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Rapid Bioassay of Pesticide Residues (RBPR) on Fruits and Vegetables[1]

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Abstract : A RBPR (Rapid Bioassay for Pesticide Residues) method is developed by TARI in integrating the AChE test and B.t. test to screen the residues of acetylcholinesterase-inhibiting (neurotoxic) insecticides, EBDC's (ethylene bisdithiocarbamates, the ETU-producing chemicals) and other B.t. inhibiting fungicides on fruits and vegetables. The RBPR (1) can complete the examination of a batch of 50 vegetable samples within 3 hrs or even faster, (2) provides toxicological indication on plants sample with residue of organophosphates, carbamates, EBDC's and other detectable fungicides, and (3) is extremely economic and the material costs only ca. N.T.$ 4.0 (= U.S.$ 0.15) per test. The sensitivities of RBPR to 20 organophosphorus compounds, 2 carbamates and 12 fungicides were examined, and a RBPR working flow chart was provided. This method is fast enough to provide information for market inspector to detect fruit and vegetable shipment with toxic response and hold from trading for further chemical analysis. The RBPR has been adopted by farmer associations, municipal markets and public health institutions in Taiwan for various functions i.e., farmer's education, preharvest inspection, market management and pesticide residues monitoring to reduce the toxic vegetable to be marketed. The RBPR can fill up informative and operational gaps between the lengthy chemical analysis of residues and the fast moving vegetable trading.

Key words : Rapid bioassay, Insecticide, Fungicide, Ethylene bisdithiocarbamates, Acetylcholinesterase, *Bacillus thuringiensis*, Pesticide residues.

Introduction

Fruits and vegetables are essential parts of the human diet. They provide carbohydrates, proteins, lipids, vitamines and mineral elements. The production nowadays depends heavily on new technologies, and pesticide usage is almost unavoidable, especially in the subtropical and tropical regions where production confronts with many insect and disease problems. Although some safe new pesticides have been introduced lately, many pest problems still remain unsolved and rely heavily on two groups of

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neurotoxic insecticides i.e., organophosphates and carbamates, as well as EBDC's, the carcinogenic ETU (ethylenethiourea) producing fungicides. Therefore, constantly monitoring the dangerous pesticide residues before marketing fruits and vegetables is absolutely needed to protect the health of general public.

The neurotoxic organophosphorus and carbamate insecticides act on the acetylcholinesterase (AChE) in animal neurosystem, block the neurotransmission and finally cause death. In the well known Ellman's test, the development of yellow color slowed down or stopped when the AChE was inhibited, and can be used to assay the presence of AChE-inhibiting insecticides (Voss, 1966 ; Voss et al., 1971).

Although the acute toxicity of EBDC's to mammals is low, the chronic effect of the carcinogenic and teratogenic ETU, a metabolite of EBDC's, is cautious (Newsome, 1976; Bontoyan and Looker,1973; Fishbein, 1977; Wagner, 1983). Other fungicides such as captan and folpet are also hazardous to human health, and their residues on vegetables should be monitored. Detection of EBDC's is usually achieved by analyzing the carbon disulfide (CS_2) that degraded from EBDC's (Razman and Thompson, 1972), or detecting another degraded product, EDA (ethylenediamine) (Newsome, 1974). Gustafsson and Thompson (1981) had chosen alkaline EDTA solution for EBDC's extraction in HPLC analysis. All those analyses are lengthy and expensive, and their usage in a rapid analysis is limited. Kao (1989) reported the entomopathogenic bacteria, *Bacillus thuringiensis,* is highly sensitive to EBDC's as well as other plant protectants such as captan and folpet, which made the development of a rapid bioassay to detect fungicide residues possible.

The goal of this research is aimed at integrating fungicide assay with AChE assay to detect the above mentioned hazardous pesticides and to keep the residues contaminated fruits and vegetables from marketing.

Materials and Methods

Chemicals, biochemicals and pesticides used

Acetylthiocholine iodine (ATCI), 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB), dimethyl sulfoxide (DMSO), ethylenediaminetetraacetic acid (EDTA), 2,3,5-triphenyltetrazolium chloride (TTC) and Triton X-100 were purchased from Sigma Chem. Company. Methanol, butanol, ethanol, acetone, sodium phosphate buffer and hydrochloric acid (fuming 37%) were supplied from E. Merck. Antibiotic medium #1 was from Difco Laboratories.

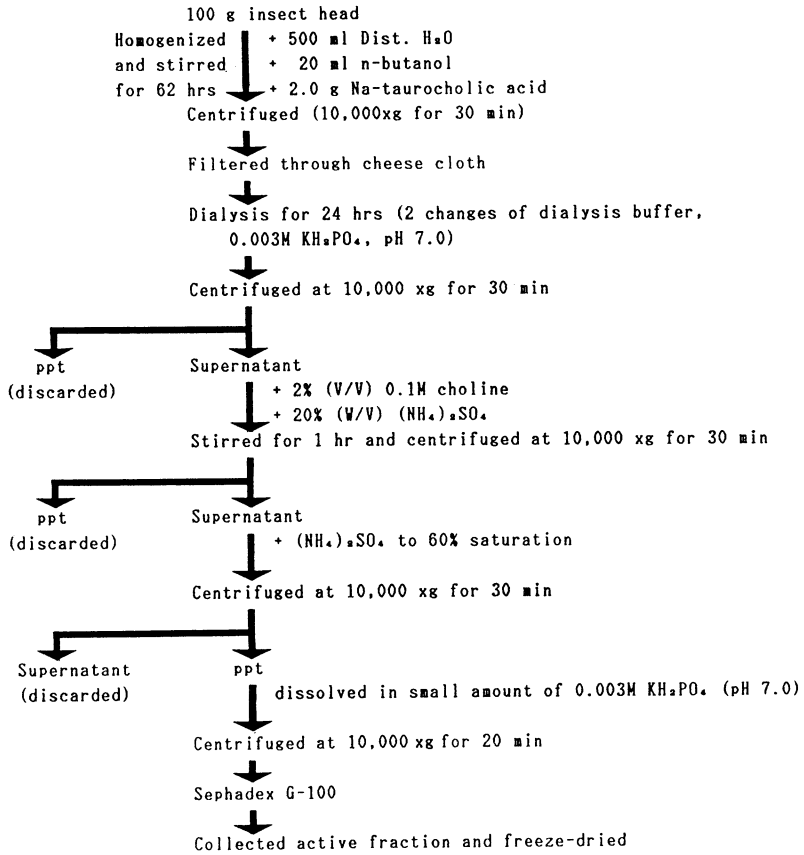
Butyrylcholinesterase of horse serum and acetylcholinesterase of electric eel were purchased from Sigma Chem. Company.

All pesticides used in experiment were commercial products, and the dilutions were made in distilled water.

Preparation of AChEs

Preparations of housefly head and honeybee head AChEs were followed the procedure outlined by Guilbault et al.(1970) up to the step of Sephadex G-100. Collected active fractions were freeze dried. Complete working scheme is presented in Chart I.

Chart I. Insect brain AChE preparation



Preparation of B.t. suspension

By transferring slant culture B.t. into a 50ml flask containing 20 ml broth medium (Salama et al., 1981) and shaking with 140 rpm at room temperature overnight, the obtained broth culture can be preserved in 5°C for a period of 5 days. Before assaying, B.t. in preserved broth culture was activated by transferring 0.8 ml suspension into a 50 ml flask with 20 ml of new broth and incubated at 37 °C with 140 rpm shaking. The new broth culture between 1.5 to 5 hrs after the start of culturing time is suitable for assaying. For the assay, the above B.t. culture in log phase is diluted with fresh broth to 18—20% T-value at 540 nm (with a Shimadzu UV-120-01 spectrophotometer) and then further diluted 10 times as the assaying solution. At this time, the concentration of B.t. is about 0.8—1.2 x 10^7 cells/ml.

Instruments and apparatus needed

Test tube mixer, visible spectrophotometer with recorder, vegetable sampler, rubber cushion, stopwatch and shake bath (with 50—60 sets of 50ml flask holders) are needed for the tests.

Working methods

1. Sampling procedures

Duplicate plant samples were prepared for insecticide and fungicide tests, respectively.

Each sample constituted of 4 leaf discs of 4.5—6 cm^2 each from separate leaves and the total leaf area used for one assay is approximately 18—24 cm^2. After chopping the leaf discs into fine pieces, the sample was transferred into a glass vial (1 cm in dia. x 7 cm in length) for extraction.

A. Insecticide assay : The plant sample was extracted with 1 ml methanol, vibrated on a test tube mixer for 30 sec and then soaked for another 2.5 min. The vials should be covered with parafilm when a longer soaking period is expected. The methanol extract was then drained into a clean vial and covered with parafilm before AChE assay.

B. Fungicide assay : All samples were extracted with 2 ml of EDTA/DMSO solution. After being vibrated on the test tube mixer for 1 min and soaked another 5 min, the extract (approximate 1.3—1.5 ml) was directly drained into a 50ml Erlenmeyer flask.

2. Insecticides residues assay :

A. Preparation of assay materials :

(1) 0.1M phosphate buffer (PBS), pH 7.0.
(2) 0.1M phosphate buffer (PBS), pH 8.0.
(3) Acetylthiocholine iodine (ATCI), 21.6 mg/ml dist. water.
(4) 5,5'dithio-bis (2-nitrobenzoic acid) (DTNB), 19.8 mg/5 ml PBS (pH 7.0)
(5) Methanol.
(6) AChE solution with enzymatic activity adjusted to suitable range for assay.

B. Assay procedures :

In a 1 cm glass cuvette, 3 ml PBS (pH 8.0), 20 μl sample extract or tested insecticide in methanol, and 20μl AChE were added. After mixing for 3 min, 100μl DTNB and 20 μl ATCI were added to start the reaction. The increasing absorbance at 412 nm was recorded and compared to the insecticide-free blank for inhibition. In a fixed reaction period, the absorbance of sample was compared to the normal AChE reaction, and the % inhibition was calculated as follow :

$$\% \text{ inhibition} = \frac{\text{Abs. change (normal)} - \text{Abs. change (sample)}}{\text{Abs. change (normal)}} \times 100\%$$

3. Fungicide residues assay:

A. Preparation of assay materials :

(1) Extracting solution : Aqueous solution of 1000 ppm EDTA containing 2% DMSO.
(2) Metabolic indicator : 2,3,5-triphenyltetrazolium chloride (TTC) in 2% (W/V) solution.
(3) Cultural medium for B.t. : The medium was prepared according to Salama et al. (1981).
(4) Stop solution : 0.5 N HCl containing 5% Triton.
(5) Slant medium : 30.5 g Difco antibiotic medium #1 in 1000 ml distilled water.

B. Assay procedures :

1. Adding 1 ml of the above prepared B.t. suspension into the flask which containing sample extract and incubated in constant temperature water bath with shaking (37°C and 140 rpm) for 90 min.
2. At the end of 90 min, 100 μl of 2% TTC solution was added into the incubation flask and continually incubated and shook for another 30 min in the dark.
3. At the end of incubation, 1 ml of 0.5N HCl in 5% Triton was added into flasks and

mixed well to stop reaction and dissolve the precipitation for red color inspection.

4. The result should be examined by naked eyes first and compared to the blank for color difference. Spectrophotometer examination is not needed for serious case since the color difference is distinct enough.
5. Only the sample with color wondering between red and yellow needs to be centrifuged and compared in a visible spectrophotometer at 484 nm for absorption difference.

$$\% \text{ inhibition} = \frac{\text{Abs. (fungicide free blank)} - \text{Abs. (sample)}}{\text{Abs.(fungicide free blank)}} \times 100\%$$

Results and Discussions

ChEs of horse serum, electric eel and housefly showed variable sensitivities to insecticides, and housefly head AChE is the most sensitive one (Table 1). The sensitivities of AChEs from housefly and honeybee to three insecticides, chlorpyrifos, carbaryl and carbofuran, were further compared (Table 2), and the results indicated that the AChE from housefly head is more sensitive to both organophosphorus and carbamate insecticides. The susceptible housefly strain used for manufacturing AChE has been reared in TARI for more than 25 years and free from insecticides resistance that occurred in wild strains.

Table 1. Comparison of carbofuran-sensitivities in acetylcholinesterases of housefly head and electric eel, and butyrylcholinesterase of horse serum

Leaf area extracted in 3 ml methanol[a]	% enzyme activity inhibited[b]		
	Housefly head	Electric eel	Horse serum[c]
1 cm^2	12.3	7.0	0
2 cm^2	39.0	18.1	0
3 cm^2	56.2	30.6	0
6 cm^2	71.1	45.8	0
9 cm^2	78.6	52.8	0
12 cm^2	85.0	63.9	0

[a] Carbofuran concentration used for spray was 200 ppm.
[b] 50 μl methanol extract was used for enzyme assay.
[c] Butyrylcholinesterase.

Table 2. Comparison of insecficide-sensitivities of acetylcholinesterases from housefly and honeybee heads

Leaf area extracted in 3 ml methanol	% inhibition[b]					
	Chlorpyrifos		Carbaryl		Carbofuran	
	H. F.	H. B.	H. F.	H. B.	H. F.	H. B.
1 cm^2	8.2	6.1	1.3	4.0	41.7	29.7
3 cm^2	16.3	9.6	16.5	33.9	66.7	55.9
6 cm^2	41.9	12.2	32.9	57.3	83.3	79.7
9 cm^2	53.5	16.5	45.6	70.2	84.5	79.7
12 cm^2	65.1	20.9	58.2	80.7	88.1	89.8

[a] Insecticide concentration used for spray were 2,000, 400, and 200 ppm for chlorpyrifos, carbaryl and carbofuran, respectively.
[b] 50 μl methanol extract was used for enzyme assay.

Organic solvents are efficient in extracting the insecticide residues, and directly incorpo-

rate the extract is the simplest way to conduct quick assay, hence the selection of a suitable organic solvent with minimum interference in AChE activity (Lewis, 1967) is important. Acetone, butanol, ethanol and methanol caused various degree of inhibition or activation in the activity of housefly AChE, and methanol had exerted the least effect compared to three other solvents (Fig. 1). Although methanol was chosen as the extracting solvent, the amount incorporated into 3 ml enzyme reaction mixture should not exceed 50 μl. When methanol content exceeded 2% in the mixture, the yellow color will slowly develop (Fig. 2).

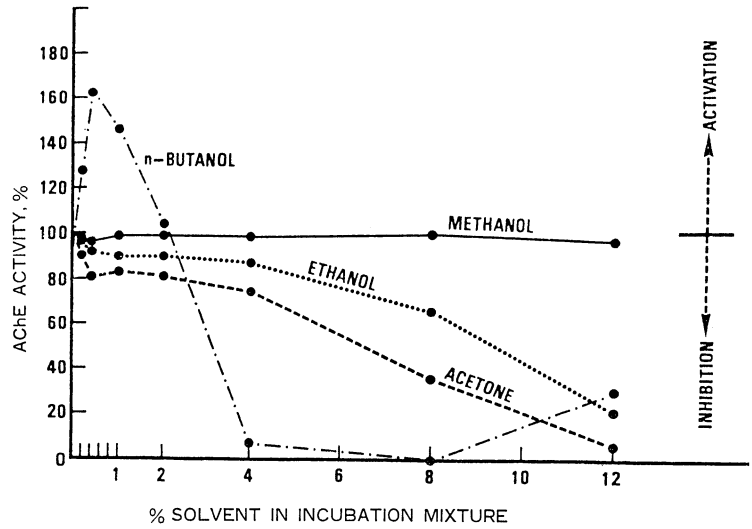


Fig. 1. The inhibition/activation of 4 organic solvents on AChE from housefly head.

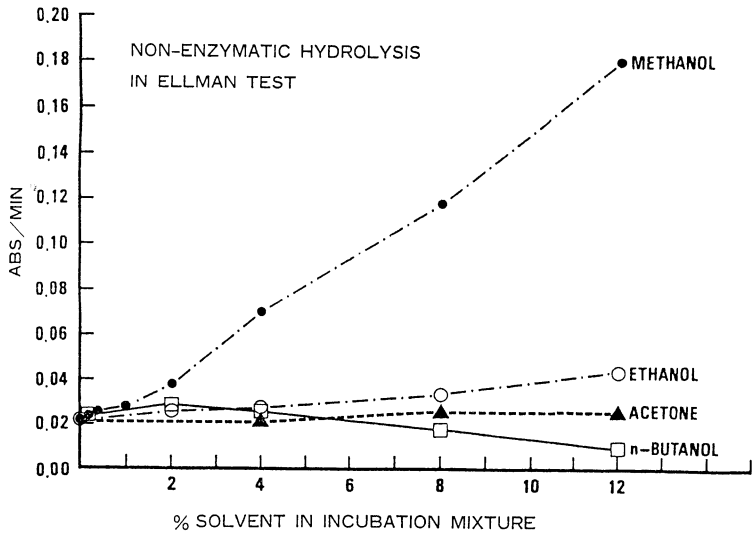


Fig. 2. The color development in the AChE-free Ellman reaction mixture by 4 different organic solvents.

Methanol is desirable for extracting both organophosphorus and carbamate insecticides, and 2 ml of methanol had been used to extract chlorpyrifos from various sizes of leaf discs ranged from 1 to 12 cm^2, and the result showed linear relationship between the amount of chlorpyrifos and the size of leaf used (Table 3). When methanol is less than 2% in the reaction mixture, protection of AChE by methanol from organophosphorus insecticide is less than 2.4% (Table 4). (Precaution: the sample vials must be covered by parafilm to reduce the vaporization of methanol at room temperature, which would result in uncertainty in concentration of extracted residue and also cause health hazard to workers.)

Table 3. The recovery of chlorpyrifos from different size of leaf in 3 ml methanol

Size of leaf	chlorpyrifos[a]/methanol μg/ml(ppm)	$\mu g/50\mu l$	% AChE[b] inhibition
1 cm^2	1.26	0.06	18.2
3 cm^2	3.74	0.19	30.0
6 cm^2	6.70	0.34	53.9
9 cm^2	12.95	0.65	77.2

[a] Determined by gas chromatography.

[b] 50 μl methanol extract was used for enzyme assay.

Table 4. The degree of protection of AChE by methanol from the inhibition of dichlorvos(DDVP)

Methanol conc. in 3ml assay solution	% AChE activity inhibited by 50μl x 0.1126 ppm DDVP	% inhibition reduced
0 %	71.4	—
2.1 %	69.0	2.4
4.1 %	66.7	4.7
7.9 %	62.9	8.5

By allowing a 25% permissible error in the AChE assay, samples with greater inhibition can be considered to have positive response, a undeniable evidence of the presence of neurotoxic pesticide residues on sampled vegetables. The sensitivities for different target insecticides based on the 25% inhibition level were listed in Table 5. The sensitivities of organophosphorus compounds such as dichlorvos, mevinphos, naled, phenthoate, profenofos, chlorpyrifos, pirimiphos-methyl, mephosfolan, methidathion, were about

0.004-5.67 ppm. For dialifor, acephate, quinalphos, fenitrothion, salithion, methamidophos, the sensitivities were 11.2 to 65.6 ppm, while sensitivities to carbofuran and methomyl were 0.059 and 3.4 ppm, respectively.

Table 5. The sensitivity of TARI's housefly head AChE to neurotoxic insecticides*

Insecticides	Detection limit ** ppm	Insecticides	Detection limit ** ppm
Dichlorvos	0.004	Mephosfolan	4.740
Carbofuran	0.059	Methidathion	5.666
Mevinphos	0.138	Dialifor	11.190
Naled	0.218	Acephate	14.770
Phenthoate	0.605	Quinalphos	27.230
Profenofos	1.387	Fenitrothion	27.380
Chlorpyrifos	1.683	Salithion	48.070
Pirimiphos-methyl	1.780	Methamidophos	65.620
Parathion	2.420	Pyridaphenthion	239.200
Methomyl	3.400	Dimethoate	299.370
Hostathion	4.290	Prothiofos	319.600

*Detectable sensitivities on different vegetables depends on the recovery of extraction.
**The detection limit set at 25%

By adapting the TTC test as the indication of B.t. growth condition, 12 fungicides were directly incorporated into B.t. suspension to screen their inhibition abilities, and the results were presented in Table 6. Due to the variation in propagation of B.t., 50 % permissible error was set in the O.D. reading of TTC reaction as detection limit, and the sample with greater inhibition can be considered as positive response which provided evidence for the presence of EBDC's and/or other detectable fungicides. Results in Table 6 showed 10 tested fungicides can be detected from 0.008 ppm to 2.5 ppm, and the sensitivities range fell into the legal tolerance range. In field practice, the sensitivities of EBDC's such as propineb, curzate -mancozeb, metalaxyl-mancozeb, mitram, mancozeb and maneb were above 0.7 ppm, and more than 12.5 ppm for zineb and Sankel. For other fungicides such as chlorothalonil and dinocap, the sensitivity exceeded 2.5 ppm.

Table 6. The sensitivity of *Bacillus thuringiensis* to commonly used fungicides[a]

Fungicides	TTC detection limit[b] (ppm)
Propineb	0.08
Curzate-M	0.16
Ridomil-Mz	0.31
Mitram	0.31
Maneb	0.31
Mancozeb	0.63
Zineb	1.56
Sankel	12.50
Chlorothalonil	1.25
Dinocap	2.50
Captan	2.50
Folpet	6.25

[a] Detectable sensitivity on different vegetables depends on the recovery of extraction.
[b] The detection limit set at 50%.

In the 2nd experiment, the same 12 fungicides were prepared into their recommended dosages and then diluted in a series of 5 to 80-folds in water. Cabbage leaves were dipped into those solutions and dried. Fungicide were extracted from vegetable leaves and tested for their inhibiting abilities on the growth of B.t. The correspondent sensitivities were indicated in Table 7. The third experiment was the field test, and cabbages were sprayed with fungicides at their recommended dosages and assayed from 0 to 9 days after treatment. The results of detectable periods were presented in Table 8.

Table 7. Detecting the residues of cabbage leaf discs dipped with various concentrations of fungicides[a]

Fungicides	Recomm. dosages (ppm)	Dilutions (TTC test in O.D. value)[b]					Check
		5 x	10x	20x	40x	80x	
Maneb	2,000	0.01	0.02	0.01	0.06	0.15[c]	0.41
Mancozeb	1,600	0.03	0.02	0.05	0.24	0.27	0.74
Curzate-M	960	—	0.04	0.06	0.07	0.21	0.74
Mitram	1,600	0.00	0.02	0.04	0.23	0.40	0.74
Chlorothalonil	1,500	—	0.05	0.07	0.08	0.77	0.74
Propineb	1,400	0.00	0.00	0.00	0.13	0.59	0.60
Ridomil−Mz	1,450	0.01	0.02	0.05	0.07	0.28	0.41
Captan	1,250	0.05	0.22	0.82	0.92	0.77	0.74
Sankel	1,625	0.07	0.51	0.60	0.72	0.32	0.60
Folpet	1,250	0.22	0.74	0.92	0.96	0.85	0.74
Zineb	1,750	0.34	0.59	0.77	0.85	—	0.60
Dinocap	195	0.19	0.26	0.28	0.29	0.36	0.32

[a] Leaf discs of 4.5 x 4 cm^2 were used.
[b] The detection limit set at 50%.
[c] Detectable range was underlined.

Table 8. The detectable periods of 12 fungicides at various days after field treatment[a]

Fungicides	Days after treatment (TTC test in O.D. value)[b]					
	0 D	1 D	3 D	5 D	7 D	9 D
Mancozeb	0.01	0.04	0.07	0.19	0.25[c]	0.62
Mitram	0.00	0.03	0.17	0.65	0.77	0.54
Curzate-M	0.00	0.06	0.02	0.06	0.05	0.77
Chlorothalonil	0.00	0.04	0.03	0.04	0.52	0.64
Propineb	0.00	0.03	0.01	0.10	0.31	0.74
Captan	0.00	0.02	0.04	0.15	0.46	0.60
Check	0.57	0.95	0.55	0.74	0.85	0.82
Maneb	0.04	0.03	0.01	0.08	0.51	—
Ridomil-Mz	0.02	0.05	0.00	0.06	0.74	—
Folpet	0.03	0.01	0.02	0.24	0.51	—
Zineb	0.08	0.05	0.20	0.52	0.89	—
Sankel	0.02	0.06	0.12	0.35	0.51	—
Dinocap	0.13	0.19	0.52	0.74	0.85	—
Check	0.40	0.32	0.66	0.74	0.89	—

[a] Leaf discs of 4.5 x 4 cm^2 were used.
[b] The detection limit set at 50%.
[c] Detectable period was underlined.

Due to the low soluability, EBDC's were difficult to extract from vegetables. By using EDTA extraction solution modified from Gustafsson and Thompson (1981), the detectable sensitivities for 12 fungicides on cabbage were listed in Table 9.

Table 9. The detectable concentrations of commonly used fungicides by *Bacillus thuringiensis* in cabbage leaf discs dipping method[a]

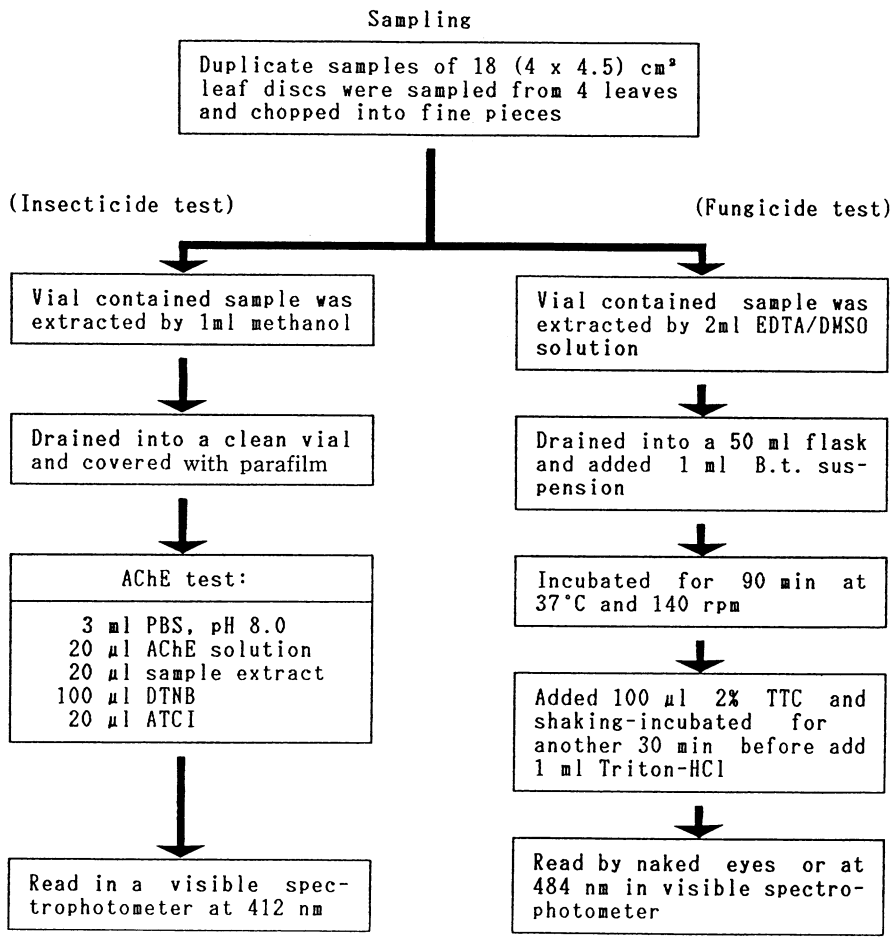
Fungicides	Detectable concentrations[b] (ppm)
Propineb	6.30
Curzate-M	2.16
Ridomil-Mz	6.53
Mitram	7.20
Maneb	4.50
Mancozeb	3.60
Zineb	63.00
Sankel	59.50
Chlorothalonil	6.75
Dinocap	7.02
Captan	22.50
Folpet	45.00

[a] Leaf discs of 4.5 x 4 cm^2 were used, and the average fungicide solution adhered was 180±36 μl.
[b] The concentrations were calculated from Table 7.

Practically, the results of both AChE and B.t. assays are in toxicological sense rather than to identify individual pesticide or to determine its concentration. The results of RBPR assay serve as a positive indication for the presence of target pesticides on sampled vegetables. Other than its simple, fast and economic aspects, the RBPR test can detect the summed toxicity of several pesticides with same toxicological action in one vegetable sample , and no separate tests are needed. If necessary, samples with positive residue response can be further analyzed by chemical method.

Both insecticide and fungicide assays have been integrated into the RBPR working scheme (Chart II), and for a batch of 50 vegetable samples, tests can be completed within 3 hrs. When a vegetable shipment shows sign of pesticide contamination, it is still in time to take proper action to stop the trading. When legal action is necessary, the suspected shipment can be examined by Public Health Bureau before further trading is permitted. Currently, the RBPR has been officially adopted in farmer's associations, wholesale markets and Public Health Bureaus in Taiwan.

Chart II. RBPR working flow chart



Special notation and appendix for RBPR

Notation :

The above mentioned rapid assay method for fungicide residues should not be used on plant materials with natural inhibition ability in B.t. growth, for example, lemon, garlic, ginger and tea.

Appendix :

Modifications in fungicide assay have to be made for plant materials with excess organic acids or anthocyanins. The low pH itself will inhibit the growth of B.t., and anthocyanins develop a red color in acidic condition which interferes the TTC reaction. Strawberry is a good example to illustrate both modifications needed. There are two major changes in the above mentioned RBRP-fungicide assay for strawberry. First, the addition of 0.1M Na_2HPO_4 buffer to neutralize the excess organic acids from strawberry extract. Second, methanol is used instead of HCl in terminating the color reaction.

Modified assay procedures are :

1. 18 cm^2 outer skin of strawberry was sampled from 4 strawberries.
2. The outer skin was extracted by 2 ml EDTA/DMSO solution as described in previous method.
3. Pipetted 0.75 ml EDTA-strawberry extract into a 50ml Erlenmeyer flask, and added 0.75 ml 0.1 M Na_2HPO_4 solution and mixed well.
4. Added 1 ml B.t. suspension and made sure the pH of mixture was between 7.2—7.4 in a preliminary run, and then incubated for 90 min at 37°C and 140 rpm.
5. Added 100 μl 2% (W/V) of TTC solution and then shaked the mixture continually for another 30 min.
6. Added 0.3 ml 10% Triton and let the color develop for 5 min.
7. Added 2 ml methanol to stop the color reaction.
8. After centrifuged at 1000xg, the O.D. value of the supernatant is measured at 484 nm and compared to the fungicide free check.
9. Sensitivities of above assay for detecting propineb and mancozeb in strawberry were 0.8—4.9 ppm and 2.8—5.6 ppm, respectively.

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蔬果農藥殘毒快速檢驗技術之研究[1]

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摘　要

臺灣省農業試驗所自民國48年推行蔬菜農藥殘毒生物檢驗以來，因農藥種類激增，蔬菜產銷流程加速且範圍擴大，故再次改進殘毒檢驗技術，期能增加檢驗農藥之種類，減少檢驗過程所需時間以及消除誤判之可能性。本項研究已完成對乙醯膽鹼酯酶（Acetylcholinesterase）造成抑制之有機磷劑、氨基甲酸鹽類及混合劑之適用農藥種類及敏感度之測試，內含18種蔬菜上常用及4種非蔬菜使用殺蟲劑。殺菌劑之檢驗主要係針對危險性最高之有機硫磺類優先進行，並以對殺菌劑極敏感之 *Bacillus* 屬細菌爲檢定菌，最後選定對哺乳類動物安全性極高之 *Bacillus thuringiensis* var. *kurstaki* 爲檢定用標準菌，以維護檢驗人員及環境之安全。12種受測之蔬菜用殺菌劑檢測之敏感度略高於法定容許範圍，故經檢出之樣品無誤判之虞。

此二項檢驗技術經整合其流程後，可在3小時之內一併完成50件蔬菜樣品之整體檢測，合乎一般市場檢驗室抽驗之要求，如用於早期快速預檢，可以過濾出含毒較高之樣品，以便通知農民延期採收，將已採收之蔬菜再清洗或送請衛生單位依法定檢驗過程查明殘毒之種類及其含量，以爲取締之依據。

上述殺菌劑之檢驗不適用於樣品含有抑制蘇力菌生長之物質，但部份樣品之抑菌物質或性狀可經由修改檢驗技術而克服。例如草莓所含之果酸會抑制蘇力菌之生長，可經由調整緩衝液之強度，修正 pH 值，使適合檢定菌之生長；而草莓所含花青素，由於在酸性情況下會自動呈現紅色，干擾檢驗結果，因此須將呈色用之酸性溶液改爲甲醇，即可進行正常之檢驗。

關鍵詞：快速檢驗、殺虫劑、殺菌劑、有機硫磺劑、蘇力菌、乙醯膽鹼酯酶、殘毒。

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1. 臺灣省農業試驗所研究報告第 1570 號。本計畫承國科會補助部份經費（NSC—71—0204—B055—01），李月嫦、李琇媛協助工作，謹此一併致謝。

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RBPR working flow chart

(Illustration on next page)

Sampling



Insecticide Test

Fungicide Test

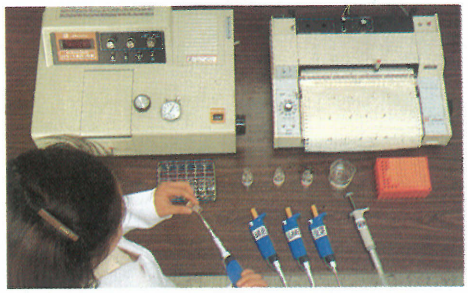
Extraction



Extraction



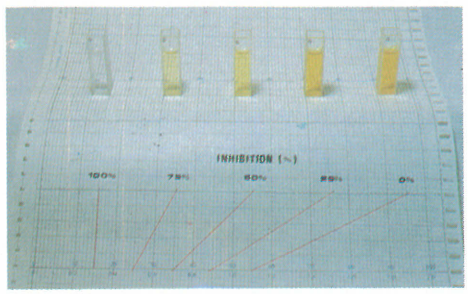
Enzyme assay



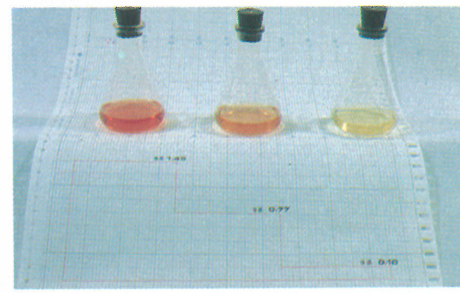
Incubation



Result／Conclusion



inhibition blank



blank inhibition

RBPR working flow chart

