CHAPTER I
INTRODUCTION

Thailand has been dealing critical economic problems since 1997. Nevertheless, the serious crisis could be alleviated by using self-dependent strategy such as improvement of crop production as rice, corn and sugar-cane. The utilization of chemicals to increase the yield of agricultural products is of utmost importance to support the economy of Thailand. As a result, the need for pesticidal chemical usage is increasing rapidly. The worldwide amount expense on pesticides has increased from 580 million dollars (in 1960) to 26.4 billion dollars (in 1990). The most important section is herbicide. In 1979, the expenditure over 10 billion dollars was distributed for pesticidal usage, 40 percent of which was on herbicides, 36 percent on insecticides, 19 percent on fungicides, and the remaining 5 percent on others, and an increasing usage rate was estimated at 3 to 5 percents annually.[1] Table 1.1 shows a total amount and value of imported pesticides for Thailand. It was found that a high tendency has been observed since 1986. Although the economy of Thailand has changed very rapidly, the value of imported pesticides, especially herbicides and insecticides, has thoroughly increased.[2]

Table 1.1 Pesticides used in 1986-1989.

Type	1986		1987		1988		1989	
	Amount	Value	Amount	Value	Amount	Value	Amount	Value
Insecticide	6,206	778	6,673	765	8,034	1,247	9,737	1,336
Fungicide	3,669	214	6,524	288	6,382	350	6,025	366
Herbicide	4,081	388	5,864	570	8,273	2,419	10,634	1,155
Total	13,956	1,380	19,061	1,623	22,689	4,016	26,396	2,857

Note : the amount in tons and the import value in million baht

Pesticides are crop protection products including all chemicals used for the control of arthropods, microorganisms, and weeds injurious to economic or crop plants. They serve to maintain crops in a healthy condition throughout their growth, as well as to reduce agricultural labor requirement and by enabling crops to reach their full yield potential, to safeguard and maintain the food supply of human beings. They can be classified as insecticides, fungicides, herbicides and plant growth regulators.

1.1 Herbicides, Plant Growth Regulators and Their Significance

1.1.1 Herbicides

According to a general definition, weeds are plants that grow where men do not wish them to be. For agriculture and horticulture, weeds are any plants other than the specific crop being grown. On railway tracks, industrial sites, airport, along the highway, and the like, the entire vegetation can be regarded as weeds. Weeds are divided into dicotyledonous plants, or broadleaf weeds, and monocotyledonous plants, or grass weeds.

Weeds are not a contemporary problem. The idea of controlling weeds with chemicals is not new. For more than a century herbicides have been employed in total weed control. Before the introduction of chemical weed killing, four measures were adopted to limit the spread of weeds. These were manual weeding, crop rotation, ploughing and various methods of preventing weed seeds from dispersing through crop seeding. These methods have a fundamental weakness, they are aids to control but cannot prevent weeds from growing along with the crop. The cost of weeding increased greatly as the cost of labor increased and selective weed control by hand today would be uneconomically feasible. Thus, the reasons for the increased use of chemical agents are manifold. Herbicides could solve many problems. However, there are needs for the development of selective herbicides that kill weeds but do not harm crop plants. The use of herbicide rotations or as mixtures in order to broaden the spectrum of weeds controlled is another alternative in utilizing chemical control method.

In the following classification of herbicides, chemicals are classified broadly into inorganics and organics, historically the inorganic chemicals are the first to be regarded.

a) Inorganic Herbicides

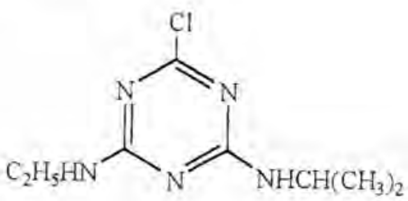
The development of weed control by chemical means is closely associated with the predominant use of inorganic chemicals that is iron (III) sulfate, sulfuric acid, and copper (II) sulfate in the control of undesired plant growth. With few exceptions, these acids and salts, produced at a very low cost, served as nonselective herbicides.

Although the inorganics are still used as total herbicides, they are steadily losing ground to the organics. Apart from the insufficient selectivity for use in crop stands and the necessarily high application rates, chemical and toxicological properties of many inorganics also hinder their wide application in modern weed control.

b) Organic Herbicides

Organic chemicals are sub-divided into families of nitrophenols and anilines, phenoxyalkane carboxylic acids (hormone-type herbicides), benzoic and phenylacetic acids, halo-aliphatic acids, amides, nitriles, aryl carbamates, substituted ureas, thiocarbamates and dithiocarbamates, bipyridylium quaternary ammonium salts, pyridines, pyridazines, pyrimidines, triazines, miscellaneous heterocyclic compounds, thiocarbonates, glycine derivatives, organo-arsenic compounds, and oils.[3]

Compounds possessing herbicidal properties are found in numerous classes. The most important commercial organic herbicides are limited to a few product groups such as 1,3,5-triazines, carbamates, arylureas, phenoxyalkane carboxylic acids, and benzoic acid derivatives. Examples of well-known and commonly used herbicides are atrazine **(I)**, paraquat dichloride **(II)**, diuron **(III)**, 2,4-dichlorophenoxyacetic acid **(IV)**, and dicamba **(V)**, respectively. Phenoxyalkanoic acids will be discussed in details.



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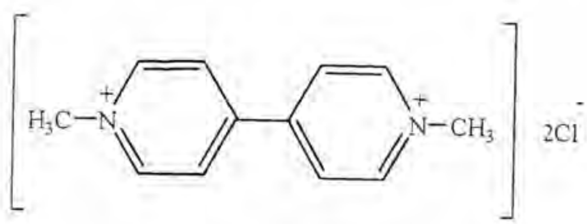

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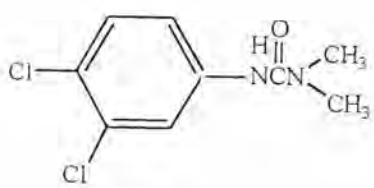

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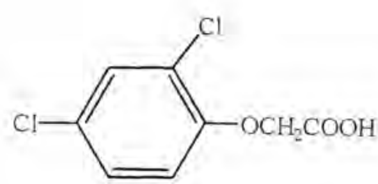

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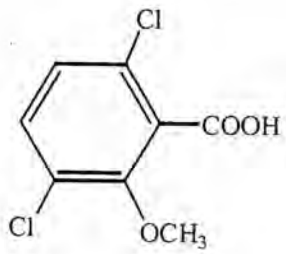

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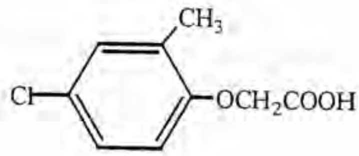

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Phenoxyalkane carboxylic acids

The introduction of 2,4-dichlorophenoxyacetic acid (2,4-D) **(IV)** and 4-chloro-2-methylphenoxyacetic acid (MCPA) **(VI)** in the mid-1940s, immediately followed World War II, had a profound effect on weed control.[4] It was demonstrated that synthetic organic compounds could be economically developed for the selective control of weeds in crops. After these compounds were introduced, the chemical industry began a major program on research and development of a wide variety of herbicides, which are commonly used today. The discovery and detail of these compounds will be discussed.

1.1.2 Plant Growth Regulators

a) Plant Hormones

Plant hormones are organic compounds which are synthesized in certain parts of a plant, translocated to and have effects on another. At very low concentrations, they cause physiological responses.[5] Traditionally plant hormones are classified into five categories, *i. e.*, auxins, gibberellins, cytokinins, ethylenes and abscisic acids.

The focus of this section will be on auxin, the first plant hormone discovered and probably best-known of all the plant hormones. Auxins play a major role in the regulation of cell elongation. In the mid-1930s, another naturally occurring compound possessing auxin activity was discovered and its structure was elucidated as indole-3-acetic acid (IAA) **(VII)**. There are several other naturally occurring auxins in higher plants, although IAA is the most important. The term "auxin" is generally used to describe both natural and synthetic chemical substances that stimulate cell elongation and growth in coleoptile and stems.

b) Plant Growth Substances

Plant growth regulators are chemical substances that exhibit their activities when utilized only in trace amount. They are currently used for practical purposes as adventitious root promotors, fruit thinning agents, fruit-drop regulators, and growth inhibitors. The effects vary according to the concentration used in treatment, growth stage of the plant, and part of the plant treated.[6]

There are many synthetic compounds which may be called auxins even though they are not plant-derived. They are instead classified as *plant growth regulators*. There are many synthetic auxins that are commercially available used and for many purposes and are classified into six groups:[7]

a) Indole derivatives: indole-3-acetic acid (IAA) **(VII)**, indole-3-butyric acid (IBA) **(VIII)**

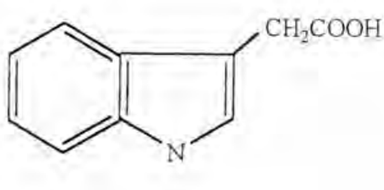
b) Benzoic acids: 2,3,6-trichlorobenzoic acid **(IX)**, 2-methoxy-3,6-dichloro-benzoic acid (dicamba) **(V)**

c) Naphthalene acids: α- and β-naphthalene acetic acid (α-NAA **(X)** and β-NAA **(XI)**)

d) Chlorophenoxyacetic acids: 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) **(XII)**, 2,4-dichlorophenoxyacetic acid (2,4-D) **(VI)**

e) Naphthoxyacetic acids: α- and β-naphthoxyacetic acid (α-NOA **(XIII)** and β-NOA **(XIV)**)

f) Picolinic acids: 4-amino-3,5,6-trichloropicolinic acid (Tordon and Pichloram) **(XV)**



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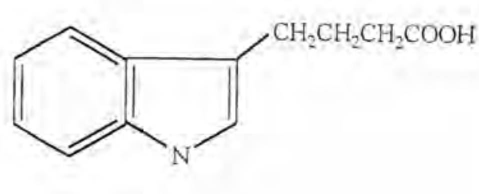

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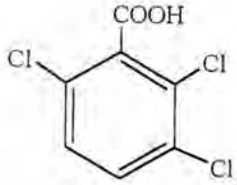

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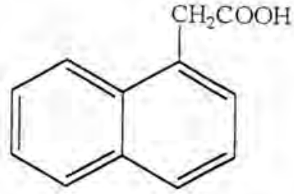

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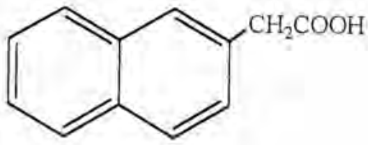

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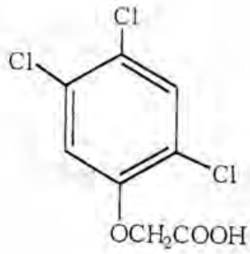

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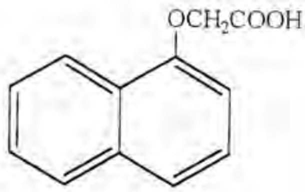

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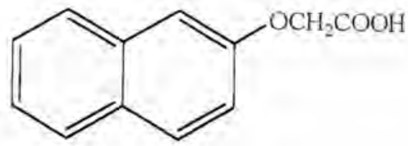

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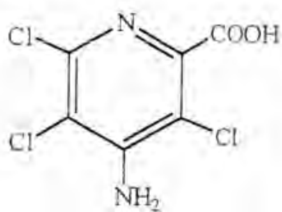


(XV)

1.2 Literature Review

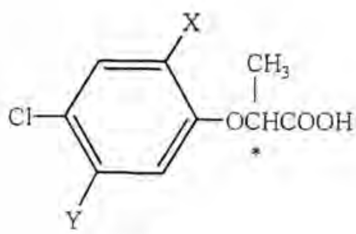
The study of plant growth regulators began with Went's discovery of auxin[8] in 1928 and Kogl's structural elucidation[9] in 1934 of the first natural phytohormone that was indole-3-acetic acid (IAA) **(VII)**. Since, the search for synthetics that could influence growth behavior of plants in the same manner as IAA to the discovery of selective herbicidal properties of many ring-chlorinated phenoxyacetic acids.

In 1941, Pokorny first synthesized 2,4-dichlorophenoxyacetic acid[10] **(VI)** (referred to as 2,4-D hereinafter) by heating a water solution of the appropriate phenol, chloroacetic acid, and sodium hydroxide for several hours. Later its activity against plants was discovered, and its selectively herbicidal activity was recognized.

In 1942, Zimmerman and Hitchcock discovered that the substitution of various groups on the ring or side chain had a profound effect on auxin activity of phenoxyalkanoic acids.[11] In addition, substituted phenoxyalkanoic acids also showed plant growth regulating properties of rooting agent,[12,13] for setting of fruit without pollination, bud formation inhibitors, and as herbicides.[14] In 1945, Zimmerman and Synerholm synthesized substituted phenoxyalkanoic acids by preparing the sodium salt of phenol in absolute alcohol, reacting with ethyl bromoacetate for one hour, followed by saponification of ester, acidification and recrystallization. Tests for determining factor for their effectiveness on plant growth regulator activity found that positions of the halogen atoms or methyl groups are the most important for cell enlargement in *Avena* coleoptiles and young tomato (*Lycopersicon esculentum* Mill.) while the halogens are generally more effective than methyl groups. The 2-, 3- and 4-positions on the benzene ring are those in which the substituents exert their greatest influence.[15]

The synthesis and toxic activity of 4-chloro-2-methylphenoxyacetic acid (MCPA) **(VI)** (LD_{50} = 700 mg/kg rat, oral, acute) was discovered in Britain and that of 2,4-D (LD_{50} = 375 mg/kg rat, oral, acute) in United State between 1941 and 1945. MCPA and 2,4-D were commonly used as selective herbicides, especially in cereals, and their advantages were low production cost and relatively low mammalian toxicity.[16] At higher application rates, these compounds not only act as growth inhibitors but can also be used in destruction of dicotyledonous plants and are tolerated by many monocotyledonous crop plants. This has been the starting point for the development of modern weed control. Furthermore, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) **(XII)**, synthesized in 1944,[14] has particularly high activity against woody plants and is used in combination with other herbicides in the control of trees, shrubs, and broadleaf weeds such as *Anthemis*, *Matricaria*, and *Galeopsis*.

In addition to the aryloxyacetic acids, 2-aryloxypropanoic acids **(XVI)** and 4-aryloxybutanoic acids **(XVII)** are also recognized for their high herbicidal activity, while 3-aryloxypropanoic acids has very low phytotoxicity. (±)-2-(2,4-Dichlorophenoxy)propanoic acids **(XVIa)** and (±)-2-(4-chloro-2-methylphenoxy) propanoic acids **(XVIb)** are mainly used for weed control in cereals. Like 2,4,5-T, (±)-2-(2,4,5-trichlorophenoxy)propanoic acids **(XVIc)** has good activity against woody plants and is used as a selective herbicide in rice and sugar-cane. In 1959, Stevenson and Brookes observed that only the D-(+) form (or R-isomer) of the propanoic acid derivatives is biologically active while the L-(-)-form or the S-isomer is inactive. However, in practice, they are used as racemates.[17]



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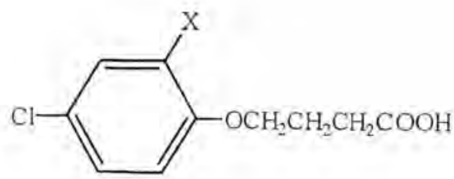
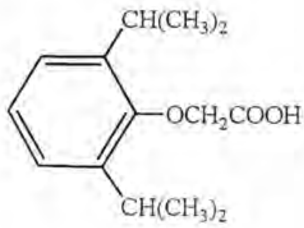


(XVII)

(XVI a); X = Cl, Y = H; *Dichlorprop*
(XVI b); X = CH_3, Y = H; *Mecoprop*
(XVI c); X = Y = Cl; *Fenoprop, Silvex*

(XVII a); X = Cl; *2,4-DB*
(XVII b); X = CH_3; *MCPB*

Furthermore, it is known that such plant growth regulating activities are potent when at least one of the *ortho*-positions on the phenyl rings is unsubstituted. 2,6-disubstituted derivatives usually show relatively weak activities.[18a] However, recent report has addressed that novel 2,6-disubstituted phenoxyacetic acid derivatives containing isopropyl groups at 2- and 6-positions on the phenyl group as 2,6-diisopropylphenoxyacetic acid **(XVIII)**[18b] exhibited excellent plant growth regulating activities. It showed a variety of activities similar to the action of gibberellin as a plant hormone at a low concentration and showed no phytotoxicity. 2,6-diisopropylphenoxyacetic acid, as well as by gibberellin A_3 (GA_3), promotes flowering of a parennial paddy weed, *Sagittaria pygmaea* Miq.[19] Only two bulky alkyl substituents, such as *iso*-propyl or *tert*-butyl groups, on the 2- and 6-positions of the phenyl ring promoted flowering. However, the active compounds, including 2,6-diisopropylphenoxyacetic acid, may be classified as anti-auxins. Typical anti-auxins, including 2,6-dichlorophenoxyacetic acid **(XIX)**, do not promote flowering.



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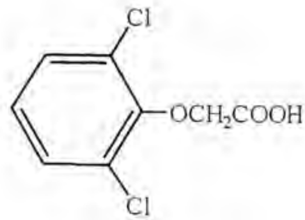
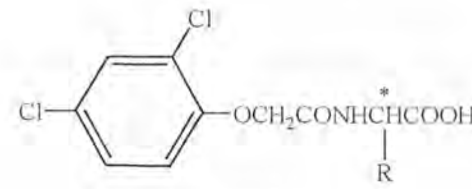


(XIX)

In 1952, Wood and Fontaine prepared a series of new halogenated phenoxyacetyl derivatives of DL-, L-, and D- amino acids and tested for plant growth regulating activity in tomato.[20] The derivatives of DL- and L- amino acids were found to be active as plant growth regulators, although derivatives of D-amino acids were relatively inactive and failed to induce formative changes in tomato plants like D-aspartic acid, D-methionine and D-phenylalanine amide. Moreover, the selective herbicide activity of the amide of 2,4-D and D-asparagine **(XXa)**[21] was observed with pig weed and mustard but not with corn. In addition, substances derived from D-amino acids acetylated with chlorinated phenoxyacetic acids, such as 2,4-dichlorophenoxyacetic acid, revealed an effect of increasing fruit size and promoting growth of tomato.

Subsequently, Feung *et al.* (1971, 1972) studied cultured callus tissues of soybean cotyledon. Their results demonstrated that 2,4-D could be transformed into corresponding amides by reacting with a number of amino acids.[22] Twenty L-form amino acid conjugates of 2,4-D were synthesized and evaluated for their effect to stimulate cell division and elongation of *Avena coleoptile* at a concentration of 10^{-5}-10^{-6} M. Like 2,4-D, elongation and growth were inhibited when high concentrations were used. All of these properties are typical of auxins and the reported metabolism of 2,4-D to conjugates of Glu **(XXb)**, Ala **(XXc)**, Val **(XXd)**, Leu **(XXe)**, Phe **(XXf)**, Asp **(XXg)** and Trp **(XXh)** cannot be considered as a detoxification mechanism.[23]



(XX)

(XXa); R = $-CH_2CONH_2$; D-isomer

(XXb); R = -H

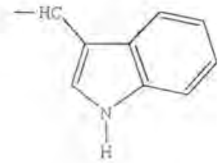
(XXc); R = $-CH_3$

(XXd); R = $-CH(CH_3)_2$

(XXe); R = $-CH_2CH(CH_3)_2$

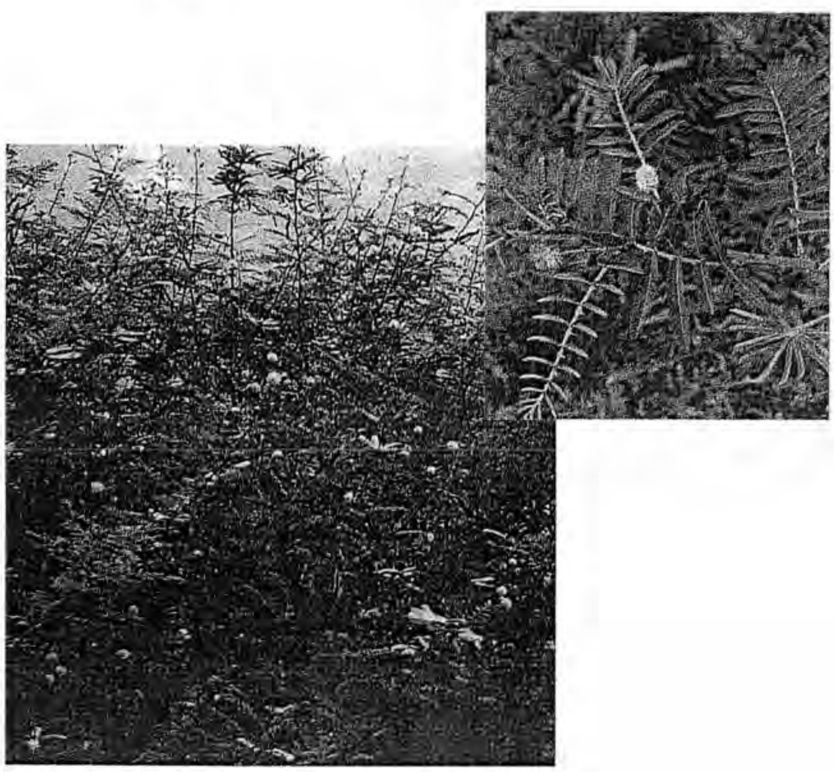
(XXf); R = $-CH_2Ph$

(XXg); R = $-CH_2CO_2H$

(XXh); R = 

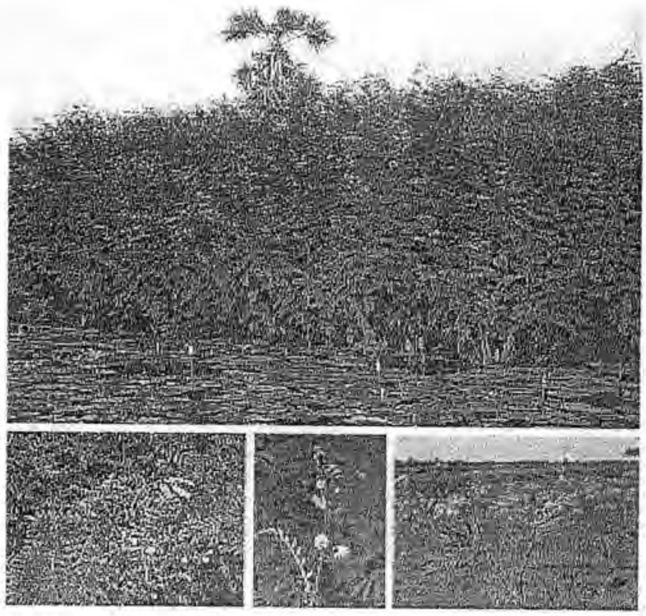
1.3 Characteristics and Information of Selected Plants

1.3.1 *Mimosa pigra* Linn.



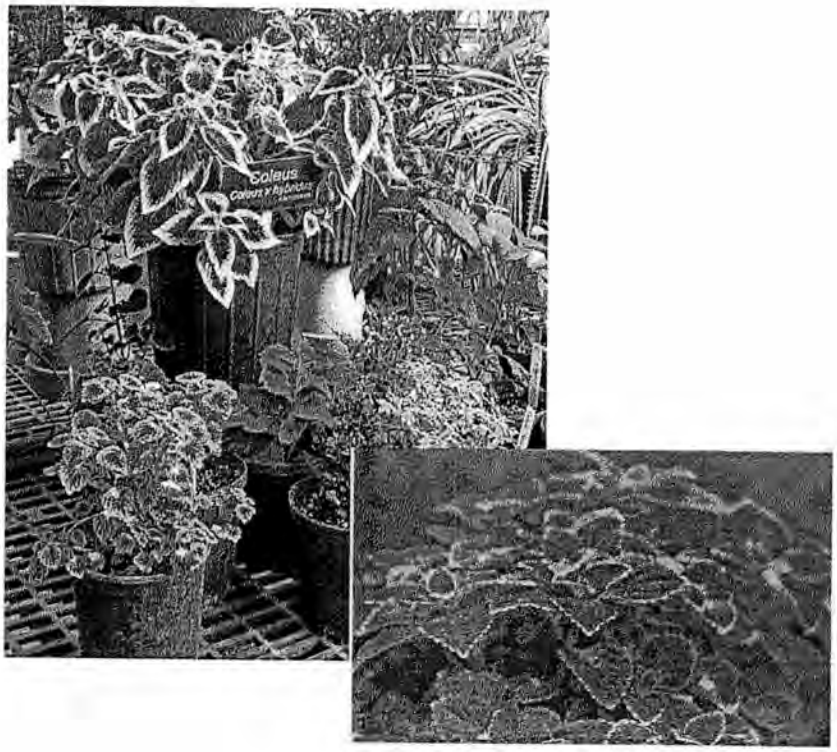
Mimosa pigra Linn. (Family: Leguminosae),[24] or Giant mimosa, or Thorny sensitive plant, and Maiyarap Yak in Thai, is a shrub native to tropical America and widely naturalized in tropical regions of Africa and Southeast Asia.

M. pigra was first known as a cover crop in a tobacco plantation by importing from Indonesia to Chiang Mai province in the early 1960s. The spread of *M. pigra* was rapid to not only around Chiang Mai and the northern provinces, but was also found in Laos and Myanmar, and to the southern provinces of Thailand such as Lamphun, Lampang, Tak and Kamphaeng Phet. It is also found in the vicinity of Bangkok in the delta of the Chao Phraya river as Saraburi and Suphanburi, as well as at Narathiwat, in the southernmost part of Thailand.[25]



This broadleaf weed grows in wet and sunny areas and can adjust itself to live in every environment growing well in paddy field, upland field or non-arable lands. Therefore, the number of mimosas increases very rapidly and invade agricultural land, dams, rivers and along highways. That is why when it forms large communities on canal and river banks, it causes potential irrigation and transportable problems.[26]

1.3.2 *Coleus atropurpureus* Benth.[27]



Coleus atropurpureus Benth. (Family: Labiatae), Coleus or Painted nettle, or Ruesee phasom in Thai, commonly called in Central part of Thailand.[28] This exotic plant is a herb and horticultural cultivar, introduced from Java. The plant has a showy leaves, square and succulent stems and white flowers. The leaves of the plant are boldly crimson-red with gold marks on finely crenate edge.[29,30]

1.4 The Goal of Research

Phenoxyacetic acids and their related compounds had been discovered, studied and reported to have broad spectrum herbicidal and plant growth regulatory activities against various plants. However, research on their biological activities is still very interesting, especially the structure-activity relationship (SAR) on growth inhibiting effect against *Mimosa pigra* Linn., which cause many problems in Thailand. Furthermore, the understanding of the SAR in stimulating root growth can be applied to other economic plants. For these reasons, the objects of this research are:

1. To synthesize substituted phenoxyacetic acids, *N*-(2,4-dichlorophenoxy acetyl)-amino acids and related compounds.

2. To study the biological activity of substituted phenoxyacetic acids and *N*-(2,4-dichlorophenoxyacetyl)-amino acids: weed growth inhibition against *Mimosa pigra* Linn. and root growth promotion in *Coleus atropurpurreous* Benth.