

TRAINING IN INSECTICIDE-KIT TECHNOLOGY AND PESTICIDE SAFETY EDUCATION FOR VEGETABLE SAFETY MONITORING IN FARMS

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ABSTRACT

The agricultural sector of Thailand composes 38% of the work force, farmers will continue to use large amounts of insecticide unless safety-monitoring schemes are developed. Organophosphate, carbamate and pyrethroid insecticides are commonly used and prevalence of insecticide-related symptoms exist among the farmer respondents. This insecticide safety-monitoring study in farms aimed to train farmers on pesticide safety and validate with farmers this insecticide-kit technology as well as obtain intellectual property petty-patent. To follow-up farmer testing competency, 67 vegetable samples' testing comparison between farmers and competent analysts from reference laboratory, post-training result was 91.9% tested accuracy by favorably acceptable competency. A quasi study in farm communities investigated intervention effectiveness through laboratory measures of testing insecticide residues in 31 intervention kale samples compared to 31 control vegetables, using test kit technology and spectrophotometry. Percent acetylcholinesterase enzyme assay inhibited by insecticides, was statistically compared by general linear model repeated measure ANOVA for intervention effect in both groups as well as evaluate effectiveness by comparing difference between baseline and follow-up measurement in both groups. Percent enzyme inhibition risk decreased 6.13 units more in the intervention than control group. Intervention effect p-value was 0.001, with statistical significance. Pesticide safety education to farmers aimed for safe farm production with less toxic pesticide use and safety awareness. The training for vegetable farm safety monitoring can be applied in other communities for agricultural safety.

Key words: training vegetable farmers, insecticide test kit, pesticide safety education, safety monitoring

INTRODUCTION

Global safety has focused on the manufacturing sector (National Sustainable Agriculture Coalition, 2009). Increased production and consumption of health promoting vegetables has been the mission but contaminated pesticides reduced the safety of many vegetables in developing countries (World Vegetable Center, 2013). An average of 134,380 tons of pesticides were imported to Thailand in 2012, compared to 52,739 tons imported in 2000 that showed 155% massive increase in quantity during 12 year span (Tirado, 2008 and Klaimala, 2013). In recent years, the four groups insecticide residues of

organophosphate, carbamate, pyrethroid and organochlorine were detected in agricultural products. The first three groups were popularly used. The organochlorine group was however banned in many countries (Leuprasert et al. 2010), but it was still being used in Thailand (Sombatsiri, 1997).

Residue testing laboratories are important instruments to screen for a better guarantee of the safety of agricultural produce but it has been difficult, complicated, expensive, and especially time consuming to use reference laboratories for the analysis. Currently, a 4 group all-in-one insecticide test kit for food safety monitoring was not available in Asian markets, before this invention. A validated test kit with petty patent from the Intellectual Property department of Thailand, was invented by authors and team for rapidly screening 4 insecticide groups in agricultural produce (about 1 hour for 10 samples). Insecticide monitoring by training 4 groups test kit technology to strengthen agriculturists, should be focused for insecticide safety (Leuprasert et al. 2010; Leuprasert et al. 2012).

Many farmers in Thailand believed that pesticide application was necessary and continued to use of large amounts of pesticides unless a campaign was conducted to educate farmers on pesticide safety attitude and proper pesticide use. Agricultural products accounted for 9% of Thailand's gross domestic product and made up approximately 19% of Thailand's total export value (Panuwet et al. 2012). In 2011, the agricultural sector of Thailand was 38% of the work force (Office of Agricultural Economics, 2013). The trained and skilled farmer force is the core of the agricultural safety system. The objective of this study was therefore aimed for farmer safety monitoring by training insecticide test kit technology and pesticide safety education to ensure vegetable safety as United Nation's key steps for safeguarding health and public well-being (Food and Agriculture Organization, 2014).

METHODOLOGY

A cross-sectional design was studied during 2012-2014 in Klongtabak community, Sikhew district, Nakhon Ratchasima province, Thailand to develop farmer safety monitoring for ensuring agricultural safety by using insecticide test kit to detect insecticide residues in field-laboratory and pesticide safety education to farmers. Pesticide use and symptoms prevalence among studied farmer group, were reviewed from the authors' previous study that identified the insecticide baseline prevalence data.

The invented test kit was validated by validation method testing in 139 vegetable and fruit samples by comparing to national standard methods (Atisook et al. 2009 and Central laboratory, 2011). Vegetables with known spiked insecticide standards using gas liquid chromatography (GLC) and high performance liquid chromatography (HPLC).

A quasi study was implemented in intervention Chinese kale farm to study effectiveness of the used insecticide test kit and pesticide safety education tools for farmer safety monitoring. The study was approved by the institutional review board of ethical committee of Chulalongkorn University, Thailand.

RESULTS AND DISCUSSION

Most used insecticides, were successively identified as percentage of farmer-users were organophosphate, 75%, carbamate, 50% users and pyrethroid, 30% users. Used pesticide kinds of the insecticide groups were counted as percentage of the total 28 used kinds, to be organophosphate 35.7%, carbamate 14.3% and pyrethroid 7.1% (Table 1).

The farmers' symptoms were neurologic, neuromuscular, eye, alimentary tract, respiratory and symptoms on skin of 80, 75, 50, 45, 35 and 25 percentage of users successively (Table 2). These symptoms were similar to symptoms caused by insecticide exposure (Leuprasert et al. 2014).

Table 1. Insecticide use and used insecticide group counted as percentage of total farmer users and used insecticide kinds

Insecticide group	Number of users	%Users of total farmers (N=20)	%Used Kinds of total Insecticide kinds (N=28)
Organophosphate	15	75	35.7
Carbamate	10	50	14.3
Pyrethroid	6	30	7.1

Table 2. Insecticide-related symptoms prevalence among intervention farmers

Insecticide-related symptoms	Number of Users (N=20)	% Users of Total farmers	Top symptoms Prevalence
Neurologic symptoms (headache, dizziness and whirling)	16	80	1
Neuromascular symptoms (fatigue, cramp, heart rate rise and weakness)	15	75	2
Eyes symptoms (blurred vision, irritation and running tears)	10	50	3
Alimentary tract symptoms (nausea, vomiting, stomach ache and diarrhea)	9	45	4
Respiratory symptoms (running nose, cough/ sore throat, chest congestion)	7	35	5
Symptoms on skin (rashes, irritation, itching and sweating)	5	25	6
Farmers (n=20) with symptoms	13 ≤ 3 7 > 3	100	1- 6

Invented Insecticide Test Kit Technology

The 4 group insecticide test kit was invented and obtained petty patent from intellectual property department, Thailand (Leuprasert et al. 2012). The prior 2 group insecticide of organophosphate and carbamate were detected by Ellman’s method of acetyl cholinesterase enzyme (AChE) based detection technique (Leuprasert et al. 1982). Organophosphate and carbamate esters that had ability to inhibit at least 50% AChE, could be detected by the test kit (Leuprasert et al. 2010 and Fucuto, 1990). The other 2 groups of pyrethroid and organochlorine were detected by using chemical technique to be photosensitive and then exposed to 254 nanometer UV light that the chemical absorbing compounds appeared as dark color. Pyrethroid and organochlorine that had UV absorbable organohalogen could be detected by the invented test kit (Thongbor et al. 2011)

The 4 group insecticide test kit used a combination of thin layer chromatography (TLC) with the AChE inhibition, chemical and physical techniques and detected organophosphate and carbamate insecticides appeared as white zone on purple TLC chromatogram background while detected pyrethroid and organochlorine insecticides showed the dark black brown zone on light brown TLC chromatogram (Fig. 1, 2).

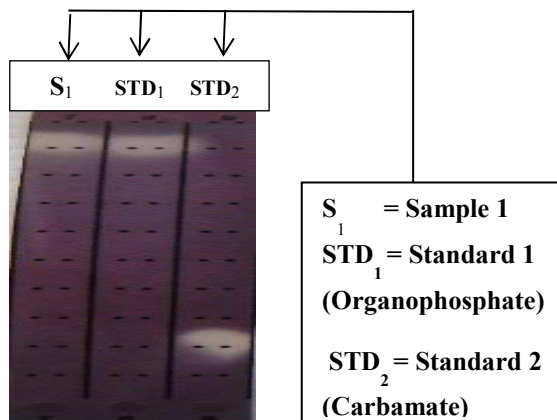


Fig. 1. The 4 group-insecticide test kit in agricultural samples by comparing test results of visible white spots on purple background comparing to standards, organophosphate and carbamate color on TLC

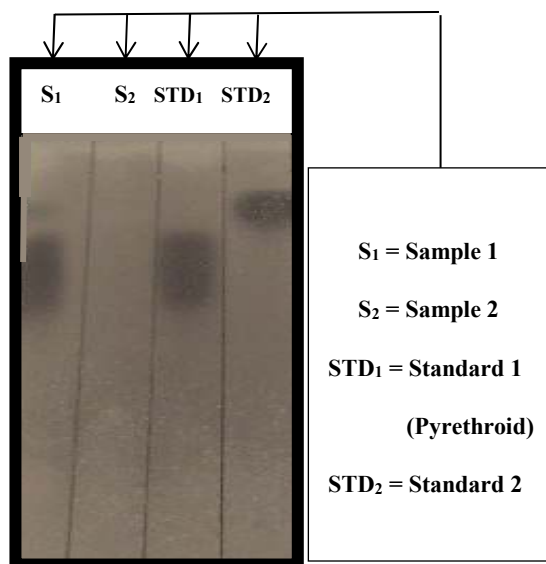


Fig. 2. The 4 group-insecticide test kit in agricultural samples by comparing results of visible black brown spots on light brown background compared to standards pyrethroid and organochlorine color on TLC

Limit of detection (LOD) of organophosphate and carbamate such as carbofuran, chlorpyrifos, diazinon, chlorfenvinphos, dicotophos, dichlorvos, monocrotophos, profenofos, methomyl, carbaryl, fenobucarb and isoprocarb in vegetable were 0.5, 4.2, 3.1, 0.04, 1.3, 0, 0.05, 1.3, 0.07, 1.6, 1.0, 8.3 and 6.0 microgram per gram successively. LOD for organochlorine and pyrethroid insecticides such as endrin,

endosulfan, DDT, cypermethrin, permethrin and deltamethrin, were 0.3, 0.7, 0.2, 1.5, 1.0 and 1.1 microgram per gram, respectively (Thongbor et al. 2011). The obtained validated data of accuracy, specificity and sensitivity were 96, 100 and 86 percent respectively (Fig. 3). The positive predictive value was 100% and negative predictive value was 94% (Table 3).

		Standard Method		
		+	-	
Test Kit	+	36 TP	0 FP	Total
	-	6 FN	97 TN	
		42	97	139

Description
 FP = False Positive
 FN = False Negative
 TP = True positive
 TN = True Negative

Fig. 3. Validated 4 group-insecticide test kit in agricultural samples by comparing test results with standard methods using gas chromatography and liquid chromatography

False positive and false negative are probability that the test kit will classify the samples as positive when they are in fact negative by standard method (false positive), or as negative by standard method- positive (false negative). True positive and true negative are the probability that the test kit will classify the samples as positive when they are surely positive by standard method (true positive), or as negative by standard method- negative (true negative).

Table 3. Validated predictive values 4 group-insecticide test kit by comparing test results with standard methods using gas chromatography and liquid chromatography

The 4 group test kit-Positive predictive value; Number of positive samples	The 4 group test kit-Negative predictive value Number of negative samples
True positive samples (TP) = 36	True negative samples (TN) = 97
False positive samples (FP) = 0	False negative samples (FN) = 6
All positive samples (TP+FP) = 36	All negative samples (TN+FN) = 103
Positive predictive value = $TP/(TP+FP)100$	Negative predictive value = $TN/(TN+FN)100$
Positive predictive value = $36/(0+36) \times 100$ = 100%	Negative predictive value = $97/(97+6) \times 100$ = 94%
Total agricultural samples = 139 (N = 139)	Total agricultural samples = 139 (N = 139)

Sample Size of Farmers and Vegetable, Sample Collection and Testing

The Chinese kale samples were purposively selected because of highly contaminated insecticide residues (Popattanachai et al. 2013) and Chinese kale is very popular vegetable among Asian consumers. Previous test measurements of cholinesterase enzyme inhibition in 23 Chinese kale vegetables by the authors, provided a basis for vegetable sample size calculation of collected 31 samples in study group and 31 samples in control group using the following equation to calculate vegetable sample size (Kadam and Bhalerao 2010).

Mean and standard deviation at 95% confidence level at power 80.

$$n = \frac{2(Z_{\alpha} + Z_{1-\beta})^2 \sigma^2}{\Delta^2}$$

Where n = sample size Z = constant (two-tailed effect)

σ = standard deviation Δ = delta (difference in pre-post survey)

$$\text{Sample size } n = 2 (1.96 + 0.84)^2 \sigma^2 / \Delta^2$$

Standard deviation of 23 trial Chinese kale analysis (σ) = 8.99

Mean %enzyme inhibition at pre-survey of previous test = 12.8

Mean % enzyme inhibition at expected post-survey 50% less (delta) = 6.42

$$n = \frac{2 (2.8)^2 (8.99)^2}{(6.42)^2}$$

Vegetable sample size (n) of each intervention and control = 31

The 31 vegetable samples in intervention Klongtabak farms and 31 samples from control farms in Janthuek community, Nahkon Ratchasima province were collected for study.

About 50 farmer stake holders (1 per household) from intervention community, were trained for general education of pesticide safety and basic insecticide test kit for safety monitoring. At least 20 purposively selected group of farmers (Wongwanich and Wiratchai 2003) by strength for intensive practice of testing 4 group residues using the insecticide test kit.

Vegetable samples were collected, tested and laboratory results were compared in both groups. The analysis was performed by the authors and medical scientists from Regional Medical Sciences Center 8, 9. Department of Medical Sciences, Thailand by using invented insecticide test kit for identification and spectrophotometer for the enzyme inhibition assay. The collected and tested 62 Chinese kale samples from both groups, one sample was collected from one vegetable plantation and Thai agricultural standard method of sampling for determination of pesticide residues was followed for vegetable sampling procedure (Ministry of Agriculture and Cooperatives, 2008).

Data Analysis

To measure the intervention effectiveness by independent t-Test and general linear model (GLM) repeated measure ANOVA by comparing the mean percentage difference of the insecticide cholinesterase inhibiting activity in Chinese kale samples between baseline and follow up tests in both groups. The GLM parameter estimates, was used to calculate magnitude of the intervention effects at the follow-up time

The licensed SPSS software for windows version 19 was used for study data analysis. The paired T-Test comparing pre intervention and post intervention %A ChE inhibition means in both groups for the direct test of intervention effect, at the follow up testing-insecticide risk of cholinesterase inhibiting activity, was statistically compared with the vegetable baseline activity in both groups and generated for quantitative data analysis, linear model parameter estimates, repeated measure ANOVA in SPSS was used to calculate the magnitude of the intervention effect for measuring the investigating effectiveness.

RESULTS AND DISCUSSION

Training Farmers Insecticide Test Kit Technology and Pesticide Safety Education

The farmer training of agricultural safety and application tools for strengthening safety monitoring activities were established by cooperation with farm safety team, supporters, leaders, trainers, advisors, medical scientists and community stake holders in the study farms. Agricultural safety was educated by the authors and agricultural experts to train agriculturists and stake holders the use of insecticide test kit technology and pesticide safety education for safe vegetable farm production, sustainable approach to manage pests and crops by use of integrated pest management and awareness of proper cultivation practice and safe produce where residues should not exceed maximum residue limit levels (IPM Danida, 2004). Participatory farmers were therefore educated with knowledge of pesticide education for proper pesticide use and good agricultural practice to reduce highly hazardous pesticide use, correct pesticides dosage and appropriate waiting period before harvest for reducing pesticide residues in agricultural product. The trained educational tools of insecticide test kit technology and pesticide safety, was prepared, produced, supplied, transferred directly and through public/social media, and educated to farmers and stake holders such as pesticide safety manual, handbook, test kit procedures, video, leaflets, poster, power point presentation etc. and also field-visits' courses to learn agricultural best practice (Leuprasert et al. 2013).

The study workshop, training, seminar, best practice-field visits and participatory learning were performed for transferring technology of the invented 4 group insecticide test kit and pesticide safety education to studied farmers and stake holders. The activities were discussed, planned, co-operatively brainstormed, organized and implemented 5 times of official classroom workshop and several individual meetings during April 2013 to October 2014. Cooperative learning among participants created work plan, priority setting, development activities and study progressive outputs for agricultural safety in their community.

Farmer Self-Testing and Competency Practice Measure for Vegetable Safety Monitoring

Self-testing establishment in this study was aimed to strengthen vegetable community to develop laboratory test of insecticide residues in vegetable farms. Management of testing process system of pesticide residue, required that the farm laboratory should have management persons who could support and technical farmers with testing competency. Klongtabak laboratory's top community management, the chief executive of sub-district administrative organization, director of Agriculture and Technology Nakhon Ratchasima College and the village chief announced and documented policies and procedures for laboratory test of pesticide residues in farms. The Klongtabak laboratory farmer groups had the competency certificate of self-test laboratory workshop training and competency testing of insecticide residues in fresh vegetable and fruit from Regional Medical Sciences Center 9, Thailand (Leuprasert et al. 2013).

The laboratory competency was measured by farmers' testing of insecticide residues in agricultural samples using the insecticide test kit. The same collected vegetable samples from studied farms were tested by farmers and competent analysts from reference laboratory for comparing farmer-testing accuracy. The collected 30 and 37 vegetable samples, were tested at baseline and follow-up testing respectively. To test the farmers' competency by inter-laboratory test comparison in the tested Chinese kale vegetable, test progress result of decreased fault tests (8.1% false tests) after follow up training was 91.9% accuracy that was favorably acceptable competency and documented, when compared with 23.3% false tests and 76.7% accuracy at baseline testing using the invented test kit (Table 4).

Table 4. Farmers’ competency test comparison with competent analyst between baseline and follow up testing by using invented insecticide test kit

Insecticide test kit; Tested by farmers	Baseline (N = 30)		Follow up (N = 37)	
	Fault Test (%)	Accuracy (%)	Fault Test (%)	Accuracy (%)
Competency test	7 (23.3%)	23 (76.7%)	3 (8.1%)	34 (91.9%)

To investigate the effectiveness of the farmer training activities for insecticide safety

To investigate the study effectiveness by testing insecticide residues in collected samples for percentage cholinesterase inhibiting enzyme activity in vegetables by the reference laboratory method (Regional Medical Sciences Center 8, 2011). The difference of insecticide inhibiting enzyme activity, mean percentage enzyme inhibition test in collected vegetable samples between pre (baseline) and post (follow-up) intervention test, was measured (Table 5, and Fig. 4). The 4 group insecticide residues were screened in the 62 vegetables by the invented insecticide test kit. Reference methods were then used to analyze all the samples in both groups at pre and post-test (Leuprasert, 2012; Regional Medical Sciences Center 8, 2011). At pre-test, 5 detected or suspected unsafe vegetable samples by the test kit were quantitatively analyzed by GC and HPLC, then compared results with international standards maximum residues limit (CODEX’s MRLs) and the 3 detected insecticide kinds (<CODEX’s MRLs) from intervention farms which were cypermethrin of pyrethroid group in two vegetable samples, methomyl and carbofuran of carbamate in one sample. The other two kale samples from the control farms had detectable chlorpyrifos residues that were greater than the CODEX MRL of organophosphate in vegetable (Central Laboratory, 2011; Steinwandter, 1989). During the follow-up test, no insecticide residues were found in both groups by the authors using the 4 groups invented test kit and the enzyme inhibition assay using spectrophotometer by Regional Medical Sciences Center 8 (2011).

Mean percentage of enzyme inhibition decreased 6.13 units more in intervention group than in control group. The - 6.13 intervention effect, the T-statistic was 3.804 and the significance (2-Tailed t-Test) p-value of this intervention effect was 0.001 (Table 5, 6 and Fig. 4). Thus the intervention was associated with a statistically significant beneficial effect on the percentage enzyme inhibition caused by the insecticide risk.

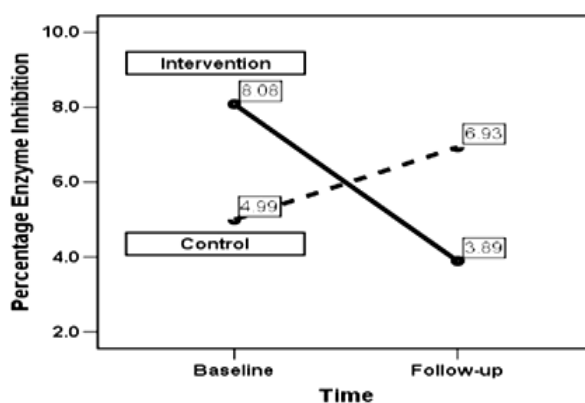


Fig. 4. Mean percentage enzyme inhibition test in collected vegetable from intervention and control groups at baseline and follow-up time of measurement

Table 5. Comparing effect size of mean % enzyme inhibition between intervention and control group at intervention periods (Baseline and Follow up)

Insecticide risk % Enzyme Inhibition (Mean %I AChE)	Control (n = 31)	Intervention (n = 31)	Difference in difference = Effect size (P- value) (Mean %Change at 95%CI)
Baseline (Pre)	8.08	4.99	
Follow-up (Post)	3.89	6.93	
Standard deviation	2.350	8.661	
Standard error mean	0.422	1.556	
Post Minus Pre (Difference in both groups)	-4.19	1.94	-4.19 – (1.04) = - 6.13 (P = 0.001) (2.857 - 9.406)

Table 6. General linear Model repeated measures ANOVA comparing mean pre and post difference between intervention and control tests

Parameter Estimates Confidence Interval			T-Test for Equality of Means			95% of the Difference	
Statistics	T-Statistic	df	Sig (2-Tailed) <i>p</i> - value	Mean Difference	Standard Error Difference	Lower Bound	Upper Bound
Post (Follow-up) Minus Pre (Baseline) Equal variance not assumed	3.804	34.393	0.001	6.131	1.612	2.857	9.406

CONCLUSION

This quasi study investigated the intervention effectiveness of the farmer-training insecticide test kit technology and pesticide safety education for vegetable safety monitoring in farms. The application of invented insecticide test kit, used instrument for safeguarding test in agricultural produce by farmers, was established by farmer-test laboratory of insecticide residues field test in Klongtabak farms with community objectives and policy support from top management. The farm laboratory had competent farmers with laboratory training certificates, had documented top management policy and procedures with available hard and electronic educational documents and insecticide test records. If tested insecticide residues were above maximum residue levels, farmers were trained to label the unsafe samples and communicated to stake holders for discussion and participation to solve problem using the training knowledge.

For follow up competency test, some insecticides particularly organophosphate and carbamate had cholinesterase enzyme inhibiting activity and %enzyme inhibition was measured in vegetable to evaluate difference of the insecticide risk variables after intervention study. General linear model repeated measure ANOVA was used to test the intervention effects which was associated with a statistically significant beneficial effect on the reduced percentage enzyme inhibition caused by insecticide residues. The insecticide risk reduction was effectively resulted by the farmer safety monitoring practice. The laboratory field test by farmers with integration of target group-trained learning materials and education. World Health Organization has mentioned the importance of educating the public as well as health-care and agriculture

workers about health risks that public education programs have been found to increase the farmers' realization of serious health consequences associated with rational use of pesticides (Mancini et al., 2005).

More intensive skill base and farmer safety monitoring is required particularly continuous support by key decision and policy makers who might reflect in budgets for sustainable practices and efficient systems to enhance safety monitoring for safer vegetable initiative on food security. The options could add value to the agricultural products and increase marketing opportunities for farmers that could be applied in other communities for beneficial agricultural safety monitoring regionally.

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