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Weed control with pre-emergence herbicides in vegetable soybean (*Glycine max* L. Merrill)

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ABSTRACT

Field and laboratory experiments were conducted in the early and late rainy seasons in Thailand to evaluate the effect of pre-emergence application of herbicides and determine the herbicide residues on vegetable soybean (*Glycine max* L. Merrill cv. No. 75) production. No visible crop injury was observed after application of alachlor 469 g a.i./ha, clomazone 1080 g a.i./ha, metribuzin 525 g a.i./ha, pendimethalin 1031.25 g a.i./ha, tank-mixed clomazone 960 g a.i./ha + pendimethalin 928 g a.i./ha, or tank-mixed metribuzin 350 g a.i./ha + pendimethalin 928 g a.i./ha. However, acetochlor 1875 g a.i./ha, isoxaflutole 75 g a.i./ha, and oxadiazon 1000 g a.i./ha caused visible crop injury. Plant bioassay of herbicide residues in the soil after harvest showed no phytotoxic effect on baby corn (*Zea mays* Linn. cv. Suwan 3), cucumber (*Cucumis sativus* L. cv. Pijit 1), pak choi (*Brassica chinensis* Jusl. cv. Chinensis), and soybean (*G. max* L. Merrill cv. CM 60). Gas Chromatography-Mass Spectrometry (GC–MS) analysis showed no significant herbicide residues on crop yield (or MRLs < 0.01 ppm) for all herbicides used in this study. The application of metribuzin at 525 g a.i./ha was sufficient to provide satisfactory full-season control of several weed species and gave the highest crop yield. In addition, pendimethalin at 1031.25 g a.i./ha, and tank-mixed metribuzin at 350 g a.i./ha + pendimethalin at 928 g a.i./ha can provide a similar level of weed control as an alternative to reduce herbicide dosage thereby increasing food and environmental safety in vegetable soybean production.

1. Introduction

Vegetable soybean (*Glycine max* L. Merrill) an important crop in the northern part of Thailand where it is grown in three main seasons. Firstly, the dry season crop is grown from mid-December to mid-January, after rice. The second crop is grown in the early rainy season from May to July, and the remaining vegetable soybean crop is grown in the late rainy season, in August (Srisombun and Shanmungasundaram, 2001). Effective weed control is an essential component of profitable vegetable soybean production. Hand weeding, which is the most common method of weed control, is now costly and sometimes not feasible due to unavailability of labor at the time of need. To avert these problems, farmers have started adopting herbicides as an alternative to the manual hand weeding. However, considerable variations occur in crop and weed response to herbicide application. At present, there is no available information on the efficacy of herbicides for early weed control while preventing crop injury and possible residual accumulation in vegetable soybean.

During the last decade, the volume of vegetable soybean exported from Thailand to the Japanese market ranked third after China and Taiwan (Lin, 2001). Frozen vegetable soybean exports are expected to grow moderately if better quality raw material and acceptable herbicide residue levels for the export market are maintained. However, lack of experience in the production of high quality vegetable soybean is a major obstacle in the early stages of development of the vegetable soybean industry in Thailand. Therefore, the objectives of this study were to evaluate the effect of pre-emergence herbicides on vegetable soybean production, observe the effect of herbicide residues in the soil on some tested plants by plant bioassay, and determine the herbicide residues on vegetable soybean product by gas chromatography-mass spectrometry (GC–MS).

2. Materials and methods

2.1. Efficacy of pre-emergence herbicides for weed control

Field trials were conducted in the early and late rainy seasons of 2008 in Mae On, Chiang Mai, Thailand. A randomized complete

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Table 1
Weed control treatments used during the experiment.

Treatment	Dose (g a.i./ha)	Trade name
Weedy control	–	
Hand weeding (at 15, 30, 45 DAP*)	–	
Acetochlor	1875	FOUR 50% EC
Alachlor	469	LASSO 10% EC
Clomazone	1080	MAGISTER 48% EC
Isoxaflutole	75	BALANCE 75% WG
Metribuzin	525	SENCOR 70% WP
Oxadiazon	1000	RONSTAR 25% EC
Pendimethalin	1031.25	HEX 33% EC
Clomazone + Pendimethalin	960 + 928	MAGISTER 48% EC + HEX 33% EC
Metribuzin + Pendimethalin	350 + 928	SENCOR 70% WP + HEX 33% EC

*DAP = days after planting.
a.i. = active ingredient; EC = emulsifiable concentrate; WG = wettable granules; WP = wettable powder.

Table 2
Conditions of GC–MS.

GC	6890N (agilent)
MS	G2579A (agilent)
Column	DB-5MS (agilent) (30 m × 0.250 mm × 0.25 μm
Column temp.	50° C (1 min) - - > 15° C/min - - > 125° C- - > 10° C/min - - > 300° C (3 min) - - > 310° C (2 min)
Injector temp.	250° C
Carrier gas	Helium gas (>99.99995%)
Injection volume	2.0 μl
Injection mode	Splitless

block design (RCBD) with four replications was used in the experiment. The vegetable soybean seeds, cultivar No. 75, were sown by hand at an inter- and intra-row spacing of 50 and 20 cm, respectively. The individual plot size was 5 × 5 m. All the pre-emergence herbicides were applied within 1 day of seeding using a Knapsack sprayer having a 8004 flat fan T-jet nozzle at a pressure of 2.1 kg/cm^2 and spray volume of 375 L/ha. The treatments consisted of a single dose of seven pre-emergence herbicides and two tank mixes, a weedy control and a hand weeding control (using hand hoe at 15, 30, 45 days after planting, DAP), making a total of 11 treatments as shown in Table 1. Two fungicides (Captan 80% WG and Mancozeb 64% WP) were used at the rate of 30-50 g/ 20 L, while the insecticides (Acetamiprid 20% SP and Imidacloprid 5% EC) were applied at 5-10 g/ 20 L. Vegetable soybean was grown to maturity and harvested according to standard agricultural practices.

The data recorded were weed cover score, crop injury and yield of vegetable soybean. Weed control and crop injury were rated visually 7, 14, 21 and 30 days after application (DAA) on a scale of 1 (no weed cover or least injured plants) to 9 (completely weedy plot or most injured plants) (Burrill et al., 1976). Yields were measured at crop maturity by harvesting 10 plants within a 1 square meter sampling area per plot. All data were subjected to analysis of variance (ANOVA) and were combined over seasons and analyzed using the Proc Mixed procedure of the Statistical Analysis System (SAS Institute, 1998). Treatment means were separated using Fisher's Protected LSD test at $P = 0.01$.

2.2. Plant bioassay for detecting herbicide residues in soil

A 2 factor experiment was laid-out at the same site in a randomized complete block design (RCBD) with four replications. Sub-plots were planted with four test crop species, whereas main-plots were assigned to herbicide treatments. In the season following each herbicide application, the trial area was disc plowed to 10 cm depth, followed by two passes with a rotavator. Herbicide residues in the soil were bioassayed using test plants such as baby corn (*Zea mays* Linn. cv. Suwan 3), cucumber (*Cucumis sativus* L. cv. Pijit 1), pak choi (*Brassica chinensis* Jusl. cv. Chinensis), and soybean (*G. max* L. Merrill cv. CM 60). The test plants were direct-seeded into soil previously treated with herbicides (Table 1) and the hand weeding and weedy control treatments. Each replicate of the experiment contained 25 seeds of the crop, which were sown in each row. The field trials were maintained weed free by hand

Table 3
Effect of pre-emergence application of herbicides on weed, crop injury and marketable yield of vegetable soybean.

Treatment	Weed cover score[a] (days after application)				Crop injury score[b] (days after application)				Yield (kg/ha)
	7	14	21	30	7	14	21	30	
Weedy control	9 a[c]	9 a	9 a	9 a	1 b	1 b	1 b	1 b	3563 d
Hand weeding (at 15, 30, 45 DAP[d])	9 a	9 a	3 b	7 a	1 b	1 b	1 b	1 b	6806 c
Acetochlor 1875 g a.i./ha	2 b	2 b	2 b	2 b	2 b	6 a	2 b	2 b	7681 c
Alachlor 469 g a.i./ha	1 b	1 b	2 b	2 b	1 b	1 b	1 b	1 b	10,075 b
Clomazone 1080 g a.i./ha	1 b	1 b	1 b	2 b	1 b	1 b	1 b	1 b	9475 b
Isoxaflutole 75 g a.i./ha	1 b	1 b	1 b	1 b	1 b	9 a	9 a	9 a	6925 c
Metribuzin 525 g a.i./ha	1 b	1 b	1 b	1 b	1 b	1 b	1 b	1 b	11,813 a
Oxadiazon 1000 g a.i./ha	1 b	1 b	2 b	2 b	6 a	7 a	3 b	2 b	7638 c
Pendimethalin 1031.25 g a.i./ha	1 b	1 b	1 b	1 b	1 b	1 b	1 b	1 b	10,544 ab
Clomazone 960 g a.i./ha + pendimethalin 928 g a.i./ha	1 b	1 b	1 b	1 b	1 b	1 b	1 b	1 b	9175 b
Metribuzin 350 g a.i./ha + pendimethalin 928 g a.i./ha	1 b	1 b	1 b	1 b	1 b	1 b	1 b	1 b	10,363 ab
LSD (0.01)	3.07	2.49	2.28	2.64	1.46	2.94	2.33	2.31	4.75
SE	0.53	0.43	0.39	0.46	0.25	0.51	0.41	0.40	0.82

SE = standard error. Degree of freedom = 30.
Remarks: Grasses: *Brachiaria mutica* (Frosk.) Stapf., *Brachiaria reptans* (Linn.) Gard & Hubb., *Cynodon dactylon* (L.) Pers., *Dactyloctenium aegyptium* (L.) P. Beauv. Ess. Agrost., *Digitaria ciliaris* (Retz.) Koel., *Echinochloa colona* (L.) Link., *Echinochloa crus – galli* (L.) Beauv., *Eleusine indica* (L.) Gaerthn., *Leptochloa chinensis* (L.) Nees., *Panicum repens* (L.), and *Rhynchelytrum repens* (wild) C.E. Hubb.
Broadleaf weeds: *Ageratum conyzoides* (L.), *Amaranthus spinosus* (L.), *Alternanthera sessilis* DC., *Boerhavia diffusa* (L.), *Borreria laevis* (Lamk.) Griseb., *Chromolaena odoratum* (L.) R.M. King & H. Robins., *Eclipta prostrata* L., *Euphorbia heterophylla* L., *Euphorbia hirta* L., *Heliotropium indicum* (L.), *Mimosa invisa* Mart., *Phyllanthus amarus* Schumach & Thonn., *Trianthema portulacastrum* L., *Tridax procumbens* L., and *Vernonia cinerea* L. Less.
Sedges: *Cyperus iria* Linn., and *Cyperus rotundus* Linn.
[a] Weed cover score using a scale of 1–9 where 1 represents no weed cover and 9 = completely weedy plot.
[b] Crop injury score using a scale of 1–9 where 1 represents least injured plants and 9 = most injured plants.
[c] Means within a column followed by the same letter are not significantly different according to Fisher's Protected LSD test at $P = 0.01$.
[d] DAP = Days after planting.

Table 4
Vegetable crop responses to pre-emergence herbicides applied in the previous season at 7 days after planting (DAP).

Treatment	*Brassica chinensis* Jusl.		*Cucumis sativus* L.		*Zea mays* Linn.		*Glycine max* L. Merrill.	
	Field emergence (%)	Crop injury score[a]	Field emergence (%)	Crop injury score	Field emergence (%)	Crop injury score	Field emergence (%)	Crop injury score
Weedy control	85 a[b]	1	85 a	1	85 a	1	85 a	1
Hand weeding (at 15, 30, 45 DAP)	85 a	1	85 a	1	84 a	1	85 a	1
Acetochlor 1875 g a.i./ha	75 b	1	80 ab	1	80 ab	1	80 ab	1
Alachlor 469 g a.i./ha	81 ab	1	85 a	1	84 a	1	84 a	1
Clomazone 1080 g a.i./ha	80 ab	1	81 ab	1	81 ab	1	82 ab	1
Isoxaflutole 75 g a.i./ha	80 ab	1	75 b	1	75 b	1	80 ab	1
Metribuzin 525 g a.i./ha	82 ab	1	85 a	1	81 ab	1	85 a	1
Oxadiazon 1000 g a.i./ha	80 ab	1	80 ab	1	80 ab	1	75 b	1
Pendimethalin 1031.25 g a.i./ha	85 a	1	85 a	1	80 ab	1	85 a	1
Clomazone 960 g a.i./ha + pendimethalin 928 g a.i./ha	85 a	1	81 ab	1	84 a	1	81 ab	1
Metribuzin 350 g a.i./ha + pendimethalin 928 g a.i./ha	85 a	1	80 ab	1	84 a	1	84 a	1
LSD (0.01)	0.09	ns	0.06	ns	0.05	ns	0.06	ns
SE	0.75	0	0.68	0	0.80	0	0.64	0

SE = standard error. Degree of freedom = 30.
[a] Crop injury score using a scale of 1–9 where 1 represents least injured plants and 9 = most injured plants.
[b] Means within a column followed by the same letter are not significantly different according to Fisher's Protected LSD test at $P = 0.01$.

weeding. The data recorded were field emergence and crop injury of tested plants. Crop injury was rated visually 7 and 14 days after emergence (DAE) on a scale of 1 (least injured plants) to 9 (most injured plants) (Burrill et al., 1976). All data were subjected to analysis of variance (ANOVA) after combining the data for two seasons and analyzed using the Proc Mixed procedure of the Statistical Analysis System (SAS Institute, 1998). Treatment means were separated using Fisher's Protected LSD test at $P = 0.01$.

2.3. Herbicide residue analysis in vegetable soybean

Herbicide residue in the vegetable soybean pods was determined using Gas Chromatography-Mass Spectrometry (GC–MS), 7 days before harvest. The analysis was carried out according to the "Multiresidue Method for Agricultural Chemicals by GC–MS (Agricultural Products)" (MHLW, 2005a).

Twenty-gram samples of vegetable soybean were homogenized with 50 mL acetonitrile and suction-filtered with a glass filter. The residue on the filter was re-homogenized with 20 mL acetonitrile and filtered. Both filtrates were mixed and made up to 100 mL with acetonitrile. A 20 mL portion was mixed with 10 g sodium chloride and 20 mL of 0.5 M phosphate buffer (pH 7.0) and shaken. After the solution was clearly separated into 2 layers, the aqueous layer was discarded and the acetonitrile layer was dehydrated with sodium sulfate (anhydrous) and filtered. The filtrate was concentrated with a rotary evaporator at −40 °C. The residue was dissolved in 2 mL of acetonitrile/toluene (3:1), applied to the mini column, and eluted with 20 mL acetonitrile/toluene (3:1). The entire eluate volume was collected and concentrated to less than 1 mL at −40 °C. Acetone (10 mL) was added to the solution and concentrated to less than 1 mL at −40 °C. A 5 mL aliquot of acetone was added to this solution and concentrated again. The residue was dissolved and made up to 1 mL with acetone/n-hexane (1:1). The solution was assayed by GC–MS. The conditions of GC–MS are shown in Table 2.

3. Results and discussion

3.1. Efficacy of pre-emergence herbicides for weed control

Pre-emergence herbicide options can be used instead of hand weeding in vegetable soybean production. Pre-emergence herbicide application was sufficient to provide satisfactory full-season control of many weed species as listed in Table 3 (remarks). Metribuzin 525 g a.i./ha was the most effective, followed by pendimethalin 1031.25 g a.i./ha, and tank-mixed metribuzin 350 g a.i./ha + pendimethalin 928 g a.i./ha, respectively. These herbicides provide efficient broad-spectrum control of broad-leaved weeds and some grasses with excellent user safety as well as the reduction of production cost. The visual injury seen in vegetable soybean soon after metribuzin application was minimal and transient and had no effect on final yield.

No visible crop injury was observed after alachlor 469 g a.i./ha, clomazone 1080 g a.i./ha, metribuzin 525 g a.i./ha, pendimethalin 1031.25 g a.i./ha, tank-mixed clomazone 960 g a.i./ha + pendimethalin 928 g a.i./ha, and tank-mixed metribuzin 350 g a.i./ha + pendimethalin 928 g a.i./ha application. However, acetochlor 1875 g a.i./ha, isoxaflutole 75 g a.i./ha, and oxadiazon 1000 g a.i./ha caused visible crop injury to vegetable soybean (Table 3). In particular, isoxaflutole and oxadiazon resulted to unacceptable crop injury.

The vegetable soybean yields of the untreated control were less than the yields of herbicide treated plots (Table 3). Herbicide application resulted in significantly higher crop yield (6925–11813 kg/ha) than the weedy control (3563 kg/ha). Application of metribuzin at 525 g a.i./ha, pendimethalin at 1031.25 g a.i./ha, and tank-mixed metribuzin at 350 g a.i./ha + pendimethalin at 928 g a.i./ha gave significantly the highest crop yield of vegetable

Table 5
Determination of herbicide residues in vegetable soybean using GC–MS.

Treatment	Level (ppm)	MRL[a] (ppm)
Weedy control	–	–
Hand weeding (at 15, 30, 45 DAP[b])	–	–
Acetochlor 1875 g a.i./ha	N.D.[c]	0.1
Alachlor 469 g a.i./ha	N.D.	0.01
Clomazone 1080 g a.i./ha	N.D.	0.05
Isoxaflutole 75 g a.i./ha	N.D.	0.01
Metribuzin 525 g a.i./ha	N.D.	0.01
Oxadiazon 1000 g a.i./ha	N.D.	0.01
Pendimethalin 1031.25 g a.i./ha	N.D.	0.2
Clomazone 960 g a.i./ha + pendimethalin 928 v./ha	N.D.	0.05 + 0.2
Metribuzin 350 g a.i./ha + pendimethalin 928 g a.i./ha	N.D.	0.01 + 0.2

[a] MRL = Maximum residue limit.
[b] DAP = Days after planting.
[c] N.D. = not detected (Detection limited < 0.01 ppm).

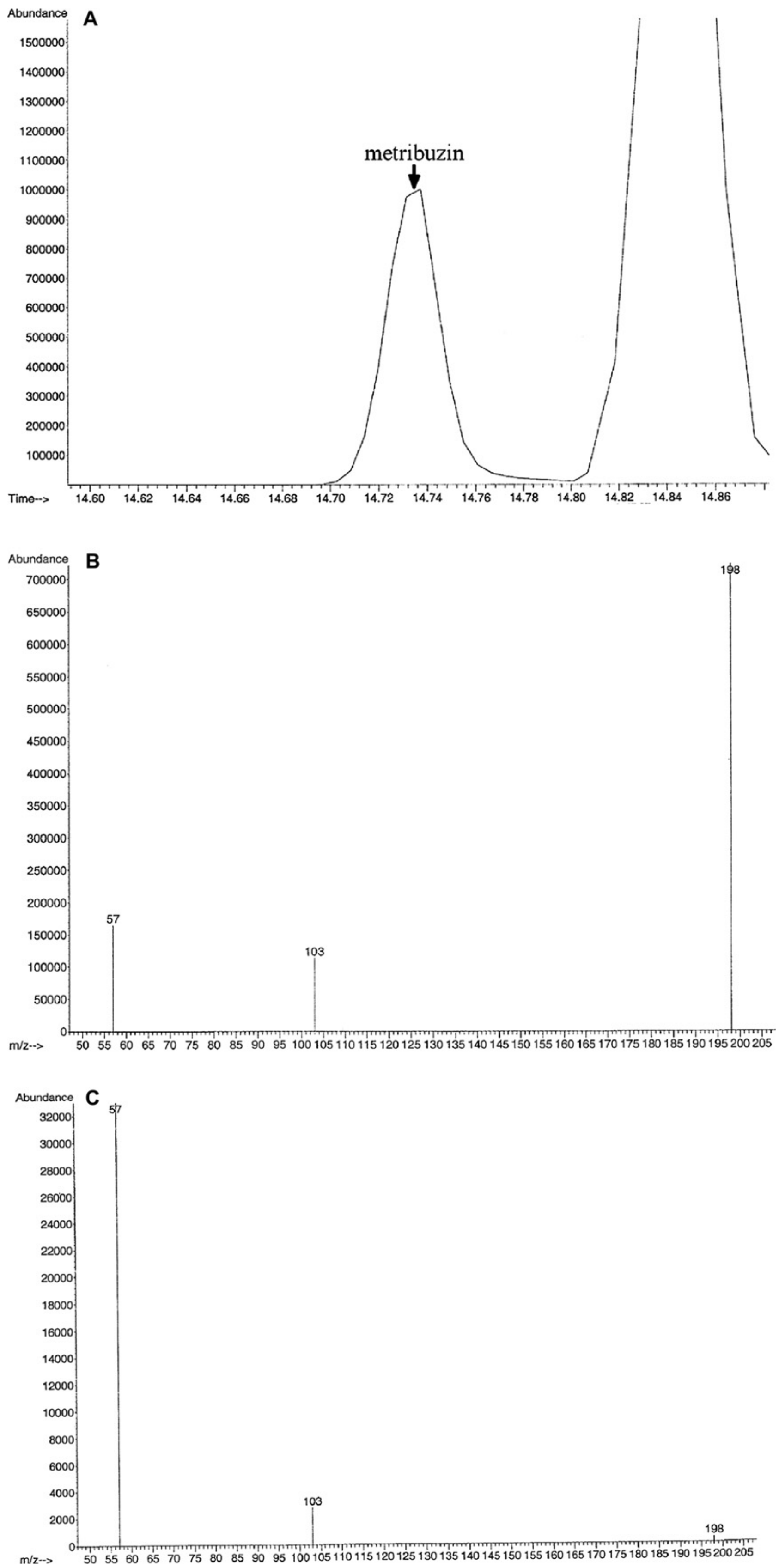

Fig. 1. Determination of metribuzin residues in vegetable soybean using GC–MS; (A) = chromatogram of metribuzin standard, (B) = Mass pattern of metribuzin standard, and (C) = mass pattern of metribuzin sample.

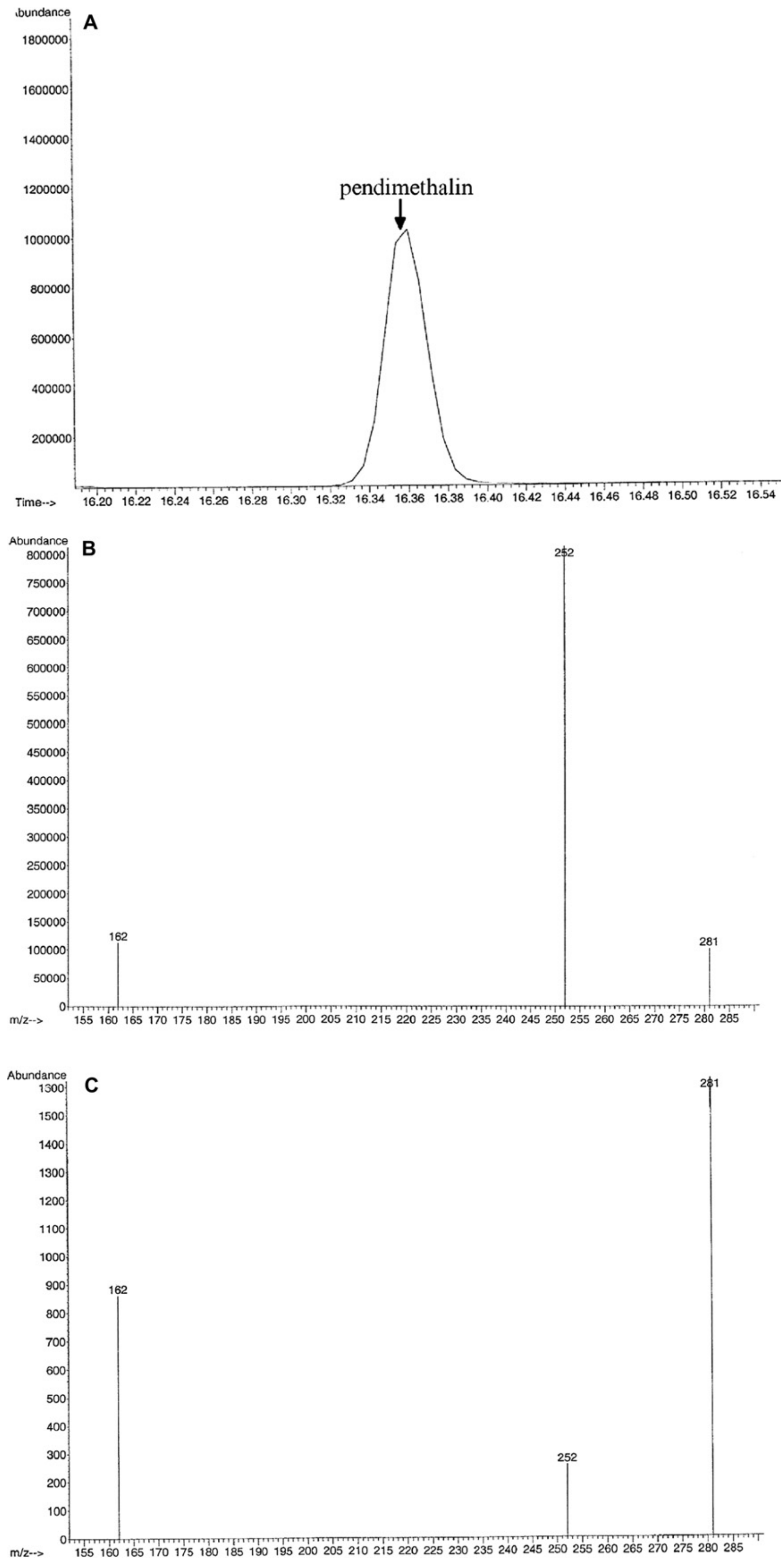

Fig. 2. Determination of pendimethalin residues in vegetable soybean using GC–MS; (A) = chromatogram of pendimethalin standard, (B) = mass pattern of pendimethalin standard, and (C) = mass pattern of pendimethalin sample.

soybean compared with all other herbicides evaluated in the trial. Hand weeding, similarly, gave high crop yield (6806 kg/ha) and was not significantly different from three herbicide treatments (isoxaflutole, 6925 kg/ha; oxadiazon, 7638 kg/ha; acetochlor, 7681 kg/ha) but significantly lower than the other herbicide treatments. Though it gave high yield, however, hand weeding had higher cost for controlling weeds (data not shown). The superior weed control and the absence of herbicide injury to the plants probably contributed to the increased crop yield in the hand weeded plots. Further research is required for acetochlor, isoxaflutole and oxadiazon to evaluate lower doses than the ones used in this study.

Considerable variations occurred in crop and weed response to pre-emergence herbicide application for early weed control in vegetable soybean. Our results clearly showed that pre-emergence herbicides alone can effectively control weeds resulting to high vegetable soybean yield. The excellent weed control afforded by pre-emergence herbicides resulted in very few weeds. Moreover, pre-emergence herbicide treatment controls weeds before or soon after the weeds emerge thereby reducing plant competition. In situations where a pre-emergence herbicide is very effective, a post-applied herbicide may not be necessary. This is in complete agreement with the results reported by Franzenburg et al. (1998) which showed that the improved weed control from the inclusion of a pre-emergence treatment, such as flumetsulam plus metolachlor, increased soybean yields compared with a single post-emergence application of glyphosate alone. Thus, any post-emergence herbicide treatment can be avoided and result to more food and environmental safety. Previous research has shown that soybean treated with pre-emergence pendimethalin followed by post-emergence imazethapyr, imazamox, or imazaquin produced yields equivalent to soybean treated with post-emergence glyphosate alone (Hofer et al., 1998; Parker et al., 1998). It is important to emphasize the fact that post-emergence glyphosate treatments are possible only to some glyphosate tolerant soybean varieties. In addition, use of pre-emergence herbicide eliminates the problems encountered with interactions between total post-emergence tank mixtures, including antagonism on some weed species, and increased vegetable soybean injury. Applying a pre-emergence herbicide also allows the grower to treat weeds before they become too large to be consistently controlled and treated when weather and field conditions allow. Therefore, the use of pre-emergence herbicide alone could provide weed control and it resulted in higher crop yield. In addition, it can reduce the chemical intensity, cost and negative environmental impacts.

3.2. Plant bioassay for detecting herbicide residues in soil

Herbicide residues in the soil was observed after the vegetable soybean crop was harvested using plant bioassay with some tested plants including baby corn (cv. Suwan 3), cucumber (cv. Pijit 1), pak choi (cv. Chinensis), and soybean (cv. CM 60). No significant effect on field emergence and presence of any crop injury due to herbicides were observed (Table 4). The results showed that herbicides, applied alone and in combination, had no phytotoxic effect or growth reduction in all tested plants. There were no visible injury symptoms at 7 DAE and no adverse effects on growth in any of the vegetable crops 1 season after herbicide was applied. In addition, a similar response was also observed at 14 DAE (data not shown).

Baby corn, cucumber, pak choi, and soybean can be safely grown following application of the pre-emergence herbicides evaluated in this study. The results clearly indicate that the residues from pre-emergence herbicides, applied alone and in combination, in the previous season caused no visible injury or growth reduction in baby corn, cucumber, pak choi, and soybean. Herbicides can be considered an effective alternative because it can control weed without leaving residues that may affect subsequent growth of crops. This might explain why vegetable crops are frequently grown in rotation with vegetable soybean in northern Thailand.

3.3. Herbicide residue analysis in vegetable soybean

The positive list system was adopted to improve the regulation of residual agricultural chemicals in foods, especially imported products, that contain above Maximum Residue Limits (MRLs) or the uniform level of 0.01 ppm if MRLs have not been established (MHLW, 2005b; Theo et al., 2006). No residue was detected in vegetable soya crop yield (or MRLs < 0.01 ppm) for any herbicide used in this study (Table 5). Separation of all herbicides used in this study was achieved under fixed gas-chromatographic conditions and, although other peaks were also detected, they showed different retention times and lower intensity than the parent compounds. Peak areas for determination of metribuzin and pendimethalin residues in green soybean using GC–MS is shown in Figs. 1 and 2, respectively. In the present study, no samples containing herbicide residues at detectable concentrations were found. These results agree with the earlier observations with other herbicides such as phenylurea from plants (Fernando et al., 2002) and phenoxy acid in water samples by GC–MS (Pereior et al., 2004). The results of this study showed that GC–MS can be effectively used to detect herbicide residues in agricultural products. The result ensured food safety after herbicide application thus increasing opportunity for exporting to other trade countries like Japan.

4. Conclusions

Many agricultural producers in the northern part of Thailand grow vegetable crops in rotation with vegetable soybean because they can generate higher income than traditional crops (corn, rice, vegetables, etc.) and can break up pest cycles where continuous vegetable soybean is grown. The application of metribuzin at 525 g a.i./ha was sufficient to provide satisfactory full-season control of several weed species and gave the highest crop yield. In addition, pendimethalin at 1031.25 g a.i./ha, and tank-mixed metribuzin at 350 g a.i./ha + pendimethalin at 928 g a.i./ha can provide a similar level of weed control as an alternative to reduce herbicide dosage. This can be used by vegetable soybean growers to reduce herbicide rates, optimize profit and minimize the environmental impact of herbicide application. The results clearly indicate that the residues from pre-emergence herbicides, applied alone and in combination, in the previous season caused no visible injury or growth reduction in baby corn, cucumber, pak choi, and soybean. Thus, herbicides can be considered an effective alternative because it can control weeds without leaving harmful residues. In addition, the judicious use of herbicide could reduce the intensity, costs and negative environmental impact of mechanical and chemical treatment.

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