# CHAPTER III
RESULTS AND DISCUSSION

The aim of this research was to synthesize substituted phenoxyacetic acids, *N*-(2,4-dichlorophenoxyacetyl)-amino acids and related compounds. These synthetic compounds were investigated for the structure and activity relationship (SAR) of weed growth inhibition against *M. pigra* and plant growth promotion of *C. atropurpurreus*.

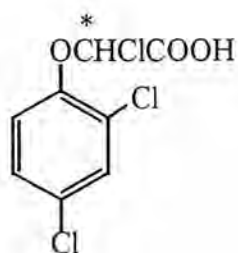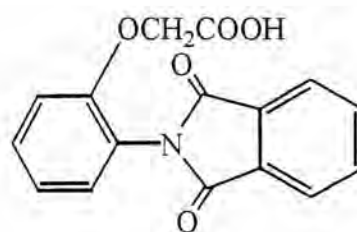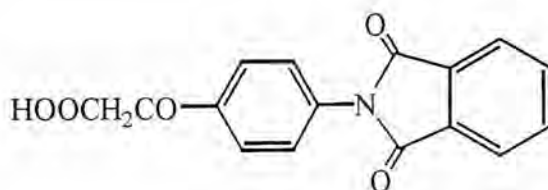### 3.1 Substituted Phenoxyacetic Acids and Their Analogues

The preparation of selected phenoxyacetic acids was performed utilizing the condensation reaction of appropriate phenols with chloroacetic acids in the presence of sodium hydroxide under refluxing for a few hours. As mentioned earlier this classical synthesis method was generally used to synthesize some phenoxyacetic acids since this method did not complicate and was convenient to obtain the products in moderate to high yield. The general equation is shown below.
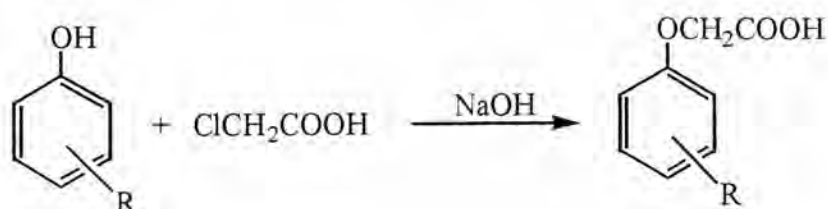
From this study, thirty-eight substituted phenoxyacetic acids were synthesized and two aminophenoxyacetic acids were prepared by reducing nitro phenoxyacetic acid with iron powder in the presence of methanol and glacial acetic acid. Two *N*-phthalimido phenoxyacetic acids were synthesized by the condensation reaction of aminophenoxyacetic acid and phthalic anhydride in the presence of acetic acid. Their physical properties are tabulated in Table 3.1. Moreover, two 2,4-dichlorophenoxy-propanoic acids and chloro-(2,4-dichlorophenoxy)-acetic acid were synthesized employing appropriate chlorocarboxylic acids. The comparative results of their physical properties are presented in Table 3.2. The ester derivatives were also synthesized using 2,4-D and appropriate alcohols catalyzed by concentrated sulfuric

acid. For amide derivatives, classical method using thionyl chloride was employed to prepare 2,4-dichlorophenoxyacetyl chloride and allowed them to react with selected amines. Their physical properties are also accumulated in Table 3.2.

Three synthesized analogues of phenoxyacetic acids (Compounds **41**, **50** and **51**) were disclosed to be new compounds based upon no report concerning these compounds in the chemical literature. The spectroscopy data of these three compounds are presented in Figures A.1-A.7 (see Appendices A).
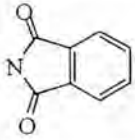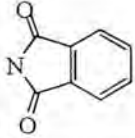

41

50

51

Table 3.1 Physical properties and % yield of synthesized phenoxyacetic acids

Compound	Substituents (R)		Physical Properties		% yield
	Position	Type	Appearance	m.p.(°C)	
1	-	-	White needle	98-99	30
2	3	F	White plate	92-93	40
3	4	F	White plate	108-110	20
4	2	Cl	White needle	142-145	52
5	3	Cl	White needle	118-119	60
6	4	Cl	White microcrystalline product	157-159	50
7	3 4	CH$_3$ Cl	White microcrystalline product	141-142	47
8	2,4	Cl	White needle	141-142	57
9	2,4,5	Cl	White plate	157-159	45
10	2,3,4,5,6	Cl	White needle	195-196	35
11	3	Br	White needle	111-113	76
12	4	Br	White plate	155-156	31
13	2,4,6 3	Br CH$_3$	White needle	230-231	38
14	2	CH$_3$	White plate	160-161	51
15	3	CH$_3$	Pale brown powder	105-106	48
16	4	CH$_3$	White needle	143-144	57
17	2,3	CH$_3$	White powder	187-189	55
18	2,4	CH$_3$	White needle	136-138	52
19	2,6	CH$_3$	White microcrystalline product	137-139	54
20	3,4	CH$_3$	White needle	161-163	61

Table 3.1 (cont.)

Compound	Substituents (R)		Physical Properties		% yield
	Position	Type	Appearance	m.p.(°C)	
21	2,3,5	CH_3	White microcrystalline product	77-79	56
22	2	NO_2	Yellowish needle	157-158	70
	5	CH_3			
23	2	$C(CH_3)_3$	White powder	>300	15
	4	CH_3			
24	2	CH_2CH_3	White microcrystalline product	140-141	42
25	4	CH_2CH_3	White needle	72-73	75
26	2	$CH(CH_3)_2$	White microcrystalline product	133-134	48
27	2	$C(CH_3)_2$	White plate	145-146	54
28	4	$C(CH_3)_2$	White plate	84-86	45
29	2	CH_2Ph	White needle	130-131	27
30	4	Ph	White plate	159-161	66
31	2	$COCH_3$	White plate	85-86	37
32	4	OCH_3	White plate	109-110	71
33	2,6	OCH_3	White plate	81-82	57
34	3,5	OCH_3	White plate	140-142	71
35	2	NO_2	Yellow plate	156-157	89
36	3	NO_2	Pale yellow powder	137-138	23
37	4	NO_2	Pale yellow plate	187-188	64
38	4	$NHCOCH_3$	Pale purple powder	165-166	74

Table 3.1 (cont.)

Compound	Substituents (R)		Physical Properties		% yield
	Position	Type	Appearance	m.p.(°C)	
48	2	NH_2	Pale yellow powder	219-220	98
49	4	NH_2	Dark brown powder	217-218	98
50	2		Brown-orange powder	238-240	63
51	4		Pale brown powder	205-206	50

Table 3.2 Physical properties and % yield of synthesized 2,4-dichlorophenoxy-alkanoic acids and derivatives

Compound	Side chain (R)	Physical Properties		% yield
		Appearance	m.p.(°C)	
39	CH_2CH_2COOH	White needle	87-89	46
40	$CH(CH_3)COOH$	White needle	115-117	50
41	$CHClCOOH$	White powder	97-98	30
42	CH_2COOCH_3	Colorless oil	-	84
43	$CH_2COOCH_2CH_3$	Colorless oil	-	97
44	$CH_2COOC_6H_5$	White powder	93-95	63
45	CH_2CONH_2	Pale brown needle	133-135	21
46	$CH_2CONHC_6H_5$	Pale brown needle	104-105	97
47	$CH_2CONHC_6H_{10}$	White needle	152-153	39

In addition, the synthesis of 2,4-D conjugated with amino acids was carried out employing the classical method using thionyl chloride to prepare 2,4-dichlorophenoxyacetyl chloride. The acid chloride was then reacted with amino acid in the presence of sodium hydroxide. Their physical properties are tabulated in Table 3.3.

Table 3.3 Physical properties and % yield of synthesized *N*-(2,4-dichloro-phenoxyacetyl)-amino acids
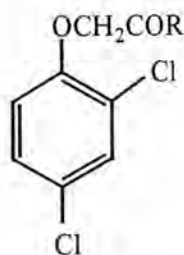
Cpd	Configuration	Substituents (R)	Physical Properties		%Yield
			Appearance	m.p. (°C)	
52	DL	NHCH(CH$_3$)COOH	White needle	213-214	69
53	L	NHCH(CH$_3$)COOH	White needle	204-205	33
54	D	NHCH(CH$_3$)COOH	White powder	208-209	40
55	L	NHCH(CH$_2$Ph)COOH	White powder	179-180	91
56	-	NHCH$_2$COOCH$_3$	Pale yellow Powder	116-117	43
57	DL	NHCH(CH$_3$)COOH	Pale brown oil	-	30
58	L	NHCH(CH$_3$)COOH	Colorless oil	-	54
59	D	NHCH(CH$_3$)COOH	Pale brown oil	-	49
60	L	NHCH(CH$_2$Ph)COOCH$_3$	White powder	112-114	79
61	L	NHCH(CH$_2$COOCH$_3$)COOCH$_3$	White needle	67-70	50
62	L	NHCH(CH$_2$CH$_2$COOCH$_3$)COOCH$_3$	Colorless oil	-	51

The last group, certain phenoxyacetate sodium salts were synthesized by refluxing phenoxyacetic acids with sodium hydroxide in toluene. Phenoxyacetate calcium salts were obtained from adding calcium chloride to a refluxing aqueous
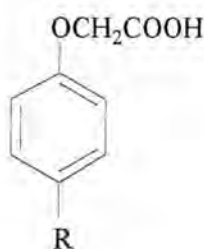
solution of phenoxyacetate sodium salts. Both phenoxyacetate sodium and calcium salts were white crystal or powder which had the melting point over 300°C.

3.2 Spectroscopic Data of Synthesized Compounds
3.2.1 Infrared Spectroscopy (IR)
Substituted Phenoxyacetic Acids

OCH$_2$COOH

R

The FT-IR spectra of substituted phenoxyacetic acids normally displayed the absorption band of O-H stretching of carboxylic acid at 3400-2500 cm^{-1}, C-H aromatic stretching vibration at 3110-3010 cm^{-1}, C-H stretching vibration of –CH$_2$, CH$_3$ at 3000-2900 cm^{-1} and the C=C ring stretching vibration at 1615-1590 and 1515-1485 cm^{-1}. Strong C=O stretching strong absorption band at 1720-1700 cm^{-1} and other absorption peaks of C-O stretching 1260-1240 and 1170-1100 cm^{-1} were also detected.

The IR absorption pattern for 2,4-dichlorophenoxyacetate displayed the characteristic of common functional group similar to those present in the structure of its corresponding acid. However, the different feature of C=O stretching in ester was shifted to 1760-1735 cm^{-1} compared with these observed in corresponding carboxylic acid (1720-1700 cm^{-1}). For 2,4-dichlorophenoxyacetamide, the C=O stretching vibration at 1700-1660 cm^{-1} and N-H stretching vibration at 3500 and 3200 cm^{-1} were attributed.

N-(2,4-dichlorophenoxyacetyl)-amino acids

The FT-IR spectra of these compounds showed the absorption pattern of common functional group as substituted phenoxyacetic acid. The notable N-H stretching peak at 3,400-2900 cm^{-1} and N-H bending peak at 1530-1500 cm^{-1} were detected.

3.2.2 Nuclear Magnetic Resonance Spectroscopy (NMR)

^{1}H-NMR

Substituted Phenoxyacetic Acids and Their Analogues

$$\overset{2}{O}\overset{1}{CH_2COOH}$$

a
f   b
e   c
d

R
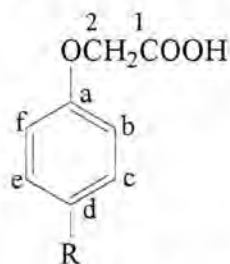
Most of substituted phenoxyacetic acids are solubled in DMSO-d_6. Their ^{1}H-NMR spectra revealed 2H integration of OCH$_2$ as a sharp singlet at 4.05-4.85 ppm depended on the substituent group on a benzene ring. The aromatic protons displayed the complicated signal around 6.70-7.50 ppm and the remaining 1H integration broad singlet signal for a hydroxy proton of carboxylic acid unusually appear in the same position and sometimes could not be detected.

For the main structure of 2,4-dichlorophenoxyacetic acid and their analogues the proton signal of aromatic ring displayed 1H integration of H-c as doublet at 7.25-7.55 ppm (J = 2.00-2.55 Hz), 1H integration of H-e was found as doublet at 7.30-7.40 ppm (J = 7.75-8.90, 2.45-2.60 Hz) and 1H integration of H-f was found as doublet at 6.70-7.05 ppm (J = 8.85-8.95 Hz).

^{13}C-NMR

Substituted Phenoxyacetic Acids and Their Analogues

$$\overset{2}{O}\overset{1}{CH_2COOH}$$

a
f   b
e   c
d

R
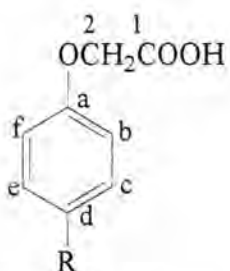
The signal of carbon atom of carboxylic acid was detected around 168.5-172.0 ppm and carbon of OCH$_2$ group appeared at 64.0-65.5 ppm. Another set of signal of aromatic carbons as C-a, C-b, C-c, C-d, C-e and C-f was detected in the range of 150.2-160.0, 112.0-116.1, 125.0-129.3, 125.0-132.0, 127.0-136.1 and 113.5-121.9 ppm, respectively. Their chemical shifts were varied depended on the substituted group on aromatic ring.

3.3 Biological Activity Test

According to the previous mention, although substituted phenoxyacetic acids are well-known herbicides and also utilized as plant growth regulators, the systematic study on their biological activities as herbicides against *Mimosa pigra* Linn. and root growth promotion activity for *Coleus atropurpurreus* Benth. have never been reported.

### 3.3.1 Weed Growth Inhibition against *Mimosa pigra* Linn.

In this study, the selected weed was *Mimosa pigra* Linn. (Giant mimosa) which is undesirable and unwholesome weed distributing in the northern part and spreading to the central part of Thailand. This plant, one of twelve noxious weeds in Thailand, is a resistant weed. It causes a serious problem not only in agriculture and transportation, but also in fish culture. This is because it forms pure communities owing to its allelopathic properties and is slightly toxic to fish. For this reason, it has been tried to get rid of by mechanical, chemical and biological control methods. For chemical method, herbicide is very popular and efficient to use and control the distribution of *M. pigra*. In this research the weed growth inhibition test by chemical mean was chosen. The test substituted phenoxyacetic acids and *N*-(2,4-dichlorophenoxyacetyl)-amino acids were conducted against *M. pigra*. The comparison of their herbicidal activities with commercially available herbicides was also performed.

Although the herbicidal activity of substituted phenoxyacetic acids against various plants have been studied and used in practice, they have never been reported in literature about the relationship between structure and herbicidal activity against *M. pigra*. In deed, there is only a report in Chiang Mai province to test the efficiency inhibition of 2,4-D, silvex and MCPA against *M. pigra*.[25]

In general, growth inhibition bioassay measures the length of root and shoot of treated plant, after planting for 7 days. The length of treated plant is then calculated as percent growth inhibition comparing with controlled plant. The results are normally justified from the length of roots more than that of shoots. This may be relied on the account of the roots that are directly contacted to the tested compound in cellulose, while the shoot growth is contributed from root and attributed to accumulated food from seed. The profound effect of tested compound in the shoot part might therefore be misinterpreted. As a consequence, the percent inhibition of root is thought to be

more important for considering than the percent shoot inhibition. At a concentration of 100 ppm, percent root inhibition would be selected and considered mainly. Due to chemical substances such as common herbicides at low concentration (1 or 10 ppm) usually show low root inhibition and sometimes display plant growth promoting effect. On the contrary, at the highest concentration (1000 ppm) substances perpetually inhibit or desist plant growth at high level showing high percent root inhibition. Thus, the consideration of tested substances at such condition, the difference of inhibition activity would probably vanish. That is why the general interpretations of the results are intensively concentrated at 100 ppm of percent root growth inhibition.

In addition, the interpretations of the results are focused on the tendency and correlation between percent root growth inhibition and concentrations-at 1, 10, 100 and 1000 ppm-of tested compounds at the first view. From Figure 3.1, the graph reveals the tendency of percent inhibition curve of a representative test substance, Compound 1 and 2. The graph illustrates percent root inhibition and concentration correlation as an increasing function in semi-logarithm graph (higher activity of each increasing one unit of logarithm concentration of dose). It could observe that Compound 1 revealed a good tendency of inhibition as good herbicide should behave. In contrast to Compound 1, Compound 2 exhibited not good trend of inhibition curve. This result is not interesting to consider as good herbicide. It should be noted at this point that the good tendency of inhibition curve will represent by symbol of "✓" while "✗" exhibits the uncertain tendency of inhibition.
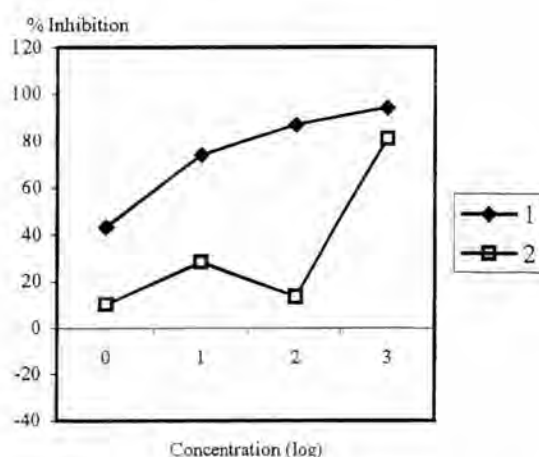
Figure 3.1 Example of results of percent root growth inhibition against *M. pigra* of Compounds 1 and 2

For the last contemplation, the level of percent root growth inhibition is divided into 4 levels as follows:

"-"   no growth inhibition activity (less than 10% inhibition)

"+"   poor growth inhibition activity (10-39% inhibition)

"++"   fair growth inhibition activity (40-69% inhibition)

"+++"   good growth inhibition activity (70-100% inhibition)

Substituted Phenoxyacetic Acids

Thirty-eight phenoxyacetic acids (Compounds **1-38**) and their analogues (Compounds **48-51**) were tested for growth inhibition activity against *M. pigra* Linn. These compounds were found to be active with *M. pigra*. The results of root growth inhibition are tabulated in Table 3.4.
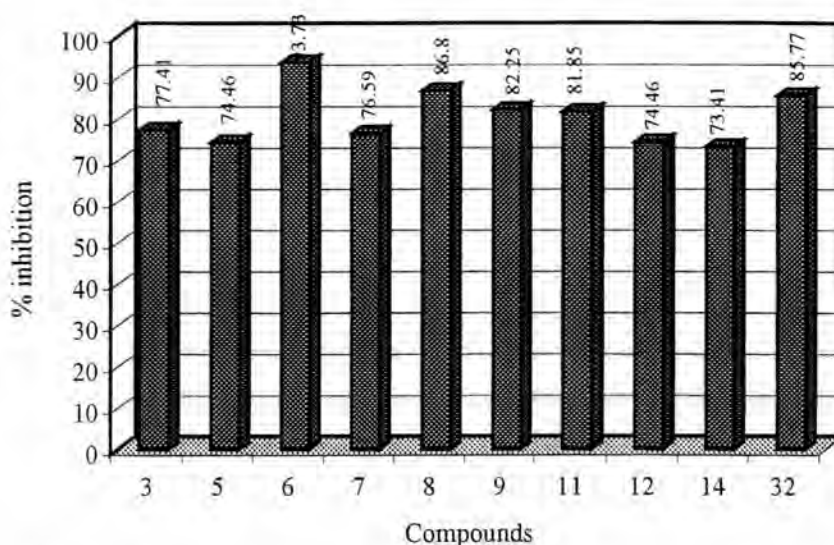
Table 3.4 Percent root growth inhibition at concentration of 100 ppm of some phenoxyacetic acids against *M. pigra*

Compound	% Root Growth Inhibition at 100 ppm	Tendency	Level
1	-46.28	✗	-
2	55.30	✗	++
3	77.41	✓	+++
4	51.04	✓	++
5	74.46	✓	+++
6	93.73	✓	+++
7	76.59	✓	+++
8	86.80	✓	+++
9	82.25	✓	+++
10	33.97	✗	+
11	81.85	✓	+++
12	74.46	✓	+++
13	52.84	✗	++
14	73.41	✓	+++
15	21.28	✗	+

Table 3.4 (cont.)

Compound	% Root Growth Inhibition at 100 ppm	Tendency	Level
16	64.90	✗	++
17	47.61	✓	++
18	39.90	✓	++
19	-60.63	✓	-
20	-68.08	✓	-
21	50.53	✓	++
22	38.46	✓	+
23	-72.01	✗	-
24	13.30	✗	+
25	17.56	✓	+
26	-24.99	✗	-
27	51.92	✗	++
28	17.31	✗	+
29	30.00	✓	+
30	-101.07	✗	-
31	9.62	✗	-
32	85.77	✓	+++
33	29.04	✓	+
34	63.85	✓	++
35	22.66	✗	+
36	32.09	✗	+
37	43.41	✗	++
38	-20.78	✗	-
48	-64.53	✓	-
49	4.20	✗	-
50	-73.18	✗	-
51	54.67	✗	++

From Table 3.4, Compounds **1**, **2**, **10**, **13**, **15-16**, **24**, **26-28**, **31** and **35-38** did not show good tendency in the correlation between the concentration and percent growth inhibition. Thus, these compounds should be excluded from the first investigation. On the other hand, even though the rest compounds revealed good tendency, Compounds **19**, **20**, **26**, **31** and **38** did not exhibit the antigrowth activity. Five Compounds-**4**, **17**, **18**, **21** and **34**-exhibited moderate activity. Very interesting, Compounds **3**, **5-9**, **11-12**, **14** and **32** exhibited the highest root growth inhibition activity above 70% inhibition against *M. pigra*. The activity of these substances is represented in Figure 3.2.

Figure 3.2 Percent root growth inhibition of active compounds with high level of activity against *M. pigra*

Based on the substituents on a benzene ring of phenoxyacetic acids, the relationship between their structures and the growth inhibition against *M. pigra* could be classified into diverse types and positions of substituents as follows:
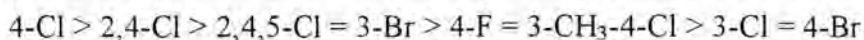
1. Halophenoxyacetic Acids (Compounds **1-13**)

Considering various types of substituents, it was observed that most compounds in the series displayed the highest inhibition activity such as Compound **3**, **5-9** and **11-12**, in a range of 75-94% growth inhibition. Especially, Compound **6** 4-chlorophenoxyacetic acid exhibited the highest percent inhibition of 94%. Among halogen substitutents studied, fluoro, bromo and chloro showed the impressive activity; however, the activity was also found depending on a position and a number

of substituents. Considering the position of fluoro, chloro and bromo at *meta* and *para* positions, it was observed that chloro group at *para* position displayed percent growth inhibition higher than fluoro and bromo which could be arranged as: 4-Cl > 4-F = 4-Br. The bromo group at *meta* position revealed slightly more active than chloro and fluoro groups, respectively, and might be represented as: 3-Br > 3-Cl >> 3-F. This means that both chloro and bromo groups could be located at either *meta* or *para* position, both of them showing high inhibitory activity while a fluoro group should be only at *para* position.

For the comparison of position of chloro group, monosubstituent at *para* position exhibited higher activity than those at *meta* and *ortho*, respectively. This was probably due to their inductive effect (4-Cl > 3-Cl > 2-Cl). In the case of disubstitution, the good inhibition activity was observed when the main structure contained a chlorine atom at *ortho* and *para* positions similar to those of 2,4-D (**8**) and 2,4,5-T (**9**). It should also be noted that pentachloro substituent in Compound **10** rendered the activity. If there was a monochlorine atom at *para* position and a methyl group at *meta* position, the inhibition activity was slightly decreased compared with 4-chlorophenoxyacetic acid (**6**). From all of the results gained, it could be concluded that halogen substituents had a markedly effect on inhibition activity and might be arranged in order from the highest to the lowest activity as:

$$4\text{-Cl} > 2,4\text{-Cl} > 2,4,5\text{-Cl} = 3\text{-Br} > 4\text{-F} = 3\text{-CH}_3\text{-}4\text{-Cl} > 3\text{-Cl} = 4\text{-Br}$$

2. Alkylphenoxyacetic Acids (Compounds **14-30**)

Among a variety of alkyl substituents studied, alkylphenoxyacetic acids did not display the interesting activity. The activity observed was ranged from medium to low inhibition, except for Compound **14** (2-Me) that exhibited high root growth inhibition of 73%. For a variation of substituents at both 2- and 4-positions as methyl, ethyl, *iso*-propyl and benzyl groups, it was observed that a methyl group which was the smallest alkyl group showed better activity than others. In the case of dimethyl substituents, it was also exhibited low to moderate inhibition activity.

3. Other Substituted Phenoxyacetic Acids (Compounds **31-38**)

The examination on the effect of methoxy groups was performed. Among three compounds (**32-34**) studied, only 4-OMe substituent showed high activity more

than 70%, while acetyl group at 2-position and *N*-acetyl amino at 4-position had no inhibition activity.

Further studies on the effect of mononitro substituent as a strong electron withdrawing group, it was found that the activities derived from these nitro derivatives were low. Corresponding to a nitro group, the derived amino group either at 2- or 4-positions had no inhibition activity. This can imply that nitro and amino substituents decreased inhibition activity. The inhibition activity of Compounds **50** and **51** containing *N*-phthalimide substituent was also not satisfied, displaying no activity and 54% inhibition, respectively.

2,4-Dichlorophenoxyalkanoic Acids

Similar to other substituents of phenoxyacetic acids, Compounds **39-41** were studied for observing the effect of substituents on carboxylic acid side chain. The data derived from these compounds are tabulated in Table 3.5.

Table 3.5 Comparative results of percent growth inhibition of 2,4-dichlorophenoxy-acetic acids at 100 ppm

Compound	% Root Growth Inhibition at 100 ppm	Tendency	Level
8	86.80	✓	+++
39	16.10	✗	+
40	48.37	✓	++
41	-101.07	✗	-

This data manifestly showed that the degree of inhibition was decreased when one additional carbon or a chlorine atom was present. For instance, Compounds **39** and **41** displayed low and no activity, respectively. For Compound **40**, the compound containing another methyl group at α-carbon of carboxylic acid side chain of 2,4-D, the inhibition activity was dropped about 1 fold to moderate activity employing 2,4-D (**8**) as reference. Thus, the activity may be arranged as follows:

$$OCH_2COOH > OCH(CH_3)COOH > OCH_2CH_2COOH >> OCHClCOOH$$
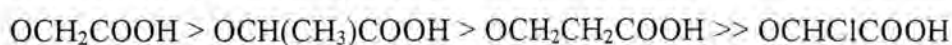
2,4-Dichlorophenoxyacetyl Derivatives

Various derivatives of 2,4-D were also studied. These groups exhibited different inhibitory effect on the growth of *M. pigra* depending on the difference in types and structures of seventeen 2,4-dichlorophenoxyacetyl derivatives. The results are tabulated in Table 3.6.

Table 3.6 Comparative results of percent growth inhibition of 2,4-dichlorophenoxy-acetyl derivatives against *M. pigra* at 100 ppm

Compound	% Root Growth Inhibition at 100 ppm	Tendency	Level
42	46.41	✓	++
43	35.69	✗	+
44	28.55	✓	+
45	91.09	✓	+++
46	82.18	✓	+++
47	-15.17	✗	-
63	88.67	✓	+++
64	87.34	✓	+++
65	78.67	✓	+++
66	88.00	✓	+++
67	63.14	✓	++
68	15.75	✗	-
69	31.54	✓	+

From the results of ester derivatives of 2,4-dichlorophenoxyacetic acid in Table 3.6, their inhibition activity was not very impressive. That was because only Compound **42** showed a moderate activity, whereas Compounds **45** and **46** which contained unsubstituted and *N*-phenyl substituted amides revealed very high inhibition activity of 91 and 82%, respectively. The change of the *N*-phenyl substituent to *N*-cyclohexyl group, the inhibitory activity was obviously dropped to no activity. The activity might be arranged as follows:

$CONH_2 > COOH > CONHC_6H_5 > COOCH_3 > COOC_2H_5 > COOC_6H_5 >$
$CONHC_6H_{10}$
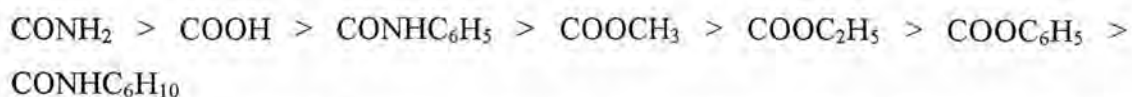
The synthesized phenoxyacetate salts were indeed studied. There are divided into sodium salt (Compounds **63-66**) and calcium salt (Compounds **67-69**). All sodium salts of substituted phenoxyacetates exhibited satisfactorily activity of more than 78% inhibition. Only one calcium salt (Compound **66**) possessed the moderate activity of 63% inhibition. This clearly showed that the inhibitory activity of sodium salt was more intense than that of calcium salt. Because of the solubility in water, the sodium salts could then be applied for a practical use very easily.

2,4-Dichlorophenoxyacetyl Derivatives of Amino Acid

Further studies on analogues of 2,4-dichlorophenoxyacetic acid, N-(2,4-dichlorophenoxyacetyl)-amino acids (Compounds **52-55**) and their methyl esters (Compounds **56-62**) were carried out. These synthesized compounds were tested for their growth inhibition activity against *M. pigra*. The ability of inhibition depends on the difference in structures of these eleven synthetic compounds. The results are presented as shown in Figure 3.3.
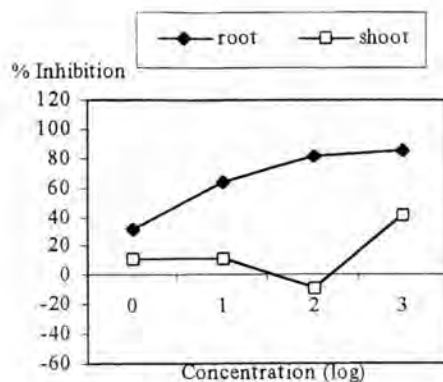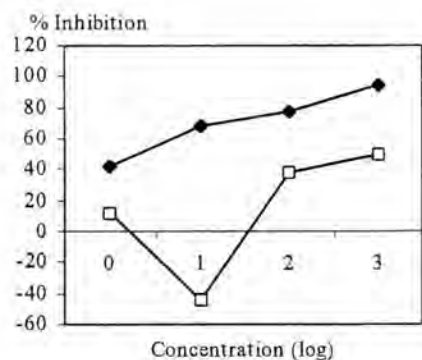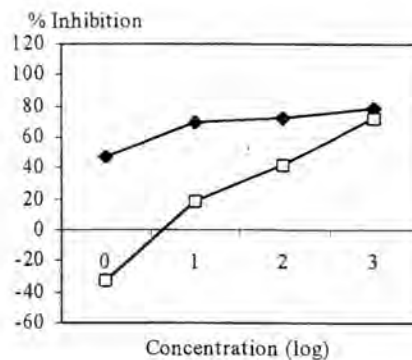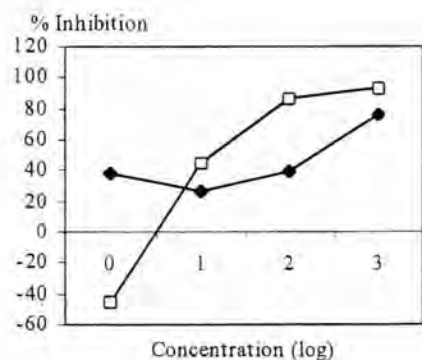
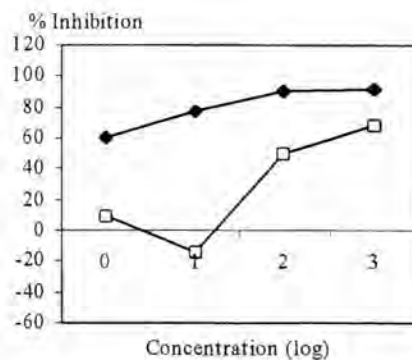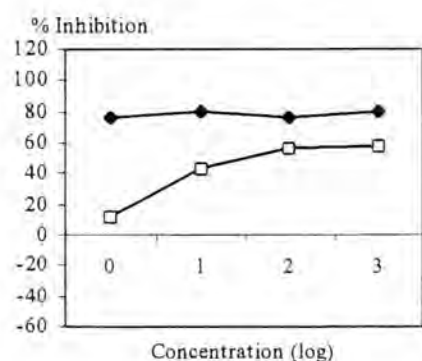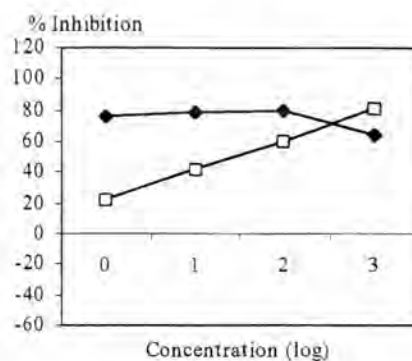**Figure 3.3** Percent root and shoot inhibition of 2,4-dichorophenoxyacetyl derivatives of amino acid against *M. pigra*

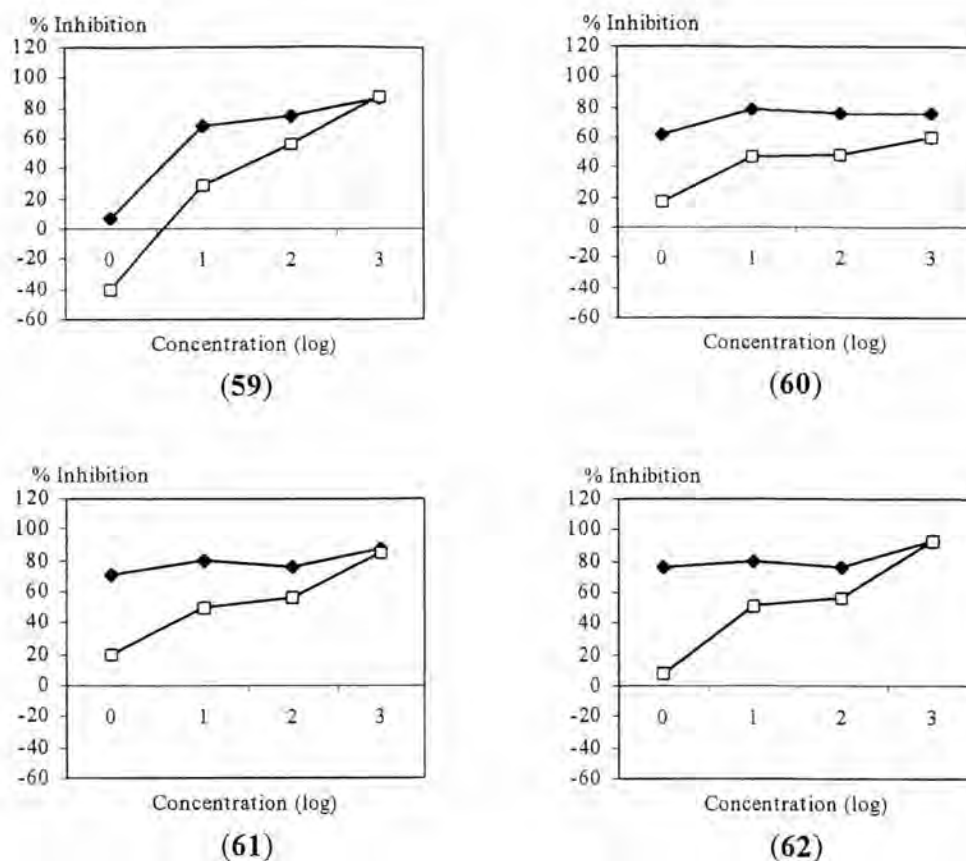(59)

(60)

(61)


(62)

Figure 3.3 (cont.)

From the results above, a variation of amino acid of 2,4-dichlorophenoxy-acetyl compound exhibited interesting inhibition activity. Good tendency of percent growth inhibition was found in many compounds including Compounds **52**, **53**, **54**, **56**, **59** and **62**. They showed highest percent growth inhibition in a range of 72-90% inhibition. The rest four compounds also exhibited percent growth inhibition more than 70% at concentration of 100 ppm; nonetheless, their tendency of inhibition did not show a good correlation curve. Therefore, these compounds were considered to reveal no activity acccording to the above mentioned general consideration.

Taking into an account of consideration on the variation of amino acids conjugated with 2,4-D, all isomers of alanine: DL-, L- and D-isomers, were firstly focussed. They exhibited high activity. In contary to alanine methyl ester, only D-isomer showed good tendency and high percent growth inhibition. Both of L-phenylalanine and L-phenylalanine methyl ester did not exhibit good tendency activity although they displayed high percent inhibition.

In summary, it can be concluded that all sixty-nine compounds were synthesized and tested for growth inhibition against *M. pigra* at concentrations of 1,

10, 100 and 1000 ppm. At concentration of 100 ppm, many impressive compounds, not only Compounds **8, 9, 11, 32, 46, 52, 53, 56, 62, 63** and **66** showed activity more than 80% inhibition, but also Compounds **6** (4-chlorophenoxyacetic acid) and **45** (2,4-dichlorophenoxyacetamide) revealed even better activity than 90% inhibition.

Furthermore, considering the inhibition of four commercially available herbicides **H1-H4** (the results as presented in Table C (see Appendices B), **H1** and **H3** displayed good tendency and high percent growth inhibition activity of 80 and 85% (at 100 ppm), respectively. The comparison of the ability of inhibition of all synthesized compounds which exhibited high activity, **H1** and **H3** is shown in Figure 3.4. It was observed that the synthesized compounds, Compounds **6, 32, 45, 56, 62, 63** and **66** could be utilized as an efficient herbicide against *M. pigra*.
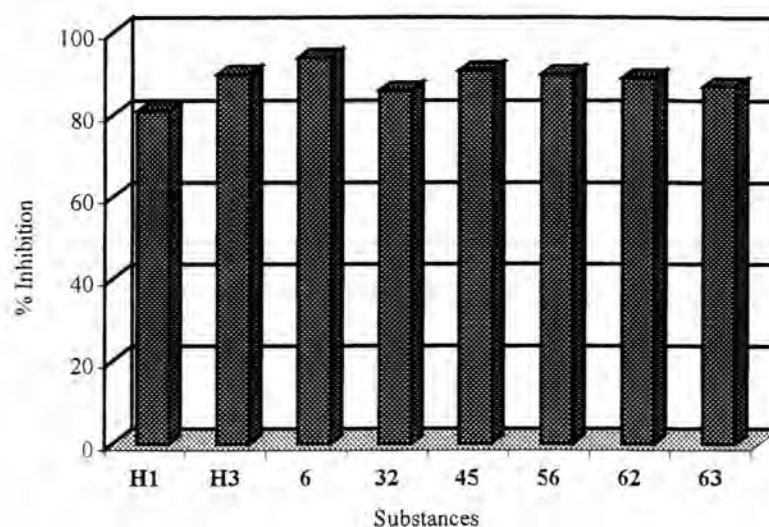
Figure 3.4 The comparison of synthesized compounds and commercially available herbicides

### 3.3.2 Root Growth Promotion Test of *Coleus atropurpurreus* Benth.

Another goal of this research is to find out the relationship between structures of substituted phenoxyacetic acids, *N*-(2,4-dichlorophenoxy- acetyl)-amino acids and their related compounds and root growth stimulation of *Coleus atropupurreus* Benth. (Ruesee phasom) which is a herbaceous plant and favorite utility as mass planting for cover the ground. Moreover, the results derived from these compounds were compared with commercially available substances such as α–naphthalene acetic acid (NAA).

At the first view, the appropriate concentration is primarily screened by using various concentrations of 10^{-10}, 10^{-8}, 10^{-6}, 10^{-4}, 10^{-2}, 1 and 10 ppm. The most appropriate concentration was considered from the number of root and the average fresh weight corresponding to the average dry weight of *C. atropurpurreus*. 2,4-D was selected as a standard compound for this purpose and distilled water was used as control. The results are shown in Tables 3.7 and 3.8 and Figures 3.5 and 3.6.

Table 3.7 Average number of root of *C. atropurpurreus* in a range concentration of 2,4-D 10^{-10}-10 ppm
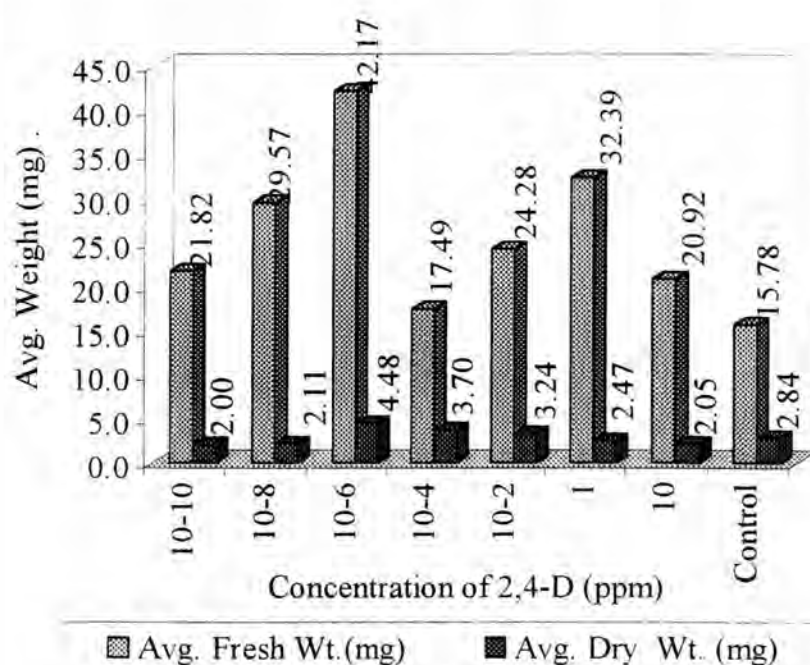
	Control	Concentration of 2,4-D (ppm)						
		10^{-10}	10^{-8}	10^{-6}	10^{-4}	10^{-2}	1	10
Average Root Number	9.1	6.9	7.6	**17.0**	15.2	13.6	12.3	1.0
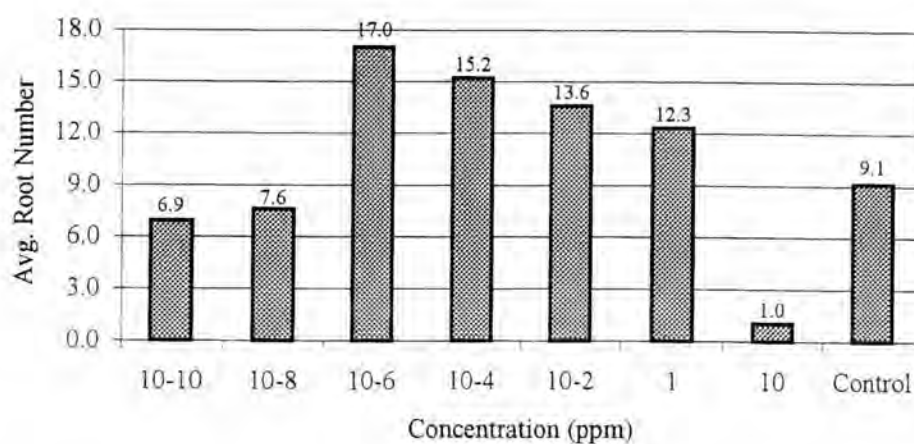
Figure 3.5 The average root number of *C. atropurpurreus* using 2,4-D in a range concentration of 10^{-10}-10 ppm

Table 3.8 Comparison of average fresh and dry weight of *C. atropurpurreus* in a range concentration of 2,4-D at 10^{-10}-10 ppm

| Average Weight (mg)	Control	Concentration of 2,4-D (ppm)						
		10^{-10}	10^{-8}	10^{-6}	10^{-4}	10^{-2}	1	10
Fresh Weight	15.78	21.82	29.57	**42.17**	17.49	24.28	32.39	20.92
Dry Weight	2.84	2.00	2.11	**4.48**	3.70	3.24	2.47	2.05

Figure 3.6 Comparison of average fresh and dry weight of *C. atropurpurreus* root in a range concentration of 2,4-D at 10^{-10}-10 ppm

Based upon the results of varying concentration of 2,4-D, it was observed that there was a correlation of average root number of *C. atropurpurreus* and the concentration of 2,4-D. The relationship was found to be in the shape of overturned bell possessing the maximum of average root number (17 roots) at concentration of 10^{-6} ppm. The average dry weight (mg) was also found to correspond to the concentration of 2,4-D with the same overturned bell-shape exhibiting the highest value of dry weight (4.48 mg) at the same concentration. Since this identical maximum value of the average number of root and dry weight at the concentration of

10^{-6} ppm was paralleled, in further study only the average dry weight would be considered and compared.

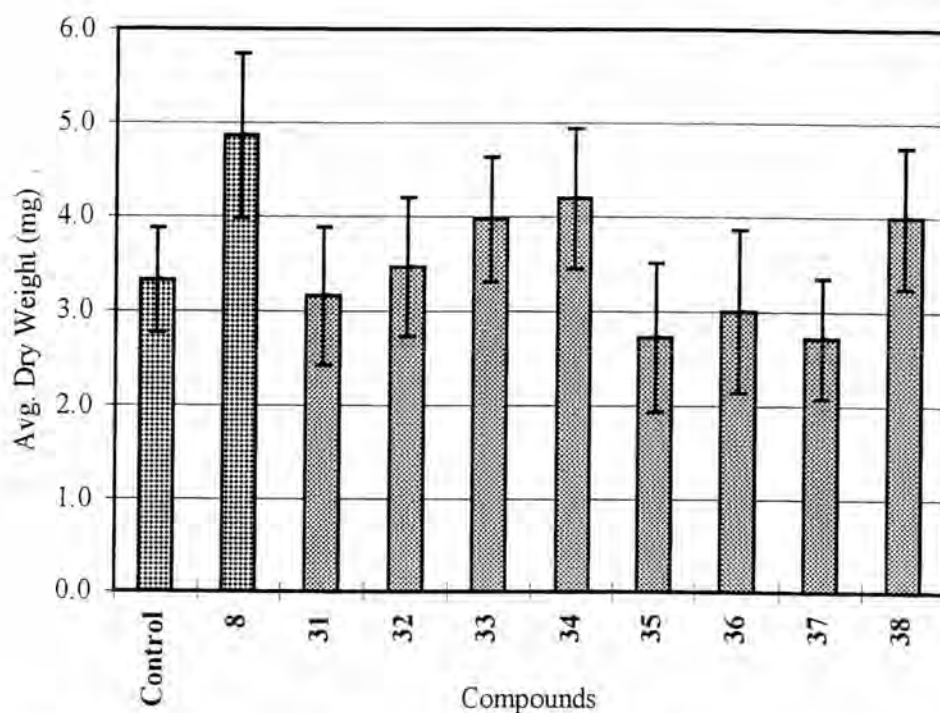### Substituted Phenoxyacetic Acids

After finding out the appropriate concentration at 10^{-6} ppm, the structure-activity relationship of substituted phenoxyacetic acids is worth considering to investigate. Thirty-eight synthesized substituted phenoxyacetic acids were subjected to test for root growth promoting activity against *C. atropurpurreus* Benth. The comparative data is presented as shown in Figures 3.7, 3.8 and 3.9.
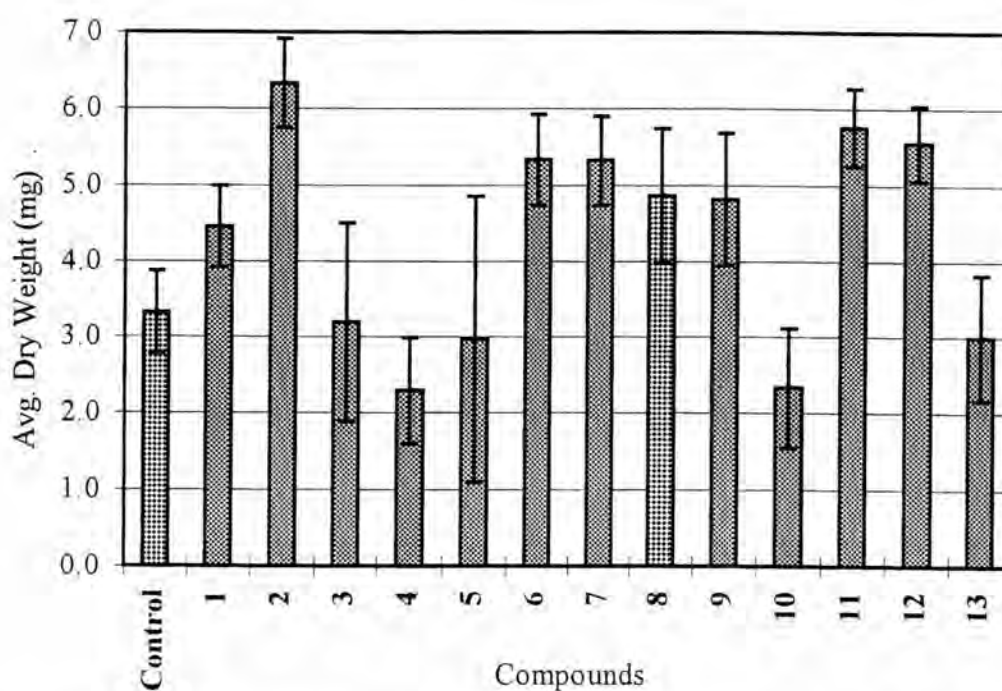
Figure 3.7 Root growth promotion of halophenoxyacetic acids at 10^{-6} ppm of *C. atropurpurreus* (The results are the means of fifteen replicates and bars represent ± SD.)
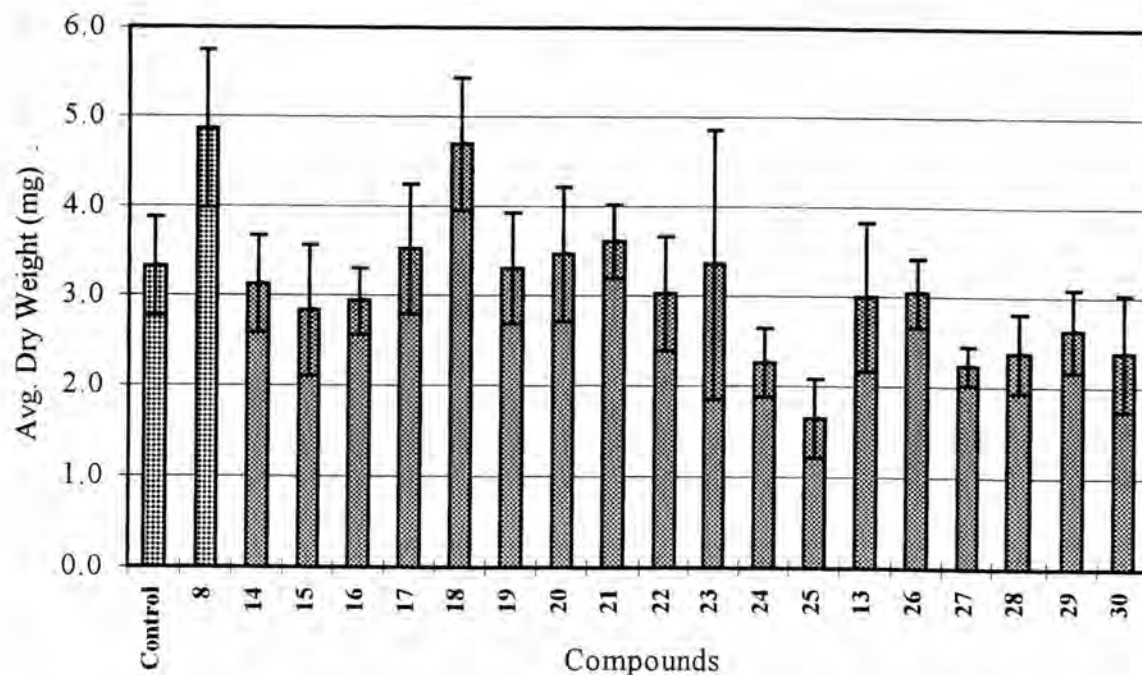
Figure 3.8 Root growth promotion of alkyl phenoxyacetic acids at 10^{-6} ppm of *C. atropurpurreus* (The results are the means of fifteen replications and bars represent $\pm$ SD.)

Figure **3.9** Root growth promotion of substituted phenoxyacetic acids at concentration of 10^{-6} ppm of *C. atropurpurreus* (The results are the means of fifteen replications and bars represent $\pm$ SD.)

From the data of this assay, it might be divided into 2 parts to consider the activity of each synthesized compound: a) a major contemplation focused on the means of fifteen replicates of dry weight data to compare the root growth promoting with other substituted phenoxyacetic acids and some commercially available substances and b) a minor consideration looked for variability of data using standard deviation value (SD), representing by the bar at the top of each data in the graph. If the bar of variation is generally narrow, it means the distribution of data is not much. On the contrary, the broad variation means a lot of distribution of that data.

In general, the root growth promotion activity may be graded into three levels using control and 2,4-D as references as follows:

Low activity: the average dry weight of root less than control.

Medium activity: the average dry weight of root in a range of control and 2,4-D.

High activity: the average dry weight of root more than 2,4-D.

For simplicity to comprehend, the relationship between structures of substituted phenoxyacetic acids, at concentration 10^{-6} ppm, and root growth promotion activity could be classified into various types of substituent groups on a benzene ring as follows:

1) Halophenoxyacetic acids

The variation of types and positions of thirteen halophenoxyacetic acids (Compounds **1-13**) was investigated as shown in Figure 3.7. These halogen substituents revealed the interesting root growth promotion activity against *C. atropurpurreus*. Their activities were found to be higher than other substituents studied. In the case of using control (distilled water) as a reference, Compounds **1**, **2**, **6**, **7**, **8**, **9**, **11** and **12** showed better activity than control. Comparing with Compound **8**, 2,4-D-a well-known synthetic auxin, as a reference compound, the data showed that Compounds **2**, **6**, **7**, **11** and **12** provided higher dry weight of root and could be classified into high activity group. Particularly Compound **2** (3-fluorophenoxyacetic acid) showed the highest activity. Focussing on the effect of the position of fluoro, chloro and bromo at 3- and 4-positions, it was found that at 3-position a fluoro group showed more activity than bromo and chloro groups, respectively (3-F > 3-Br >> 3-Cl). At 4-position, fluoro revealed less activity than bromo and chloro groups (4-F <

4-Br = 4-Cl). This implied that a flouro group should be at *meta* position which was perhaps due to its highest electronegativity. Unlike fluorine substituent, *para* chloro exhibited activity better than other positions whereas both *meta* and *para* bromo groups showed equal activity.

The activity of Compound **1**, unsubstituted phenoxyacetic acid, was in a range of control and 2,4-D. Its activity was therefore not very much attractive. Comparing with a chloro group at 2- and 4-positions, 2,4,5-T (**9**), a well-known herbicide, it showed a promoting activity nearly to 2,4-D while pentachloro substitutent (Compound **10**) gave the worst activity. The study on the variety of chloro position discovered that *para* position revealed more activity than that at *meta* and *ortho*, respectively. This may be due to their inductive effect. Thus, the promoting activity could be summarized as follows:

$$3\text{-}F > 3\text{-}Br > 4\text{-}Br > 4\text{-}Cl = 3\text{-}CH_3\text{-}4\text{-}Cl > 2,4\text{-}Cl = 2,4,5\text{-}Cl > H$$

2) Alkylphenoxyacetic acids

Considering of eighteen alkylphenoxyacetic acids (Compounds **14-30**), in Fig 3.8, the activity was found to be unattractive. Almost compounds exhibited lower activity than both control and 2,4-D (**8**), except for Compound **18** (2,4-dimethylphenoxyacetic acid) which displayed the activity nearly to 2,4-D. The effect of alkyl substitutent groups either small or bulky groups exhibited less promotion activity than a reference compound.

### 3) Oxygen-containing phenoxyacetic acids

Three methoxyphenoxyacetic acids (Compounds **32-34**) displayed moderate promotion activity. From the results, it showed that a methoxy group at *para* position, Compound **32** revealed less activity than disubstituents of Compounds **33** and **34**. Contemplation on the position of dimethoxy groups, the 3,5-position displayed more activity than the 2,6-position owing to their inductive effect of the donating group. Another studied group was an acetyl group at *ortho* position, Compound **31**, this compound showed activity nearly to control.

4) Nitro-containing phenoxyacetic acids

Further consideration was focused on the effect of electron withdrawing groups as nitro and *N*-acetylamino group (Compounds **35-38**). Three mononitrophenoxyacetic acids were synthesized and the effect of their positions was evaluated. The substituent at *meta* position exhibited more activity than other positions (position: *meta* > *ortho* = *para*). However, these nitro compounds exhibited low promotion activity comparing with control. For *N*-acetylamino group Compound **38** revealed medium activity in a range of control and 2,4-D.

From the study of the effect of substitutent groups, it could be concluded that the halogen was the best substituent group. Their activities however were depended on types and position on a phenyl ring. *N*-Acetylamino and nitro groups revealed medium and low promotion activity, respectively. In the case of electron donating group, methoxy group showed medium activity and almost alkyl groups exhibited low or same activity as control. To sum up main substituent groups that affected on promoting activity, their activity could be arranged as shown in Figure 3.10.
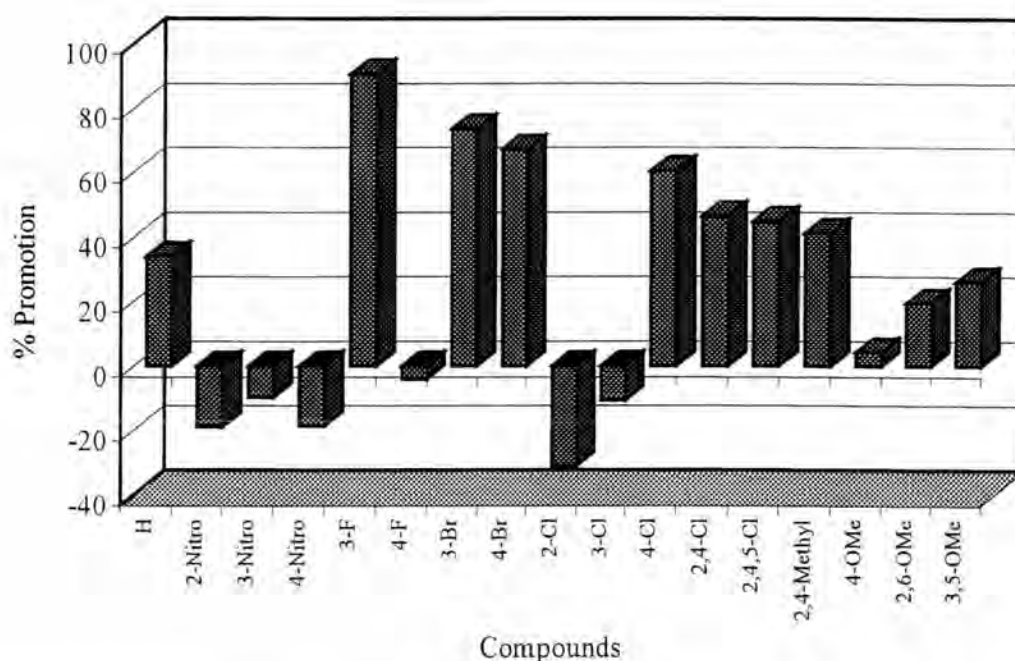
Figure 3.10 Comparison the effect of variety substituent groups of phenoxyacetic acids on root growth of *C. atropurpureus*

2,4-Dichlorophenoxyalkanoic Acids

Like other phenoxyacetic acids, 2,4-dichlorophenoxyalkanoic acids with varying side chain of carboxylic acid were also tested. In Figure 3.11, it was found that Compound **39** exhibited higher activity than Compounds **40** and **41**, respectively. This may imply that only one carbon atom in the side chain of carboxylic acid had a great potent. Compound **39** containing an additional carbon atom revealed less activity. The decrease in activity was also observed in Compound **40** which bearing one methyl group at α-carbon. The activity was dropped approximate 2 folds comparing with 2,4-D. The addition one chlorine atom instead of methyl group in Compound **41** rendered the activity about 3 folds compared with the control (distilled water). This implies that the side chain should be definitely only two carbon atom. The activity ratio significantly decreased and was arranged as follows:

$$OCH_2COOH > OCH_2CH_2COOH > OCH(CH_3)COOH > OCHClCOOH$$
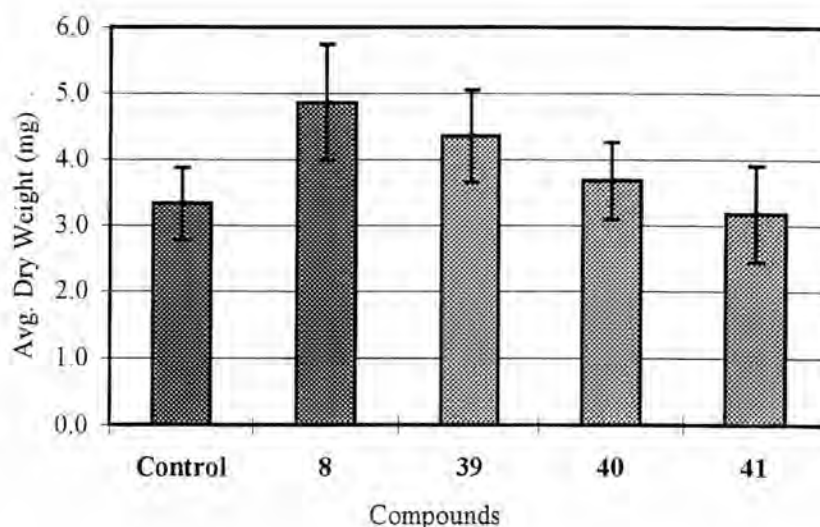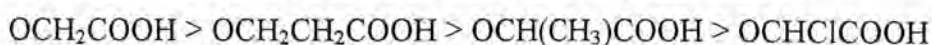
Figure 3.11 Root growth promotion of 2,4-dichlorophenoxyalkanoic acids at concentration of 10^{-6} ppm of *C. atropurpureus*

2,4-Dichlorophenoxyacetyl Derivatives

For ester derivatives of 2,4-dichlorophenoxyacetic acid, the results in Figure 3.12 showed that Compound **43** exhibited weak activity as equal to control. Compound **44** displayed medium activity with *C. atropurpureus* whereas Compound

45 showed high potential promotion activity as much as 2,4-D (**8**). Their activity could be arranged as follows:

$$COOC_6H_5 (44) > COOH (8) > COOCH_3 (42) > COOC_2H_5 (43)$$
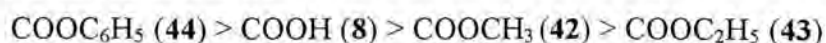
Among a variation of substituent group of amide, the results of three amides **45**, **46** and **47** exhibited high growth promotion activity of 37%, 72% and 74%, respectively, compared with control. The activity could also be illustrated as follows:

$$CONHC_6H_{10} (47) = CONHC_6H_5 (46) > CONH_2 (45) = COOH (8)$$
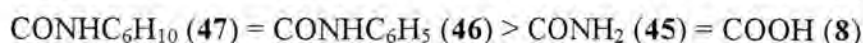
Further studies on sodium salts of 2,4-dichlorophenoxyacetic acid were observed comparing the activity with control and 2,4-D. Although Compound **66** was displayed lower activity than control about 1 fold or 39% promotion of root growth, Compounds **63**, **64** and **65** were found to be of the tendency of activity close to their parent phenoxyacetic acids. The activity of these compounds could be arranged as:

$$4\text{-Cl} > 2,4\text{-diCl} > 4\text{-OMe}$$
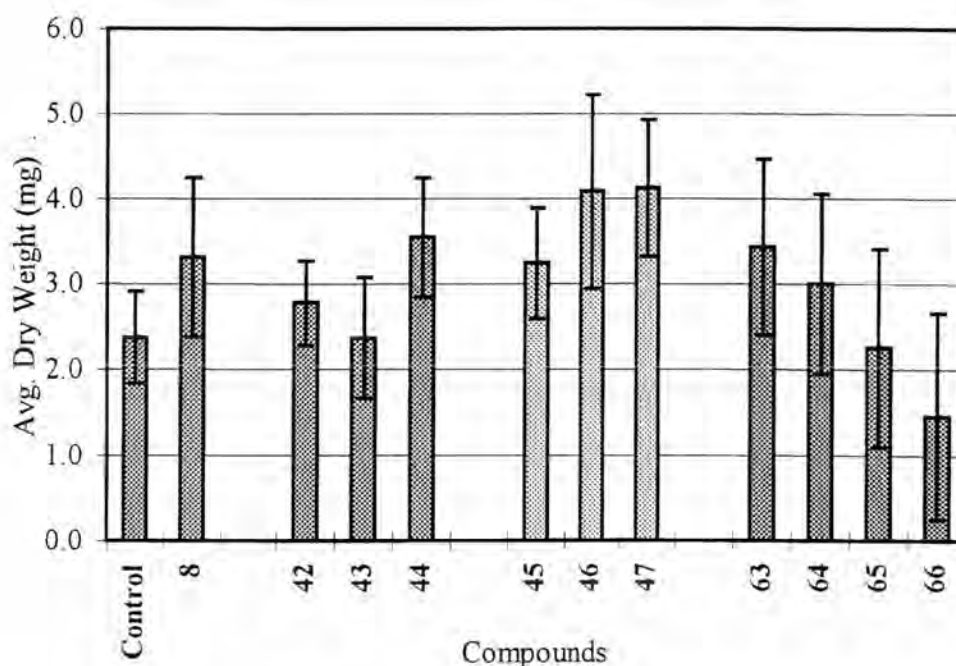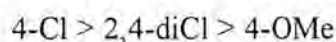
Figure 3.12 Comparison of growth promotion of 2,4-dichlorophenoxyacetic acid derivatives at 10^{-6} ppm of *C. atropurpureus*

2,4-Dichlorophenoxyacetyl Derivatives of Amino Acids

For analogues of 2,4-dichlorophenoxyacetic acids, eleven compounds in a class of *N*-(2,4-dichlorophenoxyacetyl)-amino acids (Compounds **52-55**) and their methyl esters (Compounds **56-62**) were synthesized. Both Compounds **52** and **56** showed the highest promotion activity and had percent promotion of 79% and 73%, respectively. Compounds **53, 55, 59, 60** and **61** displayed moderated activity whereas Compounds **54, 57, 58** and **62** had no promotion activity comparing with control.

Focussing on the effect of amino acids conjugated with 2,4-D, alanine were firstly discussed on their activity. It was found that DL-isomer (Compound **52**) showed more activity than L- and D-isomer (Compounds **53** and **54**, respectively), while D-isomer was less active than control. In contrast to alanine methyl ester conjugated with 2,4-D, D-isomers (Compound **59**) showed higher activity than DL- and L-isomers, which had no promotion activity, and had percent promotion nearly to 2,4-D. For L-phenylalanine conjugated with 2,4-D, it displayed the moderate activity either in amino acid form or amino acid methyl ester form. The results are shown in Figure 3.13.
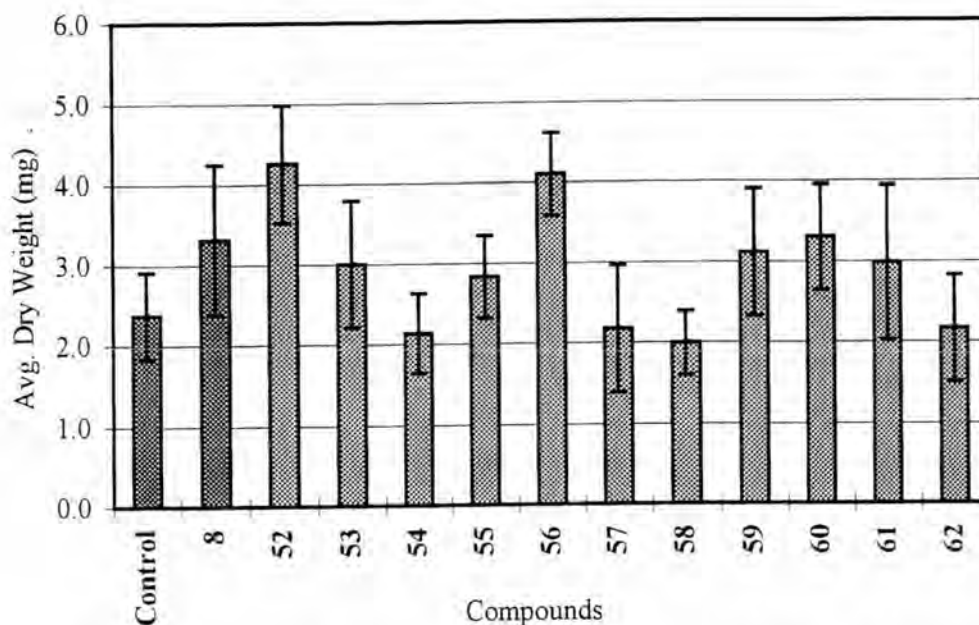
Figure 3.13 Comparison of root growth promotion of *N*-(2,4-dichlorophenoxy-acetyl)-amino acids and their methyl esters at 10^{-6} ppm of *C. atropurpureus*

As a conclusion, all sixty-two synthesized compounds were tested for root growth promotion of *C. atropurpureus* at concentration of 10^{-6} ppm. Only eleven compounds (Compounds **2**, **6**, **7**, **11**, **12**, **44**, **46**, **47**, **52**, **56** and **63**) revealed high activity and gave percent growth promotion more than 2,4-D, especially Compounds **2**, **46**, **47**, **52** and **56** revealing higher percent growth promotion than 70%.

In addition, considering the results of a commercially available root promoting substance (**C1**), as 50% of exotic brand, and NAA (**C2**), the results displayed moderate growth promotion activity of 21.5 and 35.4%, respectively. The comparison of these substances and synthesized compounds (**2**, **46**, **47**, **52** and **56**) is shown in Figure 3.14. It was found that those synthetic compounds possessing better activity than commercial substance. Thus, they can be applied to use as commercial substance to promote root growth.
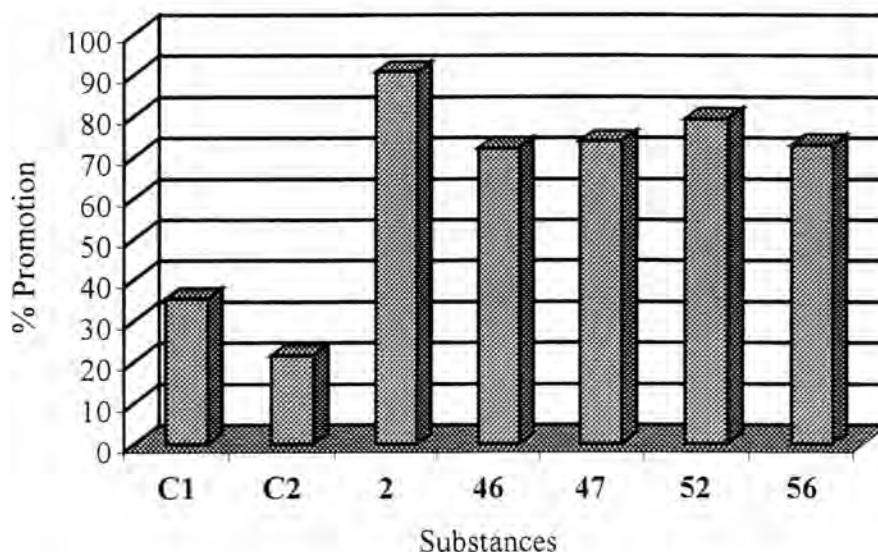
Figure 3.14 Comparison of percent root growth promotion of some commercial substances and potent phenoxyacetic acid derivatives of *C. atropurpureus*

To sum up, for the structure-activity relationship (SAR) the active structure of substitutent phenoxyacetic acids should be of one carbon atom at α–position of carboxylic acid as acetic acid. The substitutent group on a benzene ring should be a halogen group. The compounds showed high activity for both plants. An amide derivative of 2,4-D also exhibited good activity depended on their structures with each plant. Focussing on amino acid conjugated with 2,4-D, glycine (Compound **56**)

revealed good activity with both plants. Glycine derivatives (Compound **56**) could be developed to use as commercial substance to inhibit growth of *M. pigra* and promote root growth of *C. atropurpurreus.*