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The Final Report of Contract No. 95/233P

- Safety in Development: Upgrading of Lab Facilities for Monitoring Pollutant -

A. Multiple pesticide residue analysis in/on agricultural produce

1. Background

Most countries in RENPAP members as well as other part of world are operating monitoring survey on pesticide residues in agricultural produce and environmental samples. However, in general analytical practices on pesticide residue require a large number of chemicals, which are residue-grade, and long period of time. Thus, an individual residue analysis of targeted pesticide in/on agricultural crops is effectively being adopted as legislation purpose of a certain pesticide to be concerned.

Meanwhile, almost all the pesticides registered are described the waiting period from last application to harvest on the label. Agricultural climatic conditions and some commodity of vegetable crops make it difficult to follow these guidelines on waiting period. In particular, leafy or fruit vegetables such as lettuce, tomato, cucumber, etc. are continuously harvested during whole growth period of the crop; it is quite difficult to keep the waiting periods. Also various kinds of fungicides and insecticides are available for the farmers to protect their crops from diseases and insect pests. Furthermore, most farmers in east Asian countries in general do not fully acquainted with the safe use guidelines; national authority responsible for agriculture has to operate residue monitoring activity in/on agricultural products at farmer's gate so that the products over the maximum residue limit are not marketed.

In these context, simple and precise method on multiple residue analysis in/on agricultural produce are requested to evaluate the safety of the products and to inform the residue levels for the farmers.

2. Research highlights

A series of experiment were undertaken to establish the method; extraction, clean-up by catridge and glass column, and quantitative analysis by GC/ECD and GC/NPD. The involved pesticides were 45 comprising organophosphorus, carbamates, synthetic pyrethroids, herbicides, etc. The analytical parameters of GCs are; instrument (Model HP5890II), detector (ECD and NPD), GC column (HP-5, 30m x 0.25 μm thickness), column temperature programming (70 to 270 ℃ at the rate of 5 ℃/min), injector temperature (ECD 270 ℃, NPD 250 ℃), detector temperature (ECD 300 ℃, NPD 230 ℃), fuel for NPD (hydrogen 4 mℓ/min, air 175 mℓ/min), carrier gas (nitrogen 2 mℓ /min), mode (splitless mode), and injection volume (1 $\mu\ell$).

Table 1 gives the list of pesticides to be detectable by GC/ECD or GC/NPD. The pesticides which are very low sensitivity or undetectable are acephate, cartap, ethofenprox, fosetyl-Al, methamidophos, nitralin, propineb, and teflubenzuron.

Table 1. List of pesticides to be detectable and minimum detectable amount by adopted capillary GC analysis

Pesticide	Retention time (min)	Minimum detectable amount (ng)	Detector	MRL (mg/kg)
Alphmethrin	34.512	0.06	ECD	-
Befenthrin	29.373	0.06	"	0.5
Carbaryl	26.476	0.30	NPD	-
Chlornitrofen	26.932	0.30	ECD	-
Chlorpyriphos	28.001	0.03	NPD	1.0
Chlorpyriphos-methyl	26.184	0.07	"	-
Cyhalothrin	33.978	0.06	ECD	1.0
Cypermethrin	34.213	0.06	"	5.0
Deltamethrin	37.618	0.08	"	0.5
Diazinon	24.540	0.03	NPD	0.1
Endosulfan	23.703	0.005	ECD	2.0
Esfenvalerate	36.372	0.08	"	-
Etrimfos	25.093	0.10	NPD	0.1
Fenvalerate	35.927	0.02	ECD	1.0
Flucythrinate	30.445	0.004	"	0.5
Fluvalinate	36.435	0.004	"	1.0
Fonofos	24.047	0.02	NPD	-
Furathiocarb	36.851	0.10	"	-
Malathion	27.665	0.03	"	0.5
Metalaxyl	26.682	0.30	"	0.1
Napropamide	30.821	0.20	"	1.0
Pendimethalin	22.619	0.04	ECD	0.2
Phenthoate	29.582	0.30	NPD	-
Phosalone	36.987	0.20	"	2.0
Phosmet	35.649	0.09	"	2.0
Pirimicarb	25.484	0.02	"	2.0
Pirimiphos-methyl	27.331	0.04	"	2.0
Profenofos	36.161	0.04	"	-
Prothiofos	31.043	0.02	"	-
Pyraclofos	38.420	0.09	"	-
Quinalphos	33.534	0.90	"	-
Terbufos	23.975	0.02	"	0.05
Tralomethrin	37.670	0.20	ECD	0.5
Trifluralin	22.119	0.06	NPD	-

According to the polarity of tested pesticides, various combinations of eluting solvents from SPE catridge and glass column packed with florisil or silica for clean-up of crude extracts of chinese cabbage were checked their recovery from the clean-up materials. The adopted eluting solvent systems are;

A) Solvent system of SPE catridge

I. Dichloromethane/hexane (3/97, v/v) 10 mℓ
II. Ethylacetate/hexane (5/95, v/v) 10 mℓ
III. Ethylacetate/hexane (20/80, v/v) 10 mℓ
IV. Ethylacetate 10 mℓ

B) Solvent system of glass column

I. Dichloromethane/hexane (80/20, v/v) 45 mℓ
II. Dichloromethane/hexane/acetonitrile (50/49.65/0.35, v/v/v) 50 mℓ
III. Dichloromethane/hexane/acetonitrile (50/48.5/1.5, v/v/v) 50 mℓ
IV. Dichloromethane/hexane/acetonitrile (50/45/5, v/v/v) 50 mℓ

The recovered pesticides over 80 % of spiked pesticide amounts from SPE catridge and glass column packed with florisil or silica are shown in table 2 and 3.

Table 2. List of pesticides recovered over 80 % of spiked amount from the clean-up column of SFE catridge with various eluting solvent systems

Eluting solvent	SPE florisil	SPE silica
I	alpha-endosulfan, prothiophos, trifluralin	bifenthrin, chlornitrofen, chlorpyrifos, alpha-endosulfan, prothiofos, quinalphos, trifluralin
II	alphamethrin, chlorpyrifos, chlorpyrifos-methyl, cyhalothrin, cypermethrin, deltamethrin, diazinon, beta-endosulfan, esfenvalerate, etrimfos, fenvalerate, flucythrinate, fluvalinate, fonofos, furathiocarb, malathion, phenthoate, pirimiphos-methyl, profenofos, terbufos, tralomethrin	alphamethrin, cyhalothrin, cypermethrin, deltamethrin, diazinon, beta-endosulfan, esfenvalerate, etrimfos, fenvalerate, flucythrinate, fluvalinate, fonofos, phenthoate, porimiphos-methyl, terbufos, tralomethrin
III	carbaryl, napropamide, phosmet, pirimicarb, pyraclofos	carbaryl, furathiocarb, malathion, napropamide, phosmet, profenofos, pyraclofos
IV	metalaxyl	metalaxyl, pirimicarb

Table 3. List of pesticides recovered over 80 % of spiked amount from the clean-up column of glass column with various eluting solvent systems

Eluting solvent	Florisil	Silica
I	prothiofos	trifluralin
II	alphamethrin, befenthrin, chlornitrofen, chlorpyrifos, chlorpyrifos-methyl, cyhalothrin, cypermethrin, deltamethrin, alpha-endosulfan, beta-endosulfan, esfenvalerate, etrimfos, fenvalerate, flucythrinate, fluvalinate, fonofos, phenthoate, quinalphos, terbufos, tralomethrin	alphemethrin, befenthrin, chlornitrofen, chlorpyrifos, chlorpyrifos-methyl, cyhalothrin, cypermethrin, deltamethrin, alpha-endosulfan, beta-endosulfan, esfenvalerate, etrimfos, fenvalerate, fluvalinate, fonofos, pendimethalin, prothiofos, terbufos, tralomethrin
III	carbaryl, diazinon, malathion, phosalone, phosmet, pirimicarb, profenofos, pyraclofos	diazinon, malathion, phosalone, phosmet, pirimiphos-methyl, quinalphos
IV	metalaxyl, napropamide	carbaryl, furathiocarb, metalaxyl, napropamide, pirimicarb, pyraclofos

The numbers of recovered pesticides by eluting solvents of I, II, III, and IV from SPE florisil catridge are listed 3, 21, 5, and 1, respectively. Whereas, the numbers from SPE silica catridge are 7, 16, 7, and 2, respectively. In consequence, a total of 30 to 32 pesticides are able to be eluted from the catridge by adopting four types of eluting solvent system with an acceptable recovery of spiked amounts.

On the other hand, the numbers of recovered pesticides by eluting solvents of I, II, III, and IV from glass column packed with florisil are 1, 20, 8, and 2, respectively. Meanwhile, the numbers from glass column packed with silica are 1, 19, 6, and 6, respectively. A total of 31 to 32 pesticides are eluted from the glass column packed with florisil or silica by applying four types of eluting solvent system with a reasonable recovery of spiked amounts.

As a result, the established analytical method of multiple pesticide residue is an economical one because wide range of pesticides are able to be analyzed concurrently in a short period of time. Moreover, the method showed a good recovery of spiked residues as compared with conventional clean-up procedure of glass column packed with florisil or silica; therefore, the method is able to reduce the clean-up solvent by one fourth to one fifth.

B. Phytotoxic response of non-selective herbicide residues to vegetable crops

1. Background

In accordance with expansion of cropping area for orchard like apple, pear, grape, and etc., the consumed amounts and sprayed area of the non-selective herbicide; hexazinone, paraquat, glyphosate, or glufosinate, have continuously increased to control the post-emerged annual weeds. Meanwhile, those orchard fields accompany with the nearby fields of paddy rice, cash crops such as tomato, cucumber, or chilly pepper, which resulted in crop damage due to the spray drift as well as contamination of irrigation water to the crops. Even though the herbicide residue monitoring in the irrigating water gives a certain concentration, we are unable to recommend the safe crops in those area irrigated with the herbicide-contaminated water. Therefore, we executed a research project to establish the safe level of hexazinone, dicamba or imazapyr in the irrigating water for the cash crop cultivation.

2. Research highlights

The degradation rate of hexazinone in the spiked fresh water so meager, which showed that 1 ppm concentration level was maintained over 350 days after treatment regardless of the water temperature from 15 to 35C. Various concentrations of the herbicide were irrigated to the water culture and sandy loam soil pot with tomato, cucumber, or pepper. The minimal concentration of the herbicide that gave phytotoxicity in tomato, cucumber, and pepper in sand culture was 0.035ppm, 0.06ppm, and 0.90ppm, respectively as shown in Table 1. While the concentrations in the soil pot were 0.04ppm, 0.06ppm, and 0.10ppm. The phytotoxic symptom was appeared 11 days after irrigation with the relevant concentration in sand culture, whereas it was given 24 days after irrigation in the soil pot.

Table 1. Minimal phytotoxic concentrations of hexazinone in irrigation water to the target crops in different culture conditions

Test crops	Minimal conc of hexazinone to be phytotoxic in	
	Water culture	Soil culture
Tomato	0.035	0.04
Cucumber	0.06	0.06
Chilly pepper	0.90	0.10

On 15 days after crop culture, the residue amounts of the herbicide in and on the leaves and stem portions of the target crops were analyzed by means of GLC/NPD. The residue level in the crop was increased with elevating the concentration of the irrigating water. However, the level in the leaves was always higher than that in the stem irrespective to the crops and treated concentrations as illustrated in Table 2.

Table 2. Hexazinone residues in crop plant grown in water culture containing different concentration of the herbicide

Test crop	Treated conc (ppm)	Harvested portion	Residue (ppm)
Tomato	Untreated	-	<0.010
	0.035	Leaf	0.250
		Stem	0.134
	0.050	Leaf	1.251
		Stem	0.196
	0.065	Leaf	2.188
Cucumber	Untreated	-	< 0.010
	0.030	Leaf	0.720
		Stem	0.086
	0.060	Leaf	1.922
		Stem	0.127
	0.090	Leaf	2.385
		Stem	0.307
Chilly pepper	Untreated	-	< 0.010
	0.030	Leaf	0.118
		Stem	0.033
	0.060	Leaf	1.573
		Stem	0.117
	0.090	Leaf	2.016
		Stem	0.151

Meanwhile, dicamba as an selective herbicide for the control of broad-leaves weeds has also been widely used in golf course, lawn ground or forestry. However, because the chemical possesses a negatively charged molecule after spraying, it cause to leach the soil profile which resulted in phytotoxic to the crops irrigated with the contaminated leacheate or ground water.

The growth response of some crops in soil irrigated by contaminated water with dicamba was also studied. Rice growth, resistant to the chemical, was not influenced by the residue, while the growth of tomato or chilly pepper was severely retarded by 0.001 ppm to 0.003 ppm residue level in irrigation water (Table 3).

Table 3. Phytotoxic effect of dicamba concentration in irrigation water to the target crops in soil culture

Target crop	Concentration (ppm)	Growth response*
Rice	Non-treated	-
	0.10	-
	0.50	-
Tomato	Non-treated	-
	0.0005	+
	0.001	++
Chilly pepper	Non-treated	-
	0.001	+
	0.003	+

* Visual growth response was measured 50 days after treatment.

- No growth impact, + 20% growth retardation, ++ 40% growth retardation

On the other hand, imazapyr, which is newly introduced herbicide in Korean pesticide market, was also showed a severe growth inhibition effect of tomato or cucumber by irrigating with contaminated water of the chemical, 0.004 ppm, regardless of water or soil culture.

3. Research output

By monitoring the herbicide residues in the irrigating waterways, the hexazinone, dicamba or imazapyr level could be assessed the potentiality to outbreak the phytotoxicity on the basis of the current research results. The established residue level of the herbicides in irrigation water for the cultivation of the cash crops was able to be directly conveyed to the farmers to recommend the right crops in those area where the orchard field was surrounded. Furthermore, it is possible to evaluate crop monitoring data on non-selective herbicides whether the phytotoxic symptoms in the crops are caused by the herbicides or not.

Phytotoxic response of selected herbicides to dicotyledon crops

0.06ppm | 0.01ppm | 0.01ppm

0.08ppm | 0.01ppm | 0.01ppm

Hexazinone | **Dicamba** | **Imazapyr**