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APPENDIX

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX A

Azadirachtin analysis by using High Performance Liquid Chromatography (HPLC)

Azadirachtin quantity were determined by using High performance liquid chromatography of methods of Agricultural Toxic Substance Division Agricultural Department.

Adjusting condition of HPLC

Instrument : Shimadzu LC-6A
Column : RP-8 lichrospher 5 μm (Merck) 125x4 mm
Mobile phase : Acetonitrile : water, 30:70
Flow rate : 1 ml/min
detector : UV 210 nm
Attenuation : 0.02 AUFS
Elution system : Isocratic system
Sample size : 10 μl

Preparing sample to analyze

- (1). Standard solution of 95 % azadirachtin were prepared with methanol into concentration ranging 0.01-0.18 mg/ml.
- (2). Extracts samples solution 1 ml were filtered through a 0.45 μm microfilter membrane after preparing samples.

Methodology to prepare samples for HPLC analysis.

Neem seed extract

1. A suspension of ten grams of crushed Thai neem extract in 100 ml of hexane was stirred occasionally at room temperature for five hours.
2. Then, filtered through a filter paper.
3. The defatted mare was then extracted with 100 ml of methanol in the same manner as the hexane extraction for 24 hours.
4. Then, filtered through a filter paper.
5. Solution were added to 100 ml of methanol.
6. one ml of sample were prepared for HPLC analysis.

Neem oil

Follow the modified clean up procedure of Isman et al., 1990.

1. Add 10 ml of 50 % aqueous methanol and 10 ml dietheryl ether to one gram crude neem oil.
2. Mix the phases by vigorous shaking in a separatory funnel and allow to separate over night.
3. Remove the aqueous methanol layer.
4. Re-extract the diethyl ether phase twice with 10 ml of 50 % aqueous methanol.
5. Combine the aqueous methanol up to 10 ml in a volumetric flask and filter the sample.
6. For HPLC analysis dilute the sample 1: 10 with mobile phase.
7. Azadirachtin content is calculated 95 % (w/w).

Neem leave extract and commercial neem extract were dilute 1:100 . Then extracts samples solution 1 ml were filterd through a 0.45 μm microfilter membrane.

(3). Ten μl standard solution were injected until area or peak were contant and different each times less than 1 %, then sample were injected.

(4). Samples Value were compared with standard value and calculated. Quanlitative of azadirachtin in sample were determined.

Calibration curves

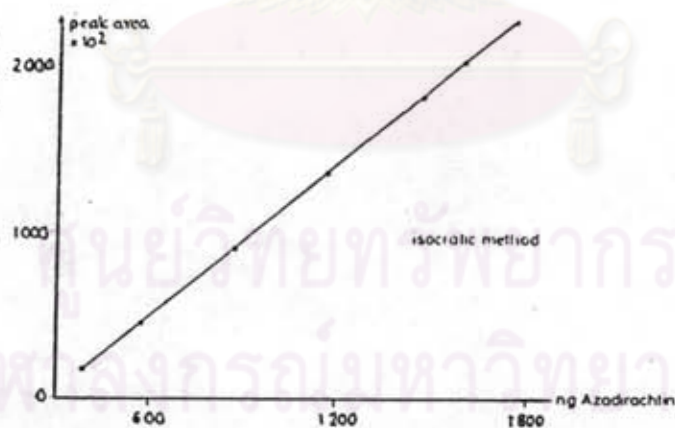


Figure 1 : Calibration curve of azadirachtin standard in average of 100 -1800 ng run under isocratic conditions. Peak area vs ng azadirachtin.

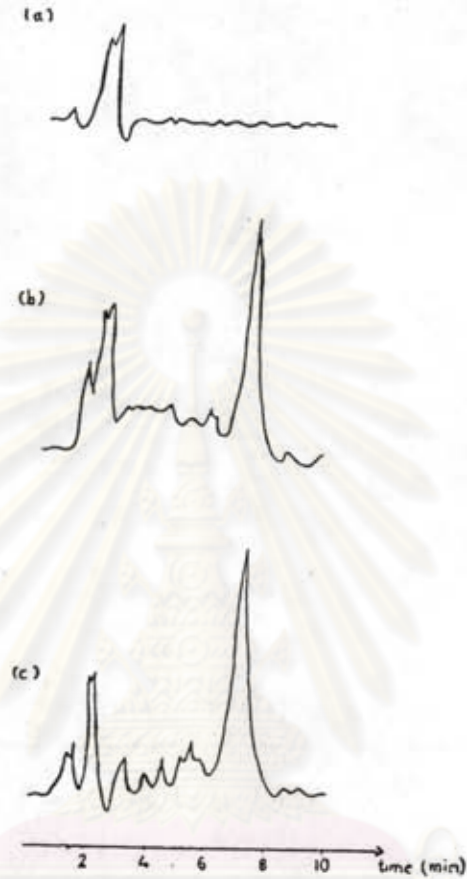
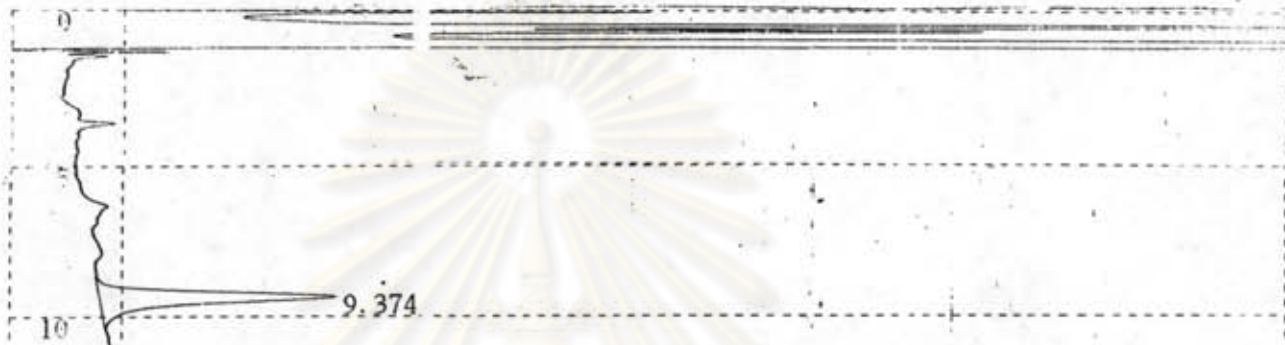


Figure 2 : HPLC chromatogram of (a). methanol control, (b). azadirachtin standard, and (c).Neem extract run under isocratic conditions. Peak area vs time.

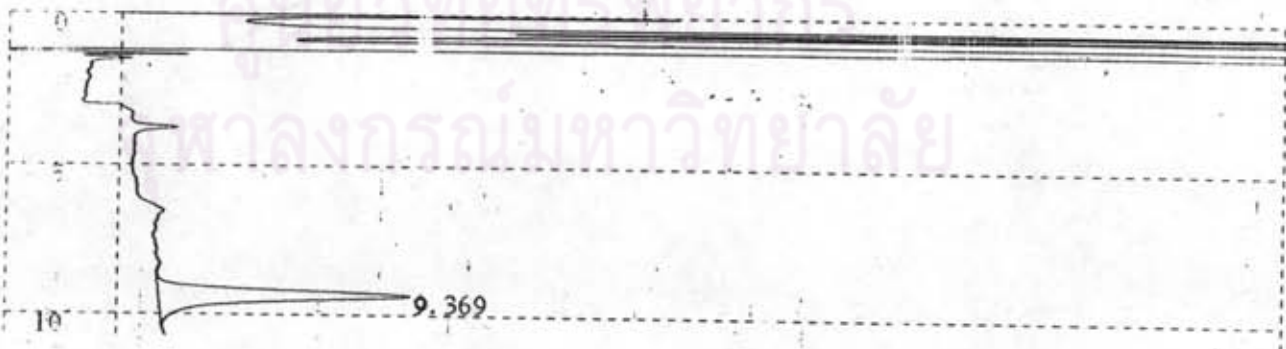
HPLC CHROMATOGRAM OF SAMPLE

Analysis File : 2:DETER.

azadirachtin determination.RP- 18 125*4mm sum CH3CN:H2O=30:70
flow 1ml/min;uv 210nm.

** CALCULATION REPORT **

| CH | PKNO | TIME | AREA | HEIGHT | MK | IDNO | CONC | NAME |
|-------|------|-------|-------|--------|----|------|------|------|
| 1 | 3 | 9.374 | 43178 | 1649 | | | 100 | |
| TOTAL | | | 43178 | 1649 | | | 100 | |

azadirachtin determination.RP- 8 125*4mm sum CH3CN:H2O=30:70
flow 1ml/min;uv 210nm.



APPENDIX B

ESTIMATION OF THE MEDIAN LETHAL DOSE (THE LD₅₀ VALUE)

The LD₅₀ value were determined by using probit analysis (Finney, 1971).

Probit analysis

Probit analysis is a system of statistical techniques chiefly concerned with the relation between the frequency of occurrence of a specific response and the numerical amount of a dose or other stimulus that tends to induce the response which known as "biological variation" that show relation in sigmoid curve.

Then probability of response on a thranformed is transformed on normal equivalent diviate (or N.E.D) scale. So that the relation between the dose parameter and the N.E.D. of the probability of response is transformed into a straight line, i.e. linear relationship.

dose is a straight line.

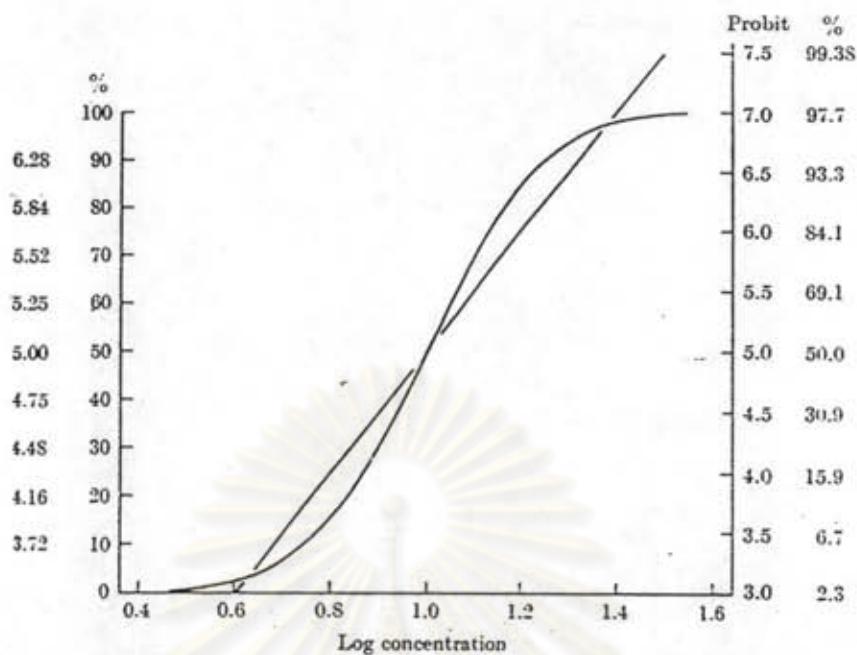


Figure 1: Effect of probit transformation. The normal sigmoid curve is transformed to a straight line when the ordinates are measured on a scale linear in probits instead of in percentages.

Table 1 : Transformation of percentages to probits.

| % | 0.0 | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 | 0.7 | 0.8 | 0.9 | 1 | 2 | 3 | 4 | 5 |
|----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|---|----|----|----|----|
| 0 | — | 1.9098 | 2.1218 | 2.2522 | 2.3479 | 2.4242 | 2.4879 | 2.5427 | 2.5911 | 2.6344 | | | | | |
| 1 | 2.6737 | 2.7096 | 2.7429 | 2.7738 | 2.8027 | 2.8299 | 2.8556 | 2.8799 | 2.9031 | 2.9251 | | | | | |
| 2 | 2.9463 | 2.9665 | 2.9859 | 3.0046 | 3.0226 | 3.0400 | 3.0569 | 3.0732 | 3.0890 | 3.1043 | | | | | |
| 3 | 3.1192 | 3.1337 | 3.1478 | 3.1616 | 3.1750 | 3.1881 | 3.2009 | 3.2134 | 3.2256 | 3.2376 | | | | | |
| 4 | 3.2493 | 3.2608 | 3.2721 | 3.2831 | 3.2940 | 3.3046 | 3.3151 | 3.3253 | 3.3354 | 3.3454 | | | | | |
| 5 | 3.3551 | 3.3648 | 3.3742 | 3.3836 | 3.3928 | 3.4018 | 3.4107 | 3.4195 | 3.4282 | 3.4368 | 9 | 18 | 27 | 36 | 45 |
| 6 | 3.4452 | 3.4536 | 3.4618 | 3.4699 | 3.4780 | 3.4859 | 3.4937 | 3.5015 | 3.5091 | 3.5167 | 8 | 16 | 24 | 32 | 40 |
| 7 | 3.5242 | 3.5316 | 3.5389 | 3.5462 | 3.5534 | 3.5605 | 3.5675 | 3.5745 | 3.5813 | 3.5882 | 7 | 14 | 21 | 28 | 36 |
| 8 | 3.5949 | 3.6016 | 3.6083 | 3.6148 | 3.6213 | 3.6278 | 3.6342 | 3.6405 | 3.6468 | 3.6531 | 6 | 13 | 19 | 26 | 32 |
| 9 | 3.6592 | 3.6654 | 3.6715 | 3.6775 | 3.6835 | 3.6894 | 3.6953 | 3.7012 | 3.7070 | 3.7127 | 6 | 12 | 18 | 24 | 30 |
| 10 | 3.7184 | 3.7241 | 3.7298 | 3.7354 | 3.7409 | 3.7464 | 3.7519 | 3.7574 | 3.7628 | 3.7681 | 6 | 11 | 17 | 22 | 28 |
| 11 | 3.7735 | 3.7788 | 3.7840 | 3.7893 | 3.7945 | 3.7996 | 3.8048 | 3.8099 | 3.8150 | 3.8200 | 5 | 10 | 16 | 21 | 26 |
| 12 | 3.8250 | 3.8300 | 3.8350 | 3.8399 | 3.8448 | 3.8497 | 3.8545 | 3.8593 | 3.8641 | 3.8689 | 5 | 10 | 15 | 20 | 24 |
| 13 | 3.8736 | 3.8783 | 3.8830 | 3.8877 | 3.8923 | 3.8969 | 3.9015 | 3.9061 | 3.9107 | 3.9152 | 5 | 9 | 14 | 18 | 23 |
| 14 | 3.9197 | 3.9242 | 3.9286 | 3.9331 | 3.9375 | 3.9419 | 3.9463 | 3.9506 | 3.9550 | 3.9593 | 4 | 9 | 13 | 18 | 22 |
| 15 | 3.9636 | 3.9678 | 3.9721 | 3.9763 | 3.9806 | 3.9848 | 3.9890 | 3.9931 | 3.9973 | 4.0014 | 4 | 8 | 13 | 17 | 21 |
| 16 | 4.0055 | 4.0096 | 4.0137 | 4.0178 | 4.0218 | 4.0259 | 4.0299 | 4.0339 | 4.0379 | 4.0419 | 4 | 8 | 12 | 16 | 20 |
| 17 | 4.0458 | 4.0498 | 4.0537 | 4.0576 | 4.0615 | 4.0654 | 4.0693 | 4.0731 | 4.0770 | 4.0808 | 4 | 8 | 12 | 16 | 19 |
| 18 | 4.0846 | 4.0884 | 4.0922 | 4.0960 | 4.0998 | 4.1035 | 4.1073 | 4.1110 | 4.1147 | 4.1184 | 4 | 8 | 11 | 15 | 19 |
| 19 | 4.1221 | 4.1258 | 4.1295 | 4.1331 | 4.1367 | 4.1404 | 4.1440 | 4.1476 | 4.1512 | 4.1548 | 4 | 7 | 11 | 15 | 18 |
| 20 | 4.1584 | 4.1619 | 4.1655 | 4.1690 | 4.1726 | 4.1761 | 4.1796 | 4.1831 | 4.1866 | 4.1901 | 4 | 7 | 11 | 14 | 18 |
| 21 | 4.1936 | 4.1970 | 4.2005 | 4.2039 | 4.2074 | 4.2108 | 4.2142 | 4.2176 | 4.2210 | 4.2244 | 3 | 7 | 10 | 14 | 17 |
| 22 | 4.2278 | 4.2312 | 4.2345 | 4.2379 | 4.2412 | 4.2446 | 4.2479 | 4.2512 | 4.2546 | 4.2579 | 3 | 7 | 10 | 13 | 17 |
| 23 | 4.2612 | 4.2644 | 4.2677 | 4.2710 | 4.2743 | 4.2775 | 4.2808 | 4.2840 | 4.2872 | 4.2905 | 3 | 7 | 10 | 13 | 16 |
| 24 | 4.2937 | 4.2969 | 4.3001 | 4.3033 | 4.3065 | 4.3097 | 4.3129 | 4.3160 | 4.3192 | 4.3224 | 3 | 6 | 10 | 13 | 16 |

For more detail see values for 95-100



Table 1 : Transformation of percentages to probits.(continue)

| % | 0.0 | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 | 0.7 | 0.8 | 0.9 | 1 | 2 | 3 | 4 | 5 |
|----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|---|---|---|----|----|
| 25 | 4.3255 | 4.3287 | 4.3318 | 4.3349 | 4.3380 | 4.3412 | 4.3443 | 4.3474 | 4.3505 | 4.3536 | 3 | 6 | 9 | 12 | 16 |
| 26 | 4.3567 | 4.3597 | 4.3628 | 4.3659 | 4.3690 | 4.3720 | 4.3750 | 4.3781 | 4.3811 | 4.3842 | 3 | 6 | 9 | 12 | 15 |
| 27 | 4.3872 | 4.3902 | 4.3932 | 4.3962 | 4.3992 | 4.4022 | 4.4052 | 4.4082 | 4.4112 | 4.4142 | 3 | 6 | 9 | 12 | 15 |
| 28 | 4.4172 | 4.4201 | 4.4231 | 4.4260 | 4.4290 | 4.4319 | 4.4349 | 4.4378 | 4.4408 | 4.4437 | 3 | 6 | 9 | 12 | 15 |
| 29 | 4.4466 | 4.4495 | 4.4524 | 4.4554 | 4.4583 | 4.4612 | 4.4641 | 4.4670 | 4.4698 | 4.4727 | 3 | 6 | 9 | 12 | 14 |
| 30 | 4.4756 | 4.4785 | 4.4813 | 4.4842 | 4.4871 | 4.4899 | 4.4928 | 4.4956 | 4.4985 | 4.5013 | 3 | 6 | 9 | 11 | 14 |
| 31 | 4.5041 | 4.5070 | 4.5098 | 4.5126 | 4.5155 | 4.5183 | 4.5211 | 4.5239 | 4.5267 | 4.5295 | 3 | 6 | 8 | 11 | 14 |
| 32 | 4.5323 | 4.5351 | 4.5379 | 4.5407 | 4.5435 | 4.5462 | 4.5490 | 4.5518 | 4.5546 | 4.5573 | 3 | 6 | 8 | 11 | 14 |
| 33 | 4.5601 | 4.5628 | 4.5656 | 4.5684 | 4.5711 | 4.5739 | 4.5766 | 4.5793 | 4.5821 | 4.5848 | 3 | 5 | 8 | 11 | 14 |
| 34 | 4.5875 | 4.5903 | 4.5930 | 4.5957 | 4.5984 | 4.6011 | 4.6039 | 4.6066 | 4.6093 | 4.6120 | 3 | 5 | 8 | 11 | 14 |
| 35 | 4.6147 | 4.6174 | 4.6201 | 4.6228 | 4.6255 | 4.6281 | 4.6308 | 4.6335 | 4.6362 | 4.6389 | 3 | 5 | 8 | 11 | 13 |
| 36 | 4.6415 | 4.6442 | 4.6469 | 4.6495 | 4.6522 | 4.6549 | 4.6575 | 4.6602 | 4.6628 | 4.6655 | 3 | 5 | 8 | 11 | 13 |
| 37 | 4.6681 | 4.6708 | 4.6734 | 4.6761 | 4.6787 | 4.6814 | 4.6840 | 4.6866 | 4.6893 | 4.6919 | 3 | 5 | 8 | 11 | 13 |
| 38 | 4.6945 | 4.6971 | 4.6998 | 4.7024 | 4.7050 | 4.7076 | 4.7102 | 4.7129 | 4.7155 | 4.7181 | 3 | 5 | 8 | 10 | 13 |
| 39 | 4.7207 | 4.7233 | 4.7259 | 4.7285 | 4.7311 | 4.7337 | 4.7363 | 4.7389 | 4.7415 | 4.7441 | 3 | 5 | 8 | 10 | 13 |
| 40 | 4.7467 | 4.7492 | 4.7518 | 4.7544 | 4.7570 | 4.7596 | 4.7622 | 4.7647 | 4.7673 | 4.7699 | 3 | 5 | 8 | 10 | 13 |
| 41 | 4.7725 | 4.7750 | 4.7776 | 4.7802 | 4.7827 | 4.7853 | 4.7879 | 4.7904 | 4.7930 | 4.7955 | 3 | 5 | 8 | 10 | 13 |
| 42 | 4.7981 | 4.8007 | 4.8032 | 4.8058 | 4.8083 | 4.8109 | 4.8134 | 4.8160 | 4.8185 | 4.8211 | 3 | 5 | 8 | 10 | 13 |
| 43 | 4.8236 | 4.8262 | 4.8287 | 4.8313 | 4.8338 | 4.8363 | 4.8389 | 4.8414 | 4.8440 | 4.8465 | 3 | 5 | 8 | 10 | 13 |
| 44 | 4.8490 | 4.8516 | 4.8541 | 4.8566 | 4.8592 | 4.8617 | 4.8642 | 4.8668 | 4.8693 | 4.8718 | 3 | 5 | 8 | 10 | 13 |
| 45 | 4.8743 | 4.8769 | 4.8794 | 4.8819 | 4.8844 | 4.8870 | 4.8895 | 4.8920 | 4.8945 | 4.8970 | 3 | 5 | 8 | 10 | 13 |
| 46 | 4.8996 | 4.9021 | 4.9046 | 4.9071 | 4.9096 | 4.9122 | 4.9147 | 4.9172 | 4.9197 | 4.9222 | 3 | 5 | 8 | 10 | 13 |
| 47 | 4.9247 | 4.9272 | 4.9298 | 4.9323 | 4.9348 | 4.9373 | 4.9398 | 4.9423 | 4.9448 | 4.9473 | 3 | 5 | 8 | 10 | 13 |
| 48 | 4.9498 | 4.9524 | 4.9549 | 4.9574 | 4.9599 | 4.9624 | 4.9649 | 4.9674 | 4.9699 | 4.9724 | 3 | 5 | 8 | 10 | 13 |
| 49 | 4.9749 | 4.9774 | 4.9799 | 4.9825 | 4.9850 | 4.9875 | 4.9900 | 4.9925 | 4.9950 | 4.9975 | 3 | 5 | 8 | 10 | 13 |
| 50 | 5.0000 | 5.0025 | 5.0050 | 5.0075 | 5.0100 | 5.0125 | 5.0150 | 5.0175 | 5.0201 | 5.0226 | 3 | 5 | 8 | 10 | 13 |
| 51 | 5.0251 | 5.0276 | 5.0301 | 5.0326 | 5.0351 | 5.0376 | 5.0401 | 5.0426 | 5.0451 | 5.0476 | 3 | 5 | 8 | 10 | 13 |
| 52 | 5.0502 | 5.0527 | 5.0552 | 5.0577 | 5.0602 | 5.0627 | 5.0652 | 5.0677 | 5.0702 | 5.0728 | 3 | 5 | 8 | 10 | 13 |
| 53 | 5.0753 | 5.0778 | 5.0803 | 5.0828 | 5.0853 | 5.0878 | 5.0904 | 5.0929 | 5.0954 | 5.0979 | 3 | 5 | 8 | 10 | 13 |
| 54 | 5.1004 | 5.1030 | 5.1055 | 5.1080 | 5.1105 | 5.1130 | 5.1156 | 5.1181 | 5.1206 | 5.1231 | 3 | 5 | 8 | 10 | 13 |
| 55 | 5.1257 | 5.1282 | 5.1307 | 5.1332 | 5.1358 | 5.1383 | 5.1408 | 5.1434 | 5.1459 | 5.1484 | 3 | 5 | 8 | 10 | 13 |
| 56 | 5.1510 | 5.1535 | 5.1560 | 5.1586 | 5.1611 | 5.1637 | 5.1662 | 5.1687 | 5.1713 | 5.1738 | 3 | 5 | 8 | 10 | 13 |
| 57 | 5.1764 | 5.1789 | 5.1815 | 5.1840 | 5.1866 | 5.1891 | 5.1917 | 5.1942 | 5.1968 | 5.1993 | 3 | 5 | 8 | 10 | 13 |
| 58 | 5.2019 | 5.2045 | 5.2070 | 5.2096 | 5.2121 | 5.2147 | 5.2173 | 5.2198 | 5.2224 | 5.2250 | 3 | 5 | 8 | 10 | 13 |
| 59 | 5.2275 | 5.2301 | 5.2327 | 5.2353 | 5.2378 | 5.2404 | 5.2430 | 5.2456 | 5.2482 | 5.2508 | 3 | 5 | 8 | 10 | 13 |
| 60 | 5.2533 | 5.2559 | 5.2585 | 5.2611 | 5.2637 | 5.2663 | 5.2689 | 5.2715 | 5.2741 | 5.2767 | 3 | 5 | 8 | 10 | 13 |
| 61 | 5.2793 | 5.2819 | 5.2845 | 5.2871 | 5.2898 | 5.2924 | 5.2950 | 5.2976 | 5.3002 | 5.3029 | 3 | 5 | 8 | 10 | 13 |
| 62 | 5.3055 | 5.3081 | 5.3107 | 5.3134 | 5.3160 | 5.3186 | 5.3213 | 5.3239 | 5.3266 | 5.3292 | 3 | 5 | 8 | 11 | 13 |
| 63 | 5.3319 | 5.3345 | 5.3372 | 5.3398 | 5.3425 | 5.3451 | 5.3478 | 5.3505 | 5.3531 | 5.3558 | 3 | 5 | 8 | 11 | 13 |
| 64 | 5.3585 | 5.3611 | 5.3638 | 5.3665 | 5.3692 | 5.3719 | 5.3745 | 5.3772 | 5.3799 | 5.3826 | 3 | 5 | 8 | 11 | 13 |
| 65 | 5.3853 | 5.3880 | 5.3907 | 5.3934 | 5.3961 | 5.3989 | 5.4016 | 4.4043 | 5.4070 | 5.4097 | 3 | 5 | 8 | 11 | 14 |
| 66 | 5.4125 | 5.4152 | 5.4179 | 5.4207 | 5.4234 | 5.4261 | 5.4289 | 5.4316 | 5.4344 | 5.4372 | 3 | 5 | 8 | 11 | 14 |
| 67 | 5.4399 | 5.4427 | 5.4454 | 5.4482 | 5.4510 | 5.4538 | 5.4565 | 5.4593 | 5.4621 | 5.4649 | 3 | 6 | 8 | 11 | 14 |
| 68 | 5.4677 | 5.4705 | 5.4733 | 5.4761 | 5.4789 | 5.4817 | 5.4845 | 5.4874 | 5.4902 | 5.4930 | 3 | 6 | 8 | 11 | 14 |
| 69 | 5.4959 | 5.4987 | 5.5015 | 5.5044 | 5.5072 | 5.5101 | 5.5129 | 5.5158 | 5.5187 | 5.5215 | 3 | 6 | 9 | 11 | 14 |
| 70 | 5.5244 | 5.5273 | 5.5302 | 5.5330 | 5.5359 | 5.5388 | 5.5417 | 5.5446 | 5.5476 | 5.5505 | 3 | 6 | 9 | 12 | 14 |
| 71 | 5.5534 | 5.5563 | 5.5592 | 5.5622 | 5.5651 | 5.5681 | 5.5710 | 5.5740 | 5.5769 | 5.5799 | 3 | 6 | 9 | 12 | 15 |
| 72 | 5.5828 | 5.5858 | 5.5888 | 5.5918 | 5.5948 | 5.5978 | 5.6008 | 5.6038 | 5.6068 | 5.6098 | 3 | 6 | 9 | 12 | 15 |
| 73 | 5.6128 | 5.6158 | 5.6189 | 5.6219 | 5.6250 | 5.6280 | 5.6311 | 5.6341 | 5.6372 | 5.6403 | 3 | 6 | 9 | 12 | 15 |
| 74 | 5.6433 | 5.6464 | 5.6495 | 5.6526 | 5.6557 | 5.6588 | 5.6620 | 5.6651 | 5.6682 | 5.6713 | 3 | 6 | 9 | 12 | 16 |

Table 1 : Transformation of percentages to probits.(continue)

| % | 0.0 | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 | 0.7 | 0.8 | 0.9 | 1 | 2 | 3 | 4 | 5 |
|------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|---|----|----|----|----|
| 75 | 5.6745 | 5.6776 | 5.6808 | 5.6840 | 5.6871 | 5.6903 | 5.6934 | 5.6965 | 5.6996 | 5.7027 | 8 | 6 | 10 | 18 | 16 |
| 76 | 5.7063 | 5.7095 | 5.7128 | 5.7160 | 5.7192 | 5.7224 | 5.7255 | 5.7287 | 5.7318 | 5.7350 | 3 | 7 | 10 | 13 | 16 |
| 77 | 5.7388 | 5.7421 | 5.7454 | 5.7488 | 5.7521 | 5.7554 | 5.7588 | 5.7621 | 5.7655 | 5.7688 | 3 | 7 | 10 | 13 | 17 |
| 78 | 5.7722 | 5.7756 | 5.7790 | 5.7824 | 5.7858 | 5.7892 | 5.7926 | 5.7961 | 5.7995 | 5.8030 | 3 | 7 | 10 | 14 | 17 |
| 79 | 5.8064 | 5.8099 | 5.8134 | 5.8169 | 5.8204 | 5.8239 | 5.8274 | 5.8310 | 5.8345 | 5.8381 | 4 | 7 | 11 | 14 | 18 |
| 80 | 5.8416 | 5.8452 | 5.8488 | 5.8524 | 5.8560 | 5.8596 | 5.8633 | 5.8669 | 5.8705 | 5.8742 | 4 | 7 | 11 | 14 | 18 |
| 81 | 5.8779 | 5.8816 | 5.8853 | 5.8890 | 5.8927 | 5.8965 | 5.9002 | 5.9040 | 5.9078 | 5.9116 | 4 | 7 | 11 | 15 | 19 |
| 82 | 5.9154 | 5.9192 | 5.9230 | 5.9269 | 5.9307 | 5.9346 | 5.9385 | 5.9424 | 5.9463 | 5.9502 | 4 | 8 | 12 | 15 | 19 |
| 83 | 5.9542 | 5.9581 | 5.9621 | 5.9661 | 5.9701 | 5.9741 | 5.9782 | 5.9822 | 5.9863 | 5.9904 | 4 | 8 | 12 | 16 | 20 |
| 84 | 5.9945 | 5.9986 | 6.0027 | 6.0069 | 6.0110 | 6.0152 | 6.0194 | 6.0237 | 6.0279 | 6.0322 | 4 | 8 | 13 | 17 | 21 |
| 85 | 6.0364 | 6.0407 | 6.0450 | 6.0494 | 6.0537 | 6.0581 | 6.0625 | 6.0669 | 6.0714 | 6.0758 | 4 | 9 | 13 | 18 | 22 |
| 86 | 6.0803 | 6.0848 | 6.0893 | 6.0939 | 6.0985 | 6.1031 | 6.1077 | 6.1123 | 6.1170 | 6.1217 | 5 | 9 | 14 | 18 | 23 |
| 87 | 6.1264 | 6.1311 | 6.1359 | 6.1407 | 6.1455 | 6.1503 | 6.1552 | 6.1601 | 6.1650 | 6.1700 | 5 | 10 | 15 | 19 | 24 |
| 88 | 6.1750 | 6.1800 | 6.1850 | 6.1901 | 6.1952 | 6.2004 | 6.2055 | 6.2107 | 6.2160 | 6.2212 | 5 | 10 | 15 | 21 | 26 |
| 89 | 6.2265 | 6.2319 | 6.2372 | 6.2426 | 6.2481 | 6.2536 | 6.2591 | 6.2646 | 6.2702 | 6.2759 | 5 | 11 | 16 | 22 | 27 |
| 90 | 6.2816 | 6.2873 | 6.2930 | 6.2988 | 6.3047 | 6.3106 | 6.3165 | 6.3225 | 6.3285 | 6.3346 | 6 | 12 | 18 | 24 | 29 |
| 91 | 6.3408 | 6.3469 | 6.3532 | 6.3595 | 6.3658 | 6.3722 | 6.3787 | 6.3852 | 6.3917 | 6.3984 | 6 | 13 | 19 | 26 | 32 |
| 92 | 6.4051 | 6.4118 | 6.4187 | 6.4255 | 6.4325 | 6.4395 | 6.4466 | 6.4538 | 6.4611 | 6.4684 | 7 | 14 | 21 | 28 | 35 |
| 93 | 6.4758 | 6.4833 | 6.4909 | 6.4985 | 6.5063 | 6.5141 | 6.5220 | 6.5301 | 6.5382 | 6.5464 | 8 | 16 | 24 | 31 | 39 |
| 94 | 6.5548 | 6.5632 | 6.5718 | 6.5805 | 6.5893 | 6.5982 | 6.6072 | 6.6164 | 6.6258 | 6.6352 | 9 | 18 | 27 | 36 | 45 |
| 95 | 6.6449 | 6.6546 | 6.6646 | 6.6747 | 6.6849 | 6.6954 | 6.7060 | 6.7169 | 6.7279 | 6.7392 | | | | | |
| | 97 | 100 | 101 | 102 | 105 | 106 | 109 | 110 | 113 | 115 | | | | | |
| 96 | 6.7507 | 6.7624 | 6.7744 | 6.7866 | 6.7991 | 6.8119 | 6.8250 | 6.8384 | 6.8522 | 6.8663 | | | | | |
| | 117 | 120 | 122 | 125 | 128 | 131 | 134 | 138 | 141 | 145 | | | | | |
| 97 | 6.8808 | 6.8957 | 6.9110 | 6.9268 | 6.9431 | 6.9600 | 6.9774 | 6.9954 | 7.0141 | 7.0335 | | | | | |
| | 149 | 153 | 158 | 163 | 169 | 174 | 180 | 187 | 194 | 202 | | | | | |
| | | | | | | | | | | | | | | | |
| % | 0.00 | 0.01 | 0.02 | 0.03 | 0.04 | 0.05 | 0.06 | 0.07 | 0.08 | 0.09 | 1 | 2 | 3 | 4 | 5 |
| 98.0 | 7.0537 | 7.0558 | 7.0579 | 7.0600 | 7.0621 | 7.0642 | 7.0663 | 7.0684 | 7.0706 | 7.0727 | 2 | 4 | 6 | 8 | 11 |
| 98.1 | 7.0749 | 7.0770 | 7.0792 | 7.0814 | 7.0836 | 7.0858 | 7.0880 | 7.0902 | 7.0924 | 7.0947 | 2 | 4 | 7 | 9 | 11 |
| 98.2 | 7.0969 | 7.0992 | 7.1015 | 7.1038 | 7.1061 | 7.1084 | 7.1107 | 7.1130 | 7.1154 | 7.1177 | 2 | 5 | 7 | 9 | 12 |
| 98.3 | 7.1201 | 7.1224 | 7.1248 | 7.1272 | 7.1297 | 7.1321 | 7.1345 | 7.1370 | 7.1394 | 7.1419 | 2 | 5 | 7 | 10 | 12 |
| 98.4 | 7.1444 | 7.1469 | 7.1494 | 7.1520 | 7.1545 | 7.1571 | 7.1596 | 7.1622 | 7.1648 | 7.1675 | 3 | 5 | 8 | 10 | 13 |
| 98.5 | 7.1701 | 7.1727 | 7.1754 | 7.1781 | 7.1808 | 7.1835 | 7.1862 | 7.1890 | 7.1917 | 7.1945 | 3 | 5 | 8 | 11 | 14 |
| 98.6 | 7.1973 | 7.2001 | 7.2029 | 7.2058 | 7.2086 | 7.2115 | 7.2144 | 7.2173 | 7.2203 | 7.2232 | 3 | 6 | 9 | 12 | 14 |
| 98.7 | 7.2262 | 7.2292 | 7.2322 | 7.2353 | 7.2383 | 7.2414 | 7.2445 | 7.2476 | 7.2508 | 7.2539 | 3 | 6 | 9 | 12 | 15 |
| 98.8 | 7.2571 | 7.2603 | 7.2636 | 7.2668 | 7.2701 | 7.2734 | 7.2768 | 7.2801 | 7.2835 | 7.2869 | 3 | 7 | 10 | 13 | 17 |
| 98.9 | 7.2904 | 7.2938 | 7.2973 | 7.3009 | 7.3044 | 7.3080 | 7.3116 | 7.3152 | 7.3189 | 7.3226 | 4 | 7 | 11 | 14 | 18 |
| 99.0 | 7.3263 | 7.3301 | 7.3339 | 7.3378 | 7.3416 | 7.3455 | 7.3495 | 7.3535 | 7.3575 | 7.3615 | 4 | 8 | 12 | 16 | 20 |
| 99.1 | 7.3656 | 7.3698 | 7.3739 | 7.3781 | 7.3824 | 7.3867 | 7.3911 | 7.3954 | 7.3999 | 7.4044 | 4 | 9 | 13 | 17 | 22 |
| 99.2 | 7.4089 | 7.4135 | 7.4181 | 7.4228 | 7.4276 | 7.4324 | 7.4372 | 7.4422 | 7.4471 | 7.4522 | 5 | 10 | 14 | 19 | 24 |
| 99.3 | 7.4573 | 7.4624 | 7.4677 | 7.4730 | 7.4783 | 7.4838 | 7.4893 | 7.4949 | 7.5006 | 7.5063 | 5 | 11 | 16 | 22 | 27 |
| 99.4 | 7.5121 | 7.5181 | 7.5241 | 7.5302 | 7.5364 | 7.5427 | 7.5491 | 7.5556 | 7.5622 | 7.5690 | 6 | 13 | 19 | 25 | 32 |
| 99.5 | 7.5758 | 7.5828 | 7.5899 | 7.5972 | 7.6045 | 7.6121 | 7.6197 | 7.6276 | 7.6356 | 7.6437 | | | | | |
| 99.6 | 7.6521 | 7.6606 | 7.6693 | 7.6782 | 7.6874 | 7.6968 | 7.7065 | 7.7164 | 7.7266 | 7.7370 | | | | | |
| 99.7 | 7.7478 | 7.7589 | 7.7703 | 7.7822 | 7.7944 | 7.8070 | 7.8202 | 7.8338 | 7.8480 | 7.8627 | | | | | |
| 99.8 | 7.8782 | 7.8943 | 7.9112 | 7.9290 | 7.9478 | 7.9677 | 7.9889 | 8.0115 | 8.0357 | 8.0618 | | | | | |
| 99.9 | 8.0902 | 8.1214 | 8.1559 | 8.1947 | 8.2389 | 8.2905 | 8.3528 | 8.4316 | 8.5401 | 8.7190 | | | | | |

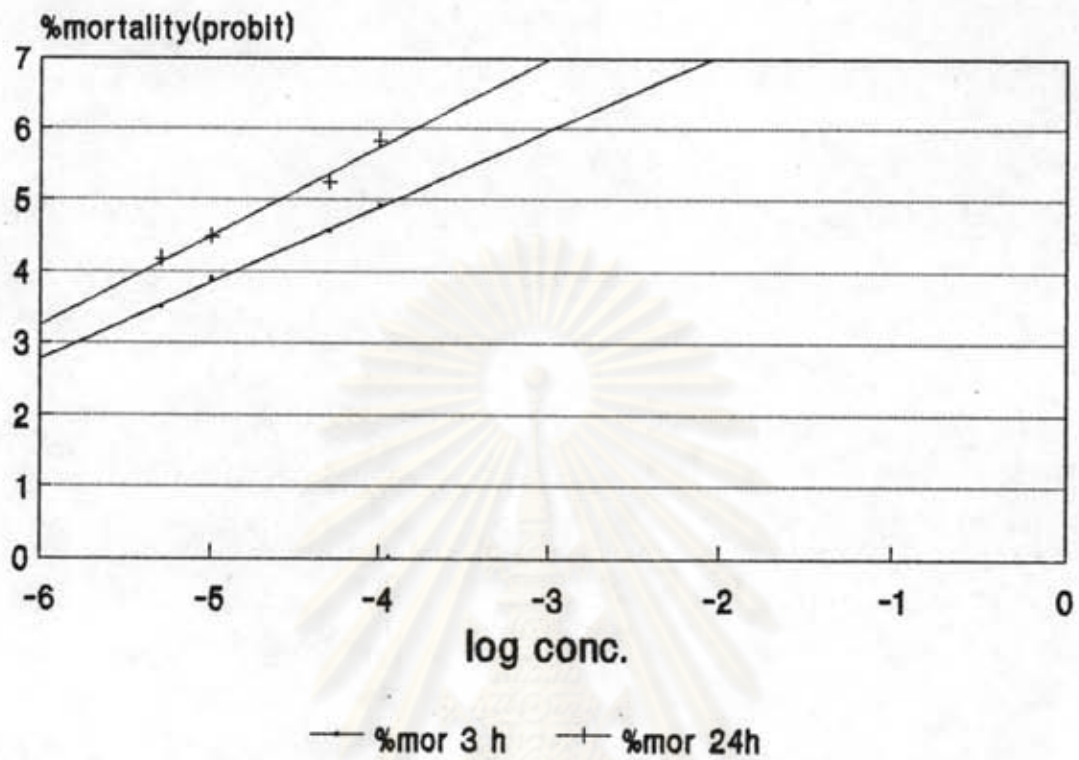


Figure 2 : Contact toxicity of cyhalothrin on *A. florea* at 3 h and 24 h

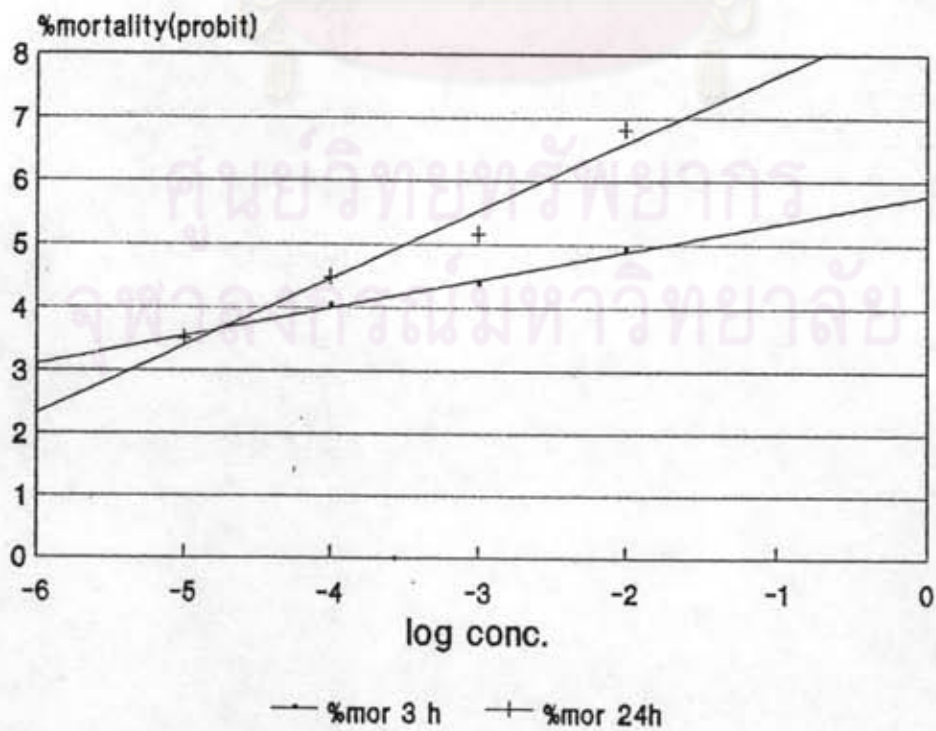


Figure 3 : Contact toxicity of cyhalothrin on *A. cerana* at 3 h and 24 h

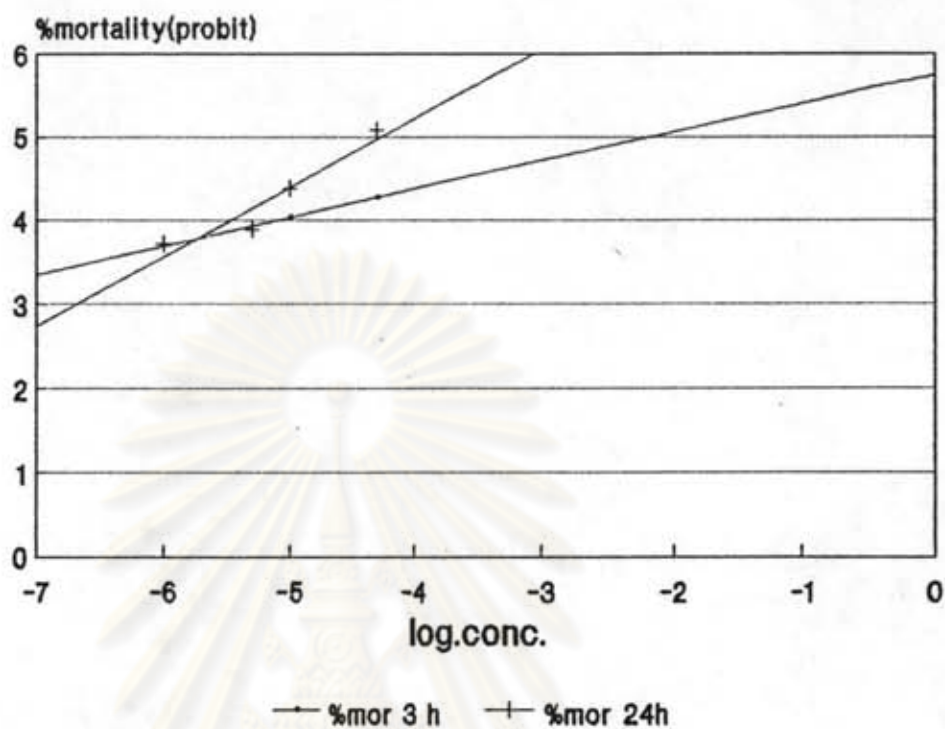


Figure 4 : Oral toxicity of cyhalothrin on *A. florea* at 3 h and 24 h

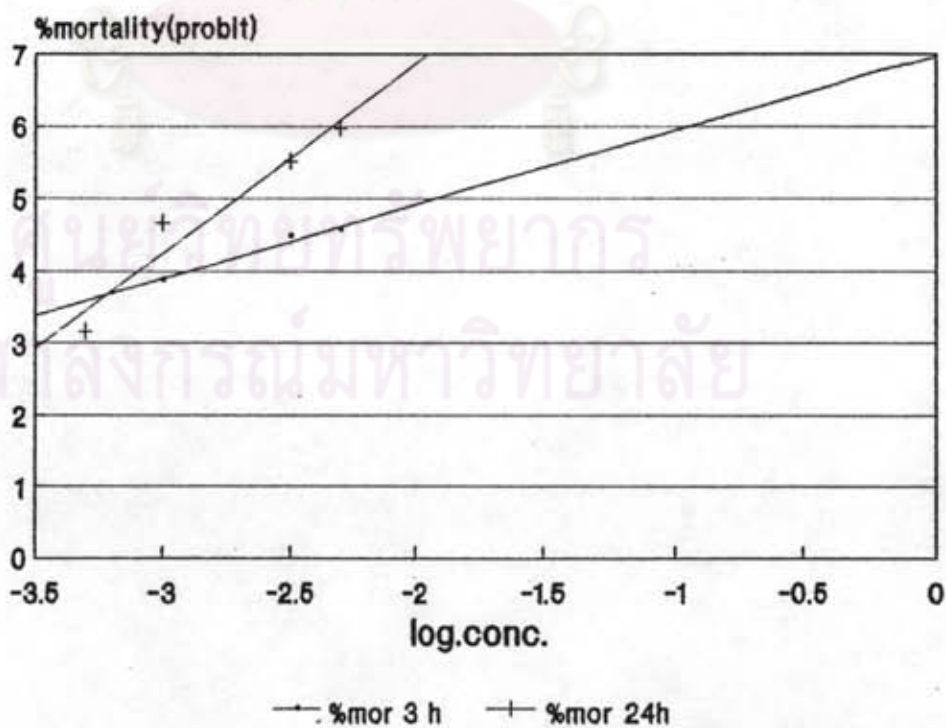


Figure 5 : Oral toxicity of cyhalothrin on *A. cerana* at 3 h and 24 h

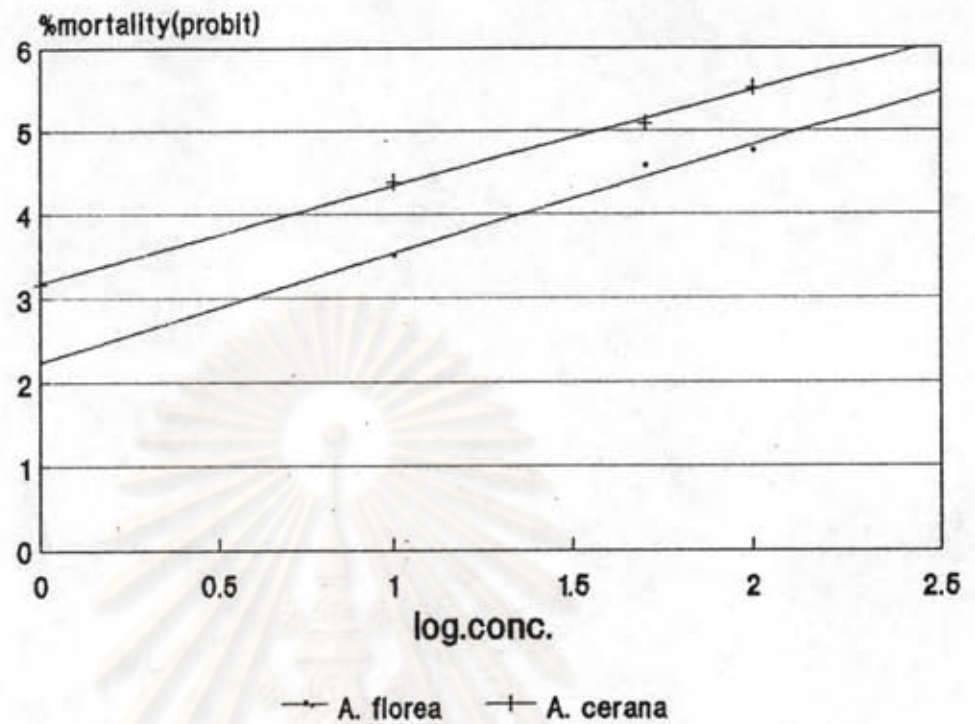


Figure 6 : Contact toxicity of neem-seed crude extract on *A. florea* and *A. cerana* at 24 h.

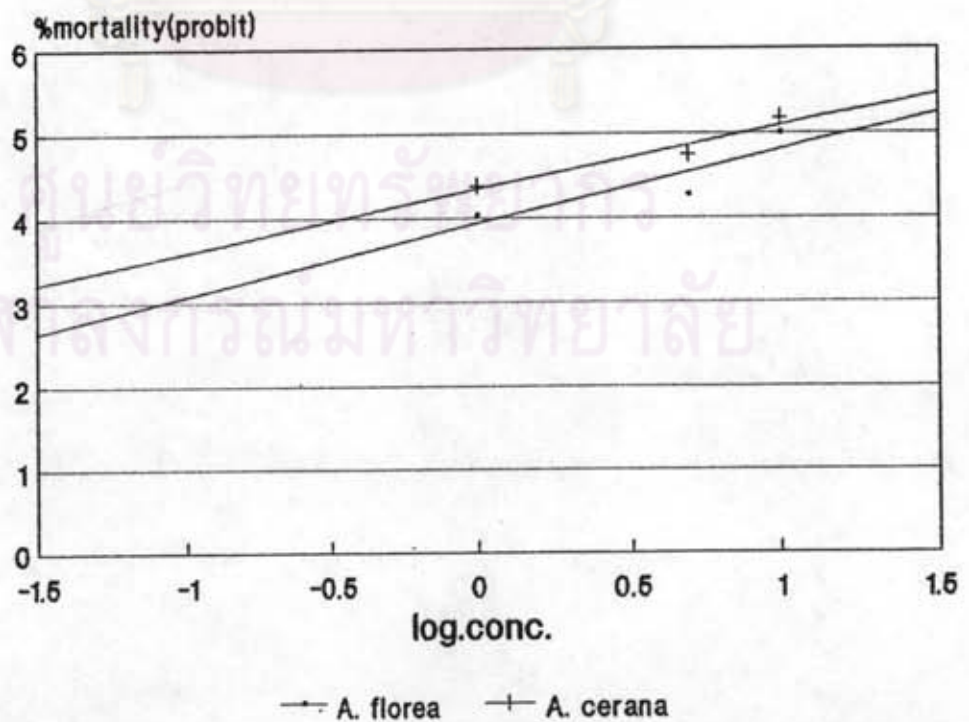


Figure 7 : Contact toxicity of neem-seed extract on *A. florea* and *A. cerana* at 24 h.

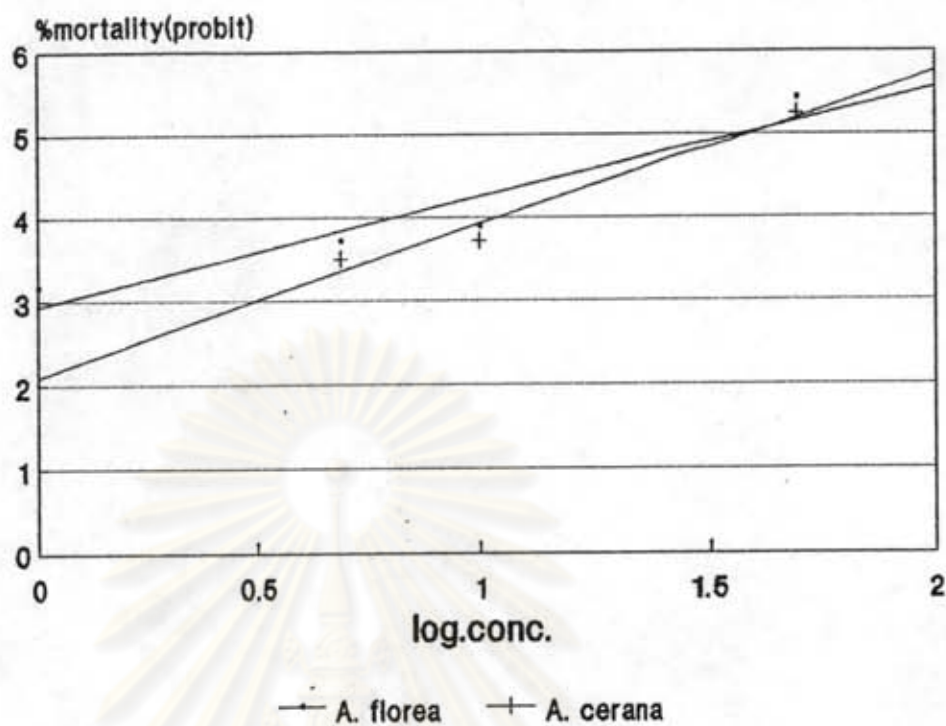


Figure 8 : Contact toxicity of neem oil on *A. florea* and *A. cerana* at 24 h.

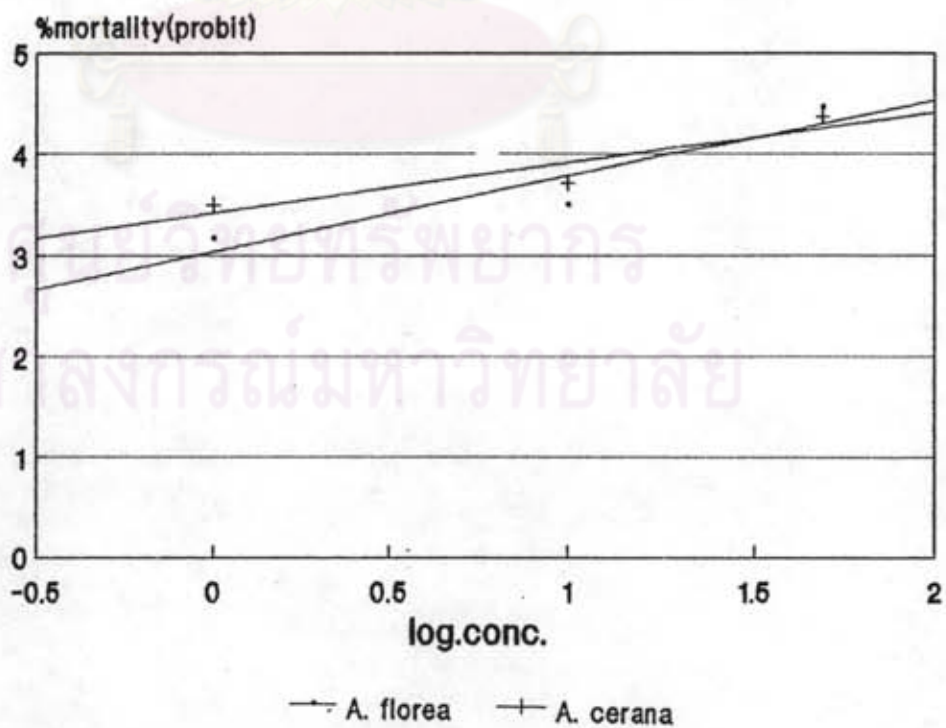


Figure 9 : Oral toxicity of neem oil on *A. florea* and *A. cerana* at 24 h.

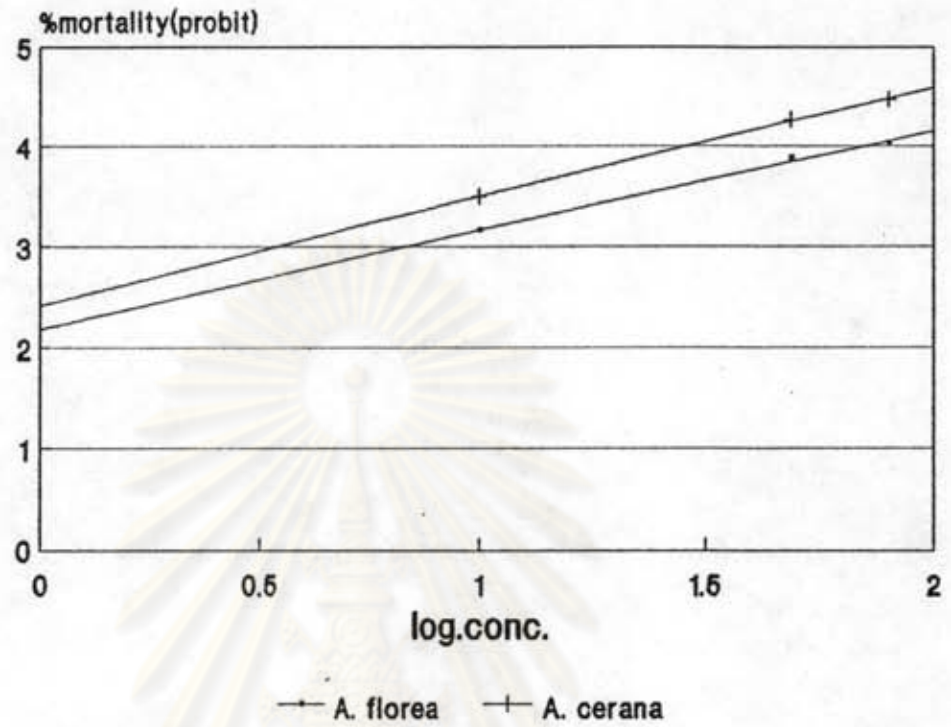


Figure 10 : Contact toxicity of Margosan-O[®] on *A. florea* and *A. cerana* at 24 h.

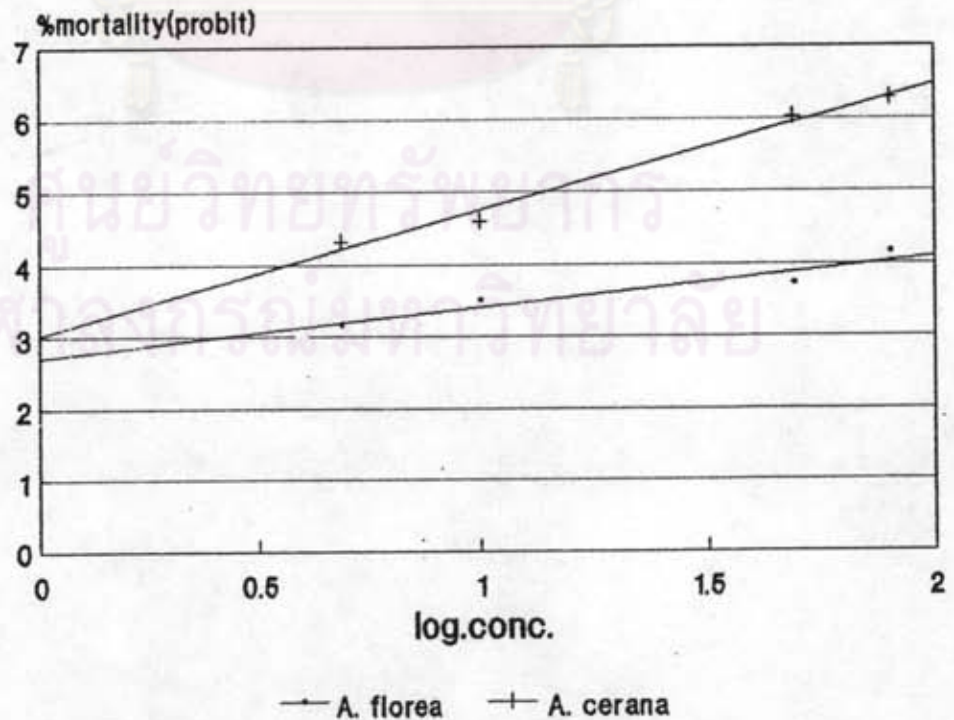


Figure 11 : Oral toxicity of Margosan-O[®] on *A. florea* and *A. cerana* at 24 h.

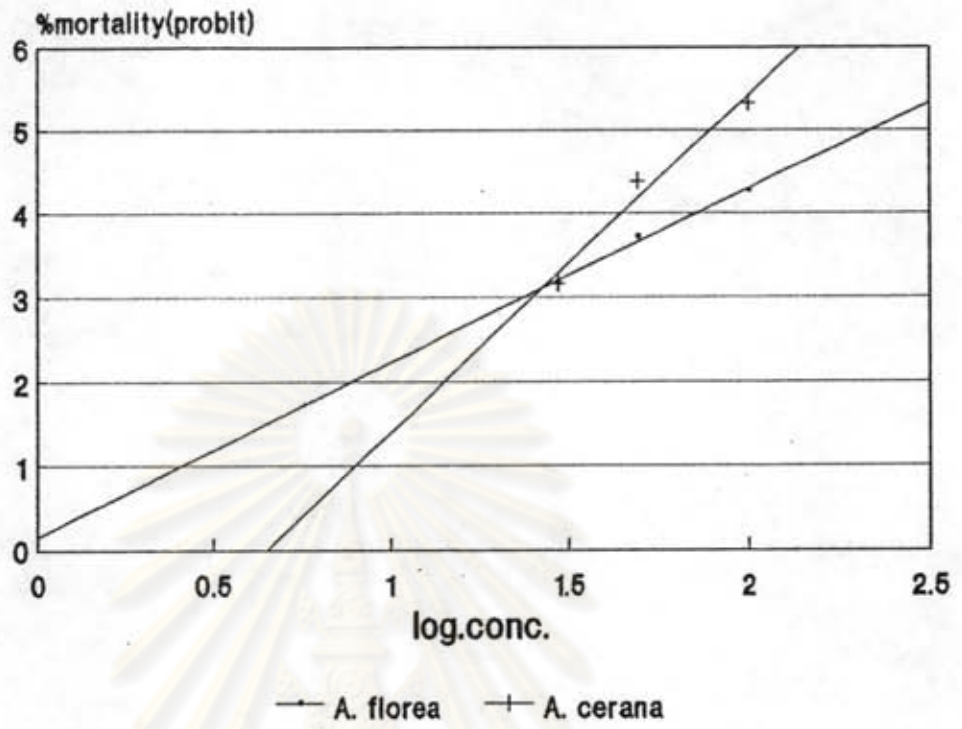


Figure 12 : Contact toxicity of Neemix[®] on *A. florea*
and *A. cerana* at 24 h.

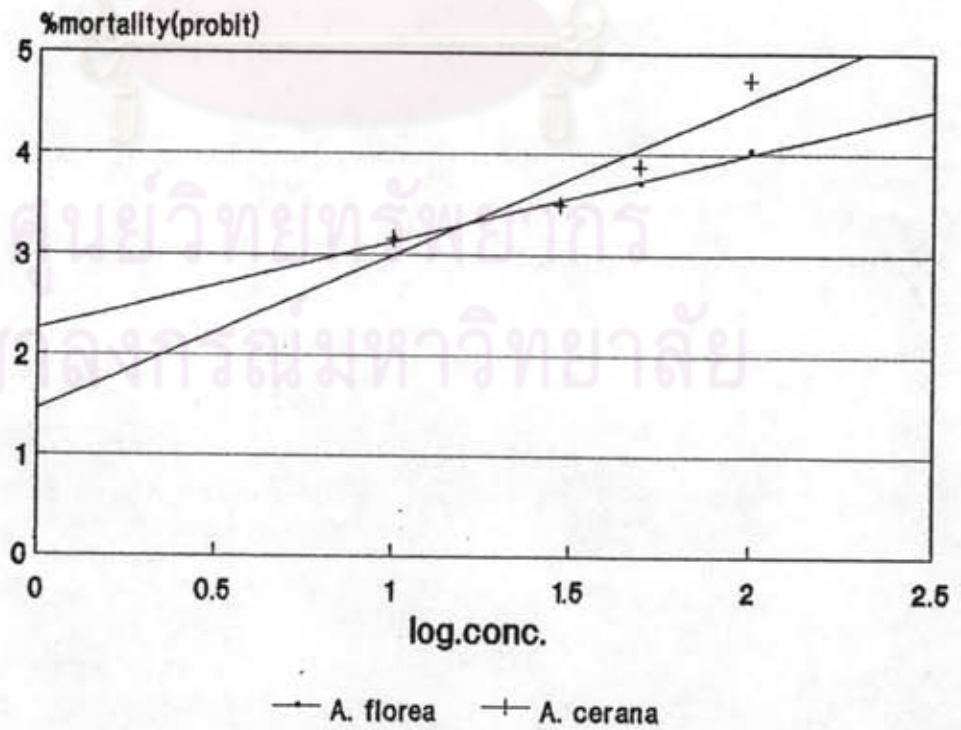


Figure 13 : Contact toxicity of Advantage[®] on *A. florea*
at 24 h.



ESTIMATION THE LD₅₀ VALUE BY USING PROBIT ANALYSIS PROGRAM (SPSS-PC
COMPUTER PROGRAM)

title A.CERANA
data list free / hr conc n RES1 .
begin data
end data
4 cases are written to the compressed active file.

This procedure was completed at 14:12:32
probit res1 of n with conc /MODEL PROBIT /PRINT ALL.

PROBIT requires 304 BYTES of workspace for execution.
A.CERANA

12/20/93

***** PROBIT ANALYSIS *****

DATA Information

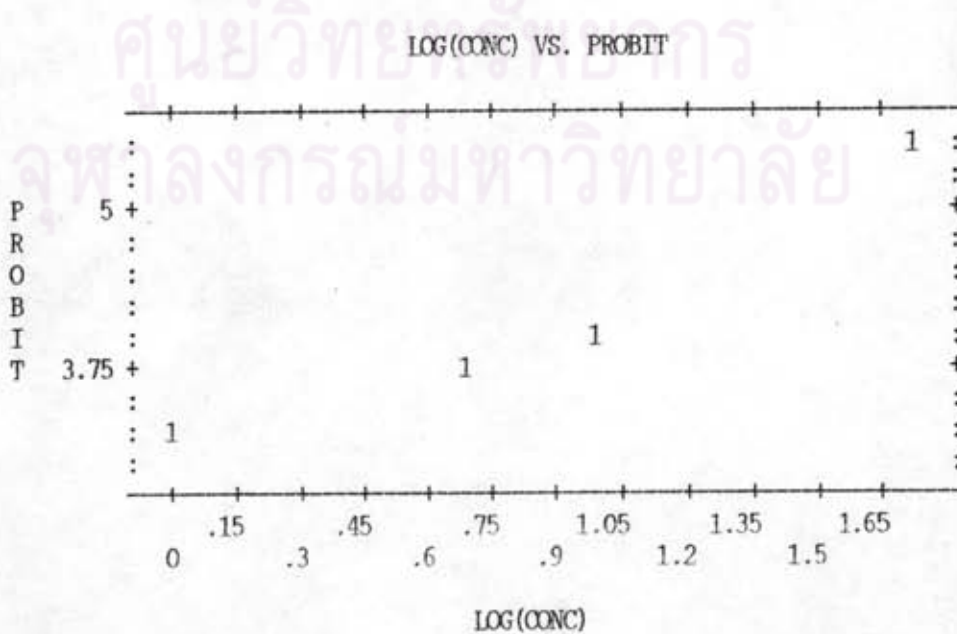
4 unweighted cases accepted.
0 cases rejected because of missing data.
0 Cases rejected because LOG-transform can't be done.

MODEL Information

ONLY Normal Sigmoid is requested.

A.CERANA

12/20/93



A.CERANA

12/20/93

Confidence Limits for Effective CONC

| Prob | CONC | 95% Confidence Limits | |
|------|------------|-----------------------|-------------|
| | | Lower | Upper |
| .01 | 1.00899 | .17289 | 2.31513 |
| .02 | 1.51599 | .33061 | 3.15047 |
| .03 | 1.96280 | .49755 | 3.84044 |
| .04 | 2.38378 | .67546 | 4.46541 |
| .05 | 2.79197 | .86489 | 5.05543 |
| .06 | 3.19402 | 1.06608 | 5.62577 |
| .07 | 3.59390 | 1.27918 | 6.18565 |
| .08 | 3.99425 | 1.50429 | 6.74127 |
| .09 | 4.39695 | 1.74145 | 7.29725 |
| .10 | 4.80343 | 1.99072 | 7.85721 |
| .15 | 6.92689 | 3.41827 | 10.81325 |
| .20 | 9.26607 | 5.13987 | 14.24437 |
| .25 | 11.89321 | 7.13256 | 18.45044 |
| .30 | 14.88156 | 9.36709 | 23.78685 |
| .35 | 18.31713 | 11.82504 | 30.69401 |
| .40 | 22.30780 | 14.51298 | 39.73619 |
| .45 | 26.99449 | 17.46727 | 51.67392 |
| | A.CERANA | | |
| .50 | 32.56720 | 20.75396 | 67.58717 |
| .55 | 39.29034 | 24.47106 | 89.08023 |
| .60 | 47.54493 | 28.75800 | 118.63940 |
| .65 | 57.90334 | 33.81691 | 160.30241 |
| .70 | 71.27094 | 39.95479 | 221.01409 |
| .75 | 89.17888 | 47.66949 | 313.64455 |
| .80 | 114.46306 | 57.84326 | 464.60291 |
| .85 | 153.11674 | 72.25077 | 736.75900 |
| .90 | 220.80510 | 95.25976 | 1320.51266 |
| .91 | 241.21764 | 101.79370 | 1521.03336 |
| .92 | 265.53731 | 109.38342 | 1773.80842 |
| .93 | 295.11747 | 118.36277 | 2100.86166 |
| .94 | 332.06561 | 129.23996 | 2538.40387 |
| .95 | 379.88383 | 142.83810 | 3150.41277 |
| .96 | 444.93330 | 160.60861 | 4061.61275 |
| .97 | 540.36113 | 185.44642 | 5552.54852 |
| .98 | 699.62419 | 224.39190 | 8418.48182 |
| .99 | 1051.17627 | 302.73697 | 16237.96980 |
| | A.CERANA | | |

12/20/93

12/20/93

This procedure was completed at 14:12:53
finish.0;37;40m

End of Include file.

APPENDIX C

Table 1 : Quantity of neem oil and crude extract in difference step of Thai neem-seed extract (method 2.2.2).

| Neem-seed kernel | Neem oil (dark green) | Crude extract (dark green) | Azadirachtin |
|------------------|-----------------------|----------------------------|----------------|
| 10 g | 3.23 g | 1.22 g | 0.0084 g |
| 10 g | 3.34 g | 1.18 g | 0.0097 g |
| average | 3.285 ± 0.08 | 1.2 ± 0.03 | 0.009 ± 0.0009 |
| percentage | 32.85% | 12.0 % | 0.09 % |

Table 2 : Characterization of neem seed.

| Type | length (cm) | weight/seed (g) | 100 g neem seed | |
|-----------------------------|-------------|-----------------|-----------------|-----------|
| | | | seed kernel | seed coat |
| - <i>Azadirachta indica</i> | 1.2 | 0.2 | 45.09 g | 54.91 g |

Table 2 (cont.): Characterization of neem seed.

| Type | length (cm) | weight/seed (g) | 100 g neem seed | |
|--|----------------|--------------------|-----------------|-----------|
| | | | seed kernel | seed coat |
| - <i>Azadirachta indica</i> var. <i>siamensis</i> | 1.5 | 0.3 | 45.20 g | 54.80 g |
| - <i>Azadirachta excelsa</i> | 2.0 | 1.1 | 44.35 g | 55.45 g |

Source : Chirathamjaree, C., Pitiyon, B., and Seangvanit, A., 1993.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX D

Neem-seed extract

Rice Insect Pests Research Unit, Entomology & Zoology Division

recommend 5% neem-seed extract for control brown planthopper, green rice leafhoppers, rice stem borers.

Agricultural Toxic Substance Division

recommend 5% neem-seed extract for control many insect pests.

Neem-leaf extract

Pimsamarn, S. (Kongarn University)

recommend 5% neem-leaf extract for control diamond back moth.

Makmon, A. (Crop Protection by Natural Method Project)

recommend 2 kg. neem-leaf and 1 kg. galanga extract per 20 litre water for control brown planthopper.

Neem oil

Sombatsiri, K. (Kasetsart University)

recommend that seed were attacked with 50% neem oil for control lesser grain borer.

Source : Farm Research Institute and Appropriated Technology Associate,
1989.



COMMERCIAL NEEM EXTRACT

Margosan-0® from Grace-Sierra Crop Protection Co., USA.

For effective management of whiteflies, thrips, mealybugs, leafminers, loopers, caterpillars, and gypsy moths, on ornamentals, tree, and shrubs in and around greenhouses, commercial nurseries, and homes.

Active ingredients : Azadirachtin.....0.3 %

Application rate : Dilute Margosan-0® to a concentration of 2.5 to 5.0 pints per 100 gallons of water (2.5 to 5 teaspoons of Margosan-0® per gallon of water) = 0.04 - 0.08 %

Neemix® from Peachpanthummachad Co., Thailand.

For effective management of oriental fruit fly, leaf-eating caterpillar, thrips, red spider mite.

Ingredients : Neem seed.....50%

Galanga.....25%

Citronella grass.....25%

Application rate : 40 - 60 ml/ 5 l of water = 0.8 - 1.2 %

Advantage® from Nonkasert Co., Thailand.

For effective management of thrips, mealybug, aphid, diamond back moth, red spider mite.

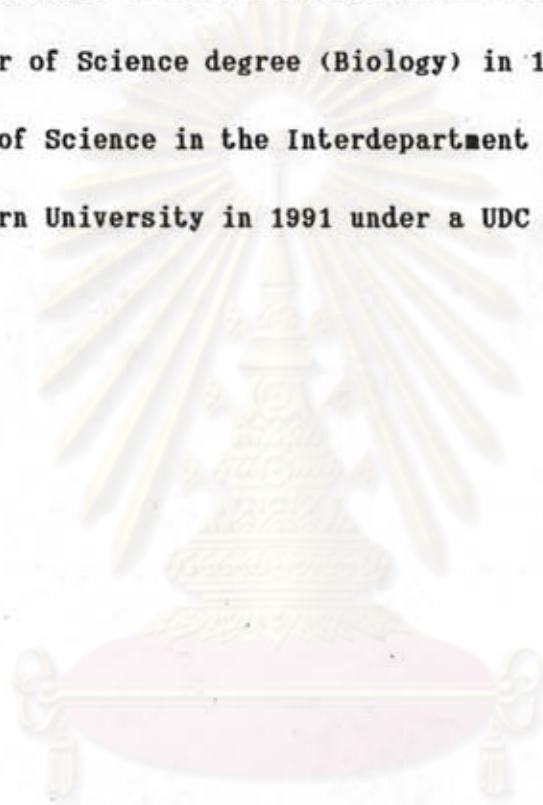
Active ingredients : Meliantriol, azadiractin, salannin, nimbidin, citronellal, geraniol.

Application rate : 40 - 80 ml/ 20 l of water = 2.4 - 4.0 %

BIOGRAPHY OF THE AUTHOR



Miss Chuleemas Boonthai was born on September 13, 1969 in Amphur Maung, Changwat Uttaradit and graduated from Chiang Mai University with a Bachelor of Science degree (Biology) in 1990. She enrolled for a Master degree of Science in the Interdepartment of Environmental Science at Chulalongkorn University in 1991 under a UDC grant and graduated in 1993.



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