PART III

RESULTS AND DISCUSSION

1. Effects of Fertilizers and Stages of Development on Plant Growth

Since plants at the preflowering stage (S_1) and the flowering stage (S_2) would be harvested before the completion of their life cycles, only the plant height at fruit-ripening stage (S_3) had, therefore, been measured, periodically. The results were shown in Table 8 (p.53) and Figure 5 (p.54), in which all treatments clearly showed no significant effect on plant growth rate. Moreover, increasing of the plant heights was faster only during the early period after transplanting but became slower during the flowering stage (S_2) and stayed relatively constant at fruit-maturing stage (S_3).

The characters and development stages of D. metel L. var. fastuosa Safford are shown in Figure 6-12 (p.55-60).

The main reasons for non significant effect of fertilizers and development stages on plant growth rate could be discussed in three categories. Firstly, it might be due to a high fertility of soil used in this study. Since the experimental plots at Department of Horticulture had been used many times for other crops. Plenty of mineral fertilizers had been repeatedly applied to the soil. The remaining elements from preceding cultivation

Table 8 Height During the Growth of O. metel var. fastuosa as Affected by Fertilizer Treatments

Fertilizer	Replicate (Block)	Height (cm) at various times									
		7/6/86		17/6/86		1/7/86		1/8/86		26/8/86	
F_1	B_1	42.25[a]		111.25		144.25		222.75		237.25	
	B_2	57.50	52.58[b]	101.25	107.92	145.00	142.00	214.00	211.25	229.00	227.33
	B_3	57.75		111.25		136.75		197.00		215.75	
F_2	B_1	51.25		94.50		128.00		209.50		229.00	
	B_2	60.25	55.17	104.25	104.92	146.25	138.83	221.25	215.75	213.75	222.42
	B_3	54.00		116.00		142.25		216.50		224.50	
F_3	B_1	56.00		111.50		158.50		219.00		233.75	
	B_2	49.75	55.75	100.75	109.66	144.25	149.42	216.25	217.00	235.75	229.83
	B_3	61.50		108.75		145.50		215.75		220.00	
F_4	B_1	53.50		111.50		153.75		225.50		238.75	
	B_2	59.75	60.50	115.00	116.50	153.25	151.92	222.00	216.58	244.00	220.00
	B_3	68.25		123.00		148.75		202.25		203.00	

[a]Mean of four determinations
[b]Mean of three replicates

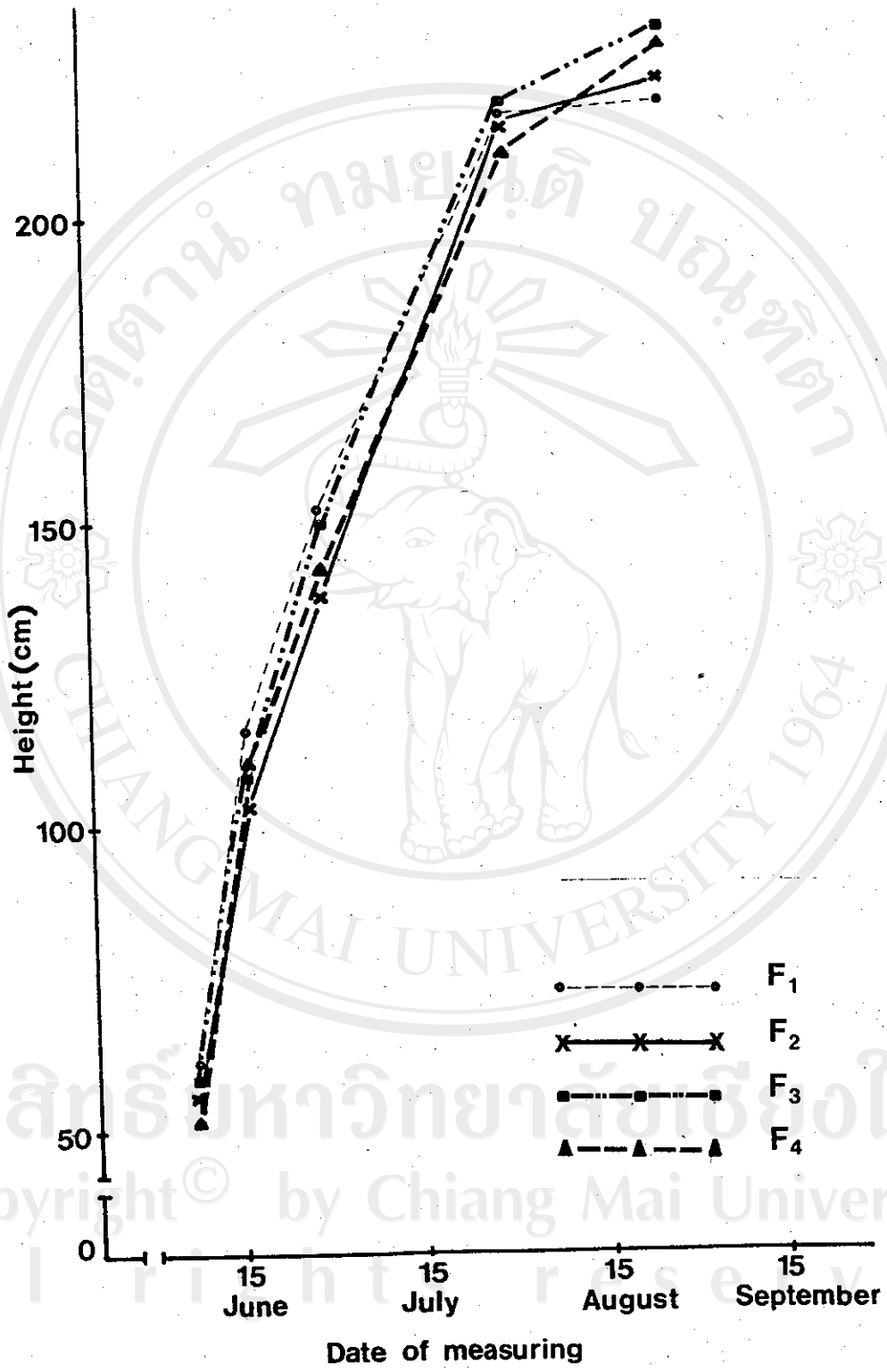

Figure 5. Height Increasing of D. metel var. fastuosa as Affected by Fertilizer Treatments.

Figure 6. Whole Plant of the Cultivated D. metel var. fastuosa

Figure 7. Leaves of D. metel var. fastuosa

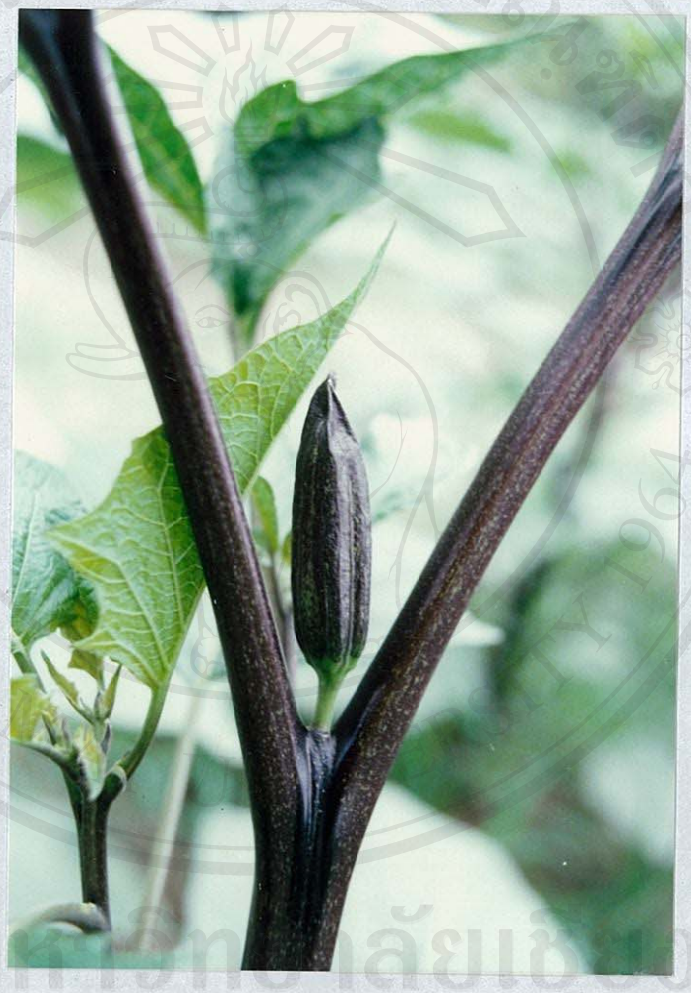

Figure 8. Preflowering (or Bud Initiation) of D. metel var. fastuosa

Figure 9. Flowers of D. metel var. fastuosa

Figure 10. Fruit setting of D. metel var. fastuosa

Figure 11. Fruit dehiscence of D. metel var. fastuosa

Figure 12. Fruit and Seeds of D. metel var. fastuosa.

might already saturate the absorption capacity of the soil particle. Secondly, the soil structure used in this study was a sandy soil with a very low content of organic matter (1.85%). The capacity of soil for absorption ions and water was, therefore, relative low. Fertilizers exceeded to the absorption capacity of soil particle would be leached down to the deeper soil layer and out of the plant root zone. The higher dose of fertilizers were applied to the plant, the higher rate of leaching would occur. Finally, A poor response of plant to the applied fertilizers might due to the nature of plant itself, by which a relative low nutrients were required for the development.

In summary, the result of soil analysis (Table 6, p.40) might be used as a basis for fertilizer application in D. metel var. fastuosa cultivation.

2. Effects of Fertilizers and Stages of Development on Dry Matter Production

The average dry matter (g/plot) of important parts at each stage of development as affected by the fertilizer treatments were shown in Table 9 (p.62). All fertilizer applications could not increase yields of the raw material from the control (no fertilizer). In contrast, the stage of plant development gave a high significance in dry matter production (using factorial design in analysis of variance, in Appendix, p.119. That was at start-blooming stage (S_2), the plant produced

Table 9 Dry Matter (g/plot) of Important Plant Parts as Affected by Fertilizers and Developmental Stages*.

Stage of development	Fertilizer			
	F_1	F_2	F_3	F_4
S_1 (Leaf)	350.0	503.0	310.0	426.7
S_2 (Leaf and flower)	853.7	1016.7	1026.3	1184.7
S_3 (Leaf and seed)	628.3	491.7	470.0	468.3

* Average data of three replications

the highest total yield (dry matter) of leaves combined with flowers followed by the fruit-maturing stage (S_3) and the vegetative stage (S_1) respectiveley.

It had been reported that some parts of Datura spp. especially leaves, flowers and seeds yielded a high content of tropane alkaloids, hyoscyamine (atropine) and hyoscine (Chaudhuri 1954, Gupta et al. 1973, Punyarajun and Tipduangta 1981, Shah and Khanna 1963). Only the dry weight of those plant parts were, therefore, measured and analysed in this study. By using the fertilizer applications at a rate upto 75-50-25 kg/ha, no increase in dry matter production was noted over the control (no fertilizer). The fertilizer treatment as suggested by Sobti and

Kaul (1982) at a dose of only 25-50-25 kg/ha was, therefore, proved to be inappropriate in Chiangmai. This might be due to the differences in soil characteristics and microclimate. The same reasons as discussed in the previous section ; a poor absorption capacity of sandy soil used in this study, the leaching of applied fertilizer out of root zone and the plant habitat of a low nutrient requirement ; were also considered to be the important factors in explaining the poor response of the plant to the applied fertilizers.

In view of industrial applications, it would be useful that plant should be harvested at start-blooming stage(S_2),because the preflowering(S_1) and fruit-ripening stages (S_3) gave much lower yield. Moreover, the fruit-ripening stage needed some labour in the process of seed separations.

3. Effects of Fertilizers and Stages of Development on Alkaloid Contents

Determination of major tropane alkaloids of D. metel var. fastuosa carried out in two steps, extraction and purification of alkaloids from crude drugs respectively. Various analytical procedures for the assessment of atropine (hyoscyamine) and hyoscine had been published. The known methods for qualitative and quantitative analysis of the tropane alkaloids were compared to examine the efficiency of different extraction procedures and various solvents for extraction. It could be seen

from the results and statistical analysis indicated in Table 19, 20 and 21 (Appendix, p.123-125), the high analytical values of the total alkaloids were obtained by extraction with either Method 1. (United States Pharmacopoeia XIX, 1979) or with methanol/hydrochloric acid (MeOH/HCl) in Method 2 (Hikino et al. 1983). Although extraction with methanol/hydrochloric acid (MeOH/HCl) in Method 2 yielded high analytical values, separation of extractives from the crude drug powder was tedious and time-consuming in evaporation steps. Fairly stable emulsions were often produced with each extraction step, so the extractions were unreliable and discouraging.

On the basis of the above findings, it was concluded that the most satisfactory results could be obtained by adopting the procedure in Method 1. The isolation method (p.45) was chosen for its simplicity and similarity to the method used in the previously successful USP collaborative study (Grady and Zimmerer 1970, United States of Pharmacopoeia XIX, 1979) of atropine (hyoscyamine) and hyoscine. It was developed for the simultaneous determination of atropine (hyoscyamine) and hyoscine in D. metel var. fastuosa.

3.1 Quantitative Determination of Total Alkaloids

Samples from each plot were assayed. Two chloroform extractions from an acidified solution of the extracts, as described in the "Procedure" (p.45), were sufficient to remove

interfering plant materials (Wyatt et al. 1976). The alkaloids were subsequently extracted into chloroform from the basified aqueous layer. Basic phosphate buffer (pH 9.5) was used instead of mineral alkali to minimize ester cleavage (Grady and Zimmerer 1970, Wyatt et al. 1976). It was claimed that at this pH the two extractions removed 99.6% of the atropine and all hyoscine (Zimmerer and Grady 1970). Emulsions were sometimes produced during aqueous solution-chloroform extraction but this problem could be solved by adding more aqueous or organic solvent. The thermal instability of the alkaloids is well-known and breakdowns of the alkaloids by heat during the assay had been documented (Solomon et al. 1969). Therefore, the final extracts were not evaporated at temperature above 45°.

The total alkaloids contents in various parts at different stages of D. metel var. fastuosa were estimated quantitatively by titrimetric method and the quantity of the alkaloids was calculated from the molar quantity of the acid which had reacted with the alkaloids to form the salts $B_2.H_2SO_4$. When the molecular weight of hyoscyamine was used in such calculations, the results were expressed as quantity (in percent) of "total alkaloids calculated as hyoscyamine", as was usually done.

l-Hyoscyamine, the most commonly occurring alkaloid from D. metel var. fastuosa is readily converted into atropine during extraction process. It is therefore highly probable that, in most case, atropine is not present in the plant but results from the

racemization of l-hyoscyamine (Holmes 1959). The quantitative estimation of the total alkaloids extracted from plant sample is based on their basicity. All observed values were compared to the fractions of atropine and hyoscine, which determined by GLC method, in the same sample code (Table 10,p.67).

3.2) Quantitative Determination of Atropine and hyoscine

For the individual alkaloid determination, GLC is one of the most rapid and accurate methods. Many workers have used different kinds of GLC column with various conditions to separate atropine and hyoscine as shown in Table 4 (p.31). In this study, the coiled stainless steel column 12 ft.x 1/16 inch (i.d.) packed with 3% OV-17 on Chromosorb W 100-120 mesh was used. The GLC condition was temperature programmed at 220-227° with the increasing rate at 1°/min. At the beginning of this experiment, 6ft. column was used to separate atropine and hyoscine from the leaf extract of D. metel var. fastuosa and homatropine was used as an internal standard. Atropine and hyoscine peaks were not completely separated from the interfering substances (Figure 13, p.69). Then the longer column (12 ft.) was tried and found atropine and hyoscine peaks were completely separated from the interfering substances. Unfortunately, homatropine which was used as an internal standard shown a little shoulder (Figure 14,p.70). Therefore several compounds such as acetanilide, pilocarpine, pilocarpine hydrochloride and methyl linoleate were investigated

Table 10 Comparisons of Total Alkaloid, Atropine and hyoscine Contents by Titrimetric and Gas Liquid Chromatographic Methods in the Same Sample Code.

Sample code[a]	Total alkaloids from titrimetric method,%[b]	Fraction of atropine from gas liquid chromatographic method,%	Fraction of hyoscine from gas liquid chromatographic method,%	Total amounts of atropine and hyoscine from gas chromatographic method,%
L-$S_1F_1B_1$	0.1928[c]	0.0632	0.0685	0.1317
L-$S_1F_1B_2$	0.1702	0.0411	0.0700	0.1111
L-$S_1F_1B_3$	0.1928	0.0520	0.0763	0.1283
L-$S_1F_2B_1$	0.1814	0.0328	0.0774	0.1102
L-$S_1F_2B_2$	0.2440	0.0702	0.1020	0.1802
L-$S_1F_2B_3$	0.1928	0.0467	0.0878	0.1345
L-$S_1F_3B_1$	0.1702	0.0379	0.0728	0.1107
L-$S_1F_3B_2$	0.2722	0.0721	0.1336	0.2057
L-$S_1F_3B_3$	0.2212	0.0562	0.0989	0.1551
L-$S_1F_4B_1$	0.2042	0.0450	0.0938	0.1388
L-$S_1F_4B_2$	0.1588	0.0446	0.0626	0.1072
L-$S_1F_4B_3$	0.1474	0.0424	0.0443	0.0867
L-$S_2F_1B_1$	0.2722	0.1302	0.0817	0.2119
L-$S_2F_1B_2$	0.3176	0.1522	0.0983	0.2505
L-$S_2F_1B_3$	0.3288	0.1353	0.1152	0.2505
L-$S_2F_2B_1$	0.3120	0.1491	0.0790	0.2281
L-$S_2F_2B_2$	0.2948	0.1335	0.0908	0.2243
L-$S_2F_2B_3$	0.2496	0.1294	0.0442	0.1736
L-$S_2F_3B_1$	0.1814	0.0753	0.0408	0.1161
L-$S_2F_3B_2$	0.2156	0.1022	0.0503	0.1525
L-$S_2F_3B_3$	0.2836	0.1244	0.0878	0.2122
L-$S_2F_4B_1$	0.2156	0.1095	0.0428	0.1523
L-$S_2F_4B_2$	0.2600	0.1161	0.0756	0.1917
L-$S_2F_4B_3$	0.2496	0.1478	0.0360	0.1838
L-$S_3F_1B_1$	0.2958	0.1462	0.0816	0.2278
L-$S_3F_1B_2$	0.2382	0.1165	0.0477	0.1642
L-$S_3F_1B_3$	0.2608	0.1334	0.0545	0.1879
L-$S_3F_2B_1$	0.2722	0.1432	0.0631	0.2063
L-$S_3F_2B_2$	0.2836	0.1615	0.0442	0.2057
L-$S_3F_2B_3$	0.3404	0.1713	0.1009	0.2722
L-$S_3F_3B_1$	0.4310	0.2282	0.1352	0.3634
L-$S_3F_3B_2$	0.3970	0.2303	0.1034	0.3337
L-$S_3F_3B_3$	0.3176	0.1833	0.0614	0.2447
L-$S_3F_4B_1$	0.3062	0.1348	0.0906	0.2254
L-$S_3F_4B_2$	0.2268	0.1088	0.0477	0.1565
L-$S_3F_4B_3$	0.3404	0.1868	0.0872	0.2748
F1-$S_2F_1B_1$	0.6580	0.3584	0.2628	0.6212
F1-$S_2F_1B_2$	0.6803	0.2920	0.3602	0.6522
F1-$S_2F_1B_3$	0.7260	0.3983	0.2973	0.6956

Table 10 (Continued)

Sample code[a]	Total alkaloids from titrimetric method,%[b]	Fraction of atropine from gas liquid chromatographic method,%	Fraction of hyoscine from gas liquid chromatographic method,%	Total amounts of atropine and hyoscine from gas chromatographic method,%
$Fl\text{-}S_2F_2B_1$	0.6352	0.3783	0.2405	0.6188
$Fl\text{-}S_2F_2B_2$	0.7260	0.4315	0.2587	0.6902
$Fl\text{-}S_2F_2B_3$	0.4424	0.2357	0.1791	0.4148
$Fl\text{-}S_2F_3B_1$	0.7374	0.4232	0.2729	0.6961
$Fl\text{-}S_2F_3B_2$	0.4652	0.1668	0.2674	0.4342
$Fl\text{-}S_2F_3B_3$	0.5672	0.3088	0.2085	0.5173
$Fl\text{-}S_2F_4B_1$	0.5558	0.3038	0.2065	0.5103
$Fl\text{-}S_2F_4B_2$	0.7146	0.3584	0.3176	0.6760
$Fl\text{-}S_2F_4B_3$	0.5558	0.2839	0.2207	0.5046
$Se\text{-}S_3F_1B_1$	0.4310	0.2057	0.1404	0.3461
$Se\text{-}S_3F_1B_2$	0.2836	0.0996	0.0949	0.1945
$Se\text{-}S_3F_1B_3$	0.4424	0.1945	0.1643	0.3588
$Se\text{-}S_3F_2B_1$	0.3744	0.1481	0.1326	0.2807
$Se\text{-}S_3F_2B_2$	0.4311	0.1924	0.1567	0.3391
$Se\text{-}S_3F_2B_3$	0.4992	0.2233	0.1816	0.4049
$Se\text{-}S_3F_3B_1$	0.4424	0.1854	0.1738	0.3584
$Se\text{-}S_3F_3B_2$	0.4318	0.1819	0.1524	0.3343
$Se\text{-}S_3F_3B_3$	0.3856	0.1727	0.1266	0.2993
$Se\text{-}S_3F_4B_1$	0.3638	0.1362	0.1077	0.2439
$Se\text{-}S_3F_4B_2$	0.4196	0.1896	0.1369	0.3265
$Se\text{-}S_3F_4B_3$	0.3914	0.1713	0.1189	0.2982

[a] L = leaf, Fl = flower, Se = Seed
[b] Calculated in term of atropine
[c] Mean of two determinations

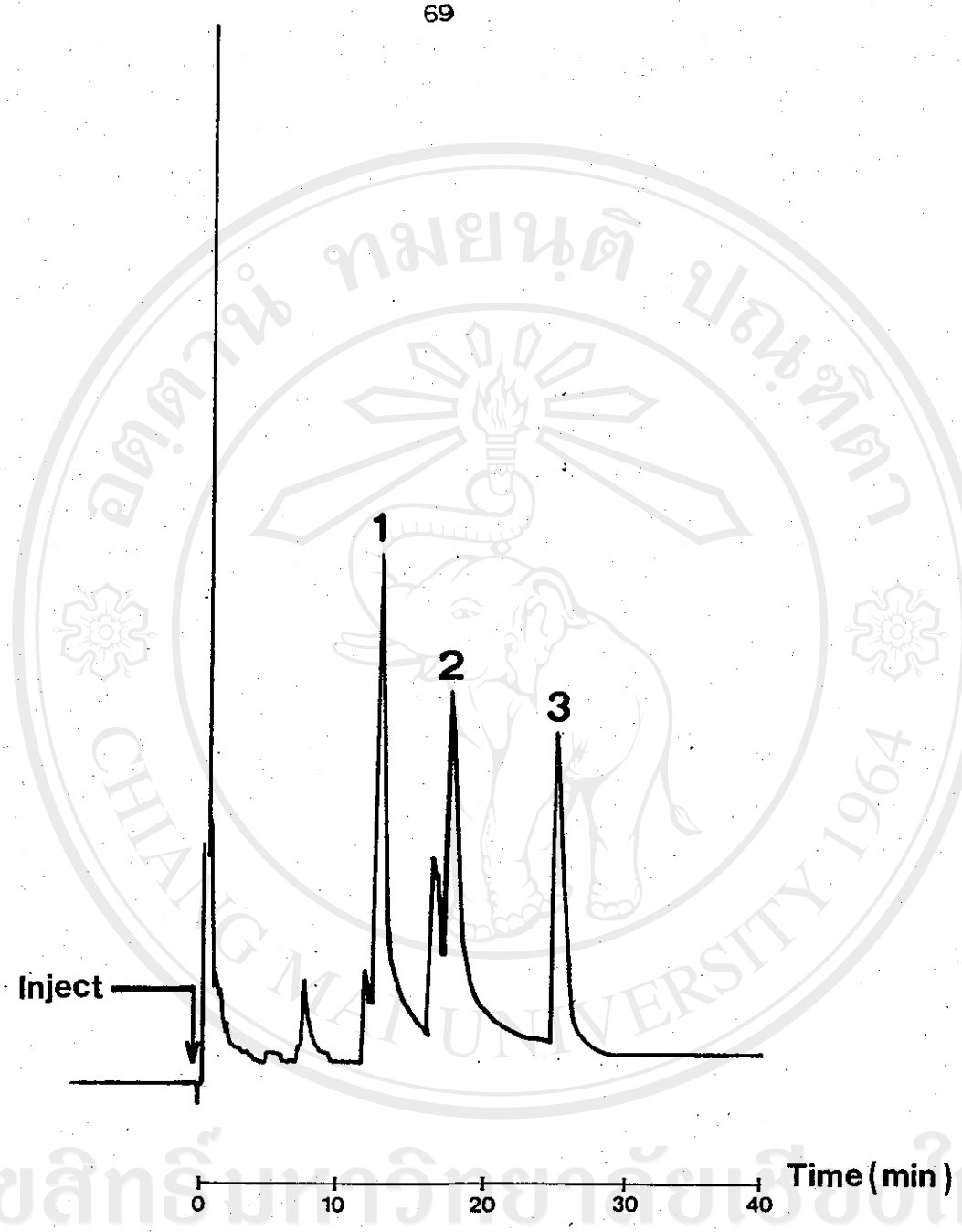

Figure 13 The GLC Analysis of the Crude Extract from D. metel var. fastuosa when 6 ft. Column was Used :

1. Homatropine (Internal Standard) ;
2. Atropine ;
3. Hyoscine.

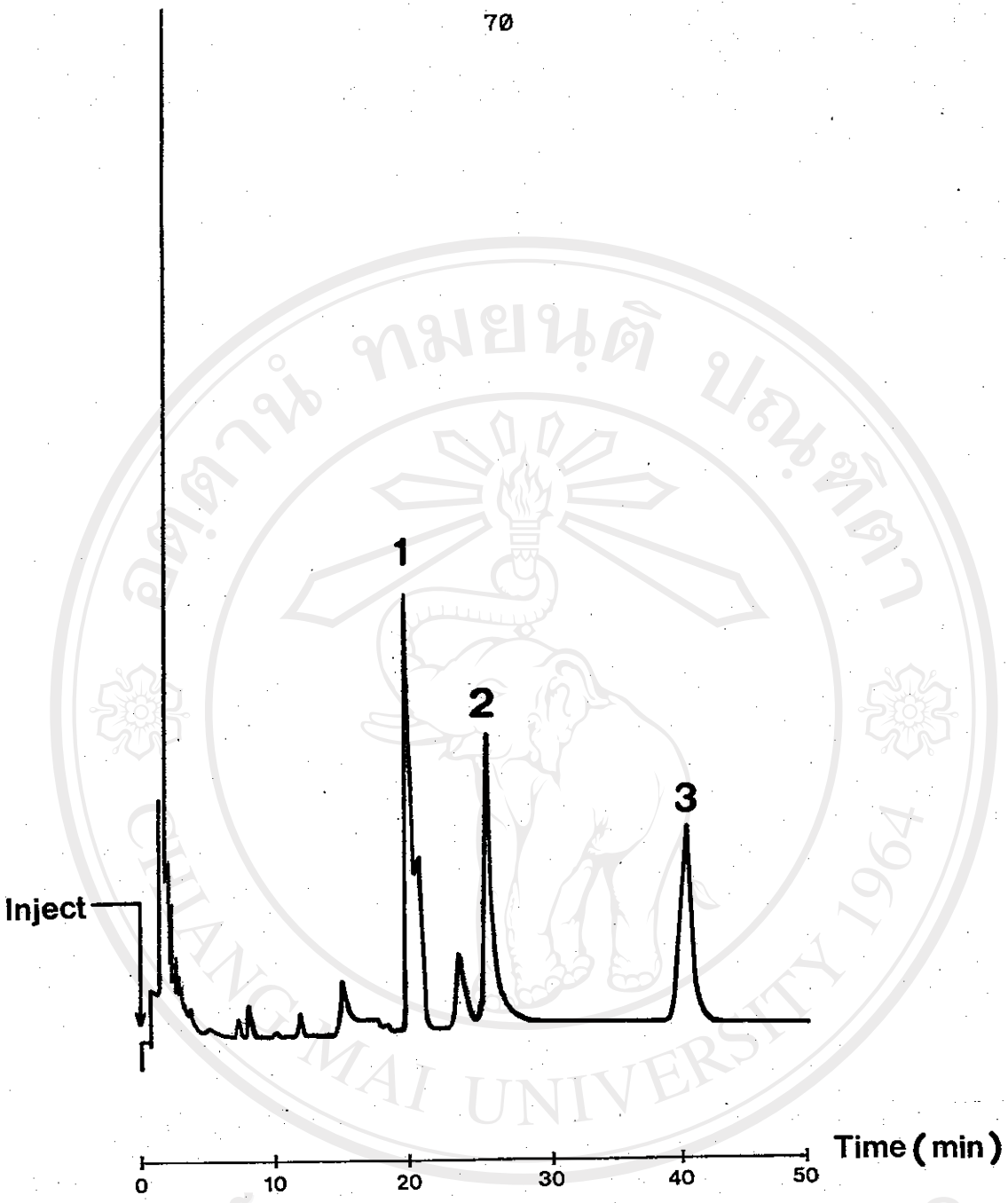

Figure 14 The GLC Analysis of the Crude Extract from D. metel var. fastuosa when 12 ft. Column was Used :

1. Homatropine (Internal Standard) ;
2. Atropine ;
3. Hyoscine.

for homatropine substituted and methyl linoleate proved to be the most satisfactory. Methyl linoleate is readily available commercially. It does not exist in plant extracts and remains stable in chloroform solution for relatively long period.

The typical chromatogram was shown in Figure 15(p.72). The three compounds were well resolved eventhough their retention times were rather long, compared to the chromatogram in Figure 13 (p.69). Methyl linoleate was found in the proximity of atropine and hyoscine. It was eluted at 10 min (220°), then atropine and hyoscine at 28 min (222°) and 42 min (227°), respectively. The condition was 220° isothermal for 27 min then programmed at 220° -227° (1°/min) and 227° isothermal for 15 min.

The calibration graphs obtained by plotting between peak height ratio of both standard atropine and hyoscine to internal standard and mass ratio of these two alkaloids to internal standard. Figure 16 (p.73) and 17 (p.74) represent calibration curves for atropine and hyoscine, respectively. Both are linear over the range 5.0-25.0 µg/ml of atropine and 2.5-7.5 µg/ml of hyoscine (the correlation coefficient of atropine and hyoscine were 0.9996 and 0.9990, respectively) and the relationship of intercept and slope are given in Table 11 (p.75). Each value shown in Figure 16 (p.73) and 17 (p.74) is the average of two independent determinations.

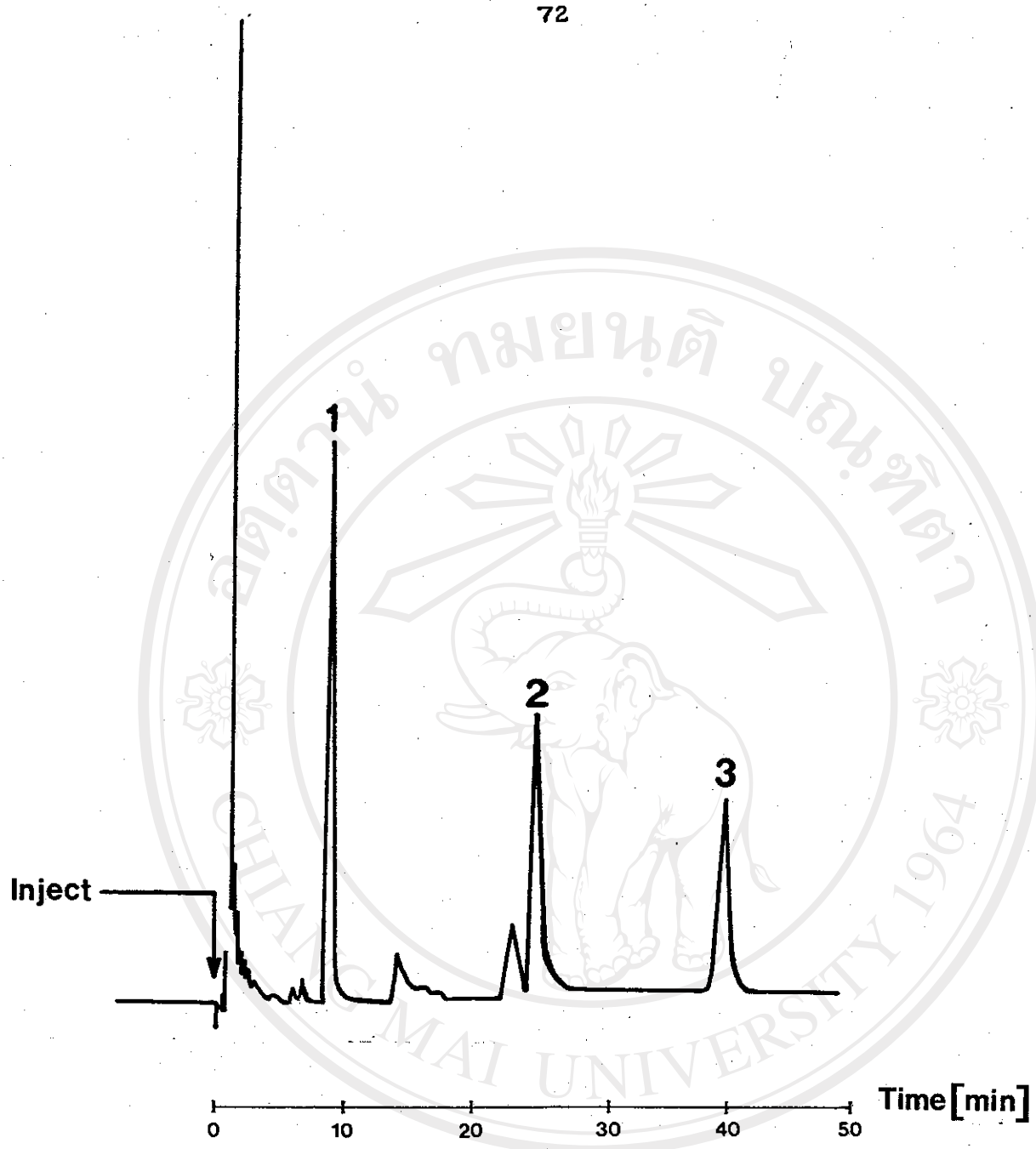

Figure 15 The GLC Analysis of the Crude Extract from D. metel var. fastuosa when 12 ft. Column was Used :

1. Methyl Linoleate (Internal Standard) ;
2. Atropine ;
3. Hyoscine.

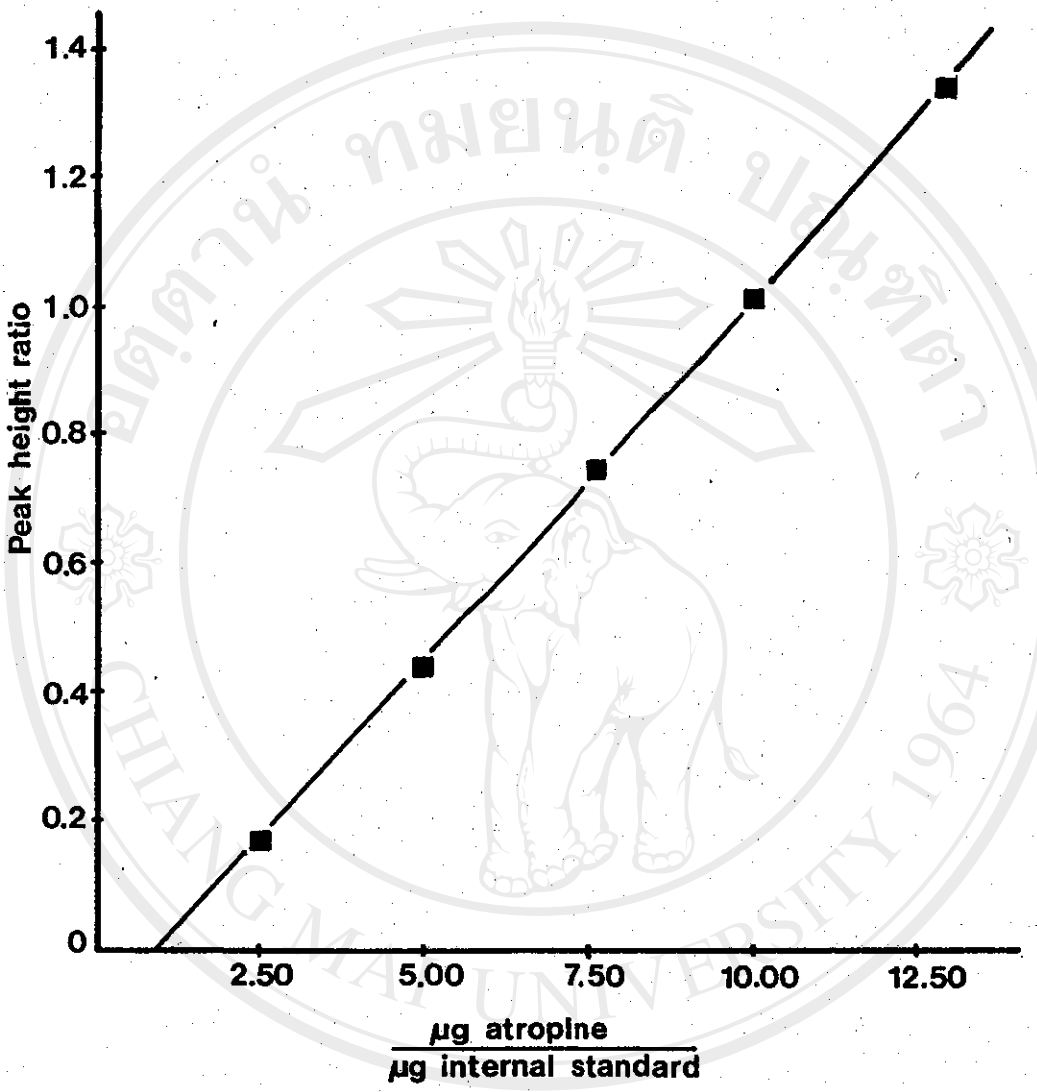

Figure 16 Calibration Graph of Atropine by GLC Method

(H = -0.1345 + 0.1174 M)

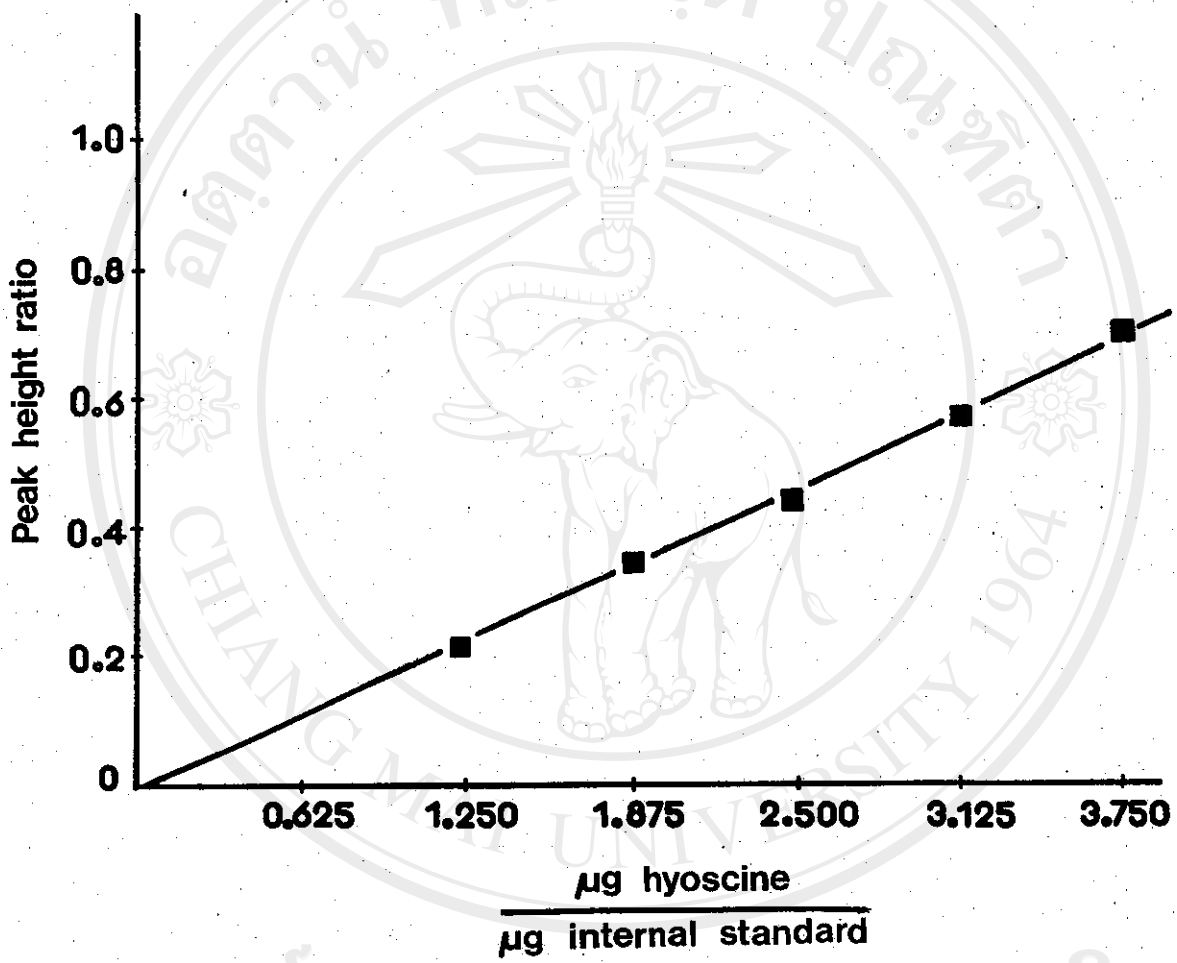

Figure 17 Calibration Graph of Hyoscine by GLC Method

(H = -0.0188 + 0.0961 M)

Table 11 Relation of Intercept and Slope of Calibration Graph Obtained by the Method of Least Squares.*

Substance	Intercept	Slope	Correlation coefficient
Atropine	-0.1345	0.1174	0.9996
Hyoscine	-0.0188	0.0961	0.9990

* calculation shown in Appendix (p124-128).

3.2.1) Recovery of Added Atropine and Hyoscine From the Method Used

The recovery was determined by adding known amounts of standard atropine and hyoscine to the sample. Adding the standard at 4.0000 mg and 2.0000 mg resulted in the recovery of 99.59 4.29 and 100 2.92% respectively (Table.12, p.76). This recovery study showed that the method used was reliable.

3.2.2 Determination of Precision of the Assay Methods of Atropine, Hyoscine and Total Alkaloids.

The precision of the assay methods, including method of extraction and methods of determinations : titrimetry and GLC, was tested by carrying out eight determinations from the same crude sample. All the assays were

Table 12 Recovery of Added Atropine and Hyoscine (In the Flowering Samples)

	Atropine (mg)			Hyoscine (mg)		
	1	2	3	1	2	3
Found[a]	7.4163	7.4163	7.4163	4.4499	4.4499	4.4499
Added	4.0000	4.0000	4.0000	2.0000	2.0000	2.0000
Calculated	11.4163	11.4163	11.4163	6.4499	6.4499	6.4499
Found[a]	11.4773	11.3778	11.3445	6.4790	6.4384	6.4384
Recovery (%)	101.52	99.04	98.20	101.46	99.42	99.42
Average (%)	99.59			100.10		
Standard deviation	1.7262			1.1778		
Coefficient of variation	1.7333			1.1766		
Confidence limit of percent recovery, p = 0.05	95.30-103.88			97.18-103.02		

[a]The average value of two determinations.

performed in duplicate in the manner described previously and the mean of both was taken for the interpretation of the results.

The statistical evaluation of these analyses (Table 13, p.78) indicated that the coefficient of variation of the GLC method in the determination of atropine and hyoscine were 0.6063 and 0.0138, respectively. In titrimetric method, the coefficient of variation for determination of total alkaloids was 1.9537. Therefore, the combination of the liquid-liquid extraction and titrimetric determination/GLC determination methods were proved to be very precise.

3.3 Statistical Evaluation of Total Alkaloid, Hyoscyamine (Atropine) and Hyoscine Contents at Different Stages of Development and as Affected by Fertilizers.

All observed values were collected and analysed statistically (calculation shown in Appendix,p.130). In these analyses, three cases were determined:

1) Comparisons of total alkaloid, hyoscyamine (atropine) and hyoscine contents in leaves at different stages of development and as affected by fertilizers.

From the data obtained for total alkaloid, hyoscyamine (atropine) and hyoscine contents as shown in Table 10, p.67 and statistically treated, it could be interpreted as follows.

Table 13 Determination of Precision of the Assay Methods from Sample L-$S_2B_2F_1$[a]

Assay no.	Gas liquid chromatographic method			Titrimetric method
	Atropine (%)	Hyoscine (%)	Atropine and hyoscine (%)	Total alkaloids (%)[b]
1	0.1522	0.0971	0.2493	0.3176
2	0.1522	0.0996	0.2518	0.3176
3	0.1522	0.0971	0.2493	0.3062
4	0.1522	0.0971	0.2493	0.3062
5	0.1522	0.0971	0.2493	0.3176
6	0.1542	0.0946	0.2488	0.3062
7	0.1522	0.0971	0.2493	0.3062
8	0.1542	0.0971	0.2513	0.3176
Mean	0.1527	0.0971	0.2498	0.3119
Standard deviation	0.0009	0.0013	0.0011	0.0061
Coefficient of variation	0.6063	0.0138	0.4411	1.9537
Confidence limit, p=0.05	0.1519-0.1534	0.0960-0.0982	0.2489-0.2507	0.3068-0.3170

[a] The average value of two determinations.
[b] Total alkaloid contents were calculated in the term of atropine.

1.1) The stages of development gave a highly significant increasing in the total alkaloid and hyoscyamine (atropine) contents ($p < 0.01$) but failed to give a significant increasing of hyoscine content. The stage of maturity thus was directly related to its alkaloid contents which attained a maximum total alkaloid and hyoscyamine (atropine) contents in leaves at the stage of fruit-ripening (S_3).

1.2) The nitrogenous fertilizer application at different levels had no significant effect on total alkaloid, hyoscyamine (atropine) and hyoscine contents. There was no significant difference in alkaloid contents among control and various fertilized plants.

2) Comparisons of total alkaloid, hyoscyamine (atropine) and hyoscine contents in flowers as affected by fertilizers.

From the data obtained (as shown in Table 10, p.67) and statistically treated, it could be interpreted that the nitrogenous fertilizer application at different levels had no significant effect on total alkaloid, hyoscyamine (atropine) and hyoscine contents in flowers. There was no significant difference in alkaloid contents of flowers among control and various fertilized plants.

3) Comparisons of total alkaloid, hyoscyamine (atropine) and hyoscine contents in seeds as affected by fertilizers.

From the data obtained (as shown in Table 10, p.67) and statistically treated, it could be interpreted that the nitrogenous fertilizer application at different levels had no

significant effect on total alkaloid, hyoscyamine (atropine) and hyoscine contents in seeds. There was no significant difference in alkaloid contents in seeds among control and various fertilized plants.

The conclusion of these statistical evaluations was summarized schematically as shown in Figure 18 (p.81).

3.4) Influences of Fertilizers and Development Stages on Alkaloid Contents in Different Parts of Plant.

In this study, leaves, flowers and seeds were systematically analysed for their alkaloidal contents starting from the preflowering stage of the plant upto the dehiscence stage of mature fruits with the objective of ascertaining which stage in the life cycle of the plant which yielded the maximum alkaloid content in the leaves, flowers or seeds and to determine at which stage either of the two major components, hyoscyamine (atropine) or hyoscine was predominantly present. It was also desirable to note at which level of fertilizers influenced the amount of alkaloids.

3.4.1) Influence of Fertilizers on Alkaloid Contents in Different Parts of Plant.

In studying the effect of different levels of nitrogenous fertilizers on alkaloid contents in D. metel var.

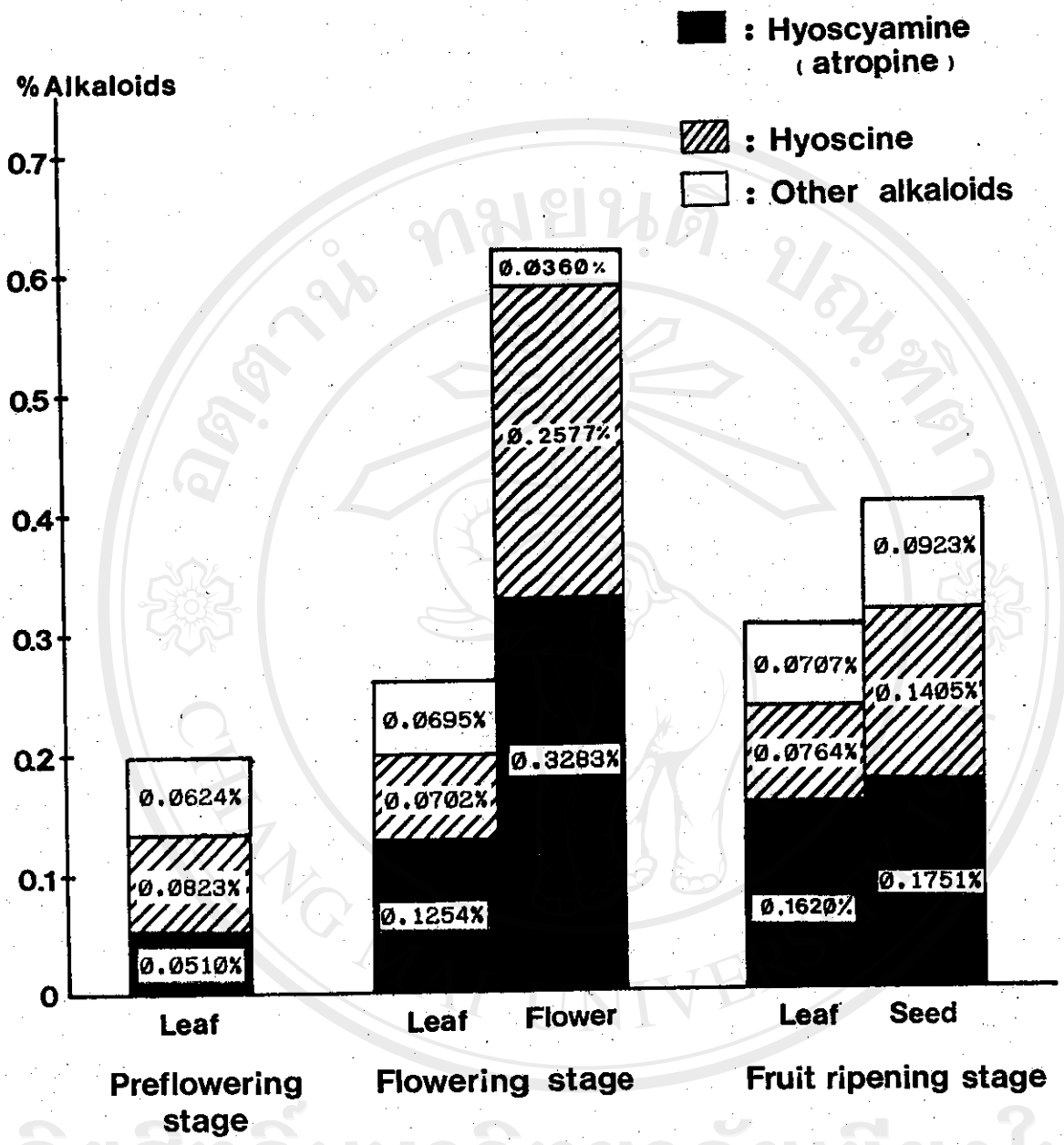

Figure 18 Comparisons of Total Alkaloid, Hyoscyamine (Atropine) and Hyoscine Contents in Various Parts at Different Stages of Development

fastuosa, treatments consisting of three levels of Nitrogen (25,50 and 75 kg N/ha) were replicated thrice in factorial design (compared with control, no treatment). Crop received uniform application of 50 kg P_2O_5/ha and 25 kg K_2O/ha. Plant parts were harvested in the preflowering, flowering and then fruit-ripening stages.

Statistical analysis showed that there was no significant difference between the alkaloid contents (total alkaloids, hyoscyamine (atropine) and hyoscine) in various parts of control and fertilized plants.

There were conflicting reports regarding the variation of alkaloids in Datura due to different levels of fertilizers. Afridi et al. (1977) reported that the alkaloid percentage in leaves was found to be significantly higher in the fertilized plant than in the control and the results showed that 60kg N/ha was the optimal conditions. Shibata et al. (1951) also showed that the maximum yield of alkaloid in plant was obtained with $(NH_4)_2SO_4$ when ratio of N : P_2O_5 : K_2O = 5:3:1.5.

3.4.2) Influence of Development Stages on Alkaloid Contents in Different Parts of Plant.

Table 10, p.67 showed an interesting periodic in alkaloidal percentage. Alkaloid contents of the plant is a consequence of interaction of many factors and the effect of any

isolated factor can not be emphasized, because the effects of other factors should not be ignored (Gupta et al. 1973). Also, information regarding the presence of the major alkaloidal component is conflicting and the fluctuations in the percentage of individual components have been mentioned (Gupta et al. 1973, Karnick and Saxena 1970). Keeping other variables in view, it was observed that the stage of development of the life cycle of D. metel var. fastuosa was the utmost importance in studying the alkaloid contents of its different organs. Hecht and Remeike had pointed out that the alkaloid content is less influenced by environment, than by genetic factors and development stages (Gupta et al. 1973). In case of leaf, the percentage of total alkaloid contents appeared to increase from the preflowering stage (S_1), reaching a comparatively high value in the flowering stage (S_2) and then attained a maximum when the fruits were ripening and at the dehiscence stage (S_3). Development stage of leaves was thus directly related to theirs alkaloid contents but the leaf size was greatly reduced in the post-flowering stage. Percentages of hyoscyamine (atropine) in leaves were not constant at different periods of growth. Hyoscine was the principal alkaloid upto the preflowering stage (S_1), and after that the hyoscyamine (atropine) content began to increase. The fluctuation pattern of the hyoscyamine (atropine) contents at different stages of plant growth was the same order as the total alkaloid contents. The controversary observation was found on the hyoscine centent ; it appeared to have no significant difference at various stages of growth. It was also found that Hyoscyamine (atropine) was the

main alkaloid in both flowers and seeds of the flowering and fruit-ripening stages, respectively. Flowers and seeds showed higher percentage of alkaloid contents than leaves at the same growth stage. The best time of harvesting D. metel var. fastuosa would be flowering stage (S_2) which produced the highest contents of total alkaloids, hyoscyamine (atropine) and hyoscine in leaves and flowers. The percentages of total alkaloids, hyoscyamine (atropine) and hyoscine in various parts of D. metel var. fastuosa at different stages of development are shown in Table 14 (p.85), which, in conclusion, showed the pattern similar results reported by Gupta et al. (1973) and is described briefly below.

According to these observations both leaves and roots of D. metel var. fastuosa contained varying amounts of hyoscyamine (atropine) and hyoscine at different stages of development. The percentages were not fixed but fluctuated. Hyoscine was usually the principal alkaloid upto the preflowering stage of plant. In succeeding stages i.e., flowering, fruit and dehiscence of fruit there was a gradual decrease in hyoscine content with a corresponding increase in hyoscyamine (atropine) content.

Most of the tracer experiment with tropane alkaloids has been applied specifically to hyoscyamine, but some to hyoscine. The metabolic relationship between these two alkaloids has been explored to some extent, but relationships involving other alkaloids of Datura spp. are mostly speculative. Interspecific

Table 14 Percentages of Total Alkaloid, Hyoscyamine (Atropine) and Hyoscine Contents in Various Parts of D. metel var. fastuosa at Different Stages of Development.

Stage of development	Plant part	Percentage of total alkaloids	Hyoscyamine (atropine) fraction (percent)	Hyoscine fraction (percent)	Hyoscyamine (atropine) and hyoscine fractions (percent)
S_1	Leaf	0.1957	0.0510	0.0823	0.1333
S_2	leaf	0.2651	0.1254	0.0702	0.1956
	flower	0.6220	0.3283	0.2577	0.5860
S_3	leaf	0.3091	0.1620	0.0764	0.2384
	seed	0.4079	0.1751	0.1405	0.3156

grafting experiments indicate that the root is the principal site of alkaloid synthesis ; however, secondary modifications of the alkaloids might occur in the aerial parts-for example, the epoxidation of hyoscyamine to give hyoscine (Trease and Evan 1985, Bernfeld. 1967). In some plants of the Solanaceae, hyoscyamine was the dominant alkaloid throughout the life cycle of the plant. The relative proportions of hyoscine and hyoscyamine in a particular species varied with not only the age of the plant, but also the other factors, including day length, light intensity, general climatic conditions, chemical sprays, debudding and chemical races (Trease and Evan 1985, Robinson 1985). Romeiky in Germany had shown that hyoscine appeared to be formed in the plant from hyoscyamine, possibly via dehydrohyoscyamine and 6-hydroxyhyoscyamine (Figure 3, p.26) (Bernfeld 1967, Trease and Evan 1985) Consequently, it could be expected that during the preflowering stage (S_1) nearly all hyoscyamine formed from the biosynthetic process had undergone to hyoscine, that was why hyoscine was the major alkaloid in this stage. After the preflowering stage (S_1) the biosynthetic process was still carried on but change from hyoscyamine to hyoscine rarely occurred. Moreover, some hyoscine might be reversed back to hyoscyamine and from workers in New Jersy (Trease and Evan 1985), some enzyme preparation might reduce hyoscine to hyoscyamine. From these points of view, more atropine could accumulate in the latter stages of development. Therefore, hyoscyamine was the major alkaloid in various parts of plant during the flowering stage (S_2) and the fruit-ripening stage (S_3). But transformation from hyoscyamine to hyoscine, or vice

versa, in various parts occurred in irregular quantities, thus, making the ratio of hyoscyamine and hyoscine in each part become fluctuated. The mechanism that produced different ratio of hyoscyamine and hyoscine in each stage was still unknown. Much more work is needed to be carried out to clarify the plausible biogenetic scheme of alkaloids upon this mechanism.