RESULTS

Part 1; Risk factors and base line malaria knowledge from different pesticide Land-use systems in malaria endemic area at Kanchanaburi Province,Thailand.

Questionnaire 1

Table 1 show names of villages, sub-districts, districts, and provinces where the survey was undertaken. Table 2 shows number of inhabitants, huts, houses (households), and questionnaire respondents in each village. Table 3 shows demographic characteristics of interviewed household heads.

A total of 232 responded to the questionnaires in Kanchanaburi Provinces.

Sixty-five percent, 155 of all respondents, were household heads and 35% were closely related to the household head, most of them being either the wife or child of the household head.

About 77% and 23% of all respondents were male and female, respectively. The average age of household heads were 47 years, ranging from 20 to 83 years.

Approximately 70% of both respondents and household heads were born in Thailand, 22% were born in Myanmar.

GENERAL INFORMATION

Table 1: Names and locations of surveyed villages.

Village	Sub-district	District	Province
Mae Nam Noi (RFV)*	Huay Kayeng	Thong Pha Phoom	Kanchanaburi
Huay Bak Kok (07)	Huay Kayeng	Thong Pha Phoom	Kanchanaburi
U-Long (04)	Ta Kanun	Thong Pha Phoom	Kanchanaburi
Thung Nang Khruan (06)	Cha Lae	Thong Pha Phoom	Kanchanaburi
Bong Ti Noi (08)	Wang Krajae	Sai Yok	Kanchanaburi

* Count only in the part of Rubber Forest Village (RFV)

Table 2: Village names, population, number of houses and huts, and total number of respondents.

Village	Population[1]	No. of huts[1]	No. of houses	Respondents	%
Mae Nam Noi (RFV)*	65	5	11	14	87.5%
Huay Bak Kok (07)	1,397	31	338	52	14.09%
U-Long (04)	2,797	92	685	77	9.9%
Thung Nang Kruan (06)	948	9	235	57	23.36%
Bong Ti Noi (08)	487	49	104	32	20.92%

[1] According to NKM, Ministry of Public Health in 2546-2003

(RFV)* = Count only in the part of Rubber forest village

Table 3: Demographic and general characteristics of interviewed household heads in Kanchanaburi.

Population characteristics	% of respondents
Age groups (years)	
20-29	8.3
30-39	21.8
40-49	27.4
50-59	20.0
60-69	16.0
70+	6.5
Gender	
Male	76.8
Female	23.2
Country of birth	
Thailand	70.4
Myanmar	21.7
No. of adults per household	
1	3.8
2	42.5
3	24.1
4	14.8
5+	15.0
No. of children per household	
1	41.6
2	34.8
3	15.6
4	6.5
5+	1.6

Table 4: Household head ethnicity

Ethnicity	Respondents	% of respondents
Thai	128	55.17
Karen	19	8.20
Mong	4	1.72
China	3	1.29
Burmese	38	16.38
Mon	24	10.34
Laos	8	3.45
Indian	1	0.43
No	7	3.02
Total	232	100

Table 5: Year living in village

Live in village	Respondents	% of respondents
Less than 1 year	5	2.2
1-5 years	32	13.8
5-10 years	48	20.68
More than 10 years	147	63.36
Total	232	100

Table 6: Number of adult and children in the village

		Respondents	% of respondents
No. adult	1.00	7	3.04
	2.00	106	46.09
	3.00	48	20.87
	4.00	37	16.09
	5.00	14	6.09
	6.00	14	6.09
	7.00	4	1.74
	8.00	0	0
	10.00	0	0
	average	32.86	
	Total	230	100
No. children	1.00	59	0.33
	2.00	66	37.29
	3.00	35	19.77
	4.00	15	8.47
	5.00	2	1.13
	12.00	0	0
	average	35.4	
	Total	177	100

Table 7: Respondents occupation

	Respondents	% of respondents
Carpenter	2	0.86
Farmer	131	56.47
Trader	5	2.16
Employed	49	21.12
Government officer	3	1.29
Orchard	25	10.78
Repairman	1	0.43
Rubber plantation worker	14	6.03
Teacher/ Volunteer teacher	2	0.86
Total	232	100.0

Table 8: Problems and diseases

PROBLEM	Respondents	Disease	Respondents
No answer	159	No answer	27
Agricultural problems[1]	25	Malaria	129 (55.6%)
Economical problems[2]	7	Fever	42
Bad communications[3]	31	HIV	0
No health center, no doctor	3	Influenza	5
Border problems[4]	3	Pink eye	1
ID Card/ Check card	1	Bone	0
Health/Sickness	0	Dengue	15
Narcotics	0	Diabetes	0
Education/Study	1	Diarrhea	7
Repellents/no net	1	Sickness	1
Destroy natural resources	0	TB	0
Difficult	0	Stomach ache	2
Electricity	1	Elephantiasis	3
Total	232	Total	232

[1] Low price for my selling products, water shortage, destroyed products.
[2] Debt, economy, no salary, no work, poor.
[3] Bad road, no transport, no bus.
[4] Minorities, war, foreigners, border areas, migration of poor people.

Table 9: Travel out of the villages

		Respondents	% of respondents
Travel frequency	Less than 1 week	121	77.42
	1-2 weeks /yr	8	5.16
	1-4 weeks /yr	16	10.32
	3-6 weeks /yr	9	5.81
	more than 1 month /yr	3	1.94
	Total	155	100
Traveled	within sub district	21	13.54
	within district	54	34.84
	within province	36	23.23
	other province	43	27.74
	outside country (to India)	1	0.65
	Total	155	100

MALARIA KNOWLEDGE

Table 10: Malaria knowledge of the household heads

		Respondents	% of respondents
Know malaria	yes	206	88.8
	no	26	11.2
	Total	232	100
Malaria frequency	No answer	3	1.29
	once	33	29.2
	2-3 times	52	46.0
	more than 3 times	113	22.1
	Never	31	13.36
	Total	232	100
Malaria symptoms	No answer	4	1.72
	Fever Headaches Shivering Muscle pain Nausea/vomiting	220	94.83
Other symptom name	Can not eat...	1	0.43
	Cough, yellow body	1	0.43
	Diarrhea	3	1.29
	Dizzy	1	0.43
	No feeling	0	0
	Stink	1	0.43
	Tired	0	0
	Yellow body	1	0.43
	Total	232	100
How malaria transmitted	No answer	29	12.5
	Mosquitoes bite	175	75.43
	Don’t know	25	10.78
	other	3	1.29
	Total	232	100
Transmitted season	rainy season	115	49.6
	Hot season	54	23.28
	Cool season	42	18.10
	Don’t know	21	9.05
	Total	232	100
Mosquito after insecticides	More	25	10.78
	Less	86	37.07
	No difference	76	32.75
	Don’t know	45	19.40
	Total	232	100

Table 11: Treatment after got malaria of the household heads

		Respondents	% of respondents
Treatment place	Malaria clinic	154	66.38
	Hospital	67	28.88
	Health clinic	5	2.16
	Health center	2	0.86
	Traditional practitioner	3	1.29
	Self treatment	1	0.43
	Total	232	100
Full course treatment	no answer	8	3.45
	yes	218	93.97
	no	6	2.59
	Total	232	100

Prevention

Table 12: The preventative of malaria (mosquitoes)

		Respondents	% of respondents
Prevention	Impregnated bed nets	193	83.20
	Non- impregnated bed nets	31	13.36
	No bed nets	8	3.45
	Total	232	100
Other preventions	long sleeve cloth	35	15.09
	Insecticides spraying	32	13.79
	Repellent	41	17.67
	Mosquito coils	81	34.91
	Cleaning around the house	25	10.78
	Cleaning water stream	11	4.74
	Other	7	3.02
	Total	232	100

Table 13: Number of bed nets in household (from 83% of 232 household head)

		Respondents	% of respondents
No. bed nets	1,00	39	20.21
	2,00	74	38.34
	3,00	40	20.72
	4,00	33	17.10
	5,00	5	2.59
	6,00	1	0.52
	7,00	1	0.52
	Total	193	100

RISK FACTORS

Work related

Table 14: Frequency of household heads sleep in field hut

		Respondents	% of respondents
Sleep in field hut	never	131	56.47
	once a year	62	26.72
	once a month	21	9.05
	2-3 nights/month	9	3.89
	4 or more/month	4	1.72
	1 or more/wk	2	0.86
	other	3	1.29
	Total	232	100
Kinds of field hut	Hut in rainy season	6	2.59
	Hut in cool season	43	18.53
	Hut in hot season	50	21.55
	Sleep in forest (no hut)	11	4.74
	Other	122	52.59
	Total	232	100

Table 15: Animal around house

		Respondents	% of respondents
Animal around house	yes	182	78.44
	no	50	21.55
	Total	232	100
Domestic animal	Cat	99	39.91%
	Dog	149	60.08%
	Total	248	100
Other Animal	Cows	128	36.36
	Buffalo	16	4.55
	Pigs	19	5.4
	Chicken	142	40.34
	Ducks	19	5.40
	Birds	3	0.85
	Fish	25	7.10
	Total	352	100

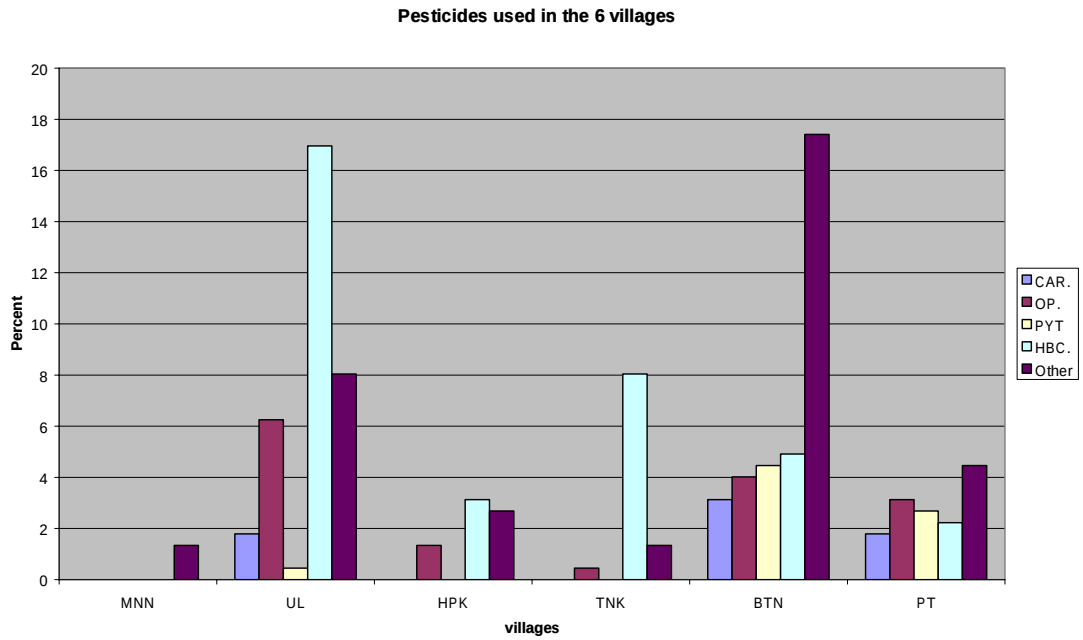
Questionnaire 2

The 1-20 most important farmers in the six selected villages were interviewed household heads specific information on agricultural pesticide used in their farms. A total of 74 respondents to the questionnaires 2, from the six villages of two districts, were shown on table 4.1. The differentiation of percentage between chemical used in each village were shown on table 4.2, 4.3 and figure 1 – 3 respectively.

Pesticides for agricultural plant protection

Table 16: Pesticides used in each village

Villages	No. of big farmer	Pesticide Groups									
		Carbamate		Organophosphate		Pyrethroids		Herbicides		Other	
		No.	%	No.	%	No.	%	No.	%	No.	%
Mae Num Noi (MNN)	1	0	0	0	0	0	0	0	0	3	1.34
U - Long (UL)	26	4	1.79	14	6.25	1	0.45	38	16.96	18	8.04
Huy Pak Kok (HPK)	12	0	0	3	1.34	0	0	7	3.13	6	2.68
Thung Nang Klourn(TNK)	15	0	0	1	0.45	0	0	18	8.04	3	1.34
Bong Ti Noi (BTN)	10	7	3.13	9	4.02	10	4.46	11	4.91	39	17.41
PU Tuey (PT)	10	4	1.79	7	3.13	6	2.68	5	2.23	10	4.46
Total	74	15	6.71	34	15.19	17	7.59	79	35.27	79	35.27
Total of chemical used	224										

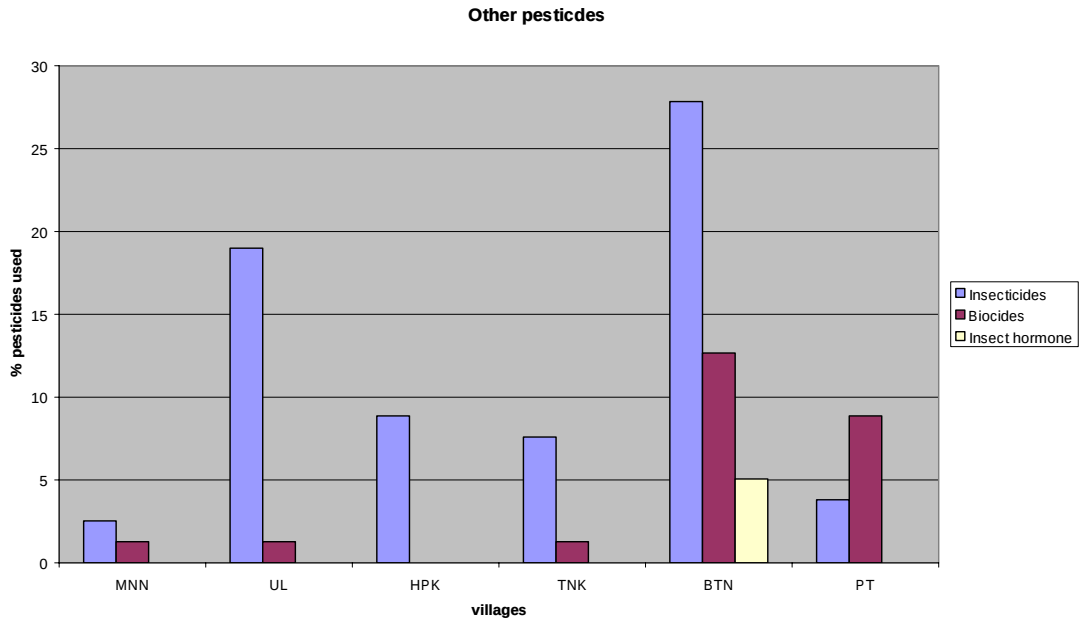


Villages: MNN = Mae Num Noi village ; Chemical groups : CAR = Carbamate
UL = U-Long OP = Organophosphate
HPK = Huy Pak Kok PYT = Pyrethroid
TNK = Tung Nang Kruan HBC = Herbicides
BTN = Bong Ti Noi
PT = Pu Tuey

Figure 19: Pesticides used in each village

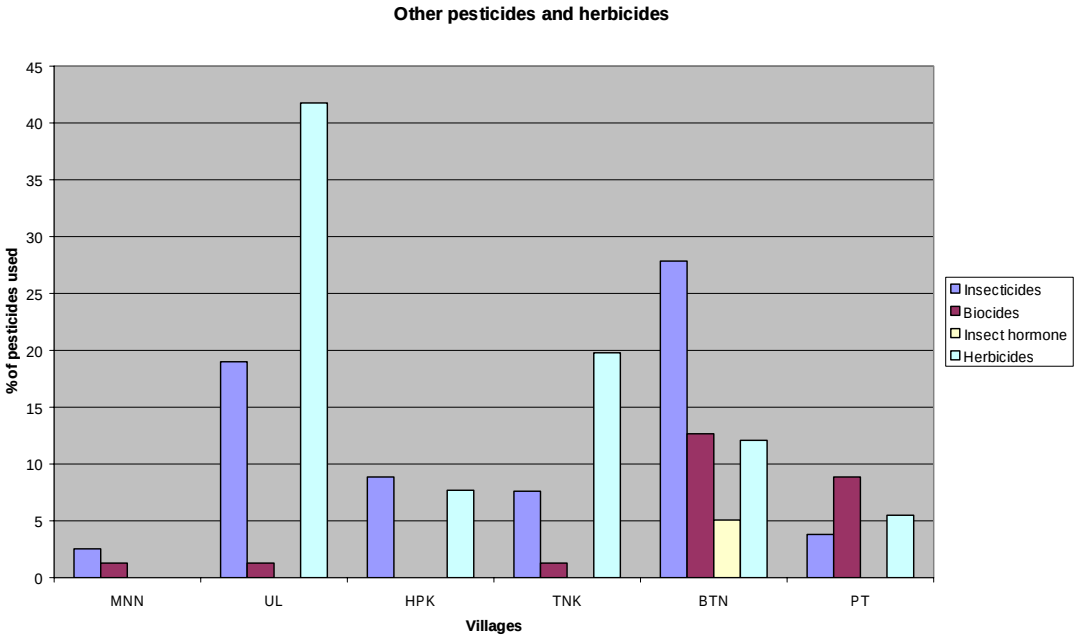
Table 17: Other pesticides used in each village

Villages	of big farmer	Other pesticides					
		Insecticides		Biocides		Insect hormone	
		No.	%	No.	%	No.	%
Mae Num Noi (MNN)	1	2	2.53	1	1.27	0	0
U - Long (UL)	20	15	18.99	1	1.27	0	0
Huy Pak Kok (HPK)	20	7	8.86	0	0	0	0
Thung Nang Klourn(TNK)	20	6	7.59	1	1.27	0	0
Bong Ti Noi (BTN)	20	22	27.85	10	12.66	4	5.06
PU Tuey (PT)	10	3	3.8	7	8.86	0	0
Total	91	55	69.62	20	25.33	4	5.06



Villages: MNN = Mae Num Noi village
UL = U-Long
HPK = Huy Pak Kok
TNK = Tung Nang Kruan
BTN = Bong Ti Noi
= Pu Tuey

Figure 20: Other pesticides used in each village



Villages: MNN = Mae Num Noi village
UL = U-Long
HPK = Huy Pak Kok
TNK = Tung Nang Kruan
BTN = Bong Ti Noi
= Pu Tuey

Figure 21: Other pesticides and herbicides used in each village

Part 2; **Biting peak and population dynamics of *Anopheles minimus* species A, from high and low agricultural insecticide area in the two villages at Kanchanaburi Province, Thailand.**

Adult *Anopheles minimus* species A collections

A total of 1,899 adults of *Anopheles minimus* species A mosquitoes were collected from the six collection sites at the two locations (Figure 4 and 7). Collection size varied from month to month. The highest numbers collected were in October at both locations (Figure22 and Table 18).

There were highly significant differences between the two locations. The total number of female mosquitoes collected per month in MNN was 1,654 and in BTN with 245 and the corresponding averages were137.83 and 20.42 females per month in MNN and BTN, respectively ($P < 0.001$). There were significant differences between the six collection sites in MNN and BTN ($P = 0.034$) (Table 19). The seasonal differences between adult females were significantly different (P=0.014). The rainy season total for MNN was 917 females (mean 229.25 females per month) and for BTN 116 females (mean 29.0 females per month). The corresponding numbers for the cool season were 403 females (mean 100.75) for MNN and 10 females (mean 2.5) for BTN (P< 0.001) and for the hot season 334 females (mean 83.5) for MNN and 119 females (mean 29.75) for BTN. Female mosquito populations from MNN and BTN decreased in abundance during the cool to hot season, but the magnitude of decrease was greater in BTN than in MNN (Figure 22).

Biting patterns of *An. minimus* A

Results of the biting cycle of *An. minimus* A between the 2 locations selected in each season did not differ considerably. In general biting peak was 18.05-23.00 h both of MNN and BTN and the second peak was 01.05-.02.00 h (Figure 23). The highest collected number of mosquitoes was recorded on 21.05-23.00 h with 220 and 37 female mosquitoes from MNN and BTN respectively. The lowest collected

number of mosquitoes were 85 and 5 from MNN and BTN respectively at 18.05-19.00 h (Table 20).

Larval populations of *An. minimus* A

Mosquito larval survey results from December 2003 to September 2004 at the four breeding sites are summarized in Table 3 and figure 8. The total number of *An. minimus* A larvae collected were 1,184. The average larval density fluctuated similarly between the two villages. Anopheles larvae were prevalent throughout the year and appeared in high densities in October to December (late rain to cool) with stream velocities between 0.025-0.092 m/s. From January to May densities decreased. within an optimum 0.017 m/s of velocities of stream in the late dry season and 0.25 m/s in early rainy season both of MNN and BTN. However, there were no larvae at BTN in August, the middle of rainy season when stream velocities were at a maximum of over 0.42 m/ s.

Table 18: Number of *Anopheles minimus* species A collected through out the year compare between the two collecting sites, MNN and BTN.

Month	Season	MNN/ low chemical	BTN/ high chemical
November	Cool	172	1
December	Cool	74	2
January	Cool	69	1
February	Cool	88	6
Mean		100.75	2.5
Total		403	10
March	Hot	46	0
April	Hot	20	0
May	Hot	150	32
June	Hot	118	87
Mean		83.5	29.75
Total		334	119
July	Rainy	202	23
August	Rainy	122	2
September	Rainy	329	15
October	Rainy	264	76
mean		229.25	29
Total in rainy		917	116

Table 19: Number of *Anopheles minimus* species A collected through out of the year compare between the six collecting sites in Kanchanaburi province, Thailand.

		Collecting sites					
Month	Season	MNN/village	MNN/rubber	MNN/forest	BTN/village	BTN/orchard	BTN/ forest
November	Cool	73	89	10	0	1	0
December	Cool	58	9	7	1	0	1
January	Cool	44	15	10	1	0	0
February	Cool	20	11	57	4	2	0
March	Hot	29	4	13	0	0	0
April	Hot	14	1	5	0	0	0
May	Hot	140	3	7	27	2	3
June	Hot	102	11	5	54	24	9
July	Rainy	118	73	11	20	2	1
August	Rainy	63	57	2	2	0	0
September	Rainy	82	241	6	6	5	4
October	Rainy	156	106	2	39	21	16
mean		74.92	51.67	11.25	12.83	4.75	2.83
Total		899	620	135	154	57	34

Table 20: Number of *Anopheles minimus* species A collected each hour through out the year compare between low chemical location (MNN) and high chemical location (BTN) in Kanchanaburi province, Thailand.

Hours	MNN / low chemical	BTN / high chemical
18.05-19.00	85	5
19.05-20.00	133	15
20.05-21.00	185	26
21.05-22.00	220	37
22.05-23.00	180	34
23.05-24.00	128	33
24.05-01.00	103	24
01.05-02.00	156	25
02.05-03.00	120	14
03.05-04.00	135	10
04.05-05.00	102	8
05.05-06.00	107	14
Mean	137.83	20.42
Total	1,654	245

Table 21: Larvae of *Anopheles minimus* species A collected each season through out the year compare between two collecting sites, MNN and BTN.

		No. of *An. minimus* A (larvae)			
		BTN		MNN	
Month	Season	Bst	Sst	HP	ST
November	Cool	75	5	21	42
December	Cool	91	25	40	52
January	Cool	54	16	28	27
February	Cool	65	8	18	41
mean		71.25	13.5	26.75	40.5
Total in cool		285	54	107	162
March	Hot	47	6	28	34
April	Hot	34	0	12	17
May	Hot	21	2	10	5
June	Hot	39	6	10	31
mean		35.25	3.5	15	21.75
Total in hot		141	14	60	87
July	Rainy	0	7	28	12
August	Rainy	0	0	13	12
September	Rainy	5	3	6	20
October	Rainy	56	30	22	60
mean		15.25	10	17.25	26
Total in rainy		61	40	69	104

Table 22: Number of female and larvae of *Anopheles minimus* species A collected through out of the year compare between two collecting sites, MNN and BTN

Month	MNN/ female	BTN/ female	MNN/ larvae	BTN/ larvae
Nov./cool	85	5	63	80
Dec./cool	133	15	92	116
Jan./cool	185	26	55	70
Feb./cool	220	37	59	73
Mar./hot	180	34	62	53
Apr./hot	128	33	29	34
May./hot	103	24	15	23
Jun./hot	156	25	41	45
Jul./ wet	120	14	40	7
Aug./wet	135	10	25	0
Sep./wet	102	8	26	8
Oct./wet	107	14	82	86
Mean	137.83	20.42	49.08	49.58
Total	1,654	245	589	595

Table 23: The statistic of the caught female of *An minimus* A comparison between MNN and BTN in each season

Source	Type III Sum of Squares	df	Mean Square	F	Significant
Corrected Model	135384.375a	5	27076.875	9.815	0
Intercept	150258.375	1	150258.375	54.467	0
Location	82720.042	1	82720.042	29.985	0
Season	30100	2	15050	5.456	0.014
Location * Season	22564.333	2	11282.167	4.09	0.034
Error	49656.25	18	2758.681		
Total	335299	24			
Corrected Total	185040.625	23			

a. Rsquared = .732 (Adjusted R Squared = .657)

Table 24: The statistic of the collected larvae of *An minimus* A comparison between MNN and BTN in each season

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	9850.833a	5	1970.167	3.222	0.03
Intercept	58410.667	1	58410.667	95.525	0
Season	8582.333	2	4291.167	7.018	0.006
Location	1.5	1	1.5	0.002	0.961
Season * Location	1267	2	633.5	1.036	0.375
Error	11006.5	18	611.472		
Total	79268	24			
Corrected Total	20857.333	23			

a. Rsquared = .472 (Adjusted R Squared = .326)

Table 25: Climatological data as recorded from the meteorological station in Thong Pha Phum (TPP) and Sai Yok (SY) district Kanchanaburi Province, average in one year.

Month	Relative humidity (average, %)		Rain fall (average, mm)	
	TPP	SY	TPP	SY
Oct-03	95	96	2.06	6.1
Nov-03	92	95	0	0
Dec-03	93	93	0	0
Jan-04	91	93	0.1	0.8
Feb-04	88	91	0.4	0.8
Mar-04	83	89	0.2	1.6
Apr-04	83	88	0.4	3
May-04	93	96	7.5	14
Jun-04	94	96	12 .8	6.7
Jul-04	95	96	5.2	4.1
Aug-04	90	96	9.19	3.5
Sep-04	90	96	5.03	5.6
Total			30.08	46.2

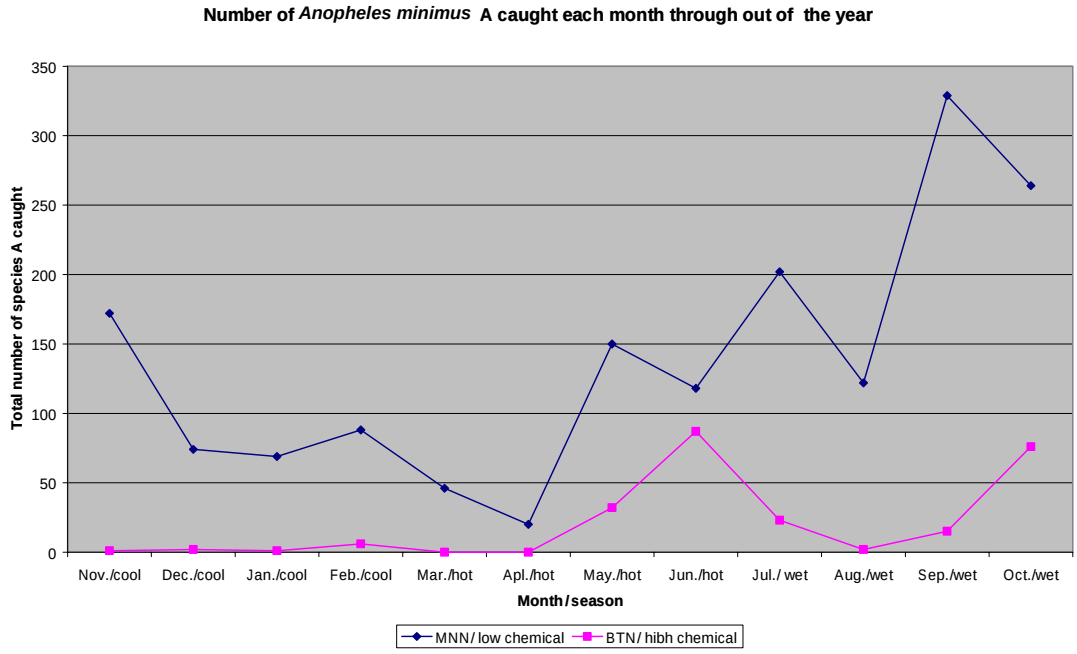


Figure 22: Number of *Anopheles minimus* species A collected through out the year compare between the two collecting sites, MNN and BTN.

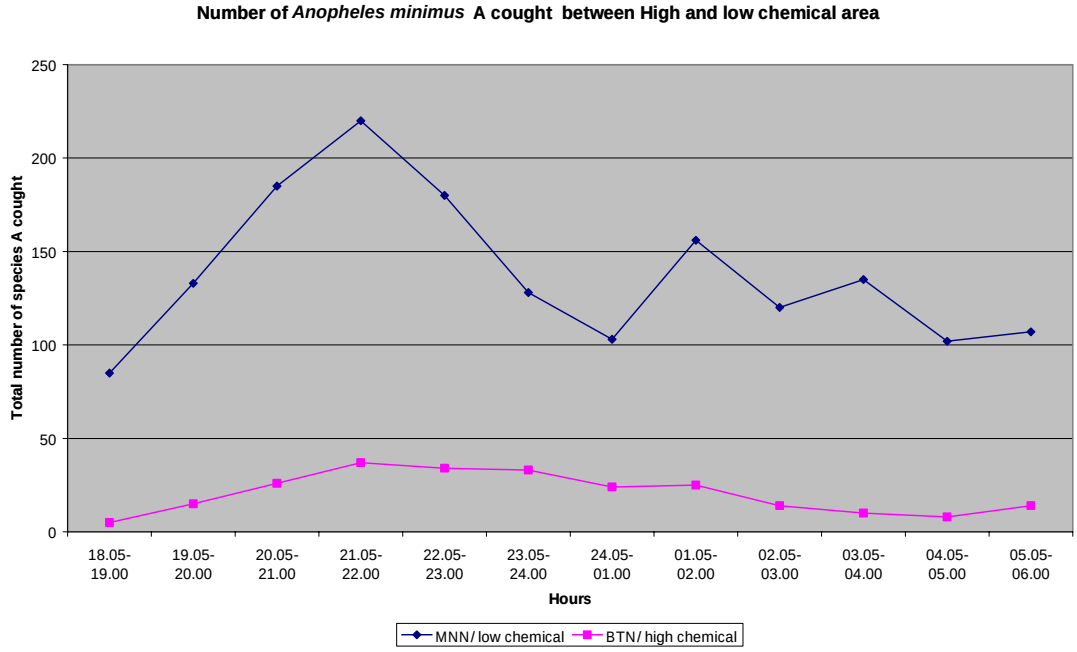


Figure 23: Number of *Anopheles minimus* species A collected each hour through out the year compare between the two collecting sites, MNN and BTN.

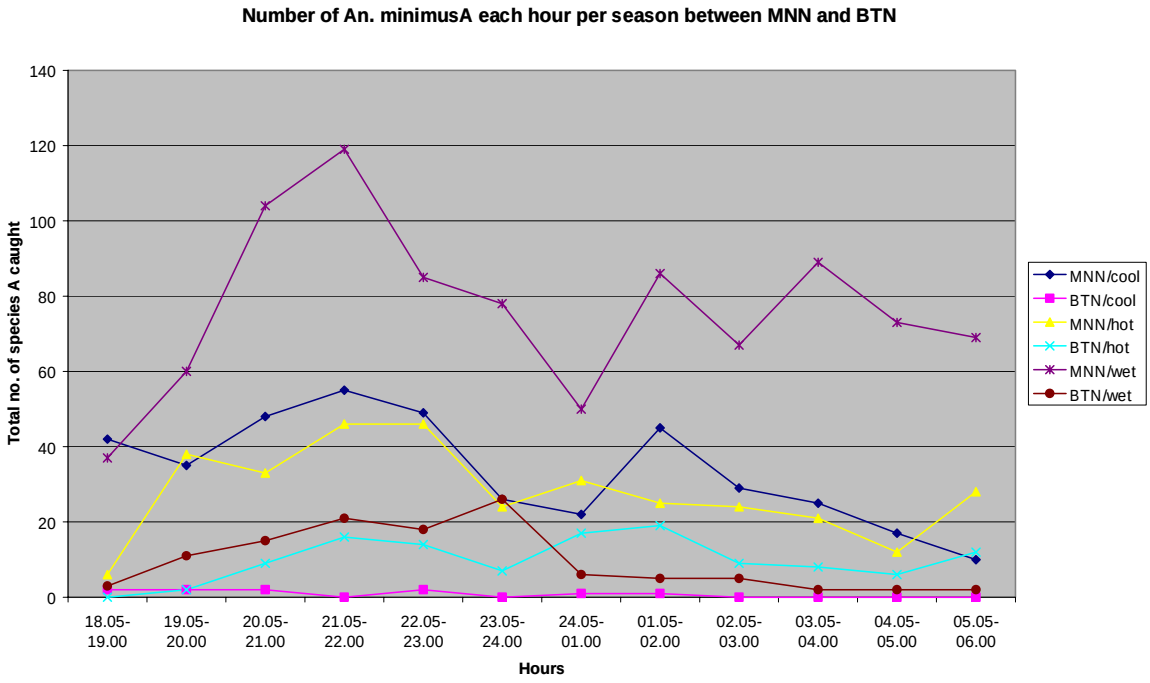


Figure 24: Number of *Anopheles minimus* species A collected each hour on three season compare between the two collecting sites, MNN and BTN.

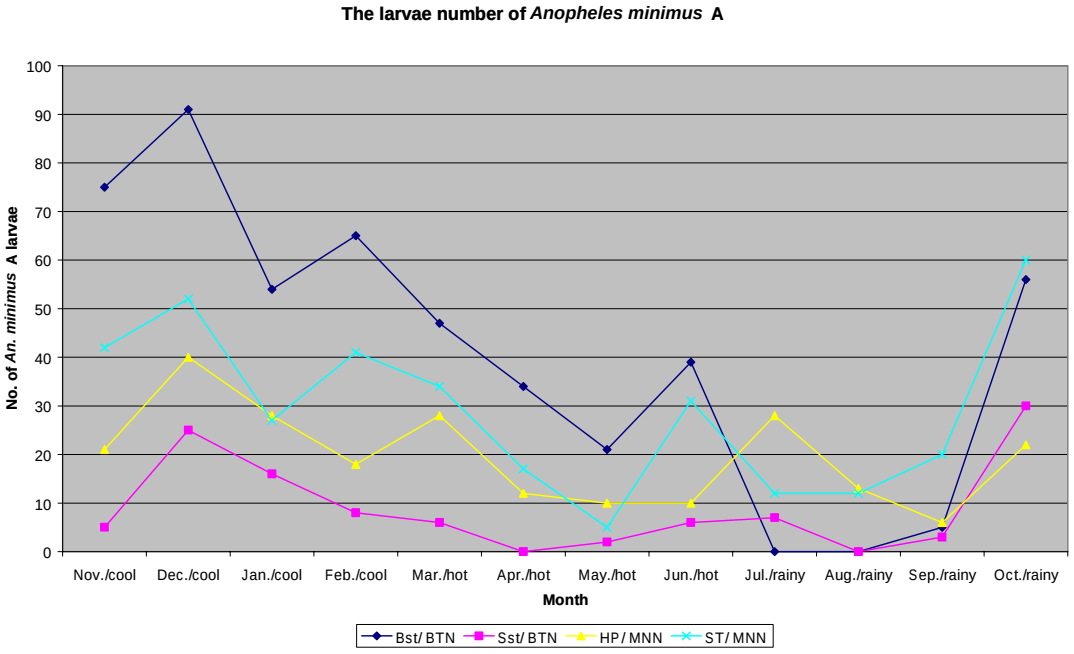


Figure 25: Larvae of *Anopheles minimus* species A collected through out the year compare between the four collecting sites in Kanchanaburi Province.

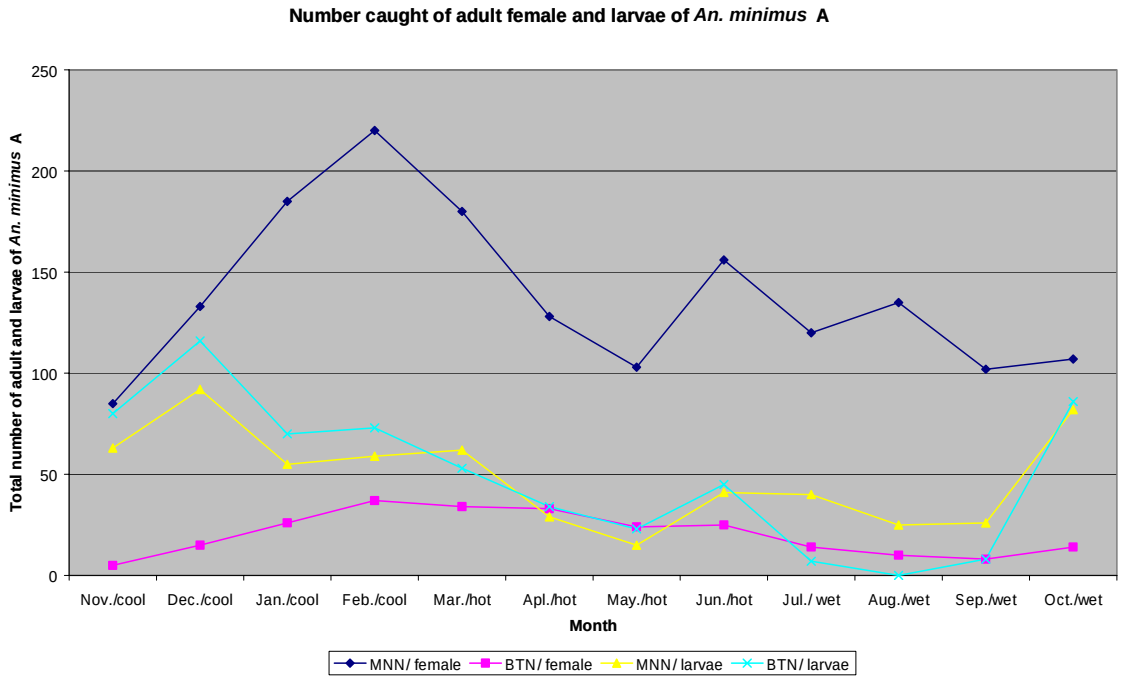


Figure 26: Number of female and larvae of *Anopheles minimus* species A collected through out the year compare between two collecting sites, MNN and BTN

Part 3; **Behavioral responses by *Anopheles minimus* species A and C to three agrochemicals.**

Presence of avoidance behavior to the tested chemicals was observed in the two strains of *Anopheles minimus* in the form of contact irritancy and non-contact repellency (Table 26). There were slightly different behaviors between the strains. In the contact trial, the escape response was stronger in species C than in species A when exposed to carbaryl (68% of species C and 46% of species A escaped; *P*=0.001) and cypermethrin (67% of species C and 58% of species A escaped; *P*=0.054) (Table 26, Figures 27 and 28). The opposite pattern was observed when mosquitoes were exposed to malathion. Here the escape response was significantly stronger in species A (85% escaped) than in species C (23% escaped) (*P*=0.0001). Similar escape patterns were observed for the repellency function in the non-contact trials. Escape response was significantly stronger in species C than in species A when exposed to carbaryl (80% of species C and 49% of species A escaped; *P*=0.001) and cypermethrin (64% of species C and 27% of species A escaped; *P*=0.001). As in the contact trial, the opposite pattern was observed for malathion. The escape response was significantly stronger in species A (52% escaped) than in species C (38% escaped) (*P*=0.001). Comparison between the contact and non-contact trials showed significant differences in escape response of *An. minimus* A and C across the three compounds *P*<0.05 (*P*=0.001- 0.0001), except for carbaryl in species A, *P*>0.05 (P= 0.105) and cypermethrin in species C, *P*>0.05 (*P*=0.205). The trend seemed to be that species A tended to escape in higher numbers in the contact trials than in the non-contact trials (malathion and cypermethrin), whereas in species C more mosquitoes escaped in the non-contact compared to the contact trials (malathion and carbaryl). Mortalities after a 24-h holding period were in general higher for species A (0-21%) than species C (0-0.2%), those for mosquitoes that remained within the chambers compared to those that escaped (Table 26). The highest mortalities were found in species A that escaped in the contact and non-contact trials with cypermethrin (21% and 10%, respectively). Of those mosquitoes that remained within the chambers, 24-h mortalities were highest to carbaryl (contact: 16.7%; non-contact: 5.6%). Low

mortalities were found in the other tests and there were no mortalities in any of the controls.

The escape times for 30%, 50%, and 70% of the two *An. minimus* strains (ET_{30}, ET_{50} and ET_{70}) can not be obtained because many mosquitoes remained inside the exposure chambers after the test had ended (Table 27). In the contact trial with malathion, 70% of species A escaped after 14 minutes, whereas it can not be estimated from the species C. Carbaryl and cypermethrin used in the contact trials were not able to force more than 70% of the two species to escape within the 30 minute test period. In the non-contact trials, only carbaryl was able to force more than 70% of mosquitoes, in this case species C, to escape; this happened after 21 minutes. In the same test with species A, only 30% of the population escaped, and after 4 minutes.

Figures 27, 28 and 29 showed the proportions of mosquitoes remaining in the excito-repellency test chambers treated with carbaryl, malathion and cypermethrin, respectively. These proportions are served as an analytical data to develop patterns of escape rates and demonstrate probabilities for escaping from test chambers in contact vs. non contact (Figures 27, 28 and 29) In contact trials, escape rate of species A with carbaryl and cypermethrin were significantly lower than species C ($P<0.05$; Figures 27 and 29). In non contact trials with carbaryl and cypermethrin, the escape rate was dramatically higher for species C than species A ($P<0.05$; Figures 27 and 29). This phenomenon was not observed in non contact trials with malathion. With malathion, the escape rate was statistically higher for species A than species C ($P<0.05$; Figure 28).

Table 26: Percentage escape response and mortality of *Anopheles minimus* A and C exposed to carbaryl, malathion and cypermethrin in contact and noncontact trials

							%Mortality			
			Treatment		Control		Treatment		Control	
Test condition	Insecticide	Strain	No. tested	% Escape	No. tested	% Escape	Escape	Not Escape	Escape	Not Escape
Contact	Cabaryl	A	77	46	76	19	8.6	16.7	0	0
		C	78	68	77	27	0.2	0.1	0	0
	Malathion	A	65	85	60	22	0	0	0	0
		C	78	23	80	12	0	0.1	0	0
	Cypermethrin	A	72	58	76	25	21	0	0	0
		C	87	67	84	23	0.1	0.1	0	0
Non-Contact	Cabaryl	A	71	49	75	10	0	5.6	0	0
		C	76	80	76	20	0	0	0	0
	Malathion	A	65	52	60	23	2.9	0	0	0
		C	80	38	78	4	0.1	0.1	0	0
	Cypermethrin	A	73	28	77	12	10	1.9	0	0
		C	85	63	83	34	0.1	0.1	0	0

Table 27: Estimated escape time (ET) at 30, 50, and 70 minutes for *Anopheles minimus A and C* in contact with 0.4 g/m^2 carbaryl, 0.19 g/m^2 malathin and 0.04 g/m^2 cypermethrin

		ET 30		ET 50		ET 70	
Species	Insecticide	Contact	Non contact	Contact	Non contact	Contact	Non contact
A	Cabaryl	10	4	-	-	-	-
	Malathion	2	4	7	16	14	-
	Cypermethrin	4	-	21	-	-	-
C	Cabaryl	4	2	18	8	-	21
	Malathion	-	18	-	-	-	-
	Cypermethrin	12	9	20	18	-	-

Table 28: Log-rank comparisons of escape responses between two species in contact and non contact trials.

Insecticide	Contact trial (*P*)	Non-contact trial (*P*)
Carbaryl	0.001	0.001
Malathion	0.0001	0.001
Cypermethrin	0.054	0.001

Table 29: Log-rank comparisons of escape responses between control and contact, contact and non-contact, and control and non contact trials for two strains of *An. minimus*

Insecticide	Test Strain	Control vs. Contact (*P*)	Contact vs. Non-contact (*P*)	Control vs. Non-contact (*P*)
Carbaryl	Species A	0.0001	0.105	0.0001
	Species C	0.0001	0.0001	0.0001
Malathion	Species A	0.001	0.001	0.001
	Species C	0.001	0.001	0.001
Cypermethrin	Species A	0.0001	0.0001	0.0001
	Species C	0.0001	0.205	0.0001

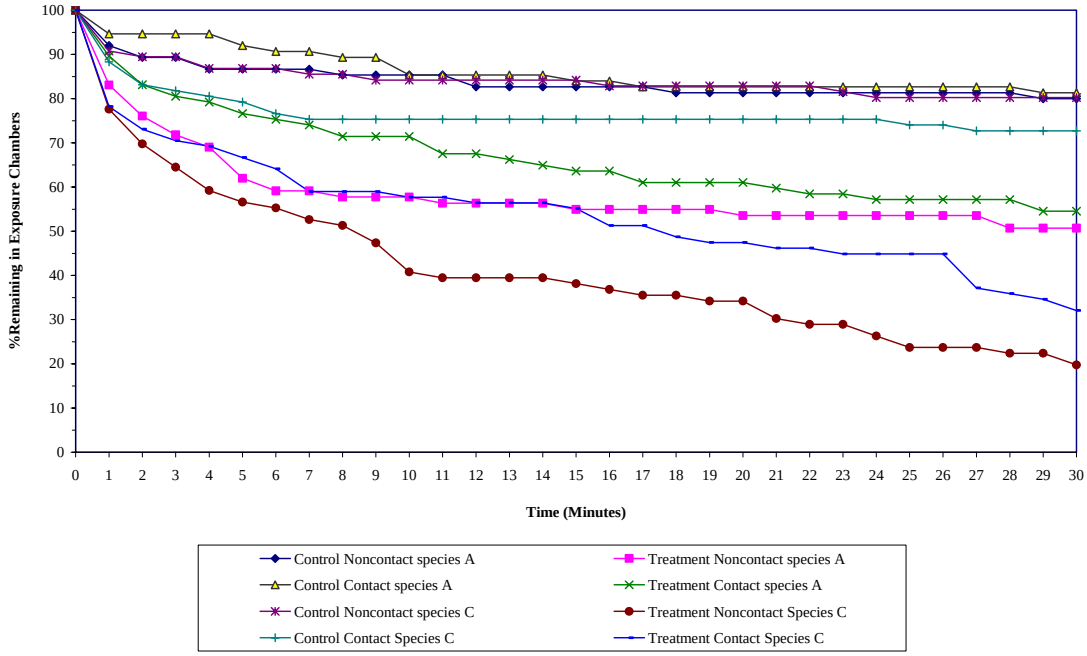


Figure 27: Escape probability of *Anopheles minimus* species A and C exposed to carbaryl and paired control chamber for contact and non-contact trials.

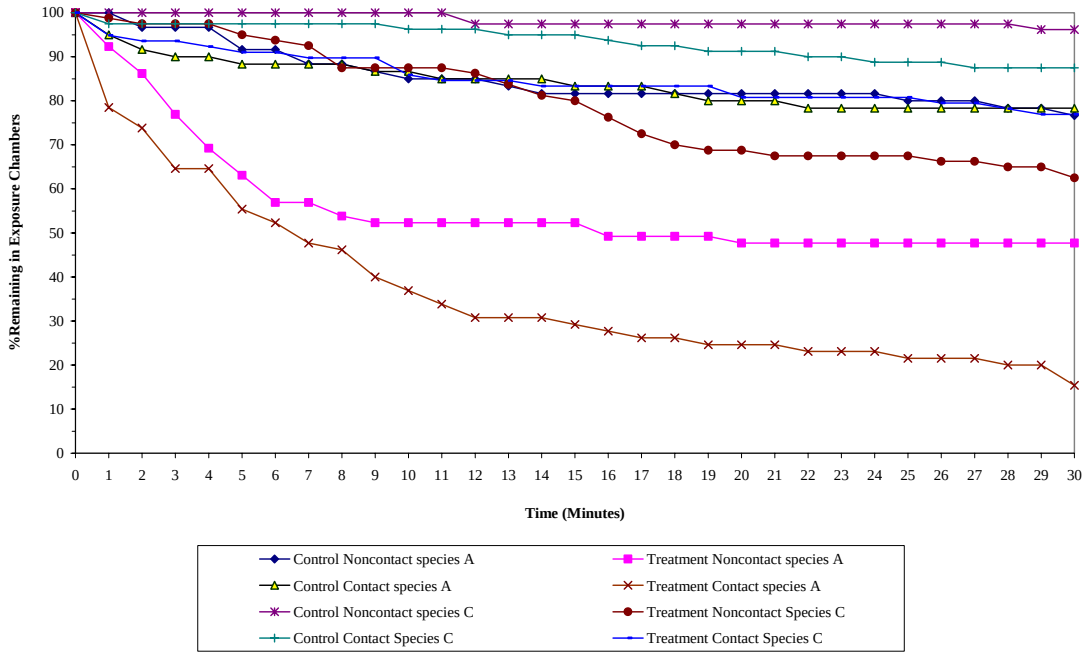


Figure 28: Escape probability of *Anopheles minimus* species A and C exposed to malathion and paired control chamber for contact and non-contact trials.

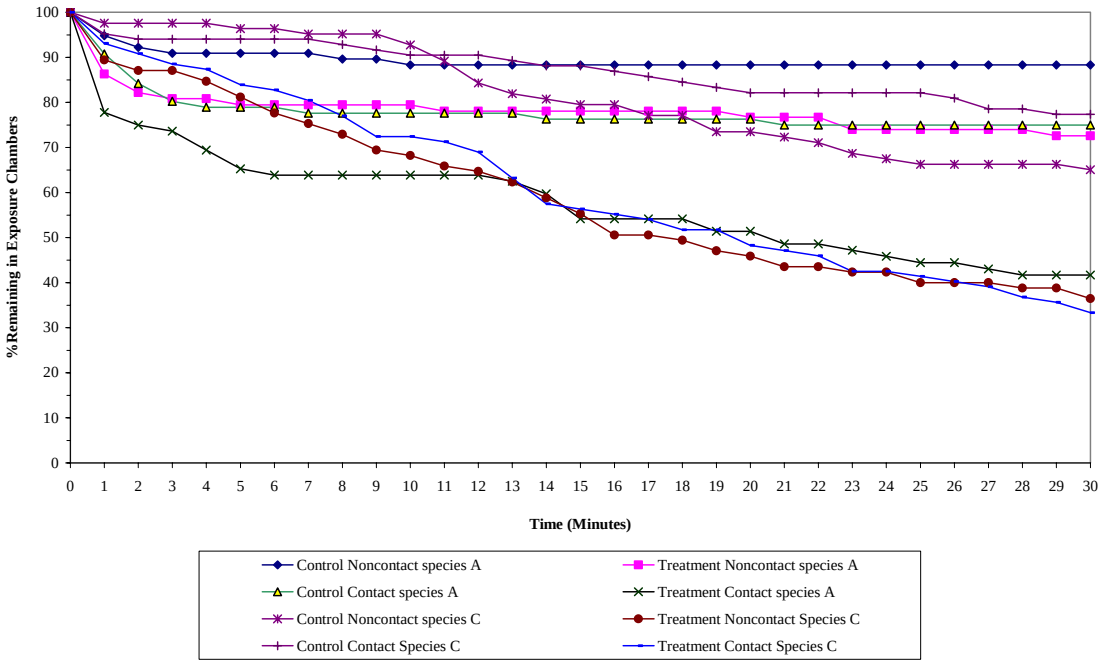


Figure 29: Escape probability of *Anopheles minimus* species A and C exposed to cypermethrin and paired control chamber for contact and non-contact trials.

Part 4; Behavioral responses by *Anopheles minimus* species A and species C to DDT and pyrethroids.

Two types of behavioral responses, contact irritancy and non-contact repellency, were observed with exposure to three insecticides and percent mortalities of escape and non-escape mosquitoes from control and treated chambers were recorded (Table 30). Patterns and rate of escape were stronger in *An.minimus* species A than *An. minimus* species C for all three compounds. In contact trials, percent escape of *An. rninimus* A (92-96%) was significantly ($P < 0.05$) higher than for *An. minimus* C (50-90%), regardless of compound used. Similarly, percent escape by species A was also greater than that by species C for the two synthetic pyrethroids. In general, a relatively low number of mosquitoes escaped from the control chambers (12-23% for contact and 10-15% for non-contact). Mortality rates of escaped mosquitoes from both test populations were low (0-13.3%), whereas those that remained in the test chamber (non-escape mosquitoes) showed much higher mortality rates (43-100%). All non-escape specimens of species A exposed to deltamethrin and lambda-cyhalothrin perished within 24 hours post-exposure (Table 30). High mortality rates (13.3%) of escaped mosquitoes from control chambers were observed with DDT. In non-contact trials, *An. minimus* species A demonstrated significantly strong escape responses to all three compounds compared with species C. After 30 minutes exposure, percent escape was approximately 96% for DDT, 92% for deltamethrin, and 87% for lambdacyhalothrin with *An. minimus* species A, while only 24% for DDT and deltamethrin and 18% for lambda-cyhalothrin with species C. Percent mortalities of escaped specimens of both populations were very low, ranging from 1.1% to 4.5%. Mortality was not seen in non-escaped *An. minimus* species A after the 24-hour holding period.

The escape patterns generated from insecticide-treated chambers are expressed in one-minute intervals for 50%, 75%, and 90% (ET_{50}, ET_{75}, and ET_{90}) of the test population to escape from exposure chambers (Table 31). In contact trials, the ET_{50}, ET_{75}, and ET_{90} for *An. minirnus* species A were 5,12.5, and 24 minutes with DDT, 2.5, 6, and 16 minutes with deltamethrin, and 7, 23.5, and 30 minutes with

lambdacyhalothrin, respectively. The ET_{50} for *An. minimus* C was 5, 8.5, and 6 minutes for DDT (0.02 g/m^2). The ET_{75}, and ET_{90} values for all three compounds for *An. minimus* species C could not be calculated (with one exception: lambdacyhalothrin ET_{75} =12.5 minutes) because of insufficient numbers of mosquitoes escaping. Similarly, ET values for *An. minimus* species C in all non-contact trials could not be estimated. For non-contact trials, the ET_{50}, ET_{75}, and ET_{90} values were 5, 4.5, and 14 minutes for DDT and 5.6, 8, and 25 minutes for deltamethrin. The ET_{50} and ET_{75} estimates were 6.5 and 23.5 minutes, respectively, for lambda-cyhalothrin.

Comparison of escape responses between *An.. rninimus* species A and C in contact and non-contact trials using log - rank analysis showed statistically significant differences in escape patterns between species in non-contact trials for all three compounds ($P < 0.05$). In contact trials, significant differences in escape responses between species were observed with DDT and deltamethrin ($P < 0.05$). Comparisons of escape responses between paired contact and control, contact and non-contact, and non-contact and control bioassays for each species for each compound were made. No significant differences between contact and non-contact escape for *An. rninimus* species A were observed ($P > 0.05$). Escape probabilities in contact and non-contact trials were significantly higher than those in paired controls for all bioassays.

Figures 2-5 show the proportions of mosquitoes remaining in the exposure and control chambers under different test conditions and chemical exposure. Strong repellency action was seen with *An.minirnus* species A against all three compounds, whereas significantly less escape reaction was observed with *An. minimus* species C (Figure 33). In non-contact tests, *An. minimus* species A demonstrated a stronger escape rate with DDT than with either deltamethrin or lambda-cyhalothrin (Figure 33). There were significant differences in escape responses seen in all contact trials compared with paired control and non-contact trials with *An. minimus* species C ($P < 0.05$). Escape patterns in all non-contact repellency trials for *An. minimus* species A were significantly greater than paired controls.

Table 30: Percentage escape response and mortality of *Anopheles minimus* A and C exposed to DDT, deltamethrin and lambda-cyhalothrin in contact and non contact trials

							%Mortality			
			Treatment		Control		Treatment		Control	
Test condition	**Insecticide**	**Strain**	**No. tested**	**% Escape**	**No. tested**	**% Escape**	**Escape**	**Not Escape**	**Escape**	**Not Escape**
Contact	DDT	A	85	92	85	12	0	42.8	0	0
		C	100	77	100	15	1.3	0	13.3	1.2
	Deltamethrin	A	76	96	75	23	0	100	0	0
		C	98	51	94	14	2	0	0	1.2
	Lambdacyha-lothrin	A	77	94	78	23	1.4	100	0	0
		C	100	90	100	18	1.1	0	0	0
Non-Contact	DDT	A	85	96	83	27	0	0	0	0
		C	100	24	100	10	0	0	0	0
	Deltamethrin	A	76	92	75	29	0	0	4.5	0
		C	100	24	100	10	0	0	0	1.3
	Lambdacyha-lothrin	A	77	87	77	27	1.5	0	0	0
		C	95	18	95	15	0	0	0	0

Table 31: Estimated escape time (ET) at 50, 75, and 90 minutes for *Anopheles minimus A and C* in contact with 2 g/m^2 DDT, 0.02 g/m^2 deltamethrin and 0.03 g/m^2 lambda-cyhalothrin

		ET 50		ET 75		ET 90	
Species	Insecticide	Contact	Non contact	Contact	Non contact	Contact	Non contact
A	DDT	5	2	12.5	4.5	24	14
	Deltamethrin	2.5	5.6	6	8	16	25
	Lambdacyhalothrin	7	6.5	23.5	23.5	30	0
C	DDT	5	0	0	0	0	0
	Deltamethrin	8.5	0	0	0	0	0
	Lambdacyhalothrin	7	6.5	12.5	0	0	0

Table 32: Log-rank comparisons of escape responses between two species in contact and non contact trials

Insecticide	Contact trial (*P*)	Non-contact trial (*P*)
DDT	0.001	0.0001
Deltamethrin	0.001	0.0001
Lambda-cyhalothrin	0.205	0.0001

Table 33: Log-rank comparisons of escape responses between control and contact, contact and non-contact, and control and non contact trials for two strains of *An. minimus*

Insecticide	Test Strain	Control vs. Contact (*P*)	Contact vs. Non-contact (*P*)	Control vs. Non-contact (*P*)
DDT	Species A	0.0001	0.205	0.0001
	Species C	0.0001	0.001	0.001
Deltamethrin	Species A	0.001	0.205	0.001
	Species C	0.001	0.0001	0.001
Lambda-cyhalothrin	Species A	0.001	0.117	0.0001
	Species C	0.0001	0.0001	0.105

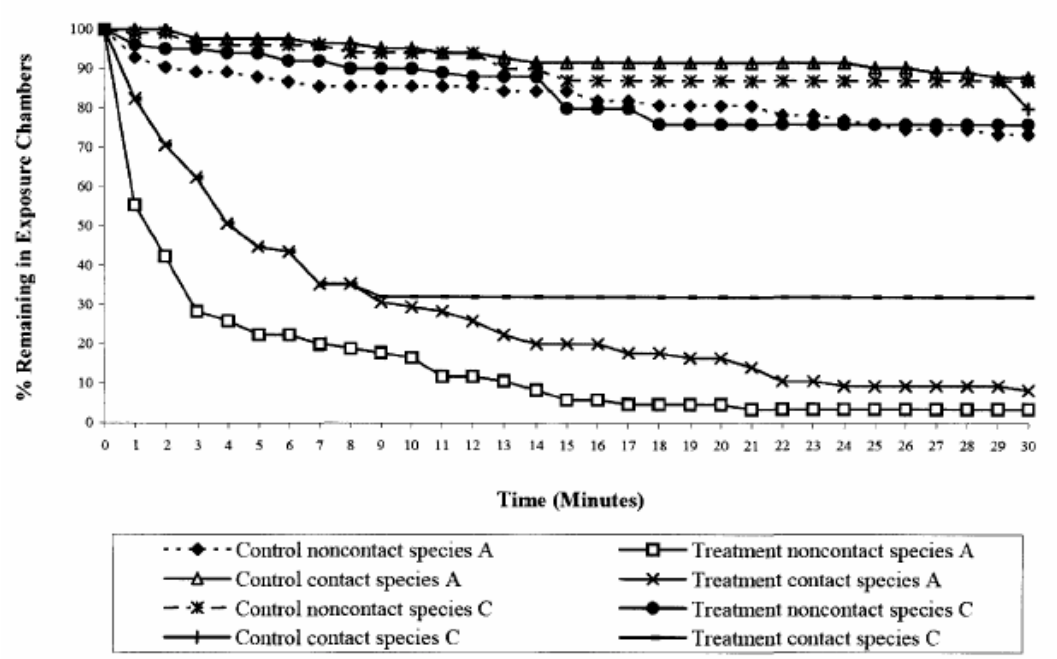


Figure 30: Escape probability of Anopheles minimus species A and C exposed to DDT and paired control chambers for contact and non-contact trials.

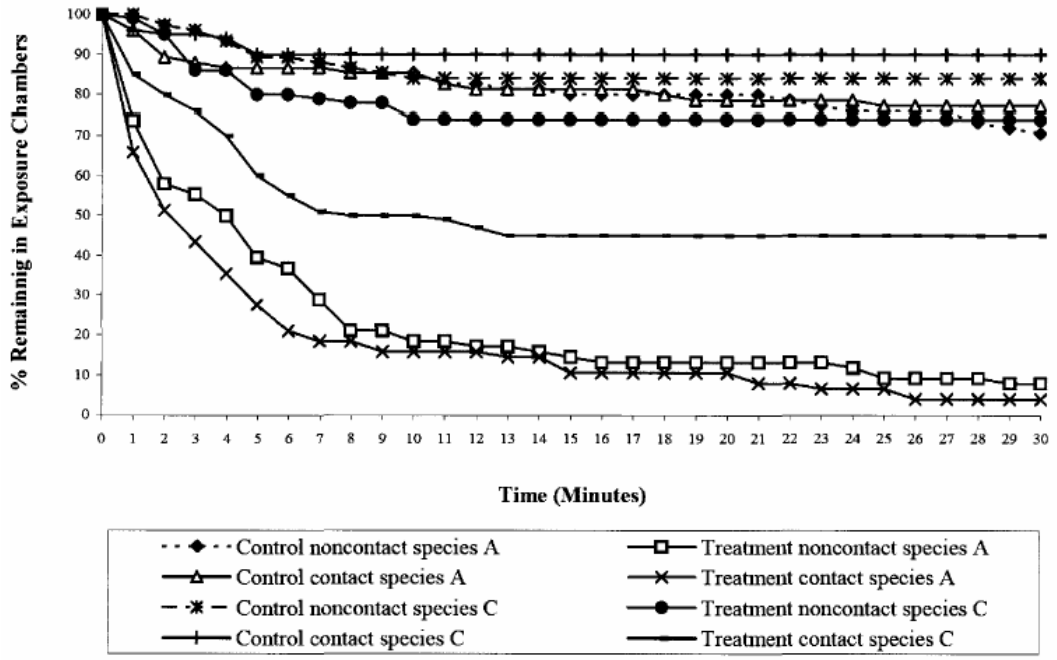


Figure 31: Escape probability of Anopheles minimus species A and C exposed to deltamethrin and paired control chambers for contact and non-contact trials.

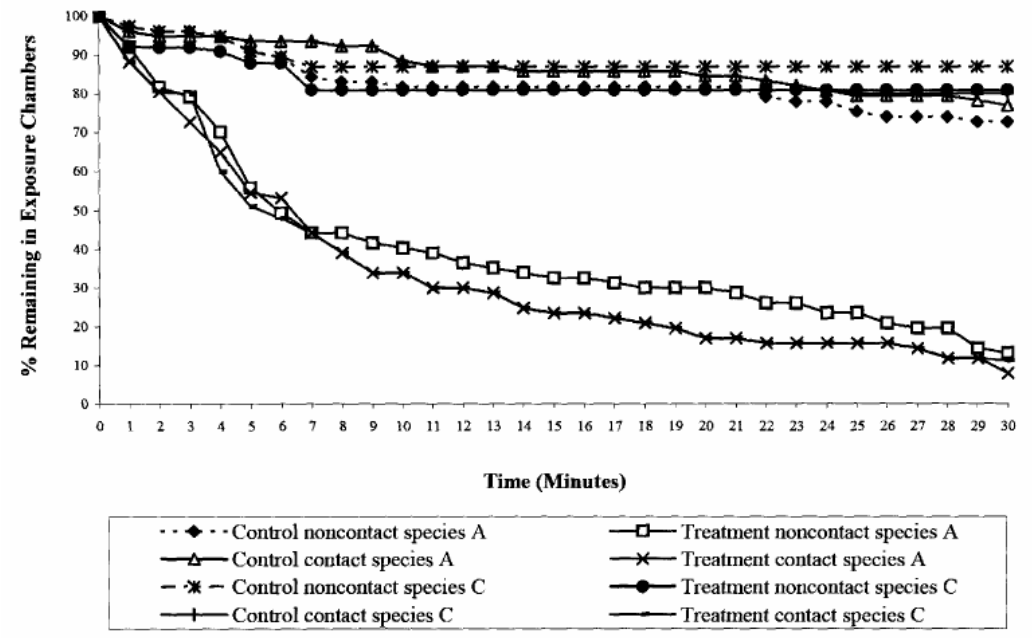


Figure 32: Escape probability of Anopheles minimus species A and C exposed to lambda-cyhalothrin and paired control chambers for contact and non-contact trials.

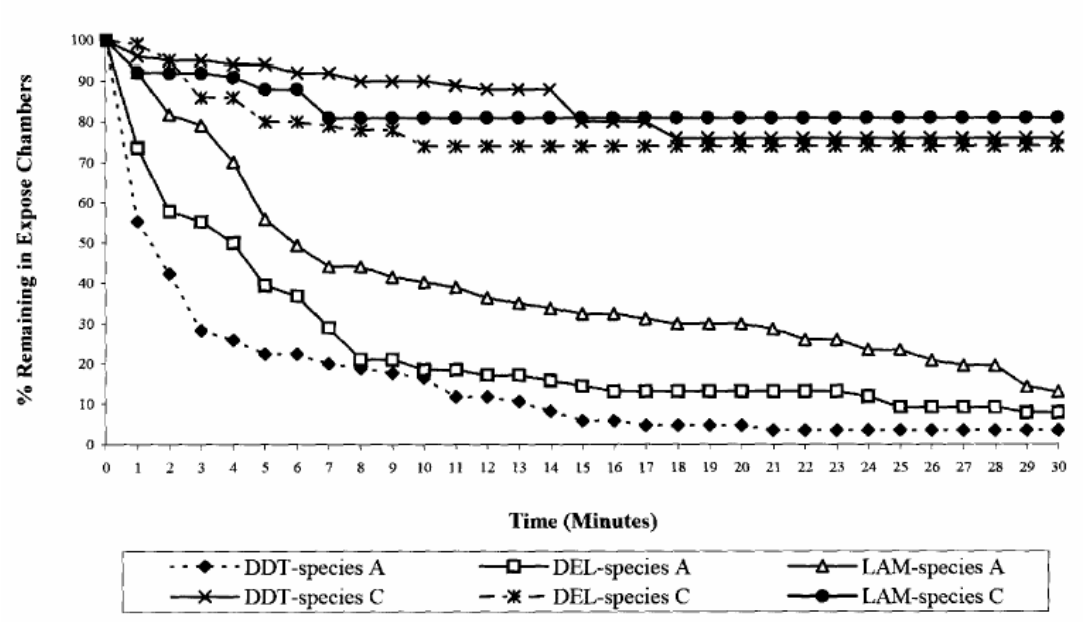


Figure 33: Escape probability of Anopheles minimus species A and C exposed to DDT, deltamenthrin (DEL), and lambda-cyhalothrin (LAM) in non-contact trials.