CHAPTER 4

RESULTS

4.1 Background data of studied subjects

The first visit was carried out in November 2003 when the strawberry growing season had begun. The farmers from 4 villages in Bo-Keaw and Mae-Sab sub districts were enrolled. All of 136 invited subjects must pass in the inclusion criteria. Three farmers had been not farmed any crop more than 2 years and six were biopesticide users, so 9 farmers were excluded from this study and only 127 farmers were qualified. The general data of these subjects are shown in the table 13.

However, 78.7 percent of subjects came back for the second follow up. Matched cases in both follow up totaled 100 subjects, all data presented in this study were analyzed and calculated based on the 100 matched cases.

Table 14 shows the mean of subjects' age, years of agriculture and growing area. Comparing the means in male and female groups, there were no significant differences between age ($p>0.05$, Unpaired t-test), year of agriculture ($p>0.05$, Unpaired t-test) and growing area ($p>0.05$, Unpaired t-test).

Table 13 Number of Subjects from 4 villages in Bo-Keaw and Mae Sab districts

Village, Sub district	Number of subjects		
	Male	**Female**	**Total**
1. Bann Pa Kiet Nok, Bo-Keaw	3	6	9
2. Bann Mae Yan Ha, Bo-Keaw	33	23	56
3. Bann Bo-Keaw, Bo-Keaw	17	15	32
4. Bann Pa Kiet Nai, Bo-Keaw	1	0	1
5. Bann Om Long, Mae Sab	24	13	37
6. Bann Mae Na Korn, Bo-Keaw	1	0	1
Excluded subjects			
1. Subjects who had not been planted or grown anything in more than 2 years.	2	0	2
2. Biopesticide users	4	2	6
3. Subjects who did not return for the second follow up study.	10	9	19
Included subjects	**53**	**47**	**100**

Table 14 Mean and standard derivation of age and year of agriculture in 100 subjects

Sex	Mean ± SD (Minimum - Maximum)		
	Age (Years)	**Year of Growing (Years)**	**Growing area (Rai)**
1. Male (n=53)	42.98±13.87 [a] (16-71)	15.13±9.35 [b] (3-50)	3.32±2.63 [c] (0-10)
2. Female (n=47)	41.15±10.46 [a] (23-75)	14.55±8.71 [b] (2-45)	3.83±2.53 [c] (1-10)
Total subjects	**42.12±12.36 (16-75)**	**14.48±9.01 (2-50)**	**3.56±2.58 (0-10)**

[a, b, c] p >0.05, Unpaired t-test

4.2 Pesticides used and exposure data

Pesticides are generally used in this area and sold in the shops and markets. The routes of exposure were due to farmer work tasks, pesticide spraying, handling pesticides, use of protective equipments and others were obtained by interview. These data are shown in table 15-16. Farmers' risk behaviors to pesticide exposure and poisoning are shown in table 17-18.

Data on pesticides used were collected by using interview and self administration forms and included amount of use per time, frequency of spraying, storage, disposal place, size of pesticide tank and others. Amount of pesticides active ingredient were calculated and presented in table 19-21.

Table 15 showed farmers work tasks. Most farmers were spraying, mixing pesticide, adding fertilizer, harvesting and packing. These were the directly exposed routes to pesticides.

Table 16 showed the use of protective equipment. Most subjects used protective equipments, but frequency of mask using in visit 2 reduced from visit 1, because visit 2 is a harvesting season and pesticides were not used as much as in visit 1, growing season.

Table 17 shows farmers risk behaviors to pesticide exposure. Data show that 19 percent frequently took food or water during spraying without changing their clothes, 11 percent got clothes wetting during spraying, 8 percent frequently got hands soaked with pesticides and 56 percent always washed their equipment after spraying pesticides. Table 18 showed most farmers thought that face and hands were frequently exposed to pesticides.

Table 15 Comparison of the frequency of farmers work tasks performed by themselves in growing and harvesting seasons

Farmer work tasks	Percentage (n = 100)	
	Growing season	Harvesting season
1. Cultivating	77.0	0.0
2. Break up soil and remove weeds	96.0	0.0
3. Fertilizer adding	96.0	92.0
4. Pesticide mixing	82.0	72.0
5. Pesticide spraying	83.0	78.0
6. Harvesting	0.0	97.0
7. Packing	0.0	94.0
8. Transportation	0.0	25.0

Table 16 Comparison of protective equipment using by farmers in growing and harvesting seasons

Protective equipments	Percentage of farmers (n=100)	
	Growing season	Harvesting season
1. Mask	75.0	46.0
2. Glove	72.0	75.0
3. Boots	76.0	78.0
4. Glasses	17.0	75.0
5. Hat	71.0	72.0
6. Long sleeve shirt	75.0	79.0
7. Rain cloth	14.0	8.0

Table 17 Frequency of farmers' behaviors that risk pesticide exposure and poisoning

Farmers' behaviors	Percentage (n=100)			
	Never	Rarely	Frequently	Always
1. Use the recently spraying coats without washing.	91	4	5	0
2. Smoking during spraying	76	23	1	0
3. Pesticide mixing with bare hands	67	23	7	3
4. Took their food or water during spraying without changing their clothes.	78	3	19	0
5. Took their food or water during spraying without washing their hand.	99	1	0	0
6. Clothes wetting during spraying	52	37	11	0
7. Hands soaked with pesticide	60	22	8	0
8. Washing spraying equipments	46	0	0	56
9. Washing their hands after spraying	32	0	0	62
10. Taking bath after spraying	24	0	0	76
11. Immediately changing their clothes after spraying	65	0	0	35
12. Immediately washing their clothes after spraying	28	0	0	72

Table 18 Frequency of farmers' pesticide exposure according to part of body

Pesticide exposure to a part of body	Frequency (n=100)
1. Face	48
2. Hands	45
3. Arms	10
4. Back	20
5. Body	10
6. Legs	10
7. Feet	8
8. Lungs	10
9. Eyes	5

Table 19 Frequency of pesticides used and total amount during two seasons

Chemical family	Common name	Total amount of pesticide (Liters)	
		Growing season	Harvesting season
1. Herbicides			
1.1 Acetamide herbicide	Propanil	0.1	
1.2 Ammonium herbicide	Paraquat	506.7	258.5
1.3 Organophosphorus herbicide	Glyphosate	178.1	142.7
1.4 Trifluoromethyl herbicide	Oxyfluorfen	0.3	30.3
1.5 Phenoxyl herbicide	Ethyl haloxyfop	0.1	-
2. Fungicides			
2.1 Acrylalanine fungicide	Metalaxyl	5.4	-
2.2 Aromatic fungicide	Chloronitrile	0.5	-
2.4 Benzimidazole fungicide	Carbendazim	11.5	65.6
2.5 Benzimidazole fungicide	Benomyl	7.1	3.4
2.6 Conazole fungicide	Triflumazole	16.9	30.7
2.7 Conazole fungicide	Diphenylkenazole	278.5	17.4
2.8 Imidazole fungicide	Prochloraz	19.5	75.5
2.10 Strobin fungicide	Azoxylstrobin	1.0	69.0

Table 19 Continued.

Chemical family	**Common name**	**Total amount of pesticide** (Liters)	
		Growing season	**Harvesting season**
3. Insecticides			
3.1 Carbamate insecticide	Carbofluran	-	1.4
3.2 Carbamate insecticide	Methomyl	1.2	44.5
3.3 Carbamate insecticide	Ethylene bis dithiocarbamate	427.4	113.3
3.4 Carbamate insecticide	Dithiocarbamate	25.0	-
3.5 Carbamate insecticide	Formetanate	-	32.2
3.6 Organophosphate insecticide	Methamidophos	1.38	2.2
3.7 Organophosphate insecticide	Methyl parathion	1.3	32.2
3.8 Organophosphate insecticide	Monocrotophos	5.3	
3.9 Pyrethroid ester insecticide	Cypermethrin	46.2	112.8
3.10 Pyrethroid insecticide	Cyhalothrin	0.3	
3.11 Organochlorine insecticide	Quintozene	-	29.7
4. Acaricide and Rodenticides			
4.1 Avermectin acaricide	Abamectin	1.0	-
Total amount		**1,534.8**	**1,091.1**

Table 20 Amount of pesticides usage categorized by active ingredients

Chemical family	Total amount (Liters)	
	Growing season	Harvesting season
1. Acetamide herbicide	0.1	-
2. Ammonium herbicide	506.7	258.5
3. Organophosphorus herbicide	178.1	142.7
4. Phenoxyl herbicide	0.1	-
5. Trifluoromethyl herbicide	0.3	30.3
6. Acrylalanine fungicide	5.4	-
7. Aromatic fungicide	0.5	-
8. Benzimidazole fungicide	18.6	69.0
9. Conazole fungicide	295.4	48.1
10. Imidazole fungicide	19.5	75.5
11. Strobin fungicide	1.0	69.0
12. Carbamates insecticide	453.6	191.4
13. Organophosphate insecticide	8.0	34.4
14. Pyrethroid pesticide	46.5	142.5
15. Organochlorine insecticide	-	29.7
16. Avermectin acaricide	1.0	-
Total	**1,534.8**	**1,091.1**

Table 21 Amount of pesticide usage categorized by type of use

Type of use	Amount of pesticide (Liters)	
	Growing season	**Harvesting season**
1. Herbicide	685.3	432.5
2. Fungicide	340.4	261.6
3. Insecticide	508.08	368.3
4. Acaricide	1.0	0.0
Total	**1,534.78**	**1,091.4**

Table 19 -21 shown that herbicides were the highest quantities of pesticide loading in growing season. The second and the third were insecticides and fungicides. The herbicide used in highest amount was paraquat in the category of ammonium herbicides (506.7 Liters), which was reduced to 258 liters in the harvesting season.

The insecticide used most in visit 1 was carbamate insecticide. Total amount was 453.6 liters, reduced to 191.4 liters in the harvesting season. While the most applied fungicide was conazole fungicide, the amount was reduced from 295.4 to 48.1 liters.

Farmers specified that herbicides were heavily loaded in farms for soil preparation and to control plant and fungal diseases before cultivating strawberry. Insecticides were sprayed after planting to control pests and insects. Fungicides were also applied for controlling fungal diseases.

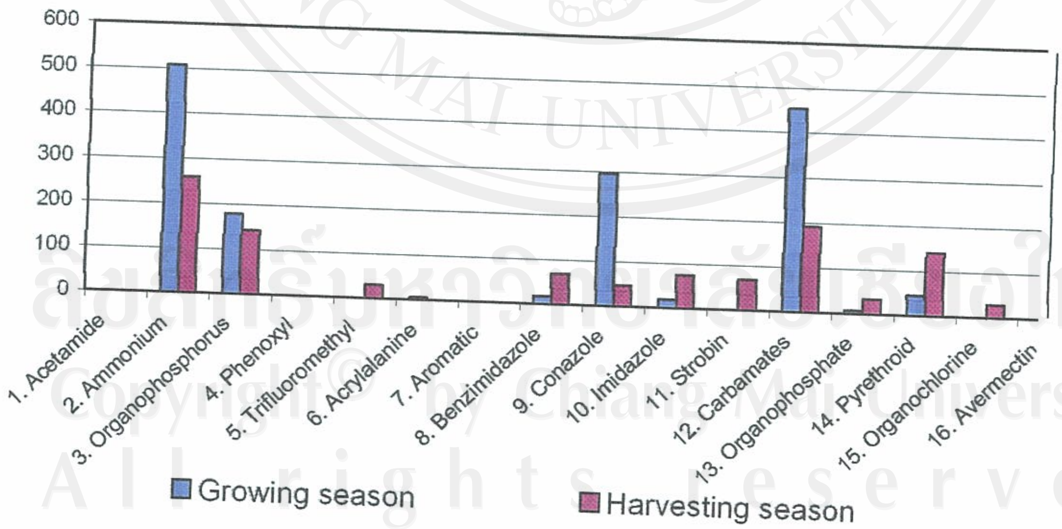

Figure 22 Bar graph showing total amount of pesticides used

4.3 Signs and symptoms of pesticide poisoning

Some signs and symptoms were observed in the low dose of pesticide exposure as farmers and occupational pesticide exposure. In this study, 21 different signs and symptoms were recorded. Table 22 shows the frequencies of 21 signs and symptoms during the observed spray operation in growing season compared with to the same subjects in harvesting season.

Signs and symptoms occurred significantly ($p<0.001$, paired t-test) more in growing season than harvesting season. In growing season, approximately one third of farmers experienced dizziness, fatigue, dry throat and sore throat. Less than 30% of farmers experienced excessive sweating, muscle weakness, red eyes, chest pain, skin redness and others.

In the following harvesting season several months later, the frequencies of signs and symptoms in farmers had returned to background levels.

Table 22 Frequency of sings and symptoms experienced by farmers in growing and harvesting season

Signs and Symptoms	Percentage of farmers(n=100)	
	Growing season[a]	Harvesting season[a]
1. Dizziness	50	13
2. Fatigue	39	6
3. Dry throat or Sore throat	37	8
4. Excessive sweating	29	1
5. Muscle weakness	23	3
6. Red eyes	22	4
7. Chest pain	18	4
8. Skin redness	17	2
9. Twitching eyelids	16	1
10. Runny nose	15	2
12. Vomiting	15	6
13. Blurred vision	12	3
14. Staggering gait	12	1
15. Excessive salivation	10	1
16. Diarrhea	10	2
17. Muscle cramps	10	2
18. Stomach cramps	9	1
19. Loss of memory	8	2
20. Cough	8	3
21. Loss of consciousness	6	1

[a] p value < 0.001, paired t-test

4.4 Cholinesterase enzyme activities and genetic variants

4.4.1 Acetylcholinesterase and butylrylcholinesterase enzyme activity

Both acetylcholinesterase and butylrylcholinesterase enzyme activities were measured in blood samples to investigate the inhibition of cholinesterase level resulting from organophosphate and carbamate pesticide exposures among the farmer groups.

Mean of AChE activity was 3.63±1.33 U/ml in growing season and 4.33±1.77 U/ml in harvesting season. Mean difference of AchE activity between growing and harvesting seasons was significant ($P<0.01$, paired t-test, n=100). AchE activity in visit 2 was higher than visit 1 (Table 23).

Mean of BuChE activity was 5.08±2.15 U/ml in growing season and 4.32±1.46 U/ml in harvesting season. Mean difference of BuchE activity between growing and harvesting seasons was significant ($P<0.01$, paired t-test, n=100). BuchE activity in visit 2 was higher than visit 1 (Table 23).

Table 23 Acetylcholinesterase and Butylrylcholinesterase activity in farmers

Enzyme Activity (U/ml)	**Growing Season**	**Harvesting Season**
	Mean (Min - Max)	Mean (Min - Max)
Acetylcholinesterase	3.63±1.33[a] (0.61-6.16)	4.33±1.77[a] (0.23 – 8.16)
Butylrylcholinesterase	5.08±2.15[b] (0.94-9.68)	4.32±1.46[b] (0.73 -12.55)

[a, b] p value < 0.001, paired t-test

4.4.2 Butylrylcholinesterase enzyme and genetic variants

Genetic variants of BuchE were determined by dibucaine number (DN) and fluoride number (FN). Frequencies of genetic variants of BuchE are presented in Table 25. The observed activities in the presence of dibucaine are thus representative of homozygosity for cholinesterase genetic variants as shown in this table.

Table 24 Cholinesterase genetic variants determination by using DN and FN

ChE Genetic variants	Dibucaine number (DN)	Fluoride number (FN)
UU	≥77	≥55
UF	72-76	≥53
UA	48-72	≥44
AF	45-59	<44
FF	64-69	<44
AA	>35	-

Sixty eight percent of subjects were UU phenotype, 17 and 15 percent were UA and AF, respectively. Pearson's correlation coefficient showed the correlation between Phenotype and cholinesterase levels at $p<0.01$. Because the correlation between genetic phenotype and cholinesterase levels were highly significant, pesticide exposure and poisoning identification should be concerned about cholinesterase genetic variants. The low level of enzyme activity is not only caused by pesticide inhibition, but also the genetic variants.

Table 25 Frequency of genetic variants of BuchE using DN and FN

Phenotype	Percentage (n=100)	BuchE (U/ml)	AchE (U/ml)
UU (n = 68)	68	6.26±1.21 [a]	3.95±1.30 [b]
UA (n = 17)	17	3.46±1.39 [a]	3.47±0.98 [b]
AF (n = 15)	15	1.56±0.41 [a]	2.33±0.97 [b]

[a, b] $p < 0.001$, ANOVA test

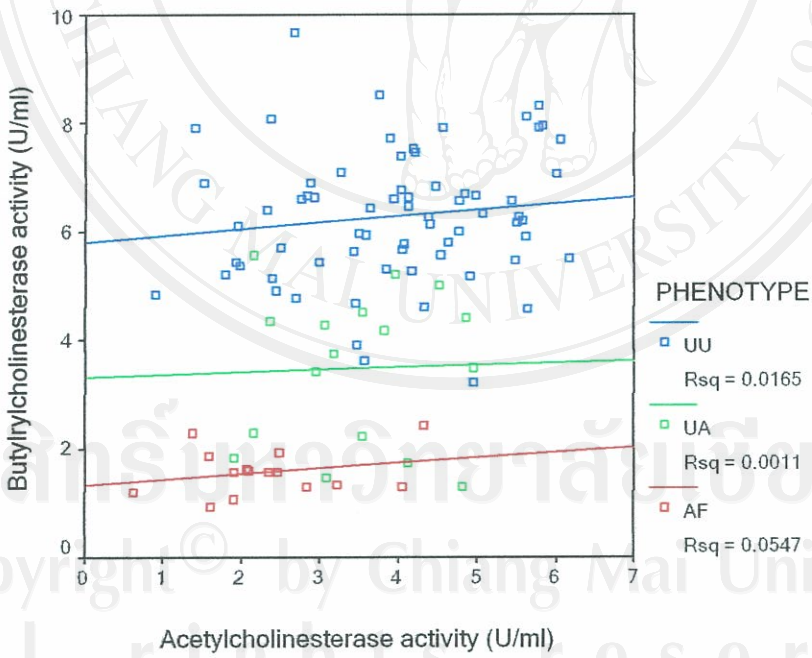

Figure 23 Scatter plot between AchE and BuchE distributed by genetic variants

Table 26 Frequency of genetic variants of BuchE in growing and harvesting season using DN and FN

Phenotype*	Percentage (n=100)	Butylrylcholinesterase enzyme (U/ml)	
		Growing season	Harvesting season
UU (n = 68)	68	6.26±1.21[a]	4.53±1.51[a]
UA (n = 17)	17	3.46±1.39	3.78±1.23
AF (n = 15)	15	1.56±0.41[b]	3.97±1.33[b]
Total	100	5.08±2.15	4.32±1.46

[a, b] p value < 0.001, paired t-test

In this table, blood BuChE activity was measured for baseline result and determined for BuChE phenotype. Decreases of BuChE activity in harvesting season were significant lower in only UU phenotype. UA phenotype subjects had no significant difference in BuChE activity in growing and harvesting season, whereas AF phenotype had BuChE activity in harvesting season higher than growing season, resulted of the variation of cholinesterase measurement and the number of subjects was quite small.

In this study, the prediction of decreasing BuChE activity after organophosphate pesticides exposure could be estimated in only UU phenotype subjects. However, the small samples size might be not enough to conclude the relationship of enzyme activity and its genetic variants between these two seasons.

4.5 Paraoxonase enzyme activity and genetic polymorphism

4.5.1 Paraoxonase activity

Mean of paraoxonase activity was 39.04±20.24 μMol/min/ml in the growing season and 35.96±17.91 μMol/min/ml in the harvesting season. Mean difference of PON activity between growing and harvesting season was not significant (paired t-test, $p>0.05$). In this study, paraoxonase enzyme provided pesticide detoxification or protective factor and it was not inhibited or reduced by pesticide exposure, as shown in cholinesterase enzyme activities.

In Table 27-29, low paraoxonase activity effects to low of pesticides hydrolysis and could be related to more pesticides inhibition of cholinesterase enzyme activity especially AChE.

Table 27 Paraoxonase enzyme activity in growing and harvesting season

Enzyme	Enzyme activity (μMol/min/ml) Mean ± SD	
	Growing season	**Harvesting season**
Paraoxonase enzyme	39.04±20.24	35.96±17.91

Table 28 The relationship between paraoxonase and cholinesterase activities in growing season

Paraoxonase activity (μMol/min/ml)	Cholinesterase activities (U/ml)	
	AchE	BuchE
Lower than 39.04	3.36±1.43[a]	5.12±2.13
Higher than 39.04	3.98±1.11[a]	5.02±2.20

[a] p value < 0.05

Table 29 The relationship between paraoxonase and cholinesterase activities in harvesting season.

Paraoxonase activity (μMol/min/ml)	Cholinesterase activities (U/ml)	
	AchE	BuchE
Lower than 35.96	4.08±1.71[a]	4.09±1.28
Higher than 35.96	4.60±1.81[a]	4.56±1.62

[a] p value < 0.05

4.5.2 Paraoxonase enzyme activity and genetic polymorphism

Frequencies of genetic polymorphisms are presented in Table 30. PON1 gene loci 192 were found in 48 percent of subjects who have QR phenotype, and in 29 and 28 percent of subjects with QQ and RR phenotype. The frequencies of Q and R alleles were 0.51 and 0.49, respectively. Mean of paraoxonase enzyme activities in QQ, QR and RR phenotype were 20.55±8.50, 36.34±10.69 and 60.07±19.28 μMol/min/ml, respectively. A pearson's value for the correlation between PON1 gene loci 192 and paraoxonase enzyme activity was $p < 0.001$ (Table 31).

PON1 gene loci 55 were found in 96 percent with the LL phenotype and 4 percent with the LM phenotype. MM phenotype was not found in this population. The frequencies of L and M alleles were 0.98 and 0.02, respectively. Mean of paraoxonase enzyme activities in LL and LM phenotype were 39.45±20.47and 29.12±10.36 μMol/min/ml, respectively. There is no correlation between PON1 loci 55 and paraoxonase level ($p>0.05$, Unpaired t-test), as shown in Table 33.

Table 30 Genetic polymorphism on PON1 loci 192 distribution

PON 192	**QQ**	**QR**	**RR**	**Total**
Number of subjects	28	42	30	100

Frequency of R allele = ((30 x 2) + 42) /200

= 0.51

Frequency of Q allele = ((28 x 2) + 42) / 200

= 0.49

Table 31 PON1 loci 192 genetic polymorphism and paraoxonase activity

Mean of Paraoxonase activity (μMol/min/ml)	PON1 loci 192 phenotype		
	QQ (n=28)	QR (n=42)	RR (n=30)
	20.55± 8.50	36.34±10.69	60.07±19.28

$p < 0.001$, ANOVA

The correlation between enzyme activity and its genetic polymorphism $r^2 = 0.736$

Table 32 Genetic polymorphism on PON1 loci 55 distribution

PON 55	**MM**	**ML**	**LL**	**Total**
Number of subjects	0	4	96	100

Frequency of L allele = ((96x 2)+4) / 200

= 0.98

Frequency of M allele = ((0 x 2)+4) / 200

= 0.02

Table 33 PON1 loci 55 genetic polymorphism and paraoxonase activity

Mean of Paraoxonase activity (U/L)	PON1 loci 55 phenotype	
	LM (n=4)	LL (n=96)
	39.04±20.24	39.45±20.47

$p > 0.05$, Unpaired t-test

The correlation between enzyme activity and its genetic polymorphism $r^2 = -0.101$

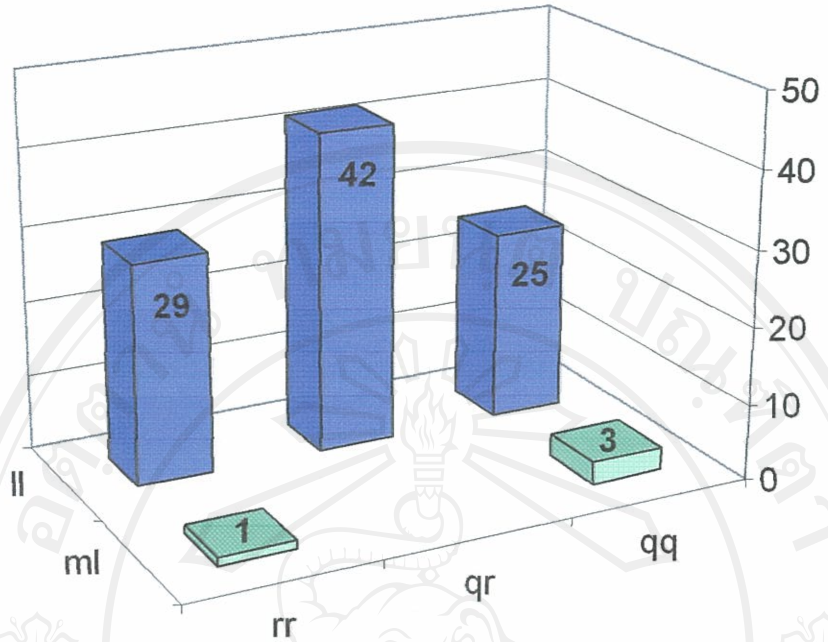

Figure 24 Distribution of PON1 loci 192 and loci 55 polymorphism in this population

Table 34 Distribution of Acetylcholinesterase activity in different PON1 loci 192 phenotype

PON 1 loci 192 phenotype N = 100	Acetylcholinesterase activity (U/ml) Mean ± SD	
	Growing season	Harvesting season
QQ (n = 28)	3.196±1.31	4.00±1.86
QR (n = 42)	3.67±1.38	4.51±1.86
RR (n = 30)	3.98±1.20	4.38±1.55

Table 35 Distribution of Butylrylcholinesterase activity in different PON1 loci 192 phenotype

PON 1 loci 192 phenotype N = 100	Butylrylcholinesterase activity (U/ml) Mean ± SD	
	Growing season	Harvesting season
QQ (n = 28)	4.57±1.99	3.85±1.22
QR (n = 42)	5.09±2.30	4.53±1.68
RR (n = 30)	5.38±2.94	4.46±1.28

4.6 Indicators of pesticide exposure and human health impact

Pesticides have an anti-cholinesterase activity and an effect on human neurological function the more of cholinesterase inhibition, the more of signs and symptoms present. Cholinesterase enzyme activities recommended a pesticide exposure indicator. Paraoxonase is also proposed as a pesticide exposure indicator, through its hydrolysis reaction. The good indicators of pesticide exposure were determined in this study and might be used to monitor farmers for pesticide exposure.

Table 36-37 shows the correlation between amount of pesticides used and enzyme activity. The pearson's correlations show the positive correlation between paraoxonase and amount of organophosphate and carbamate pesticides. In table 38-39, there is no significance between signs and symptoms correlated to enzyme activities.

Table 36 Pearson's correlation value between amount of pesticides used and enzyme activities in growing season.

	Total amount of pesticides	OP and carb	AchE	BuchE	PON
Total amount of pesticides	1.000	0.800	0.131	0.159	-0.001
Op and carb		1.000	0.070	0.022	0.870**
AchE			1.000	0.405**	0.179
BuchE				1.000	0.010
PON					1.000

** p value <0.001

Table 37 Pearson's correlation value between amount of pesticides used and enzyme activities in harvesting season.

	Total amount of pesticides	OP and carb	AchE	BuchE	PON
Total amount of pesticides	1.000	0.479**	-0.084	-0.062	-0.074
Op and carb		1.000	0.024	-0.115	0.072
AchE			1.000	0.022	0083
BuchE				1.000	0.109
PON					1.000

** p value <0.001

Table 38 Pearson's correlation value between signs and symptoms and enzyme activities in growing season.

	Subjects who had an experience of pesticide toxicity	Subjects who have more than one signs or symptoms	AchE	BuchE	PON
Subjects who had an experience of pesticide toxicity	1.000	0.495**	-0.108	-0.036	-0.072
Subjects who have more than one signs or symptoms		1.000	-0.031	-0.014	-0.075
AchE			1.000	0.315**	0.179
BuchE				1.000	0.010
PON					1.000

** p value <0.001

Table 39 Pearson's correlation value between signs and symptoms and enzyme activities in growing season.

	Subjects who had an experience of pesticide toxicity	Subjects who have more than one signs or symptoms	AchE	BuchE	PON
Subjects who had an experience of pesticide toxicity	1.000	0.752**	0.058	0.047	-0.028
Subjects who have more than one signs or symptoms		1.000	0.047	0.069	0.097
AchE			1.000	0.022	0.083
BuchE				1.000	0.109
PON					1.000

** p value < 0.001