4. RESULTS

4.1 The density and concentration of airborne bacteria in Chiang Mai city

When sedimentary method was used in the rainy season, the quantitative results of airborne bacteria were shown as density i.e. colony forming units (CFUs) per dish per minutes (Fig.5). The highest value of airborne bacterial density was 10.3 CFUs/dish/min. at grid 8 while the lowest value was 0.43 CFUs/dish/min. at gird 6. The difference between the highest and lowest density was about 24 times. The average of bacterial density in the whole area was 4.8 CFUs/dish/min.

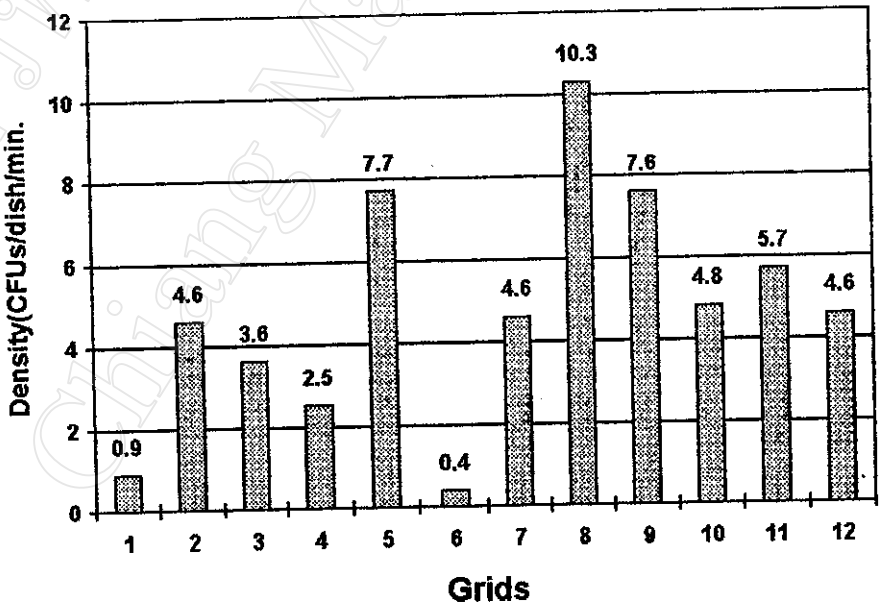

Fig.5 The density of airborne bacteria of 12 grids in the rainy season by sedimentary method

In the cool season, the air sampler was used to collect air samples. The concentration of airborne bacteria was expressed as CFUs / m^3 air. The concentration of airborne bacteria among 12 grids was shown in Figure 6. The highest concentration of airborne bacteria was 3613.3 CFUs/ m^3 at grid 12 and the lowest one was 1600 CFUs/m^3 at grid 6. The difference between highest and lowest concentration was about 2.3 times. The average of bacterial concentration was 2364.4 CFUs/m^3.

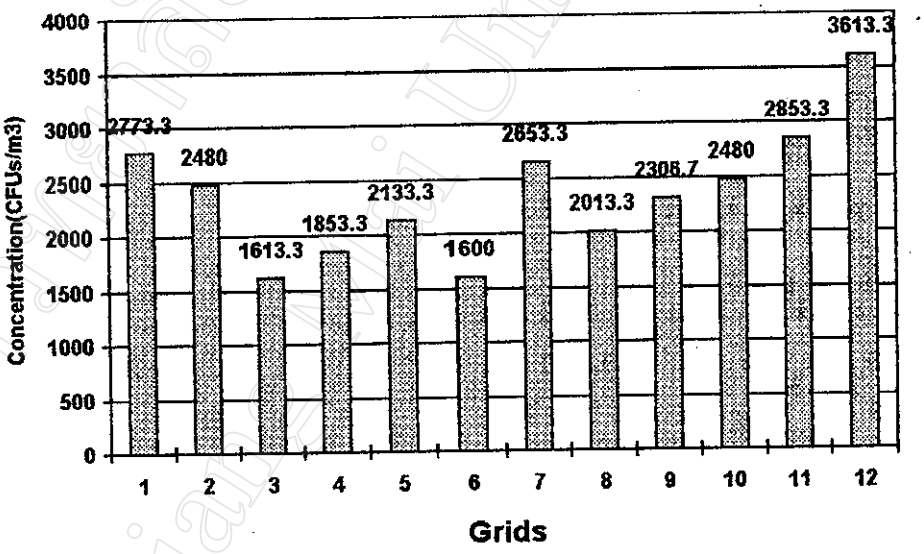

Fig.6 The concentration of airborne bacteria of 12 grids in the cool season by inertial method

4.2 The variation of bacterial density and concentration by sedimentary and inertial methods

The coefficient of variation (C.V.) demonstrates the precision of the results. The high C.V. indicates the wide range of original data or low precision and the low C.V. indicates the narrow scale of results or high precision. Table 3 shows that the highest C.V. was 140.8% at grid 8 while the lowest C.V. was 19.7 % at grid 3 by sedimentary method. The average C.V. of bacterial density in the whole area was 106.3%. When inertial method was used, the relative low value of C.V. was obtained. The data in Table 3 shows the lowest C.V. (11.3%) at grid 2 and the highest one (64.1%) at grid 11. The average C.V. of the bacterial concentration was 35.3 %, which was much lower than that of bacterial density.

Table 3 The variation of bacterial density and concentration of 12 grids by sedimentary and inertial method

		Sedimentary Method			Inertial Method		
Grid	n	Mean density	SD	C.V. * (%)	Mean conc.	SD	C.V.* (%)
1	3	0.9	0.3	33.3	2773.3	698.9	25.2
2	3	4.6	4.9	106.5	2480.0	280.0	11.3
3	3	3.6	0.7	19.7	1613.3	349.4	21.7
4	3	2.5	2.3	92.0	1853.3	625.2	33.7
5	3	7.7	3.7	48.1	2133.3	899.1	42.1
6	3	0.4	0.1	25.0	1600.0	262.2	16.4
7	3	4.6	3.8	82.6	2653.3	760.3	28.7
8	3	10.3	14.5	140.8	2013.3	362.9	18.0
9	3	7.6	2.4	31.6	2306.7	642.9	27.9
10	3	4.8	3.4	70.8	2480.0	760.0	30.6
11	3	5.7	5.6	98.2	2853.3	1829.6	64.1
12	3	4.6	3.1	67.4	3613.3	643.7	17.8
total	36	4.8	5.1	106.3	2364.4	834.3	35.3

n: the number of samples

*C.V. : Coefficient of Variation = (SD/mean) X 100

4.3 The identification of airborne bacteria

All the specimens of the airborne bacteria in this study were found to be Gram positive . Most of the specimens were Gram positive rods, which were identified to be *Bacillus* spp. based on the microscopic features and oxygen tolerance. The species of *Bacillus* spp. were identified by biochemical tests (Table 4) but no confirmation was done by referent laboratories or animal inoculation tests. Some of the specimens were Gram positive cocci, which were identified to be *Staphylococcus aureus* according to grape-like cluster, glucose and mannitol fermentation and the positive reaction in catalase and coagulase tests.

The distribution of bacteria at each grid in the rainy and cool seasons was shown in Table 5.

4.4 The density and concentration of airborne fungi in Chiang Mai city

Similar to the airborne bacteria, the quantitative data of airborne fungi in the rainy season was expressed in terms of density . The highest value of airborne fungal density was 2.8 CFUs/dish/min.at grid 7 and the lowest value was 0.6 CFUs/dish/min. at grid 9, 10, 12 (Fig.7) . The difference between the highest and the lowest density was about five times. The average of fungal density was 1.3 CFUs/dish/min.

In the cool season, the inertial method (air sampler) was used to collect air samples. The concentration of airborne fungi was expressed as CFUs / m^3 air. The concentration of airborne fungi among 12 grids is shown in Figure 8. The highest

Table 4 The identification of *Bacillus* spp. by biochemical tests

Specimen code	Biochemical tests											
	1	2	3	4	5	6	7	8	9	10	11	Species
S1	+	-	-	+	+	-	+	+	-	-	+	*B.polymyxa*
S2	+	-	-	+	-	-	+	-	-	-	-	*B.circulans*
S3	+	-	-	-	-	-	+	+	-	-	-	*B.sphaericus*
S4	+	-	+	-	-	-	+	-	-	-	+	*B.circulans*
S5	+	-	+	-	+	-	+	+	-	-	-	*B.pumilis*
S6	+	+	+	+	+	-	+	+	-	-	-	*B.cereus*
S7	+	-	-	+	+	+	+	+	-	-	-	*B.licheniformis*
S8	+	+	-	+	+	-	-	+	-	-	-	*B.mycoides*
S9	+	-	-	+	-	-	+	-	+	-	-	*B.macerans*
S10	+	-	-	+	+	-	+	-	-	-	-	*B.coagulans*
S11	+	-	-	+	+	+	-	+	-	-	-	*B.subtillis*

Biochemical test :

1. Catalase test
2. Lecithinase Production
3. Motility
4. Nitrate Production
5. V-Ptest
6. Citrate Production
7. Maltose test
8. Gelatin Hydrolysis
9. Gas from glucose
10. Indole test
11. Starch Hydrolysis

Table 5 The distribution of bacterial species at each grid in the rainy and cool seasons

Grid	Rainy Season	Cool season
1	*Bacillus circulans, B.sphaericus*	*B.sphaericus , B.mycoides B.coagulans*
2	*B.polymyxa, B. circulans, B.sphaericus*	*B.sphaericus , B.mycoides B.coagulans*
3	*B. cereus, B.polymyxa, B.sphaericus*	*B.polymyxa, B.sphaericus B.mycoides, B.coagulans*
4	*B. cereus, B.polymyxa B.sphaericus*	*B.polymyxa , B.sphaericus B.mycoides , B.coagulans*
5	*B. cereus, B.mycoides B. licheniformis, B.sphaericus*	*B.polymyxa , B.sphaericus B.mycoides , B.coagulans*
6	*B. cereus, B.mycoides B.sphaericus*	*B.polymyxa , B.sphaericus B.mycoides , B.coagulans*
7	*B. cereus, B.mycoides B. licheniformis, B.polymyxa, B.macerans, B.sphaericus*	*B.polymyxa , B.sphaericus B.mycoides , B.coagulans*
8	*B. cereus , B.mycoides, B. licheniformis, B.polymyxa, B.macerans, B.sphaericus*	*B.polymyxa , B.sphaericus B.mycoides , B.coagulans*
9	*B.subtillis, B. circulans Staphylococcus aureus*	*B.polymyxa , B.sphaericus B.mycoides , B.coagulans Staphylococci aureus*
10	*B.subtillis, B. circulans S.aureus*	*B.polymyxa , B.sphaericus B.mycoides , B.coagulans S. aureus*
11	*B.subtillis , B. circulans S.aureus*	*B.polymyxa , B.sphaericus B.mycoides , B.coagulans S. aureus*
12	*B.subtillis, B. circulans S.aureus*	*B.polymyxa , B.sphaericus B.mycoides , B.coagulans S. aureus*

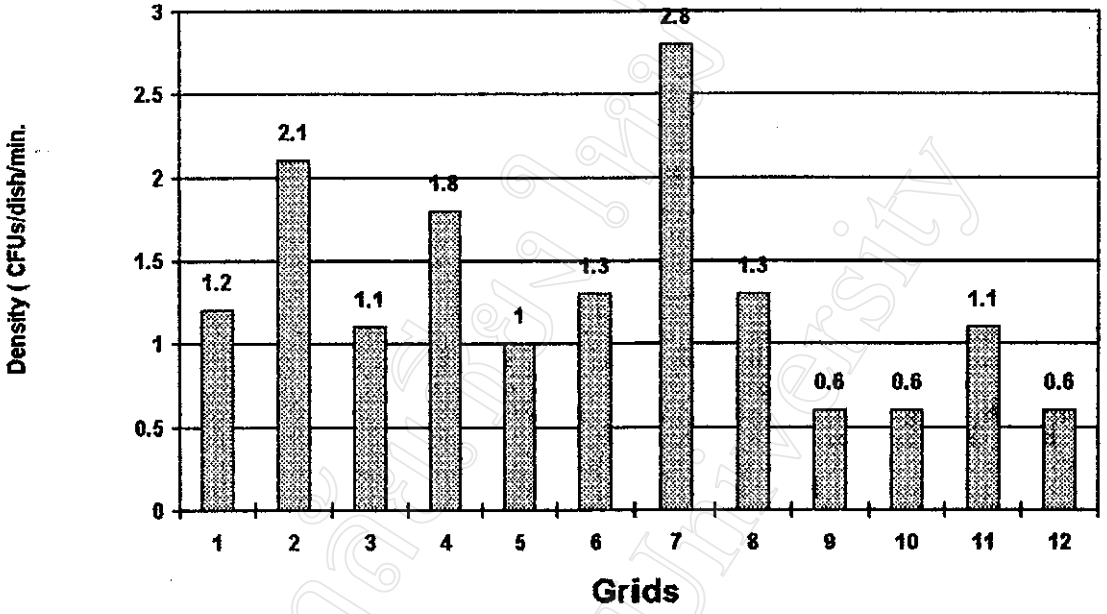

Fig 7 The density of airborne fungi of 12 grids in the rainy season by sedimentary method

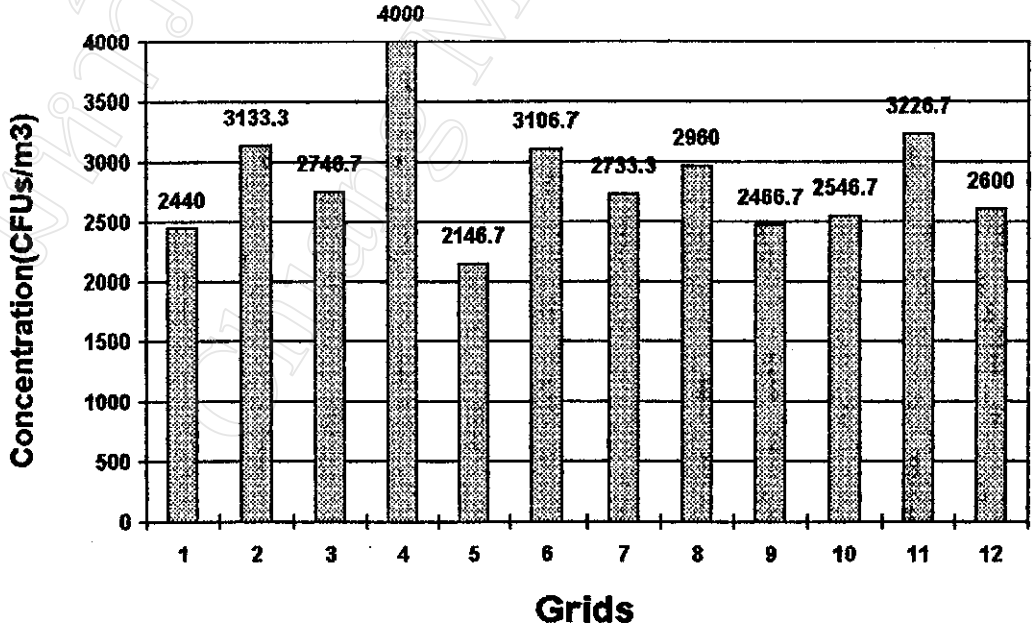

Fig 8 The concentration of airborne fungi of 12 grids in the cool season by inertial method

concentration of airborne fungi was 4000 CFUs/ m^3 at gird 4 and the lowest one was 2146.7 CFUs/m^3 at grid 5 . The difference between the highest and the lowest concentrations was about 1.9 times . The mean concentration of fungi was 2842.2 CFUs/ m^3 .

4.5 The variation of fungal density and concentration by sedimentary and inertial methods

Table 6 shows the variation of fungal density and concentration of 12 grids by sedimentary and inertial methods. When the sedimentary method was used , the highest C.V. was 127.3% at grid 3 and the lowest C.V. was 8.3 % at grid 1. The average C.V. of fungal density in the whole area was 69.2 %. When inertial method was used, the highest C.V.was found to be 40.4% at grid 4 and the lowest C.V. was 2.5% at grid 9. The average C.V. of fungal concentration was 25.3 %.

4.6 The identification of airborne fungi

The identification of airborne fungi in this study was done by observing the morphological features of colonies and microscopic features of spores. The common genera of airborne fungi were *Aspergillus* spp. , *Cladosporium* spp. , *Curvularia* spp. , *Fusarium* spp. , *Penicillium* spp. (Fig 9-18) , and Zygomycetous fungi. In addition to the common fungi, a yeast-like fungus, *Trichosporon* sp., was found also (Fig 19-20).

Table 6 The variation of fungal density and concentration of 12 grids by sedimentary and inertial methods

		Sedimentary Method(density)			Inertial Method(conc.)		
Grid	n	Mean density	SD	C.V. * (%)	Mean conc.	SD	C.V. * (%)
1	3	1.2	0.1	8.3	2440.0	277.1	11.4
2	3	2.1	1.1	52.4	3133.3	537.1	17.1
3	3	1.1	1.4	127.3	2746.7	388.5	14.1
4	3	1.8	1.0	55.6	4000.0	1614.4	40.4
5	3	1.0	0.2	20.0	2146.7	151.4	7.8
6	3	1.3	0.7	53.8	3106.7	756.1	24.3
7	3	2.8	1.6	57.1	2733.3	189.0	6.9
8	3	1.3	0.7	53.8	2960.0	211.6	7.1
9	3	0.6	0.3	50.0	2466.7	61.6	2.5
10	3	0.6	0.1	16.7	2546.7	260.2	10.2
11	3	1.1	0.6	54.5	3226.7	1020.0	31.6
12	3	0.6	0.3	50.0	2600.0	480.0	18.5
total	36	1.3	0.9	69.2	2842.2	719.3	25.3

n : the number of sample

*C.V. : Coefficient of Variation = (SD/mean)X 100

Fig 9. The colony of the *Fusarium* spp.

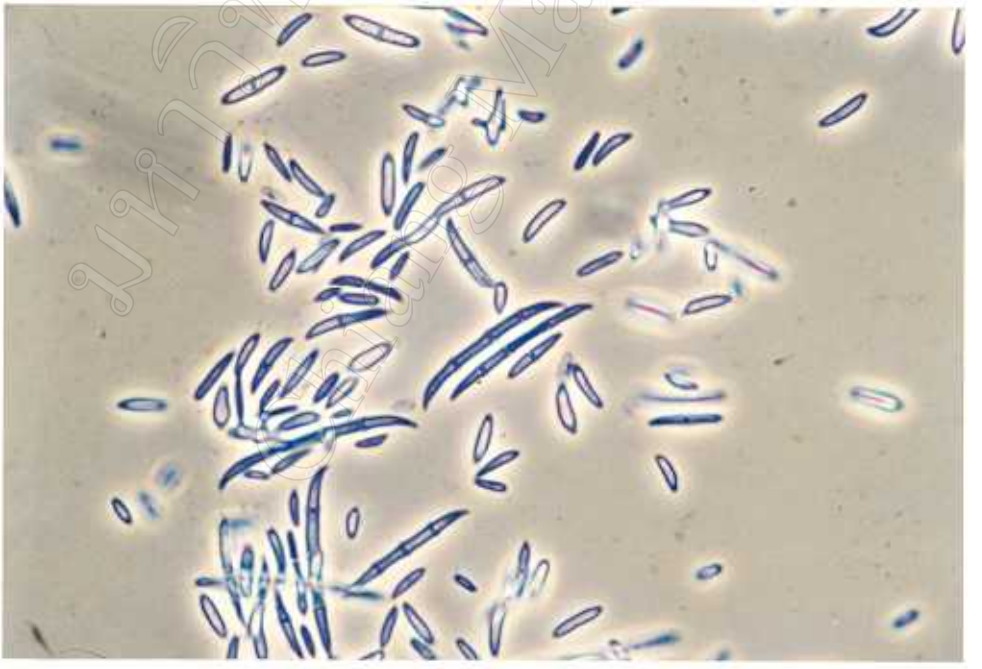

Fig 10. The microscopic features of the *Fusarium* spp. (400X)

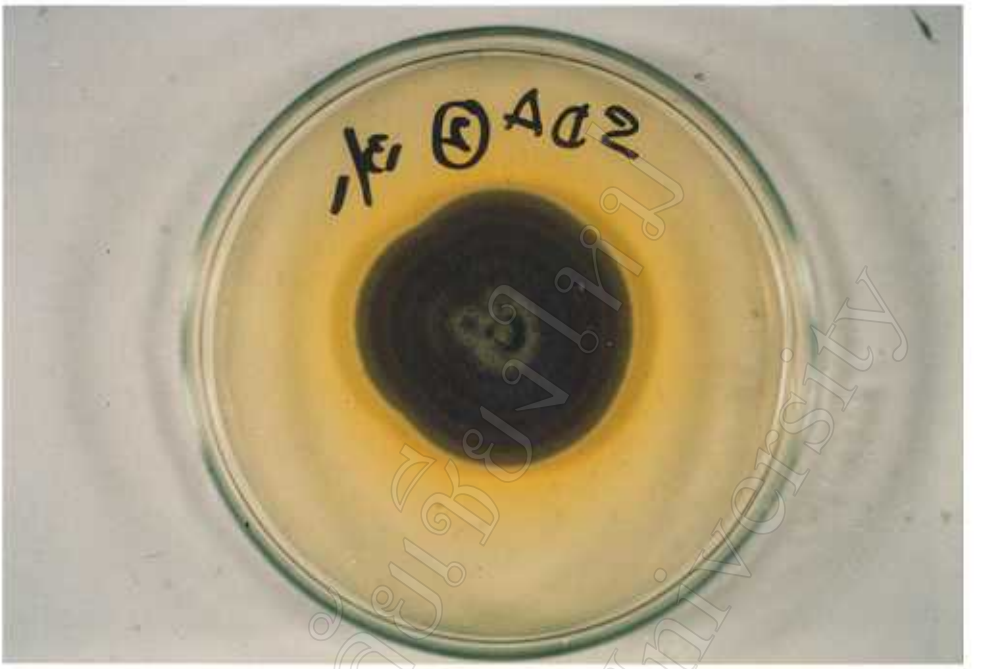

Fig 11. The colony of the *Cladosporium* spp.

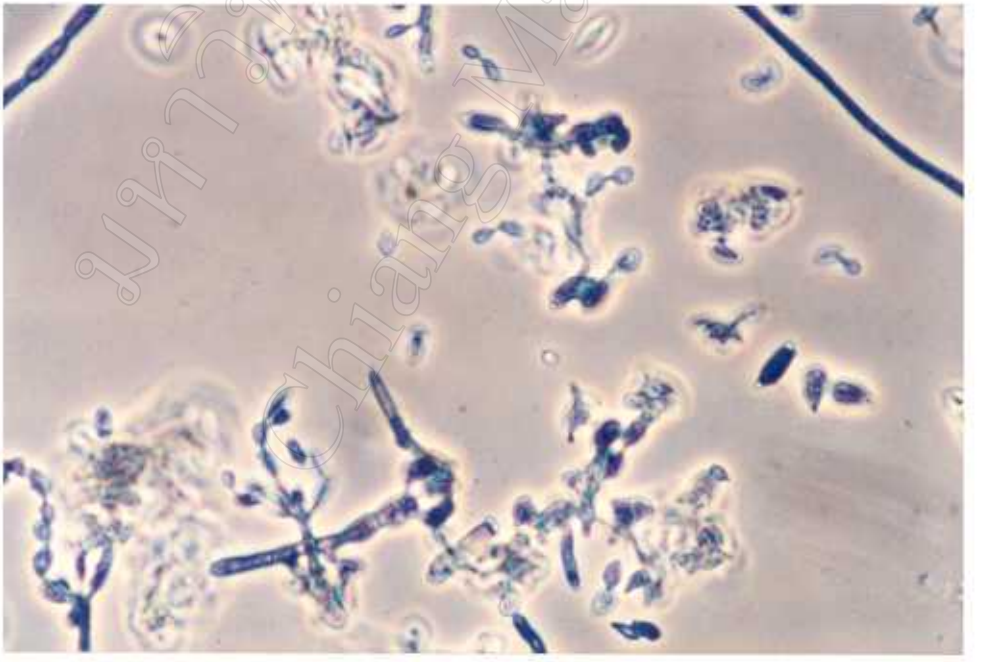

Fig 12. The microscopic features of the *Cladosporium* spp. (400X)

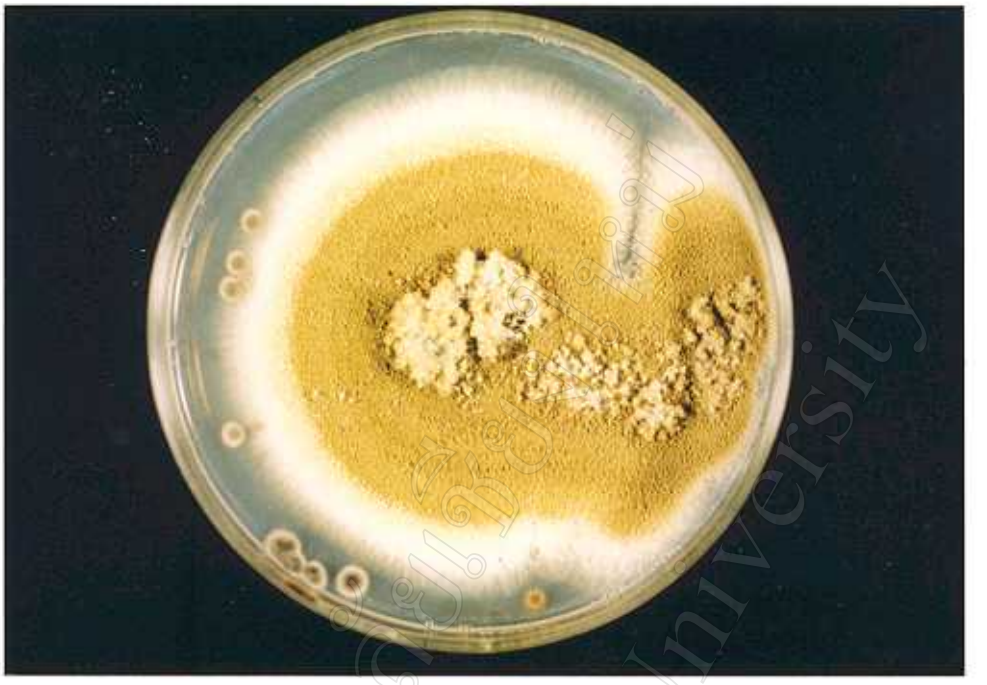

Fig 13. The colony of the *Aspergillus* spp.

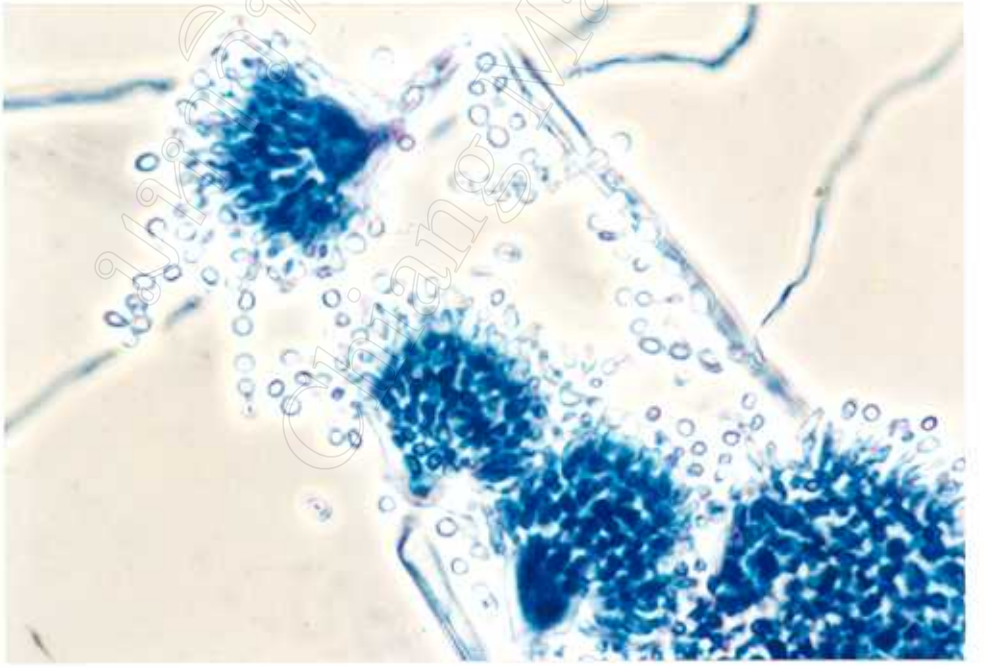

Fig 14. The microscopic features of the *Aspergillus* spp. (400X)

Fig 15. The colony of the *Penicillium* spp.

Fig 16. The microscopic features of the *Penicillium* spp. (400X)

Fig 17. The colony of the *Curvularia* spp.

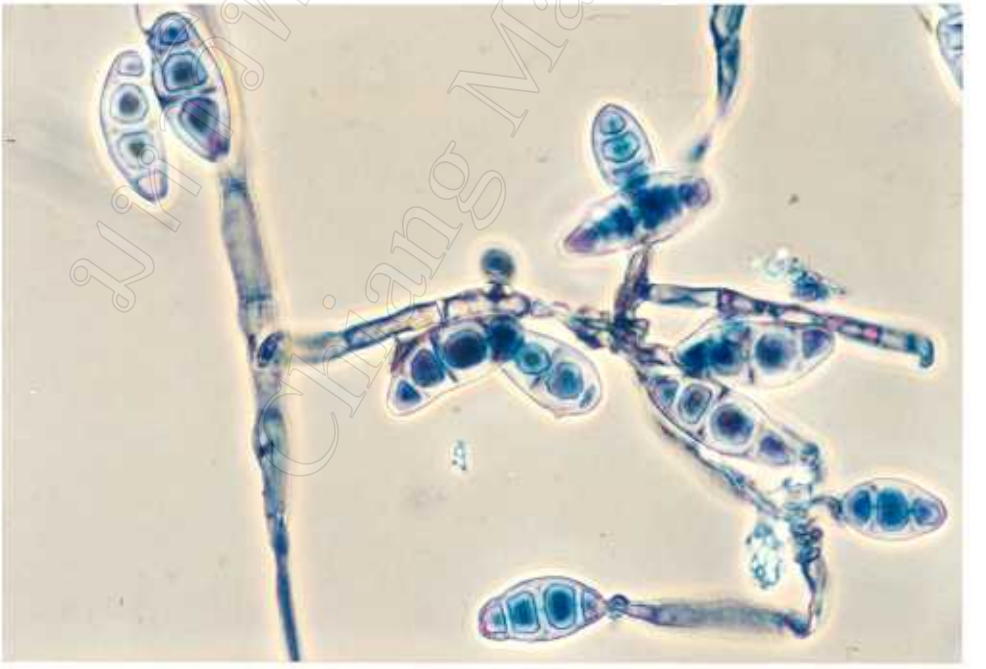

Fig 18. The microscopic features of the *Curvularia* spp. (400X)

Fig 19. The colony of the *Trichosporon* sp.

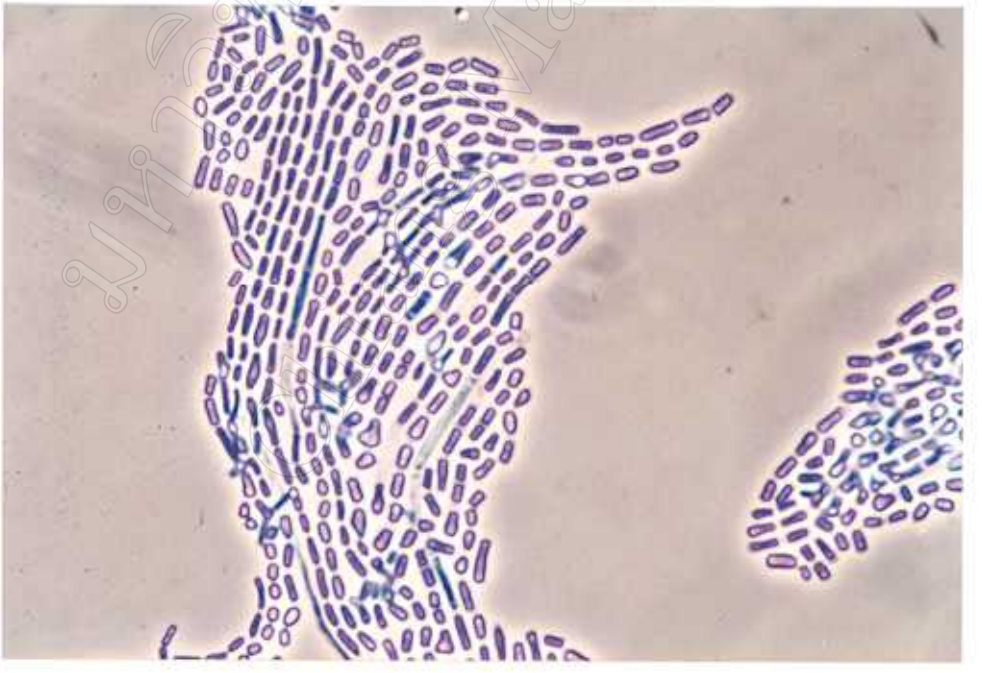

Fig 20. The microscopic features of the *Trichosporon* sp.(400X)

The distribution of airborne fungi at each grid in the rainy and cool seasons is shown in Table 7.

Table 7 The distribution of airborne fungi at each grid in the rainy and cool seasons

Grid	Rainy Season	Cool Season
1	*Fusarium* spp. *Aspergillus* spp. *Penicillium* spp.	*Cladosporum* spp.,*Fusarium* spp. , *Aspergillus* spp., *Penicillium* spp.
2	*Fusarium* spp. *Curvularia* spp.	*Cladosporum* spp.*Fusarium* spp. *Aspergillus* spp.,*Penicillium* spp. Zygomycetous fungi
3	*Fusarium* spp. *Curvularia* spp.	*Cladosporum* spp.*Fusarium* spp. *Aspergillus* spp.,*Penicillium* spp. Zygomycetous fungi
4	*Fusarium* spp. *Curvularia* spp. *Penicillium* spp.	*Cladosporum* spp.*Fusarium* spp. *Aspergillus* spp.,*Penicillium* spp. Zygomycetous fungi
5	*Fusarium* spp. *Aspergillus* spp.	*Cladosporum* spp.*Fusarium* spp. *Aspergillus* spp.,*Penicillium* spp.
6	*Fusarium* spp. , *Aspergillus* spp. *Penicillium* spp. Zygomycetous fungi	*Cladosporum* spp.*Fusarium* spp. *Aspergillus* spp.,*Penicillium* spp. Zygomycetous fungi
7	*Fusarium* spp. , *Aspergillus* spp. *Curvularia* spp. , Zygomycetous fungi	*Cladosporum* spp.,*Fusarium* spp. *Aspergillus* spp.,*Penicillium* spp. Zygomycetous fungi
8	*Fusarium* spp. *Aspergillus* spp. Zygomycetous fungi	*Cladosporum* spp.*Fusarium* spp. *Aspergillus* spp.,*Penicillium* spp. Zygomycetous fungi
9	*Fusarium* spp. *Penicillium* spp. *Trichosporon* sp.	*Cladosporum* spp.*Fusarium* spp. *Aspergillus* spp.,*Penicillium* spp. Zygomycetous fungi
10	*Fusarium* spp. Zygomycetous fungi	*Cladosporum* spp.*Fusarium* spp. *Aspergillus* spp.,*Penicillium* spp. Zygomycetous fungi
11	*Fusarium* spp. *Penicillium* spp. *Trichosporon* sp.	*Cladosporum* spp.*Fusarium* spp. *Aspergillus* spp.,*Penicillium* spp. Zygomycetous fungi
12	*Fusarium* spp. *Penicillium* spp.	*Cladosporum* spp.*Fusarium* spp. *Aspergillus* spp.,*Penicillium* spp. Zygomycetous fungi

4.7 The comparison of density and concentration between bacteria and fungi in each grid in the rainy and cool seasons.

The relationship between airborne bacterial and fungal density (or concentration) in each grid is shown in Table 8 in terms of ratio of mean density (or concentration) of bacteria to mean density (or concentration) of fungi.

Table 8 The relationship between bacteria and fungi in each grid

		Rainy Season (density)			Cool Season (concentration)		
Grid	n	Bacteria	Fungi	Ratio	Bacteria	Fungi	Ratio
1	3	0.9	1.2	0.75	2773.3	2440.0	1.14
2	3	4.6	2.1	2.19	2480.0	3133.3	0.79
3	3	3.6	1.1	3.27	1613.3	2746.7	0.59
4	3	2.5	1.8	1.39	1853.3	4000.0	0.46
5	3	7.7	1.0	7.70	2133.3	2146.7	0.99
6	3	0.4	1.3	0.31	1600.0	3106.7	0.52
7	3	4.6	2.8	1.64	2653.3	2733.3	0.97
8	3	10.3	1.3	7.92	2013.3	2960.0	0.68
9	3	7.6	0.6	2.67	2306.7	2466.7	0.94
10	3	4.8	0.6	8.00	2480.0	2546.7	0.97
11	3	5.7	1.1	5.18	2853.3	3226.7	0.88
12	3	4.6	0.6	7.67	3613.3	2600.0	1.39
total	36	4.8	1.3	3.69	2364.4	2842.2	0.83

n: the number of samples
ratio= Density (or concentration) of bacteria / density (or concentration) of fungi

The ratio represents the mathematical relation between the mean density (or concentration) of bacteria and fungi at the same grid. If the ratio is 1, the density (or concentration) of bacteria and fungi is equal. If the ratio is greater than 1, the density (or concentration) of airborne bacteria is higher than that of airborne fungi in the same grid. If the ratio is less than 1, the density (or concentration) of airborne bacteria is lower than that of airborne fungi. The results in Table 8 indicates that the ratios of densities of bacteria and fungi were greater than 1 at most grids except for grid 1 and 6 in the rainy season but the ratios of concentration of bacteria and fungi were less than 1 at most grids except for grid 1 and 12 in the cool season.

4.8 The comparison of efficiency of the two air sampling methods

The efficiency of air sampling methods was shown as the CFUs per minute. The higher the value of CFUs per minute, the higher the efficiency of air sampling method. In this study two air sampling techniques were used to carry out air sampling at grid 3 and 4 in the rainy season in order to compare these two methods (Table 9).

Table 9 The comparison of efficiency of air sampling methods

Method	Gird	n	Mean (bacteria)	Mean (fungi)
Open Plate	3,4	6	3.08	1.51
Air Sampler	3,4	4	36.00	84.00

n: the number of samples

Mean : The CFUs per minute for each plate

Table 9 shows that the sampling efficiency of the air sampler was almost 12 times higher than that of the open plate method in the bacterial sampling and almost 60 times higher than that of the open plate method in the fungal sampling.

4.9 Statistical analysis

The t-test and F-test (Analysis of Variance, ANOVA) were used to analyze the difference in density or concentration between two seasons or two methods of air sampling or among 12 grids.

4.9.1 The analysis of variance for the comparison of different density or concentration among 12 grids.

Table 10 shows that the density and concentration of bacteria and fungi among grids in the study area were not significant difference.

Table 10 One-way ANOVA results for the comparison of bacterial and fungal concentration(density) among 12 grids

Microbes	Items	D.F.	M.S.1	M.S.2	F value	p
bacteria	density	35	20.91	25.28	0.827	0.615
bacteria	conc.	35	865842	618355	1.400	0.236
fungi	density	35	1.33	0.75	1.779	0.115
fungi	conc.	35	711935	428355	1.662	0.144

D.F. (Degree of Freedom) = n-1

M.S.1 : Mean Squares of variation among grids

M.S.2 : Mean Squares of variation within grids

F value = M.S.1 / M.S.2

p : Probability

4.9.2 Comparison of differences between two sampling batches in the rainy season

Table 11 shows that the average densities of airborne bacteria and fungi between two sampling batches in the rainy season were not statistically significant ($p>>0.05$).

Table 11 Comparison of differences between two sampling batches in the rainy season by sedimentary method

	First Sampling		Second Sampling			
Microbes	n	mean (CFUs/dish/min.)	n	mean (CFUs/dish/min.)	t value	p
bacteria	36	4.79	6	4.93	0.06	0.95
fungi	36	1.31	6	1.28	0.15	0.88

n: the number of samples

4.9.3 Seasonal comparison of difference in bacterial concentration with t-test

Table 12 shows that the difference in bacterial concentration between the rainy and cool seasons was statistically significant because p was less than 0.05. The bacterial concentration in the cool season was higher than that in the rainy season.

Table 12 Seasonal comparison of difference in bacterial concentration by inertial method

Season	n	Mean($CFUs/m^3$)	S.D. ($CFUs/m^3$)	C.V.(%)
rainy	4	720.0	206.5	28.68
cool	6	1733.3	471.7	27.21

n : the number of samples

t value =3.99

p = 0.004

4.9.4 Seasonal comparison of difference in fungal concentration with t-test

Table 13 demonstrates that the seasonal difference of fungal concentration were statistically significant. The fungal concentration in the cool season was much higher than that in the rainy season.

Table 13 Seasonal comparison of differences in fungal concentration by inertial method

Season	n	Mean	S.D.	C.V.%)
rainy	4	1690.0	206.5	23.06
cool	6	3377.3	1254.7	37.15

n: the number of samples

t value =3.07

p = 0.02

4.10 The homogeneous subsets of the density and concentration of bacteria and fungi

The homogeneous subset is one way to classify the data within one group. The data in the same subset mean that the highest and the lowest averages are not significantly different. The results of homogeneous subsets are usually obtained after statistical analysis (e.g. ANOVA). The homogeneous subsets of density and concentration of bacteria and fungi in this study were listed in Table 14. The differences between low and high ends were statistically significant because the data belonged to different subsets.

Table 14 The homogenous subsets of the density and concentration of bacteria and fungi

	Bacterial density		Fungal density		Bacterial conc.		Fungal conc.	
	subset 1	subset 2	subset 1	subset 2	subset 1	subset 2	subset 1	subset 2
low end	Grid 6 0.4333 Grid 1 0.9000		Grid 10 0.6000 Grid 12 0.6000 Grid 9 0.6333		Grid 6 1600.0 Grid 3 1613.3 Grid 4 1853.3 Grid 8 2013.3		Grid 5 2146.7 Grid 1 2440.0 Grid 9 2466.7 Grid 10 2546.7 Grid 12 2600.0 Grid 7 2733.3 Grid 3 2746.7	
overlay part	Grid 4 2.5333 Grid 3 3.6333 Grid 7 4.6000 Grid 2 4.6333 Grid 12 4.6333 Grid 10 4.8333 Grid 11 5.7000 Grid 9 7.6000 Grid 5 7.7333	Grid 4 2.5333 Grid 3 3.6333 Grid 7 4.6000 Grid 2 4.6333 Grid 12 4.6333 Grid 10 4.8333 Grid 11 5.7000 Grid 9 7.6000 Grid 5 7.7333	Grid 5 1.0667 Grid 3 1.1000 Grid 11 1.1000 Grid 1 1.2333 Grid 6 1.3000 Grid 8 1.3000 Grid 4 1.8667	Grid 5 1.0667 Grid 3 1.1000 Grid 11 1.1000 Grid 1 1.2333 Grid 6 1.3000 Grid 8 1.3000 Grid 4 1.8667	Grid 5 2133.3 Grid 9 2306.7 Grid 2 2480.0 Grid 10 2480.0 Grid 7 2653.3 Grid 1 2773.3 Grid 11 2853.3	Grid 5 2133.3 Grid 9 2306.7 Grid 2 2480.0 Grid 10 2480.0 Grid 7 2653.3 Grid 1 2773.3 Grid 11 2853.3	Grid 8 2960.0 Grid 6 3106.7 Grid 2 3133.3 Grid 11 3226.7	Grid 8 2960.0 Grid 6 3106.7 Grid 2 3133.3 Grid 11 3226.7
high end		Grid 8 10.3333		Grid 2 2.1000 Grid 7 2.8333		Grid 12 3400.0		Grid 4 4000.0

Density = CFUs/dish.min.,
Conc. = CFUs /m^3

4.11 The risk assessment of airborne bacteria and fungi in Chiang Mai city

The risk assessment of airborne bacteria and fungi was done by following steps in this study i.e. Step (1) : classification of data ; Step (2) : scoring of data ; Step (3): risk assessment. The results were shown in the Table 5. The map of environmental risk assessment (ERA) was made and shown in Figure 21.

Table 15 The risk assessment of airborne bacteria and fungi at each grid

	Bacterial density		Fungal density		Bacterial conc.		Fungal conc.		Total	
Grid	class	score	class	score	class	score	class	score	score	risk
1	L	1	M	2	M	2	L	1	6	L
2	M	2	H	3	M	2	M	2	9	H
3	M	2	M	2	L	1	L	1	6	L
4	M	2	M	2	L	1	H	3	8	M
5	M	2	M	2	M	2	L	1	7	L
6	L	1	M	2	L	1	M	2	6	L
7	M	2	H	3	M	2	L	1	8	M
8	H	3	M	2	L	1	M	2	8	M
9	M	2	L	1	M	2	L	1	6	L
10	M	2	L	1	M	2	L	1	6	L
11	M	2	M	2	M	2	M	2	8	M
12	M	2	L	1	H	3	L	1	7	L

Classification of data :L = LOW LEVEL; M=MEDIUM LEVEL; H=: HIGH LEVEL

. The map of risk assessment was shown in Figure 21. The map clearly indicates the situation of the contamination of the airborne bacteria and fungi at the study area. The high risk was only shown at grid 2 (Table 15, Fig. 21).

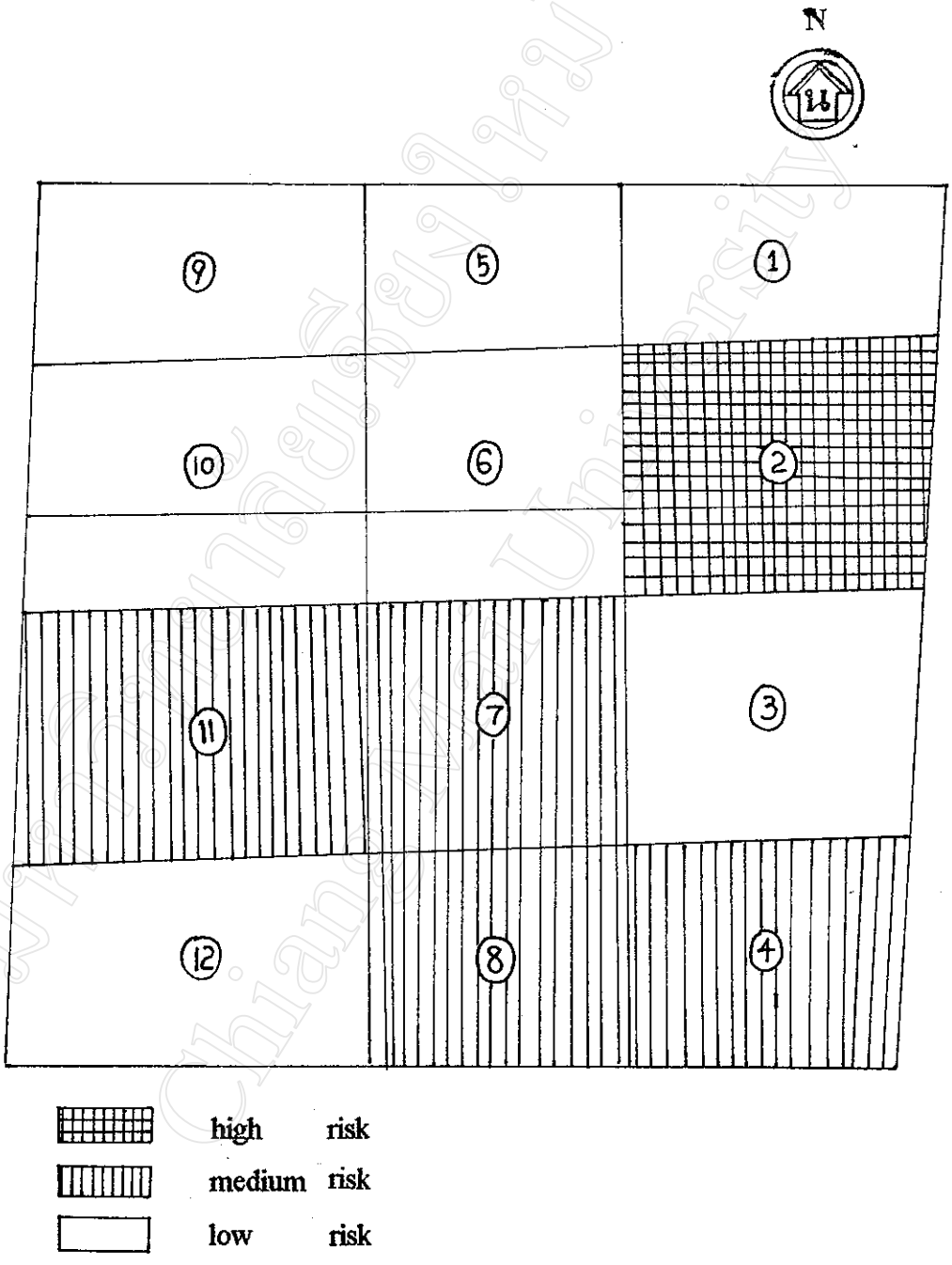

Fig 21 The map of risk assessment of airborne bacteria and fungi in Chiang Mai city