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Cypermethrin Insecticide Residues in Vegetable Soybean, *Glycine max* (L.) Merrill, at Different Days of Pre-harvest Interval

Md. Abdullah[1], Ouab Sarnthoy[1] and Suratwadee Jiwajinda[2]

ABSTRACT

The experiments were conducted to investigate cypermethrin insecticide residues in vegetable soybean at different days of pre-harvest interval for two growing seasons. Soybean pods were collected at 0, 1, 3, 5 and 10 days after last application with cypermethrin. Residues were extracted from pods and determined using gas liquid chromatography with an electron capture detector. Cypermethrin when applied at the rate of 100 g ai/ha using a knapsack sprayer showed a mean residue level of 0.71 and 0.52 mg/kg at 0 and 1 day, respectively, in dry season (December- February) whereas the residues were 1.62 and 1.17 mg/kg at 0 and 1 day, respectively, in early wet season (April-June). A pre-harvest interval of 3 days was found safe (0.42 mg/kg) for consumers in dry season whereas early wet st season soybean exhibited residue level of 0.48 mg/kg at 10 day which fell below maximum residue limit of 0.5 mg/kg as prescribed by Food and Agriculture Organization/World Health Organization. Therefore, a pre-harvest interval of 10 days should be considered for cypermethrin residues in vegetable soybean to avoid any health risk of consumers. The dissipation rates of cypermethrin on soybean pods were more or less similar at the same days for both seasons which was 70% (based on 0 day) at 10 days.

Key words: vegetable soybean, cypermethrin, pre-harvest interval, residue, gas liquid chromatography

INTRODUCTION

Vegetable soybean, *Glycine max* (L.) Merrill, is an important cash crop in Thailand. It is harvested after the R_6 and before R_7 growth stage while the pods are still green and the seeds have developed to fill 80-90% of the pod width (Fehr *et al*., 1971). Vegetable soybean is popular in China, Japan and Korea for its unique taste and high nutritional value as a source of vitamins A, B_1, B_2 and C, protein, fat, fiber and mineral such as phosphorous, calcium and iron. The on-farm yield of this crop in Asia is much lower than the potential yield. One of the major reasons for low yield is the infestation by diverse insect pests from germination to harvest. Control of these pest is essential because infestation by certain species could result in total crop loss (Talekar, 1997). As a consequence of pesticide use, the presence of residues in field crop is unavoidable.

Cypermethrin or (RS)-∝-cyano-3-phenoxybenzyl (1RS,3RS; 1RS,3SR)-3-(2,2-dichloro-vinyl)-2,2-dimethylcyclopropanecar boxylate is a digestive and contact insecticide effective against a wide range of insect pests, particularly leaf- and fruit-eating Lepidoptera and Coleoptera in cotton, fruit, vegetables, vines, tobacco

1 Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.

2 Central Laboratory and Greenhouse Complex, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand.

and other crops (Worthing, 1987). Cypermethrin is widely used widely by farmers to control insect pests of vegetables.

There is currently an increasing concern and awareness on the hazards of pesticides to consumers. Even with the adoption of integrated pest management, farmers still believe in the control of pests using pesticide because of its quick effect. The application of pesticides pre- or post-harvest could, however, leave residues on food products which pose a potential risk to the health of consumer (Lindsay, 1997). Insecticide use in controlling insect pest complex of vegetable soybean should, therefore, be examined for residues. Hence an attempt has been made to estimate the quantity of residue of cypermethrin left in vegetable soybean at different days of pre-harvest intervals for safe consumption.

MATERIALS AND METHODS

Spray treatments

Field experiments were conducted during dry (December, 1999 to February, 2000) and early wet (April to June, 2000) seasons at Kamphaeng Saen Campus of Kasetsart University, Nakhon Pathom, Thailand. Vegetable soybean variety AGS292 was planted in a plot consisted of 10 rows, 5 m long and 50 cm apart. Plants within the rows were 20 cm apart maintaining 25 hills/row. Three plots (each plot was considered as a replication) were sprayed with cypermethin 10% EC at the concentration of 100 g ai/ha using a knapsack sprayer. Adjuvant (IBA spreader) was added at the concentration of 0.5 ml/l of spray volume. Three untreated plots (each plot was considered as a replication) were also maintained to observe insecticidal contamination. Since vegetable soybean is attacked by insect pests from germination to harvesting, cypermethrin was sprayed at 10 days interval until harvest. Conditions of rainfall and sunshine data were recorded throughout the experimental periods.

Sampling procedure

Soybean pods were collected from treated and untreated plots at 0 (3 hr), 1, 3, 5 and 10 days after last spraying. Samples (600 g each) were randomly collected from the plots and poured into polyethylene bags. These bags were tightly closed with rubber band, labeled and kept in a deep frceeger at -65°C until extracted.

Analytical procedure

The procedures for cypermethrin residue estimation in vegetable soybean were as follows:

Extraction of residues from pods

The frozen samples were taken out from deep frceeger after 2 months of sample collection and allowed to set room temperature. A 100 g of sample was weighed and poured into a blender jar. Then 25 g sodium sulfate was added into the sample and blended at low speed for a few seconds. One hundred and fifty ml of petroleum ether was added into the blender jar and blended at high speed for 2 min. Decant petroleum ether was filtered through a perforated Buchner funnel using Whatman No. 1 filter paper. The blended sample was re-extracted twice with 100 ml petroleum ether in blender jar and blended at high speed for 1 min. The sides of blender jar were scraped down to break up caked materials between extractions. After last blending, blender jar and materials were rinsed with 50 ml petroleum ether. The combined extracts were then filtered through anhydrous sodium sulfate into 500 ml round-bottom flask. The extract was then evaporated by a rotary evaporator maintaining at 40°C to make a final volume of 2 ml.

Acetonitrile partitioning

Two ml extract was adjusted to 30 ml using petroleum ether, partitioned with acetonitrile saturated with petroleum ether (40 ml) in 125 ml separatory funnel, and shaken vigorously for 1 min. After allowing the layer to separate, acetonitrile was drained into 1 l separator containing 650 ml distilled water, 40 ml saturated sodium chloride solution, and 100 ml petroleum ether. Petroleum ether layer was re-extracted three times with additional 30 ml saturated acetonitrile and combined acetonitrile layers in the same separatory funnel as above. The separator was then shaken for 30-45 sec, the layer was allowed to separate and the aqueous layer was drained into 1,000 ml conocal flask. The upper petroleum ether was then transferred into a conical flask. Aqueous layer was re-extracted with 100 ml petroleum ether. The petroleum ether was combined with previous one and filtered through anhydrous sodium sulfate into 500 ml round-bottom flask. The volume of extract was reduced to 2 ml using rotary evaporator.

Clean-up with Florisil

A glass column, 50 cm × 2.5 cm id, fitted with stopcock was used where activated Florisil (90 g) (over night at 130°C) was filled to column followed by sodium sulfate (10 g). Filled column was pre-wetted with 40 ml petroleum ether. Extract solution was transferred to column with controlled flow rate (5 ml/min). The column was eluted with 200 ml 6% ethyl ether/petroleum ether followed by the same volume of 15% and 50% eluants. All of these eluates were collected separately in a 500 ml round-bottom flask and then evaporated to final volume of 0.5 ml. These were rinsed with several small portions of petroleum ether and poured into small vials to a final volume of 2 ml. All vials were kept in a refrigerator at 4°C before subjecting to GC analysis.

Gas chromatographic analysis

Sample solution in vials were reduced to a volume of 1 ml before subjecting to analysis by GC. Working standard solutions of 10 and 20 ppm were prepared in petroleum ether by dilution of appropriate volumes of the stock solution. Cypermethrin was analyzed by gas liquid chromatography using an electron capture detector. A gas chromatograph (Shimadzu Model GC-9A) was used for this purpose. All samples were injected at 1µl. The analysis conditions were as follows:

Column type: Widebore DB-1701P (1.00 µm, 30 m × 0.530 mm)
Column temperature: 250°C
Injector temperature: 270°C
Detector temperature: 270°C
Carrier gas: N_2
Flow rate: 20 ml/min.

Recovery experiment

Recovery experiment was followed to observe the efficiency of extraction, clean-up and estimation procedures, by fortifying soybean pods (100 g) with 20 µg cypermethrin on two samples.

Calculation of residues

The residue R, expressed in mg/kg were calculated using the following formula:

$$R = \frac{H_A \cdot V_{end} \cdot W_{sd}}{H_{sd} \cdot V_i \cdot G}$$

where G = sample weight (in g), V_{end} = terminal volume of sample solution (in ml), Vi = volume of sample solution injected into gas chromatography (in µl), W_{sd} = amount of cypermethrin for standard solution (in µg), H_{sd} = Peak area from W_{sd} (in cm^2), H_A = Peak area from V_i (cm^2).

RESULTS AND DISCUSSION

Recovery experiment exhibited the efficiency of procedures used for cypermethrin residues in vegetable soybean, when 20 µg cypermethrin were added to 100 g soybean pods showed an average recovery of 11.97 µg (59.85%). Cypermethrin residues on vegetable soybean at different days of pre-harvest interval were investigated for two growing seasons. Cypermethrin 10% EC when sprayed at the concentration of 100 g ai/ha showed a residue level of 0.71 mg/kg at 0 day followed by 0.52 mg/kg at 1 days in dry season

(Table 1) which were higher than the recommended maximum residue limit (MRL) of 0.5 mg/kg set for beans with pods (FAO/WHO, 1985). The residue levels at 3, 5 and 10 days decreased to 0.42, 0.30 and 0.22 mg/kg, respectively which were all below the maximum residue level in the same season. Cypermethrin residues were higher in early wet season (Table 1). Residues were 1.62 mg/kg at initial level (0 day) followed by 1.17, 0.94 and 0.69 mg/kg at 1, 3 and 5 days, respectively, in early wet season which were all above the maximum residue limit. At 10 day, the residues were 0.48 mg/kg which was below the maximum residue level (Table 1) set by FAO/WHO. No significant difference of residues were observed between different days of pre-harvest intervals in dry season. Cypermethrin residues decreased gradually in all pre-harvest intervals. The degradation of cypermethrin residue in dry season were 26.76, 40.84, 57.75 and 69.01% for 1, 3, 5 and 10 days interval, respectively, on initial residue level (0 day). In case of early wet season, the initial residue (0 day) level was 1.62 mg/kg which differed significantly from all pre-harvest intervals. No significant differences were observed between 1 and 3 day interval, 3 and 5 day interval, and 5 and 10 day interval but 10 day interval differed significantly over 1 and 3 day interval. The degradation of cypermethrin in early wet season were 27.78, 41.98, 57.40 and 70.37% at 1, 3, 5 and 10 day interval, respectively, on initial residue level (0 day). The reduction rates of cypermethrin residues were very close at the same days for all pre-harvest intervals in both seasons.

A total rainfall of 12.5 and 217.6 mm were recorded during growth period of dry and early wet season, respectively. But after last spray, dry and early wet season soybean received a total rainfall of 12.5 and 12.4 mm (Table 2), respectively, which might be the cause of the same reduction rate of residue at all pre-harvest intervals. There was no rainfall within 3 hrs of sample collection at 0 day for both seasons. So residue washing out by rain did not occur at the initial level. Cypermethrin was applied to each plot with the same amount of spray in both seasons. The higher residues in early wet season might be due to the less canopy with poor yield (5.75 t/ha) which allowed more insecticide and residues on the pods. The higher canopy in dry season with higher yield (9.65 t/ha) might be responsible for receiving less spray deposit resulting in less residues on pods. On the other hand, a total sunshine period of 593.6 and 463.7 hrs were recorded from planting to harvesting in dry and early wet season, respectively, whereas after the last spray the dry season received 81.7 hrs of sunshine while the early wet season received only 54.4 hrs sunshine period (Table 2). This might have an influence on having higher residues in early wet season. Tajeda *et al.*(1983) reported that the disappearance of residues in and on plants is the effect of the interactions of environmental conditions such as wind, rain, sun, humidity and temperature and chemical and physical factors such as volatilization and growth of the plants.

Tejada *et al.* (1989) reported an initial deposit of 0.36 mg/kg on string bean when cypermethrin was applied at the rate of 1 ml/l under field conditions. This amount is close to the residue obtained in dry season soybean of the present investigation. They also observed detectable (0.11mg/kg) residues up to 4 days after last spraying. Since in the present study the recommended dose was higher (2.5 ml/l), and gave an initial deposit of 0.71 mg/kg in dry season. The average residue level of any insecticides depends primarily on the quantity of its active ingredient. Field studies were conducted by Kumar *et al*. (1998) on chick pea where cypermethrin was applied either at 60 or 90 g ai/ha (low and high dose, respectively). Initial mean residue level on green pods were 0.42 and 0.62 mg/kg at low and high doses, respectively. These dissipated by 73.81 and 70%, respectively after 15 days which was also close to the residues at initial level (dry season) and degradation rate of both seasons. Residues of cypermethrin were determined by Singh and Kalra (1992) in the fruits and leaves of aubergines and in the soil when applied at 50 and 100 g ai/ha. After eight applications at lower dosage, initial deposits on the fruits were 0.73 mg/kg which declined to 0.08 mg/kg in 10 days. On leaves the initial deposits averaged 1.16-1.69 mg/kg which declined by 68% in the 10 days. These results are close to the present investigation where there was an initial deposit (0 day) of 1.62 mg/kg on pods which declined by 70.37% in the 10 days of early wet season. Singh and Udeaan (1989) investigated the persistence and dissipation of cypermethrin in okra fruits where 50 or 100 g ai/ha were applied three times. After 2nd and 3rd spray, the initial residues were 0.76 and 0.65 mg/kg for 50 g ai/ha

Table 1 Cypermethrin residues in vegetable soybean applied at 100 g ai/ha in dry season (February) and early wet season (June) 2000 at Kamphaeng Sean, Nakhon Pathom.

Pre-harvest interval (Days)	Cypermethin residues (mg/kg) (Mean ± S.D)	
	Dry season	Early wet season
0	0.71 ± 0.44	1.62 ± 0.21a*
1	0.52 ± 0.09	1.17 ± 0.19b
3	0.42 ± 0.36	0.94 ± 0.13bc
5	0.30 ± 0.05	0.69 ± 0.14cd
10	0.22 ± 0.14	0.48 ± 0.11d
F-test	NS	**
C. V (%)	54.2	16.6

* Means followed by the same letters are not significantly different at the 5% level by Duncun's multiple range test.

Table 2 Rainfall and sunshine data at different days of pre-harvest intervals in dry season (February) and early wet season (June) 2000 at Kamphaeng Sean, Nakhom Pathom.

Pre-harvest interval (Days)	Dry season		Early wet season	
	Rainfall (mm)	Sunshine (hrs)	Rainfall (mm)	Sunshine (hrs)
0	2.0	5.7	2.5	8.7
1	0	6.7	0	3.8
2	0	8.8	T*	0.2
3	3.9	9.3	6.0	0.2
4	0	6.1	T	4.1
5	0	9.0	0.1	5.7
6	0	9.9	T	10.4
7	0	9.7	0	7.0
8	0	9.2	0.3	4.3
9	0.1	4.2	T	2.7
10	6.5	3.1	3.5	7.3

* Trace of rainfall less than 0.1 mm

dose, respectively and 1.53 and 1.43 mg/kg for 100 g ai/ha dose, respectively. They recommended a waiting period of at least one day to let them fall below the maximum limit of 0.5 mg/kg. In the present study, the results of dry season showed a pre-harvest interval of three days for the safe use by consumers where residue fell below the maximum residue limit. Rai *et al*. (1986) observed the persistence of cypermethrin for 11 days which was below prescribed maximum residue limit within eight days on cauliflower which was close to the safe period for wet season. Kumar *et al*. (2000) reported a safe waiting period of 1.8 days when cypermethrin was applied at 300 g ai/ha on chilli which is close to the waiting period for dry season. Persistence and safe period on okra fruits were investigated by Khan *et al*. (1999). They observed an initial concentration of 1.31 mg/kg which dissipated to a mean concentration of 0.05 mg/kg after 10 days where residues existed below the maximum residue limit set in India for cypermethrin (0.2 mg/kg) at 5.91 days after final application. Awasthi (1994) reported the waiting periods for cypermethrin which was between 0 to 5 days based on the FAO/WHO prescribed tolerance limits for chilli. All of these reviews support the investigation of cypermethrin residues in vegetable soybean. In the control samples (collected from the plots where no pesticide was applied) no peaks were observed for both seasons indicating no contamination of residues on soybean pods.

From the present investigation of cypermethrin residues in vegetable soybean it was concluded that this application dose was safe for the consumption after three days of application in dry season where it fell below maximum residue limit. So a pre-harvest interval of three days might be considered for dry season. But in case of early wet season where crop canopy was smaller allowing higher deposition of cypermethrin residues on pods, it showed a pre-harvest interval of 10 days for the safe use. So vegetable soybean should be harvested after 10 days of cypermethrin application to avoid any health risk of consumers.

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