PREFACE

1. The reasons to choose the topic

Using of plant protection drugs (PPD) for pest control is one of the important measures in agriculture. Rational drug use with proper techniques will help to reduce losses, improvescrop's productivity and yield also brings high economic efficiency. So the demand of drugs increases every year in quantity and category.

However, due to their toxic characteristics,pesticides pollute the environment and affect public health. Also, due to improper techniques in using pesticides, their effectiveness is decreasing, hence the dose is increasing. It affects the environment andcausespestsresistant. Nowadays, in Vietnam there are some pesticides which are resisting pest such as biological pesticide Bacillus thuringiensis (Bt), brown plant hopper insecticide Imidacloprid.

In order to increase the efficiency of the drugsand partly overcome pest resistance, scientists have studied and proposed corrective measures.Among these, using of synergists mix with plant protection drugsis an advanced measure in pest control, bringing high economic and social benefits. Among the synergists, substances originated from fatty acids in vegetable oils have particularly got interest because they are made from natural sources which are available and environmental friendly (non-toxic, biodegradable and no residue).

Synergistic substances have been studiedaround the world and have been put into use for decades but in Vietnam there is nearly no published research in this direction.

2. Objectives of the study

The objective of the dissertation is to prepare and use some synergistic substances derived from vegetable oils, in order to raise the effectiveness of prevention of biological pesticide Bacillus thuringiensis (Bt), brown plant hopper insecticide imidacloprid. At the same time, the author puts the first step to discover the mechanism of action of synergistic substances on some detoxification enzymes and enzymes that cause insect resistance.

3. The scientific and practical meaning

The author hasselected two types of vegetable oils which are suitable sources to prepare two synergists by mixingwith BT pesticides and brown plant hopper imidaclopridinsecticides to create new products which is less toxic and more efficient in preventing some drug resisted pests in Vietnam such as diamondback moths and brown plant hopper (BPH). This is the first study to apply the principles of green chemistry in the field of sustainable agriculture in our country.

During the research, the author has successfully applied some new methods to process and prepare products from original sources, which are grains that contain vegetable oil: Useof enzymes during the extraction of oil; raising levels of methyl oleate by complexion method and 2 phases-crystallization with urea. The author also applied theexperimental planning methodology to determine the optimizing technique parameters; using ionic liquid catalyst in cross esterification reaction of vegetable oil into mixture of methylester (FAME); Study the biological effectiveness of different mixed products on a specific insect, thereby determine the optimal formula. In addition, the dissertation has studied the mechanism of action of synergists on inhibition of somedetoxified enzymes of insects thereby preliminarily explain the actionthat increases the effectiveness of prevention and elimination of pesticides. The results of this study have demonstrated the scientific value of the dissertation.

New pesticides and BPH products which are less toxic and have high biological effectiveness will bring great economic and social effects, contributing to the sustainable development of agriculture in our country. Hence the dissertation has great practical significance when applied to the production. It can create a new direction to apply synergistic substances in processing Plant protection drugs to increase the efficiency of prevention and reduce environment pollution in Vietnam.

4. Contributions of the dissertation

1. Have a full investigation of substances, that have synergistic effect with biological pesticide Bacillus thuringiensis (Bt), brown plant hopper insecticide imidacloprid then select an appropriate synergist to prevent and eliminate drug-resistant pests and hoppers in Vietnam: mixture of K-oleate and K-linoleat (approximately 54/43 w/w) is a synergist with Bt insecticide to control diamondback moths which are harmful to crucifers *(Plutellaxylostella)*and methyl oleate is a synergist with imidacloprid to control brown plant hoppers that are harmful to rice (*Nilaparvatalugens*Stal).

2. Collect and investigate content level of oil and unsaturated fatty acids C18 of 20 materials containing vegetable oil in Vietnam. Based on investigation data, the dissertation has selected peach kernel oil as a raw material suitable for the preparation of a synergist for Bt insecticides and Camellia grain oil for the preparation of a synergist for brown plant hopper insecticide imidacloprid

3. Based on the mechanism of action and the ability to break down plant cell walls of certain enzymes, the dissertation has studied, selected and used protease enzyme to extract oil from camellia grains and peach kernels. This method reduces the amount of toxic solvents needed in comparison with other methods of separating vegetable oilstherefore, it contributes in reducing environment pollution.

4. Use ionic liquid catalyst min$C_4H_8SO_3H.CH_3SO_3$to perform cross esterification of Camellia oilintomethyl ester. The process of separation and purification is simpler. This is a new research direction recommended to apply green chemistry in organic synthesis.

5. Study of the mechanism of action of synergist on some detoxifed enzymes of harmful insects. Results showed that the mixture of K-oleate and K-linoleat has strong effect on inhibiting aminopeptidase enzyme (APN) of diamondback moths (*Plutellaxylostella*); methyl oleate has strong inhibiting effect on Cytochrome P450 monooxygenase enzyme (CPY) and weak inhibiting effect onenzymGlutathione S-Tranferases (GST) of the brown planthopper (*Nilaparvatalugens*Stal.). This is a new result, published for the first time in Vietnam, contributes to explain the mechanism of action, methods of metabolism of synergist in the insects' body, thus the dissertation proposed a measure to overcome drug resistance of insect to increase biological effectiveness of plant protection drugs in Vietnam.

5. Structure of dissertation

The dissertation consists of 141 pages and is divided into the following sections: Introduction – 02 pages; overview - 37 pages; Experiment - 16 pages; Results and discussion - 70 pages; conclusion - 03 pages; new contributions - 01 page; A list of published works - 01 page; references - 13 pages (including 123 documents). The dissertation has 42 tables, 37 figures and graphs.

B - CONTENT OF THE DISSERTATION

CHAPTER 1 - OVERVIEW

This section has an overview of plant protection drugs, which focuses on BT pesticides and brown plant hopper imidacloprid, synergists, methods to prepare synergists, methods to process pesticides using synergists.

Chapter 2: EXPERIMENTS

The experimentshave been conducted in the National Key Laboratory Petrochemical and Refinery Technologies - Vietnam Institute of Industrial Chemistry and Institute of Natural Compounds Chemistry - Vietnam Academy of Science and Technology.

2.1. Investigations to determine the synergist for Bt and Imidacloprid pesticides

Explore a mixture of different substances and different ratios of K-oleate / K-linoleat to determine the synergist and optimum dose of rate for diamondback moth Bt pesticide in Vietnam. Explore and identify the synergist for brown plant hopper imidacloprid insecticidesin Vietnam. Then choose the vegetable oil that has an appropriate composition to get an original material for the preparation ofsynergist.

2.2. Extraction of vegetable oil by enzymatic hydrolysis method to be a material for synergist synthesis

Vegetable grains are shelled, finely ground, hydrolyzed in enzyme added water. After that, carry out a centrifugal process to remove sediments, other impurities and obtain vegetable oil.

2.3. Preparation of synergist for Bt pesticide

Vegetable oil is reacted with KOH in ethanol environment. Obtained products are free fatty acids.

Use the urea complexion method to separate saturated oleic and linoleic acids from acid mixture.Adjust crystallization temperature to maintainrequired ratio of oleic / linoleic acid.Then carry out acomplete reaction with KOH to get potassium oleate salt and potassium linoleate salts required to get a synergist for Bt.

2.4. Preparation ofsynergist forImidacloprid pesticide

Using of ionic liquids to carry out a cross-esterification reaction of vegetable oils, transformstriglycerideinto methyl ester mixture (FAME). Then using a 2 phase crystallization urea complex with FAME to separate and improve level of methyl oleatethat is qualified to get synergist forimidacloprid.

2.5. Making of a mixture of synergist and plant protection drug

Biological effectiveness test methodhas been used in the laboratory to determine the formula fora mixture of synergist and Bt insecticide to makeimidaclopridwhich has the highest controlling efficiency. Select and studya processing form for new pesticides and insecticides

2.6. Biological effectiveness tests of new products

Carry out a biological effectiveness test of new pesticide on Diamondback moth *Plutellaxylostella* moths and brown plant hopper *Nilaparvatalugens*Stalin Vietnam, under the provisions of QCVN 01-1: 2009 / BNNPTNT about testing pesticides, mites and QCVN 01-29: 2010 / BNNPTNT about testing of brown planthoppers insecticide. Thereby determine a synergistic factor (SF) and the effects of these synergists in improving the controlling efficiency ofcorresponding plant protection drug

2.7. Study the mechanism of action of synergists

The mechanism of action of the synergists onBt pesticide andImidaclopridinsecticide is defined by the ability to inhibit digestive enzymes APN of diamondback moths, GST detoxifying enzymes, CYP of BPH and concentration of samples to inhibit 50% of enzyme (IC_{50}).

2.8. The methods to analyze and evaluate product quality

The nature and quality of raw materials and products are characterized by popular physicochemical methods such as:

- Some physicochemical properties, iodine index, acid index, saponification index, oil content wasdetermined according to the corresponding Vietnam standards.
- IR Methods to analyze the structure, GC and GC-MS to determine the composition and content of fatty acids, methyl esters and synergists.
- Quality of new pesticide products that containBt are evaluated according to TCVN 8050: 2009 and TCCS 09: 2010 /BVTV; quality of new brown plant hopper insecticides that contain imidacloprid are evaluated according TCCS 07: 2006 and TCCS 135: 2014.

2.8. Data processing methods

Mode 5.0 software has been used to handle data and calculate empirical optimization. The test data on biological effectiveness of plant protection drugs are statistically processed by software Minitab 17 and IRISTART 4.0 soft ware.

CHAPTER 3: RESULTS AND DISCUSSION

3.1. INVESTIGATION AND DETERMINATION of SYNERGISTS FOR Bt and Imidaclorid pesticides

3.1.1. Investigationon unsaturated fatty acid C18 (oleic acid, linoleic acid) in oil seeds

Results of investigation on oil content, oleic acid, linoleic components of some nuts are presented in Table 3.1

Table 3.1. Content of oil and oleic acid, linoleic acid in some grains

No.	Name	Scientific name	Oil content (%)	Oleic acid content (%)	Linoleic acid content (%)	Harvest location	Note
1	Rubber	*Heveabrasiliensis*	40-60	35,07	42,36		[128]
2	Soybean	*Glycine max*	23,1	32,0	53,4		
4	Peanut	*Arachishypogaea*	44,5	41,6	34,9		
5	Sterculia	*Sterculiafoetida*	52,36	38,88	8,83	Sơn La	[129]
6	Aleurites	*Aleuritesmoluccana* (L)	68,93	24,26	40,17		
7	Sesame	*Sesamumindicum*	53,32	32,25	40,67	QuảngTrị	[130]
8	Camellia	*Camellia sasanqua* Thunb.*)*	60,4	79,48	9,29	QuảngNinh	
9	Canarium	*Canariumtokinensis*	2,48	14,9	81,13	Sơn La	
10	Setaria	*Setariaitalica (L.)* Beauv.	3,5	16,39	80,35	HòaBình	
11	Canarium	*Canariumtokinensis*	5,1	1,23	71,88	HàNội	
12	Llicium	*Lliciumleiophyllum*	10,2	-	72,8	Tam Đảo	
13	Brucea	*Bruceajavanica*(Bumea) Merr.	5,3	49,7	35,34	Đắklắk	
14	Sapium	*Sapiumdicolor*	21,32	52,97	30,61	HàNội	
15	Allospond	*Allospondiaslakon*	8,7	16,9	68,1	HàNội	

	ias	*ensis*					
16	Prunus	*Prunuspersica*	35,2	55 ,0	32,5	LàoCai	
17	Brassica	*Brassica napus*	43,1	60,0	19,6	HàGiang	
18	Zanthoxylum	*Zanthoxylumavicennae*	17,69	66,73	11,18		
19	Musa acuminata	*Musa acuminata*	0,58	53,47	25,62		
20	Mangifera	*Mangiferaindica L*	4,2	38,36	6,82		

3.1.2. Determination of a synergist for Bt

Table 3.2: Result of S/Bt mixture in Diamondback moth control in laboratory

Formula	**Experiment substance (S)**	**Ratio (w/w) K-oleate/K-linoleate**	**Effect (%)**	
			After 5 days	**After 7 days**
CT01	Vi-BT (for comparison), 0,25%	-	55,6abc	65,3bc
CT02	Vi-BT (for comparison), 0,125%	-	33,6e	35,7d
CT1	S1 + Vi-BT	54/43	66,7a	82,7a
CT2	S2 + Vi-BT	54/0	34,0de	37,6d
CT3	S3 + Vi-BT	0/43	19,6f	23,0e
CT4	S4 + Vi-BT	54/13	45,0cd	61,3c
CT5	S5 + Vi-BT	54/33	50,7bc	72,0abc
CT6	S6 + Vi-BT	14/43	52,0bc	63,3c
CT7	S7 + Vi-BT	34/43	54,0abc	69,9bc
CT8	S8 + Vi-BT	44/43	61,3ab	74,3ab
CT9	S9 + Vi-BT	Oleic acid/Linoleic acid: 54/43	17,7f	38,5d

CT10	S10 + Vi-BT	Peach kernel oil	17,9ef	27,2cd
CT11	S11 + Vi-BT	potassium salt of Peach kernel oil	37,6de	38,0d

From the above results, it can be seen that with the same dose,mixture of K-oleate salt and K-linoleatsalt with different ratiosall yield higher effect of eradicating diamondback moths than Vi-Bt (only Bt). At the ratio of 54/43, the result is highest. Thus, the best synergist of Bt pesticide is K-Oleate/K-Linolate at the ratio 54/43.

From table 3.1, it can be seen that peach kernel (No. 16) contains high content of oil and oleic acid, linoleic acid which is suitable to get material to prepare synergist for Bt pesticide/

3.1.3. Determination a synergist for Imidacloprid brown plant hopper insecticide

Substances to mix with Imidacloprid are: S1 – Methyl oleate; S2 – Methyl Linoleate; S3 – Oleic acid; S4 – Linoleic acid; S5: FAME of camellia oil.

Table 3.3. Results of eliminating brown plant hopper of S/I mixture

Formula	**Experiment substance (S)**	**Dose (mg/L)**	**Effect (%)**	
			After 3 days	**After 5 days**
CT1	S1 + I	0,5 S1 + 0,5 I	73,19a	84,86a
CT2	S2 + I	0,5 S2 + 0,5 I	55,36b	73,53b
CT3	S3 + I	0,5 S2 + 0,5 I	51,36b	62,31c
CT4	S4 + I	0,5 S2 + 0,5 I	48,24c	62,2c
CT5	S5 + I	0,5 S2 + 0,5 I	52,3b	63,4c
CT6	Vicondor 50EC	1,0	60,72b	69,83bc
CT7	Vicondor 50EC	0,5	41,13d	45,31d

Comment: Oleic acid, linoleic acid and corresponding methyl esters both give higher effect in eradicating hoppers comparing withVicondor 50EC, which proves, they have the effect to increasethe potency of Imidacloprid. Among them, methyl oleate (S1) is the best synergist withImidacloprid in controlling brown plant hoppers which are harmful to rice.

Based on above investigated results, we can choose camellia grain (with high oleic acid content) as raw materials for the preparation of methyl oleate as synergist withImidacloprid in order to improve the efficiency of controlling brown planthoppers *Nilaparvatalugens*Stal, which are harmful to rice in Viet Nam.

3.2. EXTRACTION OF OIL BY HYDROLYSIS WITH ENZYMES

Enzymes used for investigation are: cellulase, protease, amylase, pectinase. The results showed that when added with enzyme, the efficiency of hydrolysis reaction to separate oil from camellia grains and peach kernels increases dramatically compared with samples (without enzyme). Oil can be obtained at highest level using protease enzymes (Table 3.5).

Table 3.5: Effects of enzyme types on the efficiency of oil separation from camellia grain and peach kernel

Enzyme	Efficiency of oil separation from camellia grain (%)	Efficiency of oil separation from peach kernel (%)
Without enzyme	5	4
Cellulase enzyme	45,2	40,6
Protease enzyme	73,2	70,2
Amylase enzyme	31,9	28,7
Pectinase enzyme	50,5	46,5

The test results based oninvestigation of hydrolysis conditions (from figure 3.1 to figure 3.6) shows that:

- With camellia grains: content of enzyme is 0.6%; duration: 3 hours; temperature: 50^0C
- With peach kernel: content of enzyme is 0.8%; duration: 5 hours; temperature: 50^0C

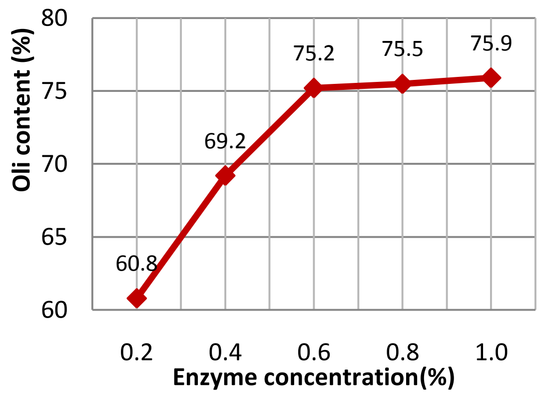


Figure 3.1. Effects of enzyme concentration on efficiency of oil separationfrom camellia grain

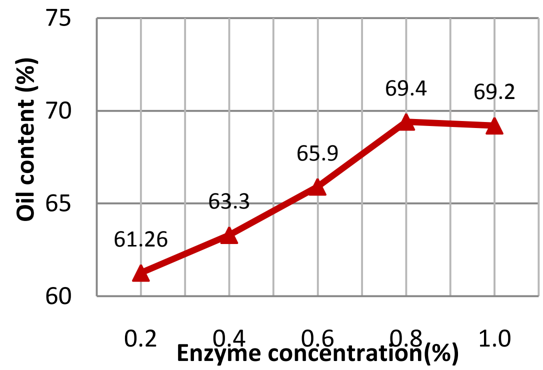


Figure 3.2. Effects of enzyme concentration on efficiency of oil separation from peach kernel

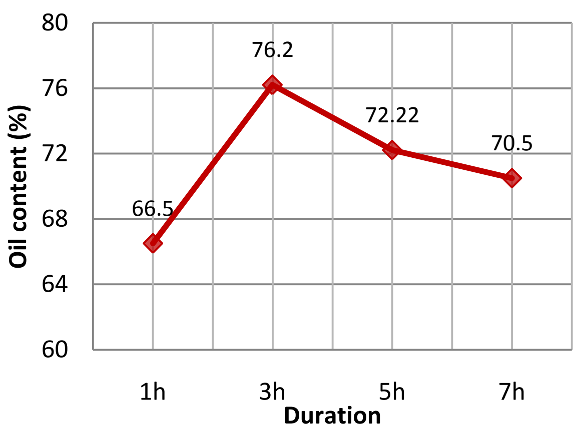
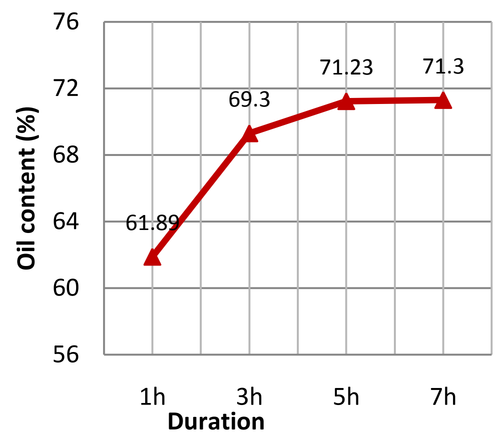


Figure 3.3. Effects of hydrolysis duration on efficiency of oil separation from camellia grain



Hình 3.4. Effects of hydrolysis duration on efficiency of oil separation from peach kernel

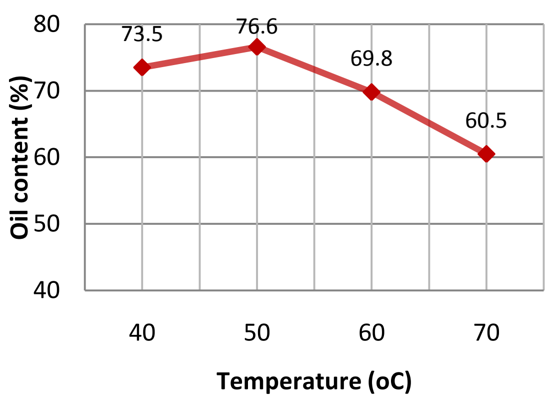
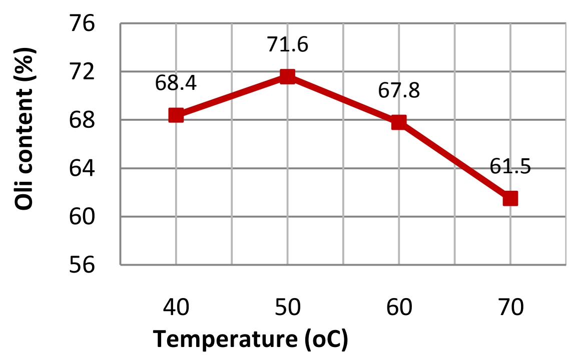


Figure 3.5. Effects of hydrolysis temperature on efficiency of oil separation from camellia grain



Hình 3.6. Effects of hydrolysis temperature on efficiency of oil separation from peach kernel

3.3.PREPARATION OF SYNERGISTS FOR BT PESTICIDE

3.3.1. Preparation of fatty acid mixture from peach kernel oil

ExploringKOH/ethanol conditions of glyceride hydrolysis in peach kernel oil to obtain fatty acid mixture, we get the following appropriate technical parameters: Ethanol and KOH/ oil ratio: 200ml/100g; Concentration of KOH content in ethanol is 1.75M; ethanol concentration: 80%; reaction temperature: 70^{0}C; reaction duration: 120 minutes. IR spectrums of oil samples and fatty acid mixture are taken.

After the reaction, the product is characterized as follows:

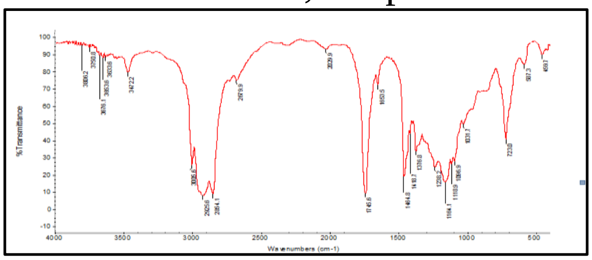


Figure 3.9. IR spectrum of peach kernel

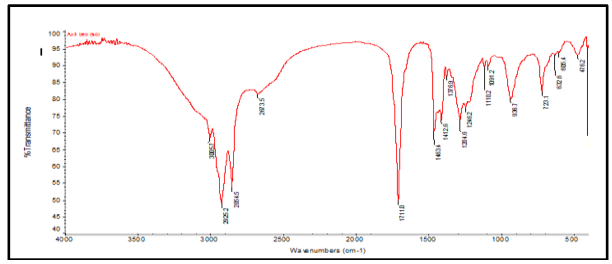


Figure 3.10. IR spectrum of fatty acid from peach kernel oil

IR spectrum (Figure 3.9 and 3.10) showsthat triglyceridewas completely transformed into fatty acids.

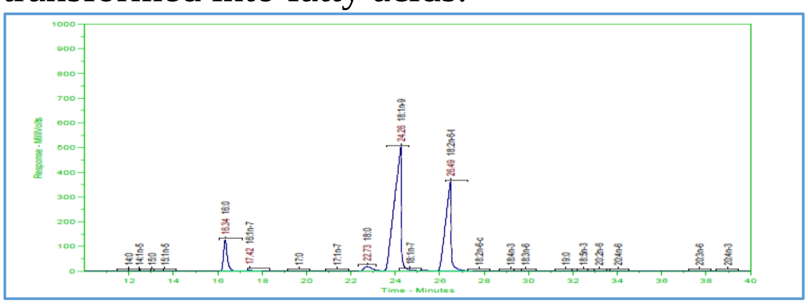


Figure 3.11. GC chromatogram of mixture of fatty acid and peach kernel oil after hydrosis

GC analysis results showed that oleic acid content is 57.6% and linoleic acid content is 32.4%. Content of remaining saturated fatty acids is 9.7%

3.3.2. Separation of mixture of oleic acid and linoleic acid

Oleic acid and linoleic acid has been separated from received acid mixture by urea complexion, crystallized in ethanol. Investigating different factors that affect the process of separation reveals a the following results: urea/FFA ratio: 1/1 - 3/1; Temperature: -5 ^{0}C to 5^{0}C; Duration: 6-18 hours (Table 3.12 - Table 3:14)

Table 3.12 Impact of urea/FFA ratio on oleic acid/linoleic ratio

urea/ FFA ratio (w/w)	Oleic acid/linoleic acid ratio (%w/w)	Content of remaining saturated fatty acid (% w/w)	Recovery Efficiency (%w)
0,5/ 1	56,8/34,6	8,6	77,3
1/1	55,4/37,9	6,7	76,9
2/1	53,9/43,2	2,9	70,6
3/1	41,27/56,73	2,0	65,6
4/1	26,73/71,68	1,59	57,5

Table 3.13. Effect of temperature on oleic acid / linoleic acid ratio

Temperature (^oC)	Oleic acid/ linoleic acid ratio (%w/w)	Content of remaining saturated fatty acid (%w/w)	Recovery Efficiency (%)
10	56,03/33,9	10,47	75,4
5	54,4/38,7	6,9	72,6
0	54,33/43,28	2,39	70,3
-5	48,27/49,23	2,5	68,9
-10	46,73/50,68	2,59	66,4

Table 3.14. Effect of crystallization time on oleic acid / linoleic acid ratio

Duration (hr)	Oleic acid/ linoleic acid ratio (%w/w)	Content of remaining saturated fatty acid (%w/w)	Recovery Efficiency (%)
2	52,34/44,31	3,35	61,4
4	52,78/44,0	3,22	62,5
6	53/43,89	2,98	65.2
8	53,27/43,76	2,97	67,9
12	54,35/43,63	2,92	70,83
16	54,36/43,62	2,02	70,83
20	54,36/43,62	2,02	70,83

To find the optimal conditions for crystallization, empirical planningmethodology level 2, orthogonal center is used to examine the dependency of technical factors on content of oleic acid and linoleic acid.

Oleic acid regression equation is as follow:

$\hat{y} = 53,089 - 4,482x_1 + 1,781x_2 - 5,11x_1^2 - 1,79x_2^2 + 1,44\ x_3^2$

Linoleic acid regression equation is as follow:

ŷ = 43,148 + 4,2x1– 1,51x2 + 0,49x3 – 2,64x12– 1,85x22

Results of optimization calculation shows: urea / FFA ratio: 2; temperature: 0 ^{0}C; Duration: 16hr. Based on these conditions, we obtain oleic/linoleic mixture with content approximately 54/43 (%), remaining saturated fatty acids is 1.13%.

3.3.3. Synthesis of Potassiumoleate and Potassium linoleate to get synergist for Bt

Bt bacteria is strong in high alkali environment, that's why when Bt are mixed with Potassium oleate and Potassium linoleate mixture, the product environment needs to be low of alkali (pH ≤ 8.5). Thus it is necessary to control the amount of KOH solution added into reaction to have a product with required pH. After studying it is found, 70ml of 20% KOH is required to neutralize 100g fatty acid. Reaction efficiency is 85.8%

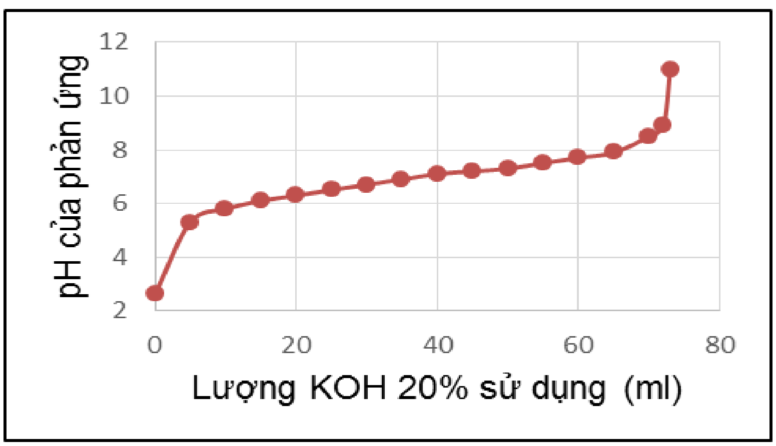


Figure 3.17. Influence of the amount of KOH solution 20% on pH

3.4. SYNTHESIS OF SYNERGIST FOR BPH IMIDACLOPRID

3.4.1. Preparation of methyl ester from Camellia oil

After studying it is found, the optimized conditions for trans-esterification of Camellia oil with methanol, using ionic liquid catalyst ($minC_4H_8SO_3H.CH_3SO_3$)to create methyl ester mixtures (FAME) are: methanol/oil molar ratio: 12/1; reaction temperature: 75^0C; Duration: 8 hours; Catalyst content: 10% of oil weight. Results are shown in figure 3.20 to 3.23. Reaction efficiency is 91%.

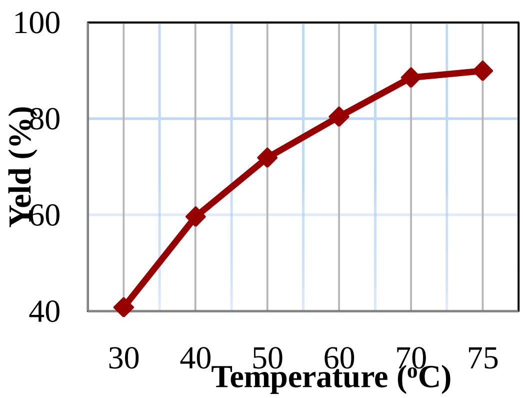
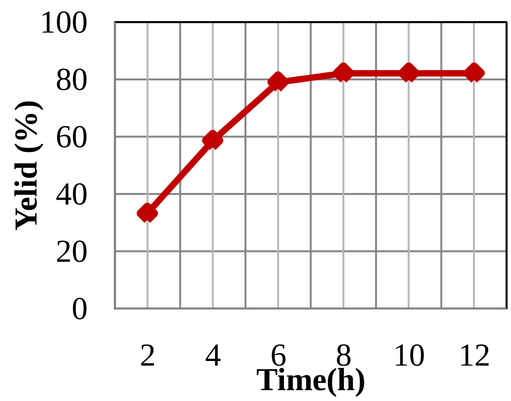
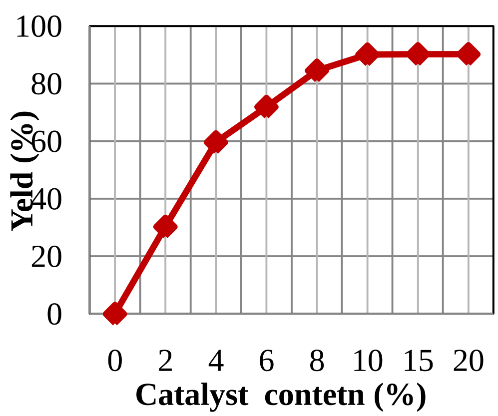


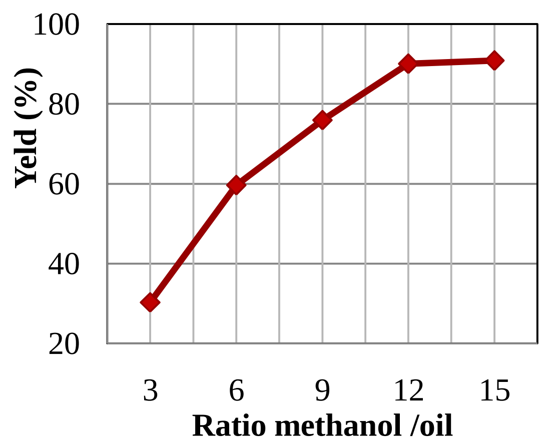
Figure 3.20.Effects of temperature on reaction efficiency



Hình 3.21. Effects of duration time on reaction efficiency



Hình 3.22. Effect of catalyst content on reaction efficiency



Hình 3.23.Effect of methanol/oil ration on reaction efficiency

Table 3:26. FAME component of camellia oil

.	**Fatty acid**	**Methyl ester**	**Content %**
1	18: 1(n-9)	Methyl oleat	**82,36**
2	18: 2(n-6)	Methyl linoleat	**9,29**
3	16: 0	Methyl palmitate	6,5
4	18:0	Methyl stearate	1,86

3.4.2. Separation and enrichment content of methyl oleate

In order to obtain high content of methyl oleate, urea crystallization method should be followed in two phases: Separate saturated FAMEs; Enrich content of methyl oleate needed to be synergist.

Like separating fatty acid, there are many factors that affect process technology, product purity and efficiency of methyl ester recovery. Result of investigation are shown in figure 3.25, 3.26, 3.28, 3.30 In summary, conditions for separating and raising content of methyl oleate are as follow:

- Phase 1 (Separating saturated FAME):
  + urea / FAME ratio: 1/1 (w / w);
  + ethanol / FAME ratio: 5/1;
  + Crystallization temperature: 10^0C;
  + Crystallizationduration time: 6hrs
- Phase 2 (Enrich content of methyl oleate):
  + urea / FAME ratio: 1/1 (w / w);
  + ethanol / FAME ratio: 5/1;
  + Crystallization temperature: 0^0C;

+ Crystallizationdurationtime: 6hrs

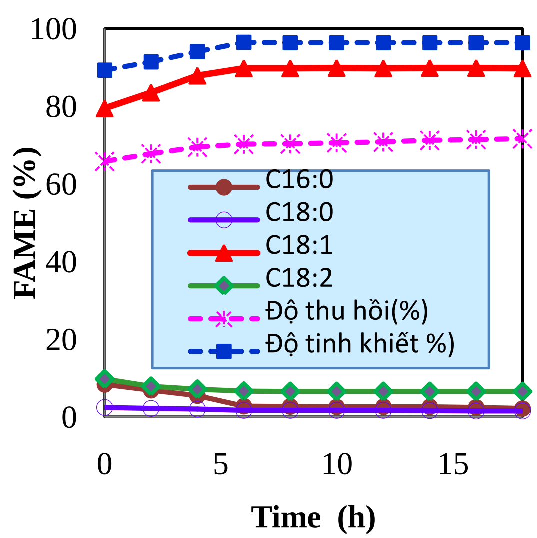


Fig3.25.Effect of crystallization time

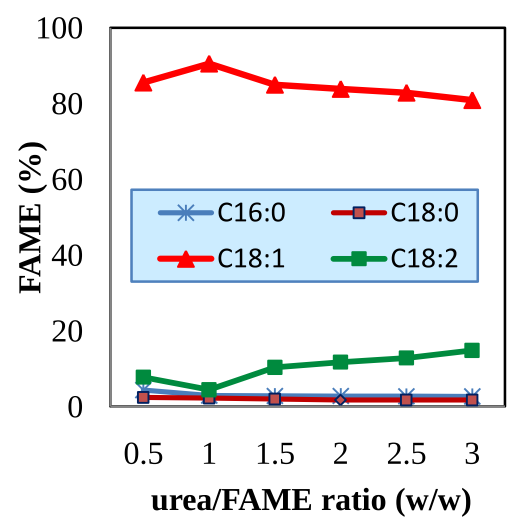


Fig3.26. Effects of urea/FAME ratio

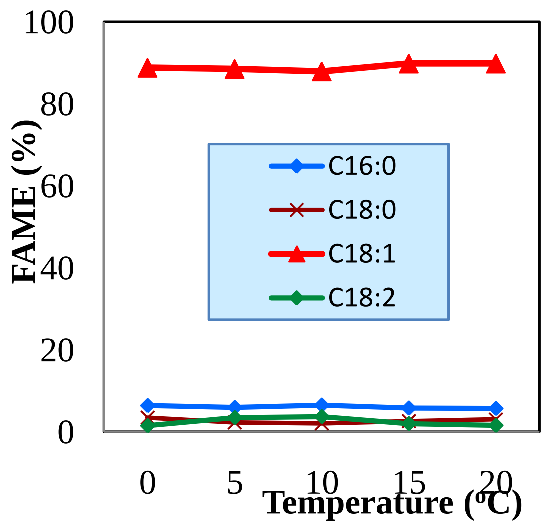


Fig 3.30. Effects of crystallization temperature

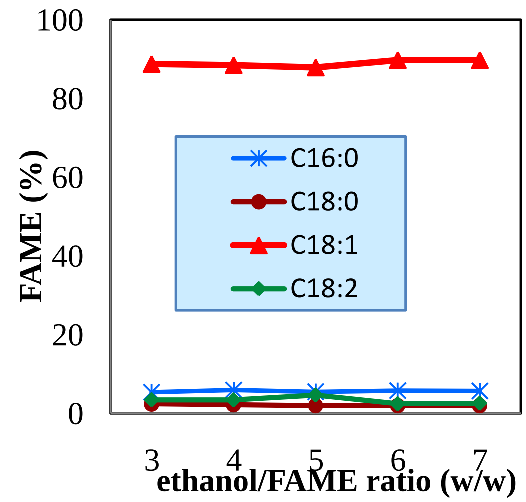


Fig 3.28. Effects of ethanol/FAME ratio

Results of GC-MS analysis of FAME component after 2 phases are shown in table 3.23 and 3.24. Content of final received methyl oleate is 98.36% which meets the standard to be synergist for Imidacloprid

Table 3.23. FAME after being separated from saturated FAME

No	Fatty acid	Scientific name	Content %
1	18: 1(n-9)	Methyl oleate	**90,53**
2	18: 2(n-6)	Methyl linoleate	**5,87**
3	16: 0	Methyl palmitate	2,18
4	18:1	Methyl stearate	1,42

Table 3:24. FAME mixture component after phase 2 separation

No	Fatty acid	Scientific name	Normal name	Content %
1	18: 1(n-9)	Octadecenoic acid	**Oleic acid**	**98,36**
2	18: 2(n-6)	Octadecadienoic acid	**Linoleic acid**	**1,64**

3.5. STUDY TO PROCESS SYNERGIST MIXTURE FOR BT AND IMIDACOPRID

3.5.1. Study to process synergist mixture forBt

3.5.1.1 Study the mixture formula

Process the mixture of synergist (S1) and Bt with different S1/Bt ratios: 0.5/1; 1/1; 1.5/1; 2/1. Obtained products are biologically tested in the laboratory on diamondback moth *(Plutella xylostella)*.

Table 3.25.Efficiency of mixture of synergist and Bt pesticide in eradicatingdiamondback moths in laboratory

Symbol	Experiment formula	Synergist/Bt ratio (w/w)	Efficiency after 7 days %)
CT1	S1 (for comparison)	1/0	12,5e
CT2	Vi-Bt(for comparison)	0/1	66,67d
CT3	S1B11	0,5/1	77,08c
CT4	S1B12	1/1	89,58a
CT5	S1B13	1,5/1	85,42ab
CT6	S1B14	2/1	81,25abc
CT7	Water	-	-
CV			*4,1%*

Thus, the mixtureof synergist S1 (K-oleate + K-linoleate) and Bt pesticides give the highest efficiency in term of ratio (w/w) is: S / Bt = 1/1.

3.5.1.2. Study to process new pesticide

Using VIPESCO Vi-BT 16.000WP to process a mixture with synergist S1. Choose WP (BT-S 16WP) to compare. Assess the quality of the product according to the TCVN 8050: 2009 and TCCS 09: 2010 /BVTV on "Pesticides containing Bacillus thuringiensis", new product is qualified.

3.5.2. Process synergist mixture with brown plant hopper insecticide Imidacloprid

3.5.2.1. Study the mixture formula

Process the mixture of synergist (S2) and Imidacloprid with different S2/I ratio: 0.5/1; 1.0/1; 1.5/1; 2.0/1; 2.5/1; 3.0/1. Obtained product is biologically tested on brown plant hopper *Nilaparvata lugens* Stal in the laboratory

Table 3.27.Efficiency of mixture between Methyl Oleate and Imidacloprid on eradicating brown plant hoppers

Symbol	Experiment formula	S2/ imidacloprid ratio (w/w)	Efficiency after 7 days (%)
CT1	$S_1 I_0$ (for comparison)	0,5/0	14,17d

CT2	$S_0 I_1$ (for comparison)	0,0/0,5	59,07c
CT3	S21I1	0,5/1	73,92b
CT4	S22I1	1/1	79,49ab
CT5	S23I1	1,5/1	83,30ab
CT6	S24I1	2,0/1	87,11a
CT7	Water	-	-
CV(%)			*4,87%*

Thus, the ratio of methyl oleate synergist and Imidacloprid 2/1 (S / I = 2/1), which gives the highest efficiency, is selected.

3.5.2.2. Study and process new BPH insecticide

Methyl oleate is water insoluble, thus it is necessary to add HĐBM Sanimal H to the mixture to make sure that new insecticide product is in EC form (Imidacloprid-S 50EC). Results show that, formula to process new product is composed of Imidacloprid: 5%, methyl oleate: 10%, Sanimal H: 9%; Solvesso solvent: full to 100%

New products Imidacloprid-S 50EC is evaluated according to TCCS 07: 2006 and TCCS 135: 2014 and are qualified.

3.6. PRELIMINARY TESTING OF BIOLOGICAL EFFECTIVENESS OF NEW PRODUCTS

3.6.1. Testing new product on *Plutellaxylostella*

Results of biological effectiveness test of new pesticide BT-S16WP on fields are shown in Table 3.30.

Table 3.30Effectivenessof BT-S16WP on diamondback moth

Formula	**Moth density before testing (moths/ 20 plants)**	**Mothdensityafterspraying (moth/20 plants)**			**Efficiency (%)**		
		1 day	***3 days***	***7 days***	**1 day**	**3 days**	**7 days**
Water (for comparison)	42,7	38,0	48,3	50,0	-	-	-
Vi-Bt	50,3	30,7	23,3	21,7	36,6a	55,2a	62,7a
S1B1	53,8	30,7	23,3	12,8	66,4b	77,9b	88,8b
Nướclã	42,7	38	48,3	50,0	-	-	-

CV (%)					*17,2*	*14,4*	*13,9*
LSD (0,05)					*3,7*	*4,4*	*4,6*

Table 3.30 shows that with only half of the amount, effect ofnew insecticide BT-S16WP on kohlrabi diamondback moth after 7 days is 88.8%, higher than the comparing sample Vi-BT (62.7%),

This demonstrates the synergistic effect of mixture of Potassiumoleate and Potassium linoleate on Bt pesticide on *Plutella xylostella* in Vietnam.

3.6.2. **Testing of new product on brownplanthoppers(*Nilaparvata lugens*Stal*)*.**

Field test results of biological effect of new BPH insecticide Imidacloprid-S 50EC are shown in Table 3.31 show that:

Compared with Vicondor 50EC, effectiveness of imidacloprid-S 50EC is significantly higher in all investigated period. Pest control effect of new product reached 83.81% after 7 days of spraying, although using lower concentrations (0.75 ml / L compared to 1.0 ml / L).

Assay results demonstrate new insecticide (mixture of synergist and imidacloprid) has better effectiveness in comparison with similar products that do not have synergists. Methyl oleate has pronounced synergistic effect with imidacloprid on BPH *Nilaparvatalugens*Stal in Viet Nam.

Table 3.31. Effectiveness of Imidacloprid-S 50EC

Formula	**Hopper density (hopper/cluster)**	**Hopper densityafterspraying(hopper/cluster)**			**Efficiency (%)**		
		1 day	**3 days**	**7**	**1 day**	**3 days**	**7 days**
Nướclã	46,6a	40,0c	47,2c	40,0c	-	-	-

Vicondor 50EC	43,5a	22,0b	19,4b	13,3b	41,08	55,97	64,38
Imidacloprid-S 50EC	48,2a	13,8a	10,4a	6,7a	66,65	78,7	83,81
CV (%)	10,4	12,6	6,5	14,8	-	-	-

3.6.3. Determination of effect of synergist

3.6.3.1. Determine synergist's effects onBtpesticide

a. Determination of synergist's effects on Bt pesticide

According to the testresults studied on a farm, effectiveness of substance with synergist BT-S after 7 days is 88.8% whileit is 62.7% when only Bt is used. Thus, synergistic effect of the BT-S withBt is(88.8 - 62.7)/88.8 x 100% = 29.39%

b. Synergistic index (SF):

According to the test results,Synergistic effect is:

SF = 88.8: 62.7 = 1.416

3.6.3.2. Determination of synergist's effect on BPH insecticide

a. Determination of synergist's effect on BPH insecticide Imidacloprid

According to the test results on the farm, effectiveness of substance with synergist Imidacloprid-S 50EC after 7 days is 83.81% while it is 64.38% when only Vicondor 50EC is used. Thus, synergistic effect of methyl oleate with Imidacloprid(83.81– 64.38)/83.81 x 100% = 23.18%

b. Synergistic index (SF):

According to the test results, Synergistic effect is:

SF = 83.81: 64.38 = 1.30

3.7. INITIAL STUDY OFSYNERGIST'S ACTING MECHANISM

3.7.1. Effect to inhibitAPN enzymeof Potassium oleate and potassium linoleate mixture

Experiments were carried out to examine effect to inhibit APN enzyme of synergist. Samples for test are: CT1: Crystal toxic strain Bacillus thuringiensis var. kurstaki (Bt); CT2: synergist S1 (mixture of potassium oleate and potassium linoleate) and CT3: Mixture of Bt and synergistS1 with volume ratio is 1/1.

Sample's ability to inhibit APN enzyme are shown in table 3.34

Table 3.34.Sample's ability to inhibit APN enzyme

Formula	Symbol	Initial concentration (mg/ml)	Inhibitation (%)	IC_{50} (mg/ml)
CT1	Bt	3	0	> 3
CT2	S1	3	78,3 ± 0,9	1,06
CT3	Bt + S1	3	70,0 ± 0,4	0,55

- Bacillus thuringiensis var. kurstaki does not have the effect to inhibit APN but synergistic substances (S1) showed the ability to inhibit this enzyme which is quite high, reaching 78.3% with IC50 = 1.06 mg / ml.
- Product mixture of Bt insecticide with (synergistic and synergist)iscapable of inhibiting the enzyme APN roughly equivalent to the synergist (70.0%), but inhibitory concentration is half (IC50 = 0.55 mg / ml).

Thus it can be seen that, a mixture of potassium oleate + potassium linoleate has quite high APN enzyme inhibitory effect. Thereby it can be concluded that, the presence of synergistic substances S1 has increased pest control efficiency of Bt pesticide. These comments conform to the results of product assay withkohlrabi diamondback moth on the farm.

3.7.2. Methyl oleate's effect to Inhibit Glutathione S-transferase enzyme

Glutathione S-transferase (GST) isan important detoxification enzyme of insects, protecting them against the toxins by binding with the thiol groups (-SH) of Glutathione to create an affinity for the toxin.

Results of tests on inhibiting GST enzyme are shown on table 3.35

Table 3.35.Ability of samples to inhibit GST enzymes

No.	Symbol	Initial concentration (mg/ml)	Inhibitation (%)	IC_{50} (mg/ml)
1	Imidacloprid	5	61,2 ± 7,4	4,28
2	Synergist S2	5	29,7 ± 1,1	> 5
3	Imidacloprid + S2	5	76,3 ± 1,2	3,34

The active ingredient Imidacloprid showed the ability to inhibit GST enzyme which reaches 61.2% at initial concentration of 5 mg/ml, proving that GST enzyme takes part in deification process but at a low level.

Methyl oleate synergist's ability to inhibit GST enzyme is not high (at 30%). However, a mixture of synergist and imidaclopridinhibits GST enzyme at 76.3%, higher than single sample's effect.

Therefore, it can be seen that methyl oleatealso has synergic characteristic with Imidacloprid to inhibit detoxifying GST enzymes, though its performance is not high.

3.7.3. Methyl oleate'seffect to inhibit Cytochrome P450-monooxygenases enzyme

Cytochrome P450- monooxygenases (CYP) enzymes are the main enzymes that cause Imidacloprid drug resistance of BPH *Nilaparvatalugens*Stal because they act as antidote to imidacloprid.

To examine methyl oleate synergist's ability to inhibit CYP enzyme,used samples are: the active ingredient imidacloprid, synergist S2 (methyl oleate) and mixture of imidacloprid with S2.

Samples' ability to inhibit CYP enzyme are shown in Table 3.36.

Table 3.36. The ability of samples to inhibit CYP enzyme

No.	Symbol	Initial concentration (mg/ml)	Inhibitation (%)	IC_{50} (mg/ml)
1	Imidacloprid	5	50,2 ± 7,4	2,58
2	Synergist S2	5	85,7 ± 1,1	1,02
3	Imidacoprid + S2	5	86,3 ± 1,2	0,95

The differences between the formulas are as follows:

- At a concentration of 5 mg/ml, Insecticide Imidacloprid inhibitsenzyme CYP at 50.2%, with IC_{50} = 2.58 mg/ml. The result shows that imidacloprid is resisted by brown plant hopper in certain level.
- Synergist S2 (methyl oleate) is more capable of inhibiting enzyme CYP (85.7%, with IC_{50} = 1.02 mg/ml) and equivalent to the ability of mixture ofsynergist andimidacloprid (86.3%, IC_{50} = 0.95 mg/ml).

With above results, it can be preliminarily concluded that:

- The active ingredient imidacloprid is resisted by brown plant hoppers
- Concerning drug resistance of brown plant hoppers, cytochrome P450 monooxygenase enzyme plays a major role, while Glutathione S-transferase enzyme has minor influence.

- Mixture of methyl oleate andImidacloprid is also well capable of inhibiting Cytochrome P450 monooxygenase enzyme, therefore it is able to overcome Imidacloprid resistance of hoppers, thereby increasing the pest control effectiveness of the mixed product

CONCLUSION

1. Dissertation has examined and finalized two synergists suitable for two plant protection drugs which effectiveness have been being losing in Vietnam: mixture of potassium salts of oleic acid and linoleic acid for Bt pesticide to prevent and eradicate diamondback moth *Plutella xylostella*; methyl oleate for BPH insecticide Imidacloprid to prevent and eradicate BPH *Nilaparvata lugens* Stal. in Việt Nam
2. It is the first study that collects and examines content of oil and some unsaturated fatty acids C18 among 20 raw materials which contain vegetable oil in Vietnam and has selected peach kernel oil to be appropriate material to process synergist for Bt pesticide and camellia grain oil to process synergist for BPH insecticide Imidacloprid.
3. Studied method uses enzymes to extract camellia grain oil and peach kernel with the following parameters: With camellia grains: temperature: 50 ^{0}C; protease enzyme concentration: 0.6% (w / w); extraction time 3hr; Oil yield: 76.9%. With peach kernels: temperature: 50 ^{0}C; protease enzyme concentration: 0.8% (w / w); extraction time 3h; Oil yeild: 71.3%. After separating, oleic acid/linoleic acid, ratiois 55/32(%) in peach kernel oil and 82.3/77(%) in camellia grain oil, which has been qualifiedas raw materials to prepare synergists for Bt and imidacloprid.
4. From peach kernel oil, a mixture of K-oleate / K-linoleat (54/43% ratio) was prepared to be the synergist for Bt pesticide, through the following steps: 1) vegetable oil hydrolysis with KOH in ethanol; 2) Separate oleic acid, linoleic acid from the obtained mixture by crystallization methods through complex formation with urea. Using experimental planning method to optimize the separation process, optimum parameters were obtained: ratio of urea / FFA: 2/1, crystallized at 0 ° C, duration: 16 hours. The result wasthe mixed fatty acid containing primarily oleic acid (53.9%), linoleic acid (43.62%) and small amount of saturated fatty acid remainder

(over 1%). 3) The obtained fatty acid mixture was transformed into potassium salt in weight and pH proportion qualified to be used as synergist for Bt pesticide.

5. From camellia grain oil, methyl oleate was prepared to be synergist for BPH insecticide Imidacloprid through the following steps: 1) Synthesize methyl ester of fatty acid by Camellia oil trans-esterification reaction at 75^0C, duration: 8 hours, using an amount of ionic liquid catalyst $mimC_4H_8SO_3H.CH_3SO_3$equal to 10% oil volume. 2) Separate and enhancecontent of methyl oleatethrough complex formation with urea through 2 stages: crystallized at 10°C and 0°C.Obtained products has content> 98%, qualified to be synergist. The fatty acids and methyl esters is defined by GC-MS spectrum.
6. Has made 2 new plant protection drug products which are qualified: Pesticide BT-S 16WP (mixture of K-oleate / K- linoleat with Vi-BT 16.000WP pesticide with the rate of 1/1 w/w) and BPH insecticides imidacloprid-S 50EC (mixture of methyl oleate with imidacloprid in proportion 2/1)
7. Have the biological effect tests of two new pesticide products. The results showed that, the effect to eradicate diamondback moth on vegetable of BT-S 16WP is 29.9% higher than the control, synergistic index (SF): 1,416; effect ofImidacloprid-S 50EC to eradicate BPH on rice is 23.18% higher compared to the control, SF: 1.30.
8. For the first time in Vietnam, the dissertation has studied the mechanism of action of synergistsand hadpreliminary conclusions: mixture of K-oleate salt/K- linoleat salt has stronger inhibitory effect on APN enzymes of diamondback moth *Plutellaxylostella* (78.3% with IC50 = 1.06 mg / ml); Methyl oleatehas weak inhibitory effect on detoxifying GST enzymes but has strong inhibitory effect on CYP enzyme of BPH *Nilaparvatalugens*Stal (85.7%, with IC50 = 1.02 mg/ml). Thus the efficiency of new products to eradicate pests and BPHs is enhanced. The research results help to explain the mechanism of action of selected synergists on some pesticides to control the effectiveness of pesticides, whichhas been decreased in Vietnam.