl acetate was then added into the round bottom flask and the volume decreased using a flash evaporator, with the temperature below 40 °C. The residue was transferred into a new 5 mL volumetric flask using ethyl acetate (PR). The extracted solution was divided into two parts. The first part was utilized for the organophosphorous (OP) residue analysis.

For another part, an extract sample of 2 mL was placed in a 5 mL volumetric flask; 2–3 drops of iso-propyl alcohol were added to the flask and blown dry with N_2. The sample was cleaned up again and then collected in a 2 mL round bottom flask before passing through flash evaporation. The last process was rinsing with n-hexane (PR) and the final volume was adjusted to 2 mL before being subjected to pyrethroid (PY) residue analysis.

Gas chromatography analysis

Extracted samples of sweet basil were analyzed by gas chromatography (GC) following the method of Steinwandter (1985). GC analyses were performed in an isocratic system using a GC Model 6890 (Agilent Technology; Santa Clara, CA, USA) fitted with a flame photometric detector (DB-1701; Agilent Technology; Santa Clara, CA, USA) and an electron capture detector (DB-1; Agilent Technology; Santa Clara, CA, USA) analytical column. The injection mode was splitless 1 µL and helium and nitrogen were used as the mobile phase. A 20 µL sample was injected through the auto sampler. The column temperature and pressure were kept at 250 °C and 148.1692 × 10^3 Pa, respectively. The flow rate of gas was 20 $mL.min^{-1}$, for hydrogen 90 m $L.min^{-1}$ and in the oxidizer using air at 80 m $L.min^{-1}$.

RESULTS

Field survey

The conventional production field under investigation was treated with agrochemicals including inorganic and organic fertilizers. Most of the sweet basil produced by conventional systems was used for local consumption. For insect pest control, fields were sprayed preventatively with the following insecticides: cypermethrin (Cypermethrin 10), lambda-cyhalothrin (Karate 2.5®)) and deltamethrin (Decis®)). Cypermethrin is a synthetic pyrethroid widely used by both large-scale commercial agricultural production as well as by farmers with small holdings; it behaves as a fast-acting on the axons in the peripheral and central nervous system by interacting with sodium channels in insects (International Programme On Chemical Safety, 2013). Lambda-cyhalothrin is a contact insecticide against sucking and chewing herbivores (Bert, 2007). Other insecticides used in the conventional production systems were chlorpyrifos (Chlorpyrifos 40®)), dimethoate (Dimethoate 40®)) and pirimiphos-methyl (Pirimiphos-methyl®)). They are organophosphorus compounds which bind to acetylcholinesterase and other cholinesterases resulting in disruption of nervous impulses, killing the insect or interfering with its ability to carry on normal functions (Bert, 2007). The application rates of the various insecticides used on sweet basil under different production systems are shown in Table 1.

Most of the sweet basil produced under the GAP system will be exported to foreign countries. Under this system, some fields were treated with both inorganic and organic fertilizers although no OP's were allowed to be used. For insect pest control, fields were sprayed preventatively with cypermethrin, lambda-cyhalothrin, deltamethrin and indoxacarb (Avatar®). Indoxacarb was used for controlling Lepidopteran pests in certain vegetable and fruit crops.

For the organic production system, only organic fertilizer was applied and no synthetic pesticides were allowed to be used under this system.

The numbers of applications for each insecticide during the study period are shown in

Table 2. Under conventional systems, insecticides were applied on average 3.67 times per month. Synthetic pyrethroids accounted for 88.6% of those applications while the remaining 11.4% was organophosphate insecticides. Among the pyrethroids, cypermethrin and lambda-cyhalothrin were the most frequently used insecticides. For the GAP system, fields were sprayed with insecticides on average 2.42 applications per month. Similarly, synthetic pyrethroids accounted for 86.2% but cypermethrin and deltamethrin were often selected. Lambda-cyhalothrin was rarely used under this system. The remaining 13.8% was the application of indoxacarb.

The results of the residue analysis are shown in Table 3. A total of 93 out of 360 samples had detectable levels of pesticide residues. However, 87 samples out of those 93 were from fields produced under conventional systems while only 6 samples with detectable residues were produced from the GAP farm. No residues were found on samples collected from the organic production system. Out of those 93 samples, 16 were basil plants with residue levels higher than those specified in the European Pesticide Database (European Pesticide Database, 2013). Pyrethroids were most frequently found on sweet basil with cypermethrin found on 51 samples of conventionally grown basil and another 6 samples were from the GAP system. The residue level of cypermethrin on basil collected from conventional farms, however, exceeded that of the EU MRL (2 ppm) in one sample. Another pyrethroid (deltamethrin) was detected on 20 samples all of which were basil samples from conventional fields. There were four samples with the detected concentration above that of the EU MRL (0.5 ppm).

Three organophosphorus pesticides were found, but only on conventionally produced basil. Chlorpyrifos residues were found on five samples all of which were below the EU MRL (0.05 ppm). Dimethoate residue was found only once during November at a level of 0.302 ppm and this value

Table 1 Application rates of various insecticides used on sweet basil under different production systems in Latbualuang district, Phra Nakhon Si Ayutthaya Province.

Insecticide	Group	Target Insects	Application rate (g active ingredient.ha^{-1})		
			Conventional	GAP	Organic
Cypermethrin (Cypermethrin 10 EC®)	Pyrethroid	chewing insects	104.16	83.31	N/A
Lambda-cyhalothrin (Karate 2.5 EC®)	Pyrethroid	chewing insects, thrips	34.72	31.25	N/A
Deltamethrin (Decis®)	Pyrethroid	leaf miner fly	20.81	18.75	N/A
Chlorpyrifos (Chlorpyrifos 40 EC®)	OP	cotton aphid	694.44	N/A	N/A
Dimethoate (Dimethoate 40 EC®)	OP	cotton aphid	347.22	N/A	N/A
Pirimiphos-methyl (Pirimiphos-methyl®)	OP	Mealybug	694.44	N/A	N/A
Indoxacarb (Avatar®)	Oxadiazines	Lepidopteran pests	no application	31.25	N/A

EC = Emulsifiable concentrate, OP = Organophosphorous, N/A = Not allowed to be used.

Table 2 Number of insecticide applications and number of contaminated sweet basil samples from different production systems in Latbualuang district, Phra Nakhon Si Ayutthaya province, Thailand from February 2010 to January 2011.

Production system	Insecticide	Number of insecticide applications[a]												Total no. of applications for each insecticide during the study[b]
		Feb 2010	Mar 2010	Apr 2010	May 2010	Jun 2010	Jul 2010	Aug 2010	Sep 2010	Oct 2010	Nov 2010	Dec 2010	Jan 2011	
Conventional	Cypermethrin	1	1	1	2 (3)	2 (5)	1 (5)	1 (6)	1 (6)	1 (3)	0 (5)	1 (8)	2 (10)	14 [31.82%]
	Lambda-cyhalothrin	1	1	1	1	1	1	2	2	1	2	1	0	14 [31.82%]
	Deltamethrin	0	1 (10)	1 (10)	1	1	1	1	1	1	1	1	1	11 [25%]
	Chlorpyrifos	1 (5)	0	0	0	0	0	1	0	0	0	0	0	2 [4.55%]
	Dimethoate	0	0	0	0	0	0	0	0	0	1 (1)	0	0	1 [2.27%]
	Pirimiphos – methyl	0	0	0	0	0	0	0	0	0	1 (3)	1 (7)	0	2 [4.55%]
	Total no. of applications during the month	3	3	3	4	4	3	5	4	3	5	4	3	44 [100%]
GAP	Cypermethrin	1 (3)	0	1 (3)	1	2	1	1	1	1	1	1	1	12 [41.38%]
	Lambda-cyhalothrin	0	1	0	0	0	0	0	0	0	0	0	0	1 [3.45%]
	Deltamethrin	1	1	1	1	1	1	1	1	1	1	1	1	12 [41.38%]
	Indoxacarb	1	0	0	0	0	0	1	0	1	1	0	0	4 [13.79%]
	Total no. of applications during the month	3	2	2	2	3	2	3	1	3	3	2	2	29 [100%]
Organic	Synthetic insecticide	0	0	0	0	0	0	0	0	0	0	0	0	0

GAP = Good agricultural practice,

[a] Numbers in parentheses indicate the number of insecticide-contaminated basil samples detected within the month.

[b] Numbers in square brackets indicate the percentage of that particular insecticide of the overall insecticide applications during the course of study.

was above the EU MRL (0.02 ppm). All 10 samples containing pirimiphos-methyl residues were found to exceed the EU MRL (0.05 ppm). This pesticide should be closely monitored since one residue level of this substance was the highest concentration recorded at 11.85 ppm on sweet basil produced under a conventional production system.

Overall, more than 93% of the samples with insecticide residues came from conventional system samples while less than 7% came from the GAP production system. However, all 16 samples with residue levels exceeding those of the EU MRL values were from conventional farms. Although pyrethroid insecticides were found more frequently, the greater persistence and the higher application rates of organophosphates have caused this group of insecticides to be more likely to exceed the EU MRLs. Pirimiphos-methyl alone was detected with levels above the EU MRL in 10 samples.

The Pearson correlation value between the total numbers of contaminated samples (regardless of production system) and the weather data are shown in Table 4. The results indicated that the total number of contaminated samples was significantly ($P < 0.05$) correlated with the monthly rainfall and the average humidity. As the rainfall increased, the number of contaminated samples reduced. Rain washes away the residues from plants and thus reduces the number of contaminated samples. However, in this study, the average daily temperature did not show any correlation with the insecticide residues.

DISCUSSION

These results indicated that the consumer of basil sourced from Central Thailand has a high risk of exposure to pesticide residues. Even though the detected concentrations of cypermethrin, deltamethrin and chlorpyrifos on the basil samples were mostly below the MRLs, the continuous consumption of the herb even with moderate contamination levels can result in the accumulation of the pesticides in the consumer and may have up to 179 adverse effects on the human population in the long term (Farag, *et al*., 2011).

For GAP systems, the use of pesticides is officially recommended or authorized under practical conditions and such regimes are certified by the Department of Agriculture (DOA). Reducing the application rate and the frequency of pesticide use can reduce the residue level. On the contrary, in conventional systems, the use of pesticides is determined by the farmers who tend to apply higher concentrations of pesticides and spray more frequently; therefore, the amount of pesticide residues left on the crop is higher (Muhummad *et al*., 2011).

Overall, Thai farmers may lack knowledge of pests, diseases and their management options which ultimately causes them to rely solely on pesticides. The violation of MRLs observed in this study might indicate deviation from the conventional production system where the pre-harvest intervals are not followed or the rate of application and the concentration are not adjusted to the recommendations of the DOA. In conclusion, the farmers need to use the appropriate doses of pesticides and restrict the spraying of pesticides just before harvesting the crop or during transportation in order to reduce the level of residues in vegetables (Barbara, 2003; Iqbal *et al*., 2009; Farag *et al*., 2011; Frederick, 2011; Muhummad *et al*., 2011; Rohan *et al*., 2012).

Although organic farming was superior to the other production systems in terms of having fewer contaminated samples, this study did not consider the production cost and yield analysis or both. Therefore, no conclusion could be drawn in terms of the economic benefit of organic farming.

CONCLUSION

The results clearly showed that the certified GAP production system was a very

Table 3 Levels of pesticide residue found in sweet basil collected from different production systems.

Pesticide group	Detected pesticide	No. of contaminated samples			Minimum residue detected			Maximum residue detected			EU MRL (ppm)	No. of samples where residue > EU MRL
		Conventional	GAP	Organic	Conventional	GAP	Organic	Conventional	GAP	Organic		
Pyrethroids	cypermethrin	51	6	0	0.01	0.01	0	3.98	0.11	0	2	1
	deltamethrin	20	0	0	0.13	0	0	0.71	0	0	0.5	4
Organophosphates	chlorpyrifos	5	0	0	0.02	0	0	0.03	0	0	0.05	0
	dimethoate	1	0	0	3.02	0	0	3.02	0	0	0.02	1
	pirimiphos-methyl	10	0	0	7.14	0	0	11.85	0	0	0.05	10

GAP = Good agricultural practice, EU MRL = European Union maximum residue limits specified in the European Pesticide Database (2013).

Table 4 Weather conditions and total of pesticide residue during the period of experiment (February, 2010-January, 2011) (Meteorological station of Latbualuang district, Phra Nakhon Si Ayutthaya province).

Weather parameters	Month												Pearson correlation[a]	*P*-value
	Feb 2010	Mar 2010	Apr 2010	May 2010	Jun 2010	Jul 2010	Aug 2010	Sep 2010	Oct 2010	Nov 2010	Dec 2010	Jan 2011		
Monthly Rainfall (mm)	0	0	1.8	76.7	137.8	294.7	228.8	222.2	193.3	0	28.1	0	-0.660	0.020*
Mean Relative humidity (%)	73.86	66.58	69.17	74.26	75.40	80.55	82.03	83.03	81.52	67.5	69.03	59.71	-0.675	0.016*
Mean Temp (°C)	23.71	24.00	25.87	25.49	24.56	23.99	23.48	23.44	23.21	22.17	21.69	20.39	-0.317	0.315
No. of contaminated samples	8	10	13	3	5	5	6	6	3	9	15	10		

[a] Correlations between weather parameters and number of contaminated samples detected within the month.

* = Significant at $\alpha = 0.05$.

useful tool in reducing the pesticide usage and hence reducing produce contamination from pesticide residues. The data indicated a pressing need to adopt the GAP production system and to adhere to the official recommendations on the use of the authorized pesticides. This could be achieved through educating and licensing farmers and applicators, especially for the application of high risk pesticides. Based on this information, researchers and government agencies in the agricultural sector need to work with farmers in developing integrated pest management strategies that will reduce the heavy use of pesticides.

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