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HERBICIDAL POTENTIAL OF LEMONGRASS (*Cymbopogon citratus*) OIL AND ITS MODE OF ACTION

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A Thesis Submitted to the Graduate School of Naresuan University
In Partial Fulfillment of the Requirements
for the Doctor of Philosophy in Agricultural Sciences
July 2014

Thesis entitled "Herbicidal Potential of Lemongrass (*Cymbopogon citratus*) Oil and Its Modes of Action"

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has been approved by the Graduate School as partial fulfillment of the requirements for the Doctor of Philosophy in Agricultural Sciences of Naresuan University

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ACKNOWLEDGEMENT

I would like to acknowledge the Faculty of Agriculture, Natural Resources and Environment, Naresuan University to support by my Ph.D. research scholarship.

I would like also to acknowledge the Royal Initiative Discovery Foundation which also supported my scholarship.

I would like to thank and offer my sincere gratitude to my advisor, Associate Professor Dr.Udomporn Pangnakorn and co-advisors, Associate Professor Dr.Umporn Suwannamek, Associate Professor Dr.Chamroon Laosinwattana and Dr.Anusorn Worasingha. I greatly appreciated the guidance, support, and encouragement that were offered throughout the length of the study. I also would like to give special thanks to all thesis committee members, and with deeply grateful for their helpful comments.

I wish to acknowledge the laboratory staff of the Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand, who was my co-advisors, Associate Professor Dr.Chamroon Laosinwattana, Assistant Professor Dr.Montinee Teerarak, Assistant Professor Dr.Patchanee Charoenying, Department of Organic Chemistry, Faculty of Science, and Miss Jantanee Sonti, Miss Kanokporn Changsawake and Miss Pattharin Wichittrakarn, Masters students of the Department of Plant Production Technology, Faculty of Agricultural Technology. I am most for their help and assistance.

The friendship and encouragement given to me by my classmates is also appreciated, and I would like to give especial thanks to my father, mother, brothers, uncle for their endless love, understanding and support.

I would like thanks Mr.Roy Morien to improve my thesis grammar.

Finally, I would like to express my gratitude to all others for all support to make me complete this thesis but are not named in this acknowledgement.

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Academic Paper	Thesis Ph.D. in Agricultural Science, Naresuan University, 2013
Keywords	Allelopathy, Essential oil, *Cymbopogon citratus* (DC.) Stapf, Weed, Natural Herbicide

ABSTRACT

The study investigated the development of a natural herbicide based on essential oil extracted from plants. A preliminary investigation of 18 essential oils was conducted for their phytotoxic activities. It was found that the essential oil extracted from lemongrass (*Cymbopogon citratus* (DC.) Stapf) showed the greatest inhibitory effect. The essential oil was identified by Gass chromatography–Mass Spectrometry (GC-MS) for major compound constituents; six compounds representing 92.59% of the total essential oil were identified. The main constituent was citral (76.00%). The combination of major constituents had showed more phytotoxic effect than single compound. In a laboratory bioassay, high concentration of essential oil significantly inhibited seed germination and seedling growth of *Echinochloa crus-galli* by affecting α-amylase activity of seeds. In a glass house bioassay, essential oil at concentrations of 0% (control), 1.25%, 2.5%, 5% and 10% (v/v) was foliar-applied on *E. crus-galli* at 28 days after sowing at spray volume of 1,000 L/ha (160 L/rai). A visual injury level was noted at 6 h after treatment. Chlorophyll a, b and carotenoid content of *E. crus-galli* was decreased with increasing concentrations of essential oil, indicating that essential oil interferes with photosynthetic metabolism. Lemongrass essential oil caused an electrolyte leakage indicating membrane disruption and loss of integrity. Treated leaves exhibited an increase in thiobarbituric acid reactive substances (TBARS),

suggesting lipid peroxidation. The herbicide formulation prioritized on lemongrass was prepared by mixing 60% w/w of lemongrass oil, white oil by 15% w/w, coconut diethanolamide by 15% w/w, Tween20 by 5% w/w, sodium lauryl sulfate by 1.25% w/w and water by 3.75% w/w. The herbicide action was found to be more effective when applied post-emergence better than pre-emergence. The herbicide was also tested post-emergence on 2 – 3 leaf stage of some crops and weeds. The results indicated that the oil is contact and non-selective herbicide. All crops and weeds were completely killed at 8% ai while lower dosages were slightly effective and non-affective at 1% ai. This result indicated that the natural herbicide based on lemongrass essential oil could be applied as a direct herbicide for weed control at the 2 – 3 leaf stage.

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CHAPTER I

INTRODUCTION

Rationale for the significance of the study

Weeds are a major problem in crop growth causing huge economic losses to farmers. They compete with crop plants for different factors such as light, water, nutrients, carbon dioxide, etc. For example; soybean (*Glycine max*) growth interfered by pigweed (*Amaranthus* spp.) and barnyardgrass (*Echinochloa crus-galli*) can lose yield ranging from 32% to 99% (Cowan, et al., 1998). *Phalaris minor* Retz., *Rumex dentatus* L., *Coronopus didymus* (L.) Sm., *Medicago denticulata* Willd., *Chenopodium album* L., and *Poa annua* L., are major weeds in wheat field which can cause maximum yield loss of 76% (Siddiqui, et al., 2010). Redroot pigweed (*Amaranthus retroflexus* L.) at the density of 4.7 plants/m^2 decreased crop yield of peanut (*Arachis hypogaea*) by 63.9% (Bukun, 2011). In the United States, weed infestion in 46 crops caused monetary loss of $4.1 billion, and $984 million in Canada (Anderson, 1996a). Various chemical, biological and cultural methods have been achieved to control weeds (Singh, et al., 2005). To overcome weed infestation in modern agriculture a large variety of herbicides have been heavily used (Cheema, et al., 2008). Herbicides have helped farmers to increase yields while reducing labor. Indeed, without herbicides, labor would be a major cost of crop production in developed countries (Macias, et al., 1999b). In Thailand, the value of imported synthetic pesticides is more than 10,000 billion baht per year, of which about 60% was herbicides. This suggests that Thailand heavily relies upon the synthetic herbicides. However, the widespread use and over use of synthetic herbicides causes negative impacts on human health and on the environment. It also leads to an increase of herbicidal resistance in many weed species (Vyvyan, 2002).

Allelopathy means any process involving secondary metabolites produced by plants, microorganisms, viruses or fungi that influence the growth and development of agricultural and biological systems. Allelopathy has the potential to enhance crop production and leads to the development of more sustainable agriculture. Allelopathy includes weed and pest control, crop rotation, residue management and a variety of

approaches in biocontrol. All of which enhance sustainability in agriculture, natural compounds released from allelopathic plant residues may help to reduce and replace the use of synthetic pesticides for pest management and therefore cause less pollution as well as being safer on humans, animals, and agricultural products (Singh, et al., 2003; Khanh, et al., 2007; Sodaeizadeh, et al., 2010).

Allelochemicals, chemical compounds released from allelopathic plants, were classified into several groups such as glucosinolates, phenolic compounds, terpenoids, alkaloids and hydroxamic acids. (Haig, 2008). However, the terpenoid group seemed the better group for phytotoxic activities against plants. This group is well known for the essential oils found in aromatic plants. Among the natural plant products, essential oils constitute an important group that provides a characteristic odor to the aromatic plants (Singh, et al., 2002). Recently, interest in exploring essential oils from aromatic plants for weed control has increased tremendously (Kaur, et al., 2010) for example; eucalypt oil (*Eucalyptus* spp.), clove oil (*Syzygium aromaticum*), cinnamon oil (*Cinnamomum zeylanicum*), Manuka oil (*Leptospermum scoparium*) (Tworkoski, 2002; Batish, et al., 2004; Singh, et al., 2005; Bainard, et al., 2006; Dayan, et al., 2011; Stokłosa, et al., 2012). They do not persist in soil and do not leach into ground water (Singh, et al., 2002; Singh, et al., 2005). The essential oils were also safe for human and animals. For these reasons, essential oils can be a source of new natural herbicides.

Thailand is a tropical country where many plants are available, and numerous species among them may possess strong allelopathic properties able to be developed as natural herbicides. Observation of new plants containing strong allelopathic potential, which may result in greater weed control, is very important to reduce dependence on synthetic herbicides in agricultural production in Thailand, as well as being of world-wide interest for the same reasons.

Research and development of natural herbicides produced from essential oils extracted from plants is the most promising way to control weeds and help to reduce the use of synthetic herbicides.

Objectives of the study

1. To investigate allelopathic plants and compounds.
2. To develop allelopathic plants and compounds as natural herbicide.
3. To evaluate herbicidal efficacy on weed control and its physiological mechanisms.

Expected benefits and application

1. To recognize the natural product of herbicide based on essential oil.
2. To realize the application method of natural herbicide.
3. To understand the physiological mechanisms on inhibiting weeds of natural herbicide.

CHAPTER II

LITERATURE REVIEWS

Weeds

The Oxford English Dictionary (Little, et al., 1973) defines a weed as an "herbaceous plant not valued for use or beauty, growing wild and rank, and regarded as cumbering the ground or hindering the growth of superior vegetation."

H.G. Baker defined weed as "a plant is a "weed" if, in any specified geographical area, its populations grow entirely or predominantly in situations markedly disturbed by man (without, of course, being deliberately cultivated plants). Thus, weeds include plants which are called agrestals by some writers of floras (they enter agricultural land) as well as those which are ruderals (and occur in waste places as well as along roadsides). It does not seem to me necessary to draw a line between these categories and accept only the agrestals as weeds (although this is advocated by some agriculturally oriented biologists) because in many cases the same species occupy both kinds of habitat. Ruderals and agrestals are faced with many similar ecological factors, and the taxa which show these distributions are, in my usage, weedy" (Baker, 1965; Baker, 1991).

Weeds infest crop production fields causing crop loss. Studies have shown that average yield losses due to weeds alone have approached 80% in processing tomatoes and 60% in cabbages (Tolman, et al., 2004). Weeds can reduce yields in agricultural fields in several ways such as competing for nutrients, light, spacing, CO_2, etc. Weeds not only interfere in commercial agricultural production but they also cause problems for general public in many other ways - for example, in regard to health (infestation of small seeds and spores) and maintaining home landscaping recreational areas and other non-crop areas. Specific problems such as being poisonous and cause allergies, acting as hosts or attractants for pests and diseases, tainting the taste of meat and milk from contaminated animals, interference with cultivation, water management problems cause soil erosion and aesthetics (Monaco, et al., 2002c; Mason, 2003).

Thus, weed control programs are essential for successful crop production. Weed control practices can be grouped into six categories (Anderson, 1996b; Monaco, et al., 2002b; Zimdahl, 2007)

1. Scouting
2. Prevention
3. Mechanical practices: hand pulling, mowing, hoeing, artificially high temperatures: hot water, burning, solarization, sound and electricity, flooding, salting water, draining, chaining, mulching, and tillage,
4. Cultural practices: crop rotation, crop selection, crop varieties, planting date, plant population and spacing, fertility and irrigation, no tillage system, and cover crops or living mulching
5. Biological control

Plant pathogens: *Alternaria alternata* ITCC4896 for controlling *Parthenium hysterophorus* (Saxena and Kumar, 2010), *Alternaria pellucida* for controlling *Sagitaria trifolia* (Motlagh and Javadzadeh, 2010), *Fusarium oxysporum* and *Curvularia lunata* for controlling *Echinochloa crus-galli* (Jyothi, et al., 2010), etc

6. Chemical control: herbicides

Herbicides

An herbicide has been defined as "any chemical substances or cultured biological organism used to kill or suppress plant growth" (Anderson, 1996a). Nowadays, herbicides seem to be the best way for weed control because they are more effective, more convenient, more economical and faster than other ways. Today, more than half of the volume of all agriculture pesticides applied world-wide were herbicides (Dayan, et al., 2009). In Thailand, there has been significant use of herbicides around 70% of total pesticides in 2002 – 2012 (Table 1).

Table 1 Quantity and value of imports of pesticides in Thailand in 2002 – 2012

Years	Insecticides		Fungicides		Herbicides		Others***		Total	
	Volume*	Value**	Volume	Value	Volume	Value	Volume	Value	Volume	Value
2003	9,790	3,136	6,732	1,678	31,879	6,101	1,930	426	50,331	11,341
2004	16,731	2,835	10,108	1,719	55,649	6,080	4,417	502	86,905	11,135
2005	18,529	3,322	9,052	1,716	48,841	5,806	3,744	516	80,166	11,360
2006	20,487	3,856	9,383	1,722	62,129	6,821	3,764	499	95,763	12,899
2007	21,590	3,746	10,626	1,833	79,239	8,914	4,869	533	116,323	15,026
2008	25,332	4,577	11,255	2,537	68,825	11,487	4,497	580	109,908	19,182
2009	19,709	3,972	8,485	2,968	85,821	9,338	4,137	537	118,152	16,816
2010	23,417	4,669	9,670	3,859	80,278	8,845	4,450	583	117,815	17,956
2011	34,672	5,938	12,179	3,875	112,176	11,480	5,356	751	164,383	22,044
2012	16,796	3,686	6,971	3,883	106,860	11,293	3,750	495	134,377	19,357

* Volume: tons

** Value: million baths

*** Others including: PGR, Fumigant, Mollussicide, Acaricide, Rodenticide, Nematocide and Bio-pesticide

Source: www.oae.go.th

Although the use of synthetic herbicides for controlling weeds is effective and convenient, the side effect of herbicides can also strongly negatively impact humans, animals and environment. For example; 2, 4-D and 2, 4, 5-T caused the reduction of the population of the tortoise *Testudo hermanni* in Greece (Willemsen and Hailey, 2001). The contamination of water by cyanazine, diclofop, prometryn, simazine and simetryn caused photosynthesis-inhibiting, ACCase inhibitor, protox inhibiting herbicides of cyanobacteria, (*Anabaena flos-aquae*, *Microcystis flos-aquae* and *M. aeruginosa*) (Ma, et al., 2010). Glyphosate, a systemic herbicide widely used in the world, can be responsible for oxidative damage to human epidermal cells and causes a reverse of the effect of vitamin C and E on human skin (Gehin, et al., 2005). Glyphosate has also been to reduce a population of *Chordodes nobilii* (Gordiida, Nematomorpha), a poorly known group of worm-like animals similar to nematodes. (Achiorno, et al., 2008). Paraquat, a cationic nonselective contact herbicide widely

used in agricultural production, produces toxicity in dopaminergic neurons of the rat and mouse brain, such that it can be a risk factor in the incidence of Parkinson's disease (PD (Yang and Tiffany-Castiglioni, 2005).

Long term herbicide application also causes resistance and tolerance in weeds. In 1968, the first report was published about herbicide resistance. Common groundsel (*Senecio vulgaris*) was resistant to simazine and atrazine (Ryan, 1970). Currently, about 233 species had reported resistant to more than 16 herbicide chemical families (Monaco, et al., 2002a).

Allelopathy

The word "Allelopathy" was coined in German by the eminent Austrian plant physiologist Hans Molisch, in his last book, Der Einfluss einer Pflanze auf die andere – Allelopathie1, published shortly before his death in 1937. The word originates from the Greek roots, allelon, meaning 'mutual' or 'among each other', and pathos, meaning 'suffering' or 'feeling' (Willis, 2007). In 1984, E.L. Rice, the famous American allelopathy researcher, defined the term of allelopathy as "any direct or indirect harmful or beneficial effect by one plant (including microorganisms) on another through production of chemical compounds that escape into the environment" (Rice, 1984). In 1996, the International Allelopathy Society definded the meaning of allelopathy as "Allelopathy means any process involving secondary metabolites produced by plants, microorganisms, viruses or fungi that influence the growth and development of agricultural and biological systems".

Allelopathy is involved with the chemicals that one organism produces and releases into the environment. These compounds are released by volatilization from leaves, leaching from leaves by rain, fog and dew, root exudation from roots, decomposing sloughed roots, and decomposing leaves, fruits and twigs (Figure 1) (Xuan, et al., 2005).

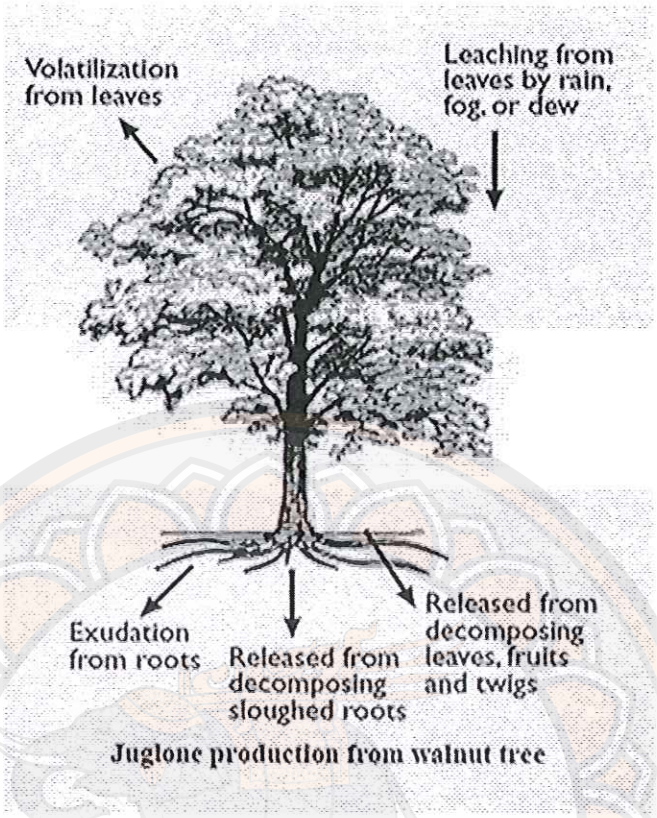


Figure 1 The release of juglone produced from walnut tree through the enviroment by the ways

Source: http://pubs.ext.vt.edu/430/430-021/430-021.html

Allelochemicals

Secondary metabolites that occurred from on allelopathy phenomenon are called "allelochemical", "allelopathic compound", or "allelochemic" (Putnum and Tang, 1986). A classification of allelochemicals can be divided into six groups (Haig, 2008), for example;

1. Glucosinolates

These compounds are mainly found in plants of the order of Cappareles. Sinigrin, Gluconasturtiin, Glucobrassicin and Glucoerucin that are from Brassica species are always represented in this group.

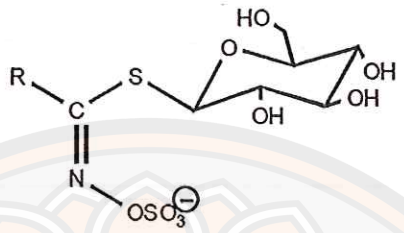


Figure 2 General glucosinolate structure

2. Phenolic compounds

This compound group is commonly found in the higher plants. Particularly cinnamic acid is always found in higher plants. The structures of compound include simple aromatic phenols, hydroxy and substituted benzoic acids and aldehydes, hydroxy and substituted cinnamic acids, coumarins, tannins, and perhaps a few of the flavonoids (Figure 3).

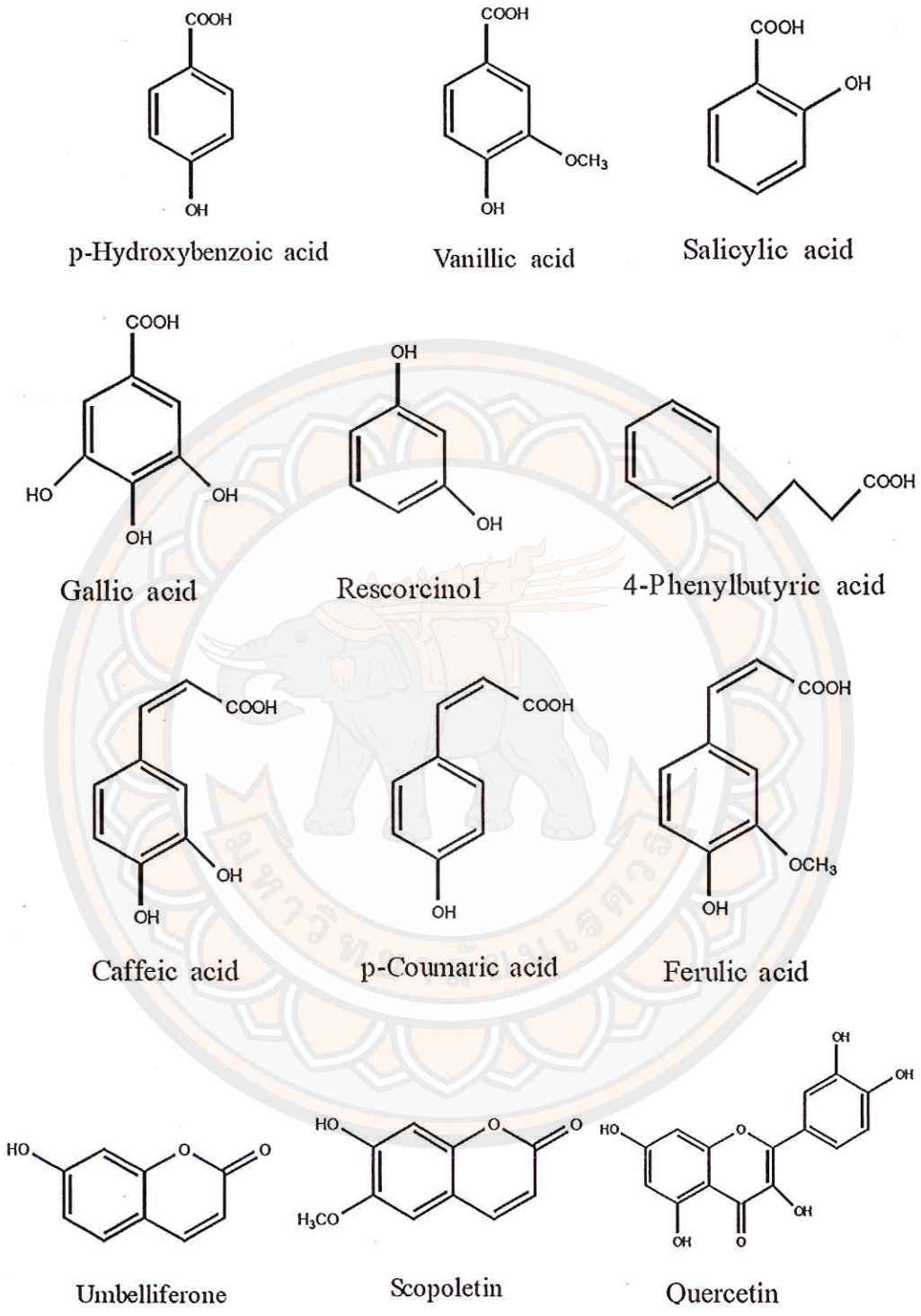


Figure 3 Typical structures among the allelochemical phenolics

3. Terpenoids

Plants containing essential oils are always found in this chemical class. It has been known that the monotepenes have strong inhibitive effect on plant. The terpenoids are distinguished by their origin from the biochemical pathway via mevalonic acid and isopentenyl pyrophosphate (a basic C-5 building unit) which combines with itself to produce C-10 monoterpenes, C-15 sesquiterpenes, C-20 diterpenes, and C-30 triterpenes (Haig, 2008).

The monoterpeniod, 1,4-cineole (Figure 4), is the basis of the structure of cinmethylin (Figure 4), the commercial herbicide.

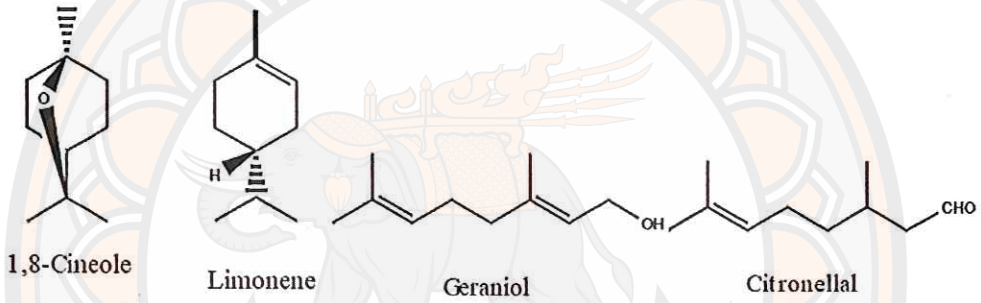


Figure 4 Typical of monotepenoids

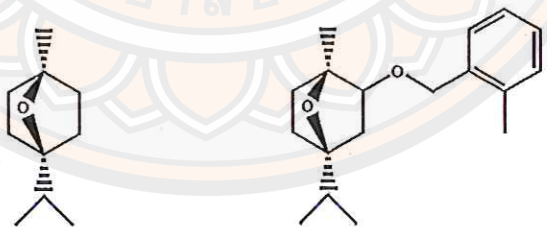
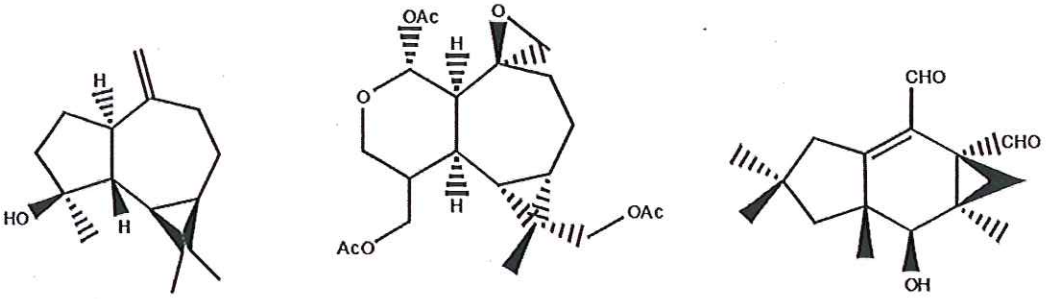


Figure 5 The monoterpeniod, 1,4-Cineole, and Cinmethylin, the commercial herbicide



Spathulenol Plagiochilin Merulidial

Figure 6 Typical of sesquiterpenoids

4. Alkaloids

These substances are usually cyclic or polycyclic, and are mostly derived through biosynthetic pathways which begin with a natural amino acid. The families Fabaceae, Apocynaceae, Asteraceae and Borginaceae always contain alkaloids. They have also often fulfilled the role of providing plant protection from fungi, viruses, microorganisms, competing plants and herbivores.

The example compounds of plant alkaloids such as nicotine, morphine, caffeine, piperine and colchicine (Figure 7) have long been found. Piperine, nicotine and caffeine have insecticidal properties.

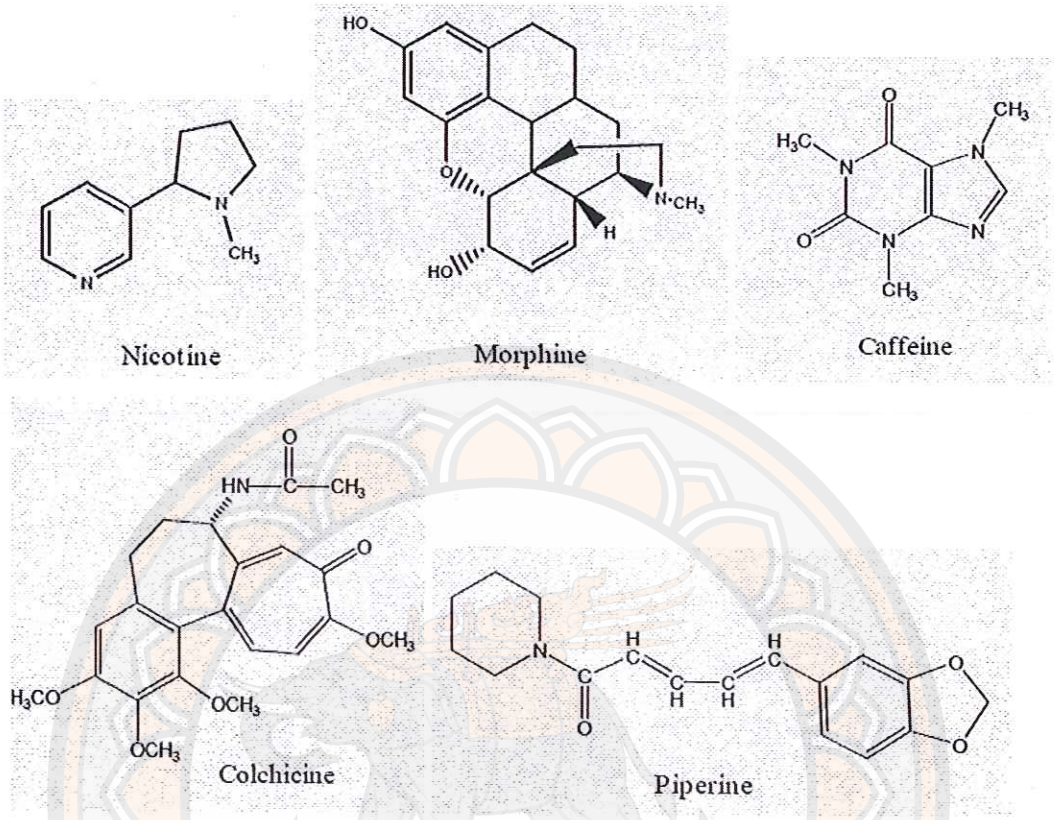


Figure 7 Typical of plant alkaloids

5. Hydroxamic acids

The allelochemicals often called "cyclic hydroxamic acids" are actually a small part of the broader group of naturally occurring benzoxazinones which possess the 2- hydroxy-2H-1, 4-benzoxazin-3-(4H)-one skeleton as shown in Figure 8 (Sicker and Schulz, 2002). Plant families of Gramineae, Ranunculaceae, and Scrophulariceae commonly contain benzoxazinones, especially DIBOA [2, 4-dihydroxy-2H-1, 4-benzoxazin-3-(4H)-one], DIMBOA [2, 4-dihydroxy-7-methoxy-2H-1, 4-benzoxazin-3-(4H)-one] showed in Figure 9 .

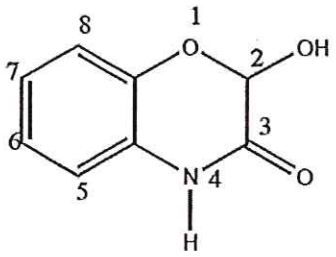
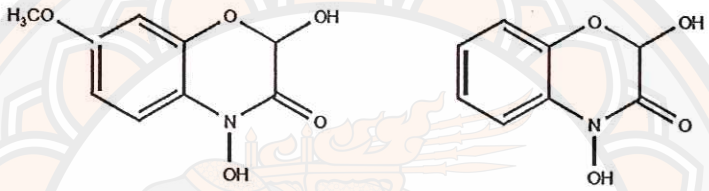


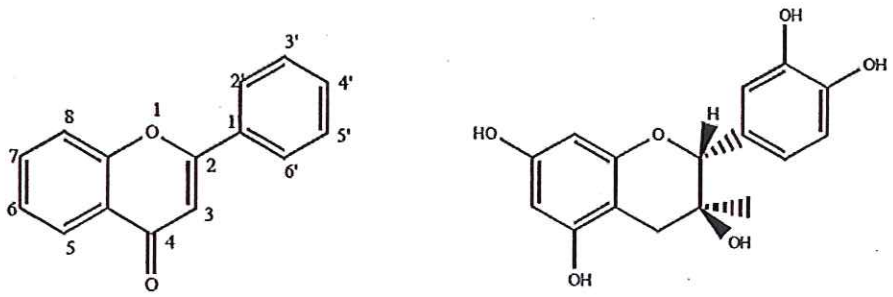
Figure 8 Cyclic hydroxamic acid skeleton



(a) DIMBOA (b) DIBOA

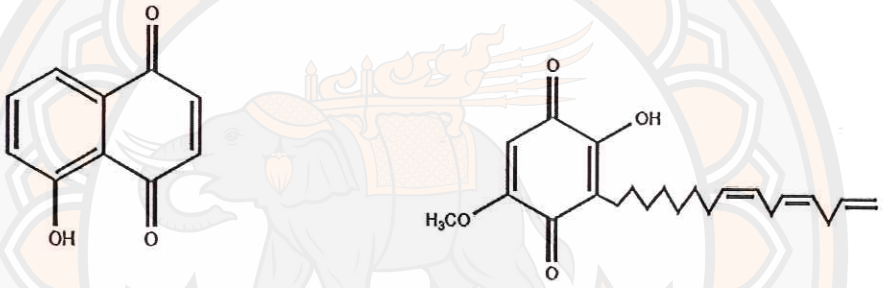
Figure 9 Prominent hydroxamic acid allelochemicals, DIMBOA and DIBOA

6. Other Compounds
   - 6.1 Flavonoids such as flavone and catechin (Figure 10)
   - 6.2 Quinones such as juglone and sorgoleone (Figure 11)
   - 6.3 Polyacetylenes (Figure 12)
   - 6.4 Miscellaneous compounds such as pisatin and thiocyanate (Figure 13)



Flavone (+)-Catechin

Figure 10 Typical strutures of flavonoids



Juglone Sorgoleone - quinone form

Figure 11 Typical strutures of quinones

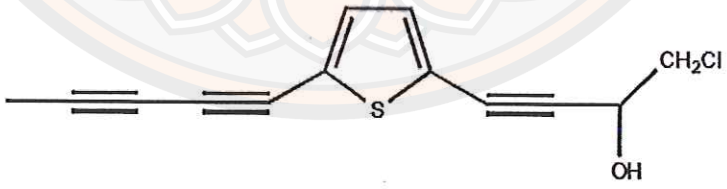
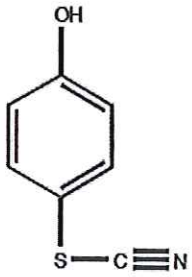
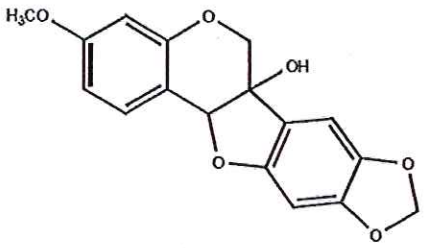


Figure 12 Polyacetylene found in in the roots of Centaurea repens (Haig, 2008)



Thiocyanate



Pisatin

Figure 13 Miscellaneous; thiocyanate exudated from cucumber, and pisatin from Pisum sativa

Allelochemicals effect the development and growth of organisms such as; (Rice, 1984)

1. Cell division, cell elongation, and ultrastructure of cell
2. Hormone-induce growth
3. Membrane permeability
4. Mineral uptake
5. Easily available phosphorus and potassium in soils
6. Stomata opening and photosynthesis
7. Respiration
8. Protein synthesis and changes in lipid and organic acid metabolism
9. Possible inhibition of porphyrin synthase
10. Inhibition and stimulation of specific enzymes
11. Corking and clogging of xylem elements
12. Stem conductance of water and internal water relations
13. Miscellaneous mechanisms

S.O. Duke and F.E. Dayan (Duke and Dayan, 2006) have divided the sites of action of allelochemicals on plants including;

1. Photosystem II (PSII)
2. Photosystem I (PSI)
3. Photophosphorylation

4. Protoporphyrinogen oxidase and other enzymes of chlorophyll synthesis,
5. Singlet oxygen generators
6. HPPD – *p*-hydroxyphenylpyruvate dioxygenase
7. Respiration
8. H-ATPase of the plasma membrane and tonoplast
9. Mitotic inhibitors
10. Protein synthesis and non-amino acid antimetabolites
11. Glutamine synthetase
12. Acetolactase synthase
13. Asparagines synthase
14. L-galactano-*y*-lactone dehydrogenase

Allelopathic plants and allelochemicals for weed control

The potential for allelopathic plants and microorganisms to control crop diseases, increase disease resistance in crops through signal transduction, development of biocontrol agents, biofumigants and plant growth promoters is endless but little explored (Mallik, 2008).

However, the target of research in allelopathy has been mostly for weed control. Some new techniques involving allelopathy have been suggested for weed suppression (Macias, et al., 1999a)

1. The use of natural or modified allelochemicals as herbicides.
2. The transfer of allelopathic traits into commercial crop cultivars.
3. The use of allelopathic plants in crop rotation, companion planting, and smother crops.
4. The use of phytotoxics mulches and covers crop management, especially in notillage systems.

Juglone, isolated from walnut trees has been found to be effective against redroot pigweed, velvetleaf and barnyard grass (Weston, et al., 1987). Artemisinin, is a sesquiterpene endoperoxide lactone isolated from the shoots of *Artemisia annua*. Effects of which artemisinin is most evident on root growth and chlorophyll content. Inhibition of mitosis is dose-dependent and is accompanied by abnormal mitotic conjurations (Chen and Leather, 1990; Delabays, et al., 2008).

2-Benzoxazolinone (BOA), is a well-known allelochemical with strong phytotoxicity exudated by the root of grass species. It is a potential herbicide candidate. It's mode of action was unknown. However, several researches had been reported that BOA interfere with both electron transport and ATPase activity in mitochondria as well as plasma membrane H+- ATPase functions (Dayan and Duke, 2009; Dayan, et al., 2012).

Nowadays, the successful of identification and purification of allelochemicals from organisms are used as natural herbicides. For example, some of the natural products exploited as commercial herbicides are triketone, cinmethylin, bialaphos, glufosinate and dicamba. Bialaphos, a microbially originated herbicide produced by *Streptomyces viridochromogenes*, is a type of product in which phosphinothricin inhibits glutamine synthetase, accumulates ammonia, and inhibits photosynthesis in plants. However, it has limited use in Japan. Two natural fatty acids, pelargonic acid and maize gluten (a byproduct of the maize-milling process) are mainly used in organic farming but have limited use in USA (Duke, et al., 2000; Bhowmik and Inderjit, 2003; Dayan, et al., 2012).

There are examples of the successful use of allelopathic plants on weed control. Application of dry root powder of kava (*Piper methysticum*) at 1 ton/ha 6 days after saturating paddy soil with water caused around 80% reduction of natural paddy weed growth and increased tillering and root number of rice (Xuan, et al., 2003b). Alfalfa (*Medicago sativa* L.) and rice by-product of *Oryza sativa* L. cv. *Japonica*, hull, applied at a dose of 1 ton/ha significantly reduced weed growth and weed species (Xuan, et al., 2003a). The application of dry leaf powder of *Biden pilosa* and *Tephrosia candida* at the rate of 2 ton/ha after transplanting seedling rice in paddy fields reduce weed numbers and weed dry weight by more than 80% and increased the rice yield by 20% (Hong, et al., 2004). The upper parts of *Stylosanthes guianensis*, grown as a popular pasture legume and cover crop, applied at 1 ton/ha reduced paddy weed biomass by 80% and increased rice yield by 40% (Khanh, et al., 2006). Some aromatic and medicinal plants have been reported as heavy allelopathic activity on weed control. These included *Anisomeles indica*, an erect camphor-scented perennial woody shrub growing wild in Southeast Asia, with the leaf and root powder being applied as mulch at 1 and 2 ton/ha, significantly reduced the emergence and growth of

Phalaris minor and other weeds of wheat crops similar to herbicides, without any negative effect on the wheat growth and yield (Batish, et al., 2007).

Examples of allelopathic plants used in crop rotation include, incorporation of aromatic plants; *Pimpinella anisum* L., *Foeniculum vulgare* P. Mill., *Ocimum basilicum* L., *Anethum graveolens* L., *Coriandrum sativum* L., *Petroselinum crispum* (P. Mill.) Nyman ex A.W. Hill, *Phacelia tanacetifolia* Benth., *Mentha Xverticillata* L., *Origanum vulgare* L., and *Melissa officinalis* L. as green manure in maize crops this reduced weed numbers by 11%-83% while the emergence and growth of the maize was not affected. After harvesting, the grain yields of maize were increased by 10% – 43% when compared with plots for free green manure (Dhima, et al., 2009). *Eucalyptus globulus* fresh-leaves when incorporated into soil as green manure reduced the emergence of monocot and dicot weed species. However, it reduced biomass of the corn, but increased the final yield of corn (Puig, et al., 2013).

Essential oils

Plant essential oils are obtained from non-woody parts of the plant, particularly foliage, through steam or hydrodistillation. Terpenoids, particularly monoterpenes (C10) and sesquiterpenes (C15), play a central role in generating the chemical diversity of plant volatiles (Baldwin, 2010). The characteristic aroma and odour of the plant are determined from the specific mixture of a variety of aromatic phenols, oxides, ethers, alcohols, esters, aldehydes and ketones (Langenheim, 1994; Batish, et al., 2008). Plant volatile oils play important roles in many aspects of human behavior and industries such as drugs in food acquisition for humans, pharmaceuticals, nutritions and perfumes in industries (Baldwin, 2010).

Essential volatile oils are released to the environment through the membranes of the epidermal tissues, where they are synthesized, or through structures such as trichomes, hair, or osmophores. They are also released through opening stomata. The essential volatile oils can be stored in vacuoles in conjugated form. (Baldwin, 2010, pp. R392-R397)

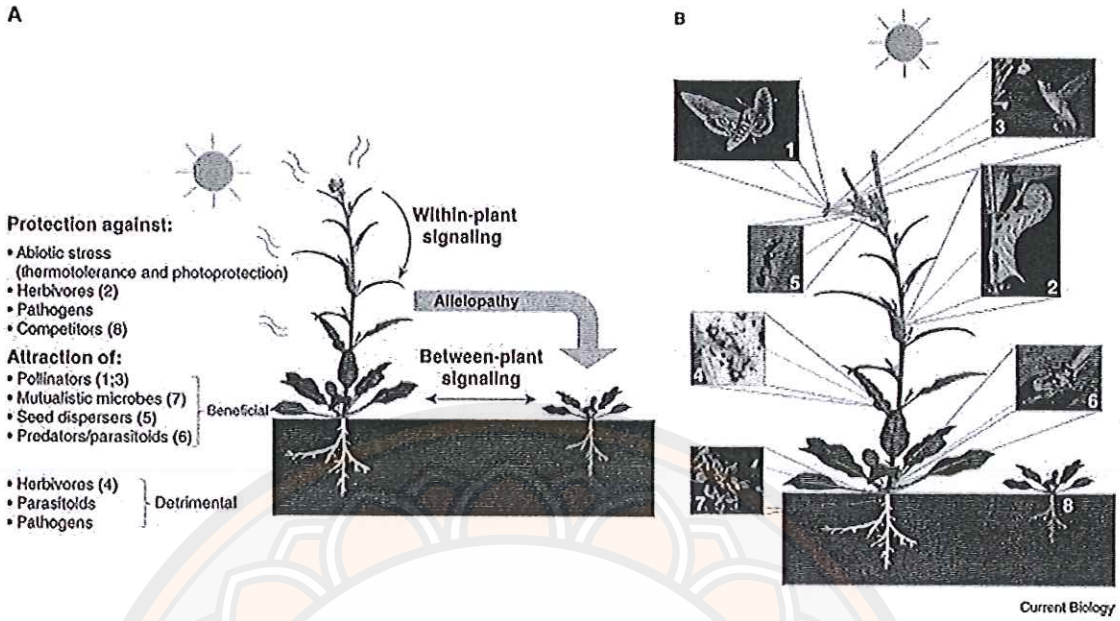


Figure 14 Plant volatiles function in protecting against biotic and abiotic stresses and in signaling that can be both beneficial and detrimental to the emitting plant

Source: Baldwin, 2010

The essential oils that are being trialled as potential candidates for weed control (Singh, et al., 2003; Batish, et al., 2004; Batish, et al., 2007). As well, essential oils are also used in pest and disease management (Isman, 2000; Abad, et al., 2007; Pawar, et al., 2011). Tworkoski (2002) demonstrated that essential oils can be used in organic farming systems and provided prototypes for the synthesis of available herbicides.

Essential oil for weed control

The allelopathic and herbicidal effects of essential oil have been studied by researchers. For example, essential oil from *Ruta graveolens* inhibits seed germination and radical growth of radish (*Raphanus sativus*) (De Feo, et al., 2002). Essential oils obtained from rosemary (*Rosmarinus officinalis* L.), thyme (*Thymus vulgaris* L.), and savory (*Satureja montana* L.) and the four monoterpenes that are their major constituents, α-pinene and 1,8-cineole, were studied for inhibitory effects on weeds. Results showed that the oils inhibited seed germination and seedling growth of *Chenopodium album*, *Portulaca oleracea*, *Echinochloa crus-galli*, *Raphanus sativus*, *Capsicum annuum*, and *Lactuca sativa* (Angelini, et al., 2003). Volatile oils extracted by *Coriandrum sativum* L., *Satureja montana* L., *Santolina chamaecyparissus* L., and *Thymus vulgaris* L. have been reported on inhibiting germination and seedling growth of *Zea mays* L., *Triticum durum* L., *Pisum sativum* L., *L. sativa* L., *P. oleracea* L. and *Vicia sativa* L. (Grosso, et al., 2010). Clove oil from *Syzygium aromaticum* plants has been to have herbicidal activity on several broccoli (*Brassica oleracea* var. *italic*), redroot pigweed (*Amaranthus retroflexus* L.) and common lambsquarters. (*Chenopodium album* L.). The clove oil displayed the activity similar to paraquat, a commercial contact herbicide, but acted both in light and dark conditions, whereas paraquat was only viable under light conditions (Bainard, et al., 2006; Stokłosa, et al., 2012). Volatile essential oil from the leaves of *Anisomeles indica* have been reported as inhibiting the radicle and seedling elongation and dry weight accumulation of *Bidens pilosa*, *Cassia occidentalis*, *Amaranthus viridis* and *E. crus-galli*. Essential oil from *Baccharis trimera* (less.) DC influenced on percentage emergence, the rate of speed emergence and speed emergence index of *Vigna unguiculata* (L.) Walp (Batish, et al., 2012)

After previous published examples of allelopathic and herbicidal activity of essential oils such as: essential oil from kenaf (*Hibiscus cannabinus* L.) leaves (Kobaisy, et al., 2001); *Tagetes minuta* L. and *Schinus areira* L. (Lee, et al., 2002; Scrivanti, et al., 2003); *Chromolaena odorata* (Ling, et al., 2003); four sweet basil (*Ocimum basilicum* L.) cultivars and six oreganos or marjoram (*Origanum* spp.) (Vasilakoglou, et al., 2007); leaf of *Agastache rugosa* (Kim, 2008); essential oils from *Salvia hierosolymitana* Boiss. and *Salvia multicaulis* Vahl. var. *simplicifolia* Boiss

(Mancini, et al., 2009); *Lantana camara*, *Eucalyptus camaldulensis* and *Eriocephalus africanus* (Verdeguer, et al., 2009); *Cinnamomum zeylanicum* L., *Lavandula* spp. And *Mentha* x *piperita* L. (Cavalieri and Caporali, 2010); *Schinus molle* (Zahed, et al., 2010); *Peumus boldus* and *Drimys winterii* (Verdeguer, et al., 2011); the essential oils from the aerial parts of catmint (*Nepeta meyeri* Benth.); manuka tree (*Leptospermum scoparium* J.R. and G. Forst) (Dayan, et al., 2011); *Prangos ferulacea* L. (Razavi, 2012); *Lolium rigidum* Gaudin (Vasilakoglou, et al., 2013), etc.

The number and proliferation of these reports strongly suggests that essential oils are of significant interest as to their use as natural herbicide. This provides a strongly reason for my current research.

CHAPTER III

METHODOLOGY

Screening allelopathic potential of 18 essential oils

1. Preparation of essential oils

The vegetative stages around 3 – 6 months of growth of the mature plants in this category, lemongrass (*Cymbogopon citratus* (DC.) stapf), citronella grass (*Cymbogopon nardus* Rendle), bush tea (*Hyptis suaveolens* (L.) Poit), sweet basil (*Ocimum basilicum* L.), holy basil (*Ocimum tenuiflorum* L.), hairy basil (*Ocimum americanum* L.), and white sage (*Lantana camara* L.) were extracted the essential oils from fresh leaves. The oils from tangerine (*Citrus reticulate* Blanco), kaffir lime (*Citrus hystrix* DC.), pummelo (*Citrus maxima* Merr.) and lime (*Citrus aurantifolia* Swing.) were extracted from cells inside the rind of a fruit.

The fresh leaves or cells inside the rind were cut into 1 – 2 cm. They were dipped into distilled water at the ratio of 1: 10 and then the essential oils were extracted by stream distillation in a conventional Clevenger-type apparatus (Figure 15) for 3 – 6 hours, depending on species. The essential oils from anise (*Pimpinella anisum* L.), wintergreen (*Gaultheria procumbens* L.), pine (*Pinus* sp.) cajeput (*Melaleuca leucadendron* L.), bay (*Pimento racemosa* (Mill.) J.W.Moore), black pepper (*Piper nigrum*) and ajowan (*Trachyspermum copticum*) were purchased from Honghuat®, Bangkok, Thailand.

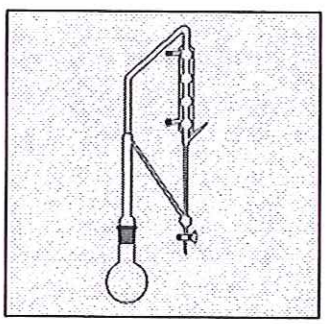


Figure 15 A stream distillation in a conventional Clevenger-type apparatus

2. Preparation of bioassay seeds

Seeds from a monocot weed; barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) and a dicot weed; slender amaranth (*Amaranthus viridis* L.) were collected from agricultural fields in Phitsanulok Province, Thailand. The barnyard grass seeds were aired under full light for 72 h, incubated at 50°C for 72 h to break their dormancy and then used for the experiments. The slender amaranth seeds were aired under full light for 48 h. All the seeds were kept at 12°C for the experiments. Before experimenting, both seed species were sterilized with 1% sodium hypochlorite for 15 min and washed several times in distilled water.

3. Petri-dish test bioassay

Under laboratory conditions, 30 of the barnyard grass seeds or the slender amaranth seeds were placed into Petri dish lined with 2 germination paper sheets and 5 mL of distilled water. Then, different amounts of each oil (5 and 10 µL) were loaded (using a calibrated glass micropipette) onto a piece of filter paper attached (by double-sided adhesive tape) to the inner side of the cover of the Petri dish and sealed with Parafilm®. The Petri dishes were placed in a laboratory room. Four Petri dishes were maintained as replicates for each treatment in a completely randomized design. The 37 treatments included 2 amounts of 18 essential oils (5 and 10 µL) and another was distilled water used as control. After 7 days, the germination, hypocotyls length, and roots length were measured. The inhibition on seed germination and seedling of test weeds were the indicator following by the formulated:

% Inhibition = (1-sample) x100/ control

4. Phytotoxic effect by foliar application

In glasshouse experiment, 20 seeds of the barnyard grass and the slender amaranth, were sown in 15-cm-diameter plastic pots that contained soil [pH: 6.53; NO_3-N (mg 100/g dry soil): 20.0; OM (mg 100/g dry soil): 2.21; exchangeable K_2O and available P_2O_3 (mg 100 /g dry soil): 103.42 and 7.51]. Two weeks after sowing, the pots were thinned to 10 equal-sized healthy plants per pot. When the plants were 2-weeks old, oil solution was applied to the foliage using hand sprayer. The same 18 essential oils used as experiment 3.1 were dissolved in Tween20 5,000 ppm giving a concentration of 2% and 4% using a water spray volume of 1,000 L/ha. The pots were placed in a glass house. Four pots were maintained as replicates for each treatment in a

completely randomized design. There were 38 treatments including two concentrations of 18 oils solution viz., 2% and 4% ai., no spray (control), and tween20 5,000 ppm (check). A visual injury level adjusted from the European Weed Research System EWRS classification scale (CIBA-GEIGY, 1981) that showed in Table 2 was evaluated at 5 days after treatment.

Table 2 The European Weed Research System (EWRS) classification scale

Score	Phytotoxic
1	No symptoms/healthy plants
2	Very mild symptoms, slight stunted
3	Mild but clearly recognizable symptoms
4	More severe symptoms (e.g. chlorosis) not necessarily with negative effect on yield
5	Thinning out, heavy chlorosis or stunting, reduction in the yield to be expected
6 7 8 9	Heavy damage to total kill

Source: CIBA-GEIGY, 1981

Analysis of lemongrass (*C. citratus*) essential oil by GC-MS

In previous study, lemongrass essential oil showed the highest inhibitory activities on seed germination, seedling growth and the visual injury level of bioassay weeds Thus, it was selected for this study. Lemongrass essential oil was prepared as same as the experiment 3.1. Gas chromatography (GC) analysis of the essential oil was performed on a Dani Educational gas chromatograph fitted with a Carbowax column (30 m X 0.25 mm i.d., film thickness 0.325 µm). The oven temperature was programmed to rise from 50 ˚C to 215 ˚C at 4 ˚C/min then remain isothermal at 215 ˚C for 20 min. Typically, a 0.2 µL sample was injected with a split ratio of 1:30. Injection

and detector were maintained at 250 ˚C. The carrier gas was helium at a pressure of 1.4 bar. Peak area and retention time were calculated with a Shimadzu CR6A data processor, while the Kovĭats retention indices (KI) were determined on a series of nalkanes. GC–MS analysis was performed on a Carlo Erba HRGC 5160 Mega series coupled to a Finnigan MAT quadruple ion trap detector (ITD), operating at 70 eV with an ion source temperature of 220 ˚C. The GC was fitted with a 30 m × 0.25 mm i.d. Carbowax column. The carrier gas was helium at a pressure of 0.8 bar. The GC operating parameters were identical with those of GC analysis.

Comparative allelopathic effect of lemongrass essential oil and its major constituents against weeds by bioassay test

After the experiment 3.2, the major constituents of lemongrass essential oil were the monoterpene, citral, as a mixture of the sterioisomers geranial (41.94%) and neral (34.06%) as well as β-myrcene (10.39%), Z-β-ocimene (0.22%) and geraniol (4.63%). This chapter aimed to compare the activities of major constituent's viz., citral, myrcene and geraniol on seed germination and seedling growth of barnyard grass and slender amaranth by Petri dish test and on the visual injury level of these weeds by foliar application. Citral, geraniol and myrcene were purchased from Sigma Aldrich®, St. Louis.

1. Petri dish bioassay

At a laboratory condition, 20 seeds of barnyard grass or slender amaranth were placed into Petri dish lined with 2 germination paper sheets and 5 mL of distilled water. After that, different amounts of each compound were loaded (using a calibrated glass micropipette) on a piece of filter paper attached (by double-sided adhesive tape) to the inner side of cover of the Petri dish and seal with Parafilm®. The Petri dishes were placed in laboratory room. Four Petri dishes were maintained as replicates for each treatment in a completely randomized design. According to previous study, major constituents of lemongrass essential oil were citral ~ 75%, myrcene ~ 10%, geraniol 5%. Thus, the treatments were lemongrass essential oil at 1, 2, 4 and 8 µL, citral at 0.75, 1.5, 3 and 4.5 µL, myrcene at 0.2, 0.4 and 0.8 µL, and geraniol by 0.2 and 0.4 µL. Distilled water was used as control. After 7 days, the germination, hypocotyls

length, and roots length were measured. The inhibition on seed germination and seedling of test weeds were the indicator.

2. Phytotoxic effect by foliar application

A glass house experiment, 20 seeds of barnyard grass and slender amaranth, were sown in 15-cm-diameter plastic pot. The soil type was prepared as same as the experiment 3.1: foliar application. Pots were thinned to 5 equal-sized healthy plants per pot at 10 days after sowing. They were foliar application by each compound solution when plants were 15 day-old after sowing. Each compound was dissolved in using water spray volume of 100 ml/m^2. Pots were placed in a glass house. Four pots were maintained as replicates for each treatment in a completely randomized design. The treatments were lemongrass essential oil at 2%, 4% and 6%, citral at 1.5%, 3% and 6%, myrcene at 0.15%, 0.3% and 0.45%, geraniol at 0.075%, 0.15% and 0.3%, distilled water (control), and tween20 5,000 ppm (check). The visual injury level was evaluated at 5 days after treatment like experiment 3.1.

Study on the effect of lemongrass essential oil on α-amylase activity during seed germination

In order to understand the mechanisms effect on inhibited seed germination, α-amylase activity was analyzed. Barnyard grass was used as the model weed for studying this experiment. 60 seeds of barnyard grass were placed in Petri dish (9-cm diameter) lined with two layers of germination paper moistened with 5 mL of distilled water. Different amounts of lemongrass essential oil (1, 2, 4 and 8 μL) were loaded (using a calibrated glass micropipette) on a piece of filter paper attached (by double-sided adhesive tape) to the inner side of the cover of the Petri dish and then sealed with Parafilm®. α-Amylase activity was measured at 12, 24 and 36 h after treatment. Four Petri dishes were maintained as replicates for each treatment in a completely randomized design.

Extraction and measurement of activity of α-amylase (EC3.2.1.1) was performed by following the method of (Bernfield, 1955) and (Sadasivum and Manickam, 1996). Barnyard grass seeds (60 seeds for one determination) were ground to a fine powder in a mortar using a pestle. The powder was then homogenized with a 4-mL ice-cold solution of 0.1 M $CaCl_2$ and centrifuged at 9600 × g for 10 min.

Supernatant was used as the enzyme extract. The α-amylase activity was then assayed by measuring the rate of generation of reducing sugars from soluble starch. The reaction medium (3 mL) contained 1 mL of 1% soluble starch in acetate buffer solution at pH 5.5 and 1 mL of the supernatant. After incubation at 37 °C for 15 min, the reaction medium was terminated by adding 1 mL DNS reagent (40 mM 3,5 dinitrosalicylic acid, 0.4 M NaOH and 1 M K-Na tartrate), and immediately heated in a boiling water bath for 5 min. The mixture was cooled under running tap water. After dilution with distilled water, the intensity of color was measured at absorption at 560 nm in a spectronic GENESYS 20 spectrophotometer (Thermo Electron Corporation, USA). A standard graph was prepared using maltose, and the amount of α -amylase present in the sample was calculated from the standard curve and expressed as μmol maltose/min g (FW).

Study of physiological mechanisms on weed injury by foliar application

The effects of foliar-applied by lemongrass essential oil on growth and physiological mechanism of barnyard grass were studied. Plastic pots 15-cm in diameter and 15-cm in height were filled with soil prepared as same as the experiment 3.1. In each pot, 15 seeds of barnyard grass were sown at 1-cm depth. Pots were placed in experimental house with natural light conditions and irrigated daily with tap water. Emergent barnyard grass plants were thinned to 5 equal-sized healthy plants per pot at 14 days after sawing (DAS). Treatments used in this experiment were 1.25, 2.5, 5 and 10% (v/v) solution of essential oil or distilled water (control). A hand pressure sprayer filled with solid cone nozzle was used for spraying at a rate of 1,000 L/ha. Four pots were maintained as replicates for each treatment in a completely randomized design. All treatments including distilled water were sprayed in the randomized pots 28 DAS. The visual injury level of phytotoxic, chlorophyll a, b and carotenoid content, relative electrolyte leakage and lipid peroxidation were determined at 6 h after spray.

1. Estimation of chlorophyll and carotenoid content

Fresh leaves of barnyard grass cut at 0.5 cm diameter about 100 mg fresh leaf samples in all treatments were extracted with aqueous 80% acetone in a mortar using a pestle for 3 h and the suspension was filtered through a Whatman filter paper No. 1. Chlorophyll and carotenoid contents were determined spectrophotometrically

using spectronic GENESYS 20 spectrophotometer (Thermo Electron Corporation, USA) at 3 wavelengths: 663 nm for chlorophyll a, 647 nm for chlorophyll b and 470 nm for carotenoids. Calculations were completed using Lichtenthaler's equation (Lichtenthaler, 1987) and expressed as mg/g dry weight.

2. Membrane integrity

Membrane integrity of leaves was measured by electrolyte leakage. For each treatment, 5 leaf discs (0.5-cm diameter) were cut and floated abaxial side up on 5 mL of distilled water for 30, 60, 90 and 120 min at room temperature. After incubation, the conductivity was measured with a Consort C860 conductivimeter (Consort, Belgium).

3. Lipid peroxidation

The method of (Heath and Packer, 1968) was used to measure the thiobarbituric acid reactive substances (TBARs), a secondary product of lipid peroxidation. Fresh leaves in the control and treated leaves (nearly 0.3 g) were homogenized in 3 mL of 20% (w/v) trichloroacetic acid (TCA) and centrifuged at 10,000 ×g for 20 min. One milliliter of 20% TCA containing 0.5% (w/v) TBA and 100 µL 4% butylated hydroxytoluene in ethanol was added to 1 mL aliquots of the supernatant. The mixture was heated at 95 ˚C for 30 min and then quickly cooled on ice. The contents were centrifuged at 6000 ×g for 15 min and the absorbance was measured at 532 nm. The value for non-specific absorption at 600 nm was subtracted. The concentration of TBARS was calculated using an extinction coefficient of 155 mM/cm. The results were expressed as nmol/g FW.

Glasshouse evaluation of herbicidal formulation based on lemongrass essential oil

1. Preparing the natural herbicide

The natural herbicide based on lemongrass essential oil (NHL) was prepared by the mixing of lemongrass essential oil by 60 % w/w, white oil by 15% w/w, coconut diethanolamide by 15% w/w, Tween20 by 5% w/w, sodium lauryl sulfate by 1.25% w/w and water by 3.75% w/w that giving 60% active ingredient (ai). The NHL was kept in dark condition at 14 °C before use.

2. Pre and post emergence efficacy in the glasshouse

A flat 20 x 40 x 10 cm size containing 1.2 kg dried field soil [pH: 6.53; NO_3-N (mg 100/g dry soil): 20.0; OM (mg 100 g/ dry soil): 2.21; exchangeable K_2O and available P_2O_3 (mg 100 g/ dry soil): 103.42 and 7.51] was planted by 3 crops including corn (*Zea mays* L.), mung bean (*Vigna radiata* L.) and radish (*Raphanus sativus* L.), and 3 weeds including barnyardgrass, slender amaranth and finger grass). For pre-emergence test, the flats were sown with one row with following seeds: 10 corn and mungbean, 20 radish, 50 slender amaranths, barnyardgrass and fingergrass. After the seeds planted and watered for 1 day, the various concentrations of NHL solution was sprayed on soil surface using a water spray volume of 1,000 L/ha. There were 5 treatments viz., four concentrations of formulation (1%, 2%, 4%, and 8% (1, 2, 4 and 8 kg ai /ha) and distilled water (control). Four flats were maintained as replicates for each treatment in a completely randomized design. The emergence at 3, 7, 14 and 21 days after treatment was collected. The dried shoot and root biomass were collected at 14 days after treatment with oven-drying at 80 °C.

For post emergence test, the seeds of weeds and crops that were similar with pre emergence were sown in flats. The resulting seedlings were sprayed with the solution of formulation based on lemongrass essential oil at 14 days after seeded. These flats were thoroughly watered before treatment and not watered again until 2 days after spraying. The formulation was diluted in distilled water and sprayed using the water spray volume of 1,000 L/ha. The flats were placed in the glasshouse. Four flats were maintained as replicates for each treatment in a completely randomized design. The treatments were the concentrations of formulation viz., 0% (control), 1%, 2%, 4% and 8% ai (10, 20, 40 and 80 kg ai/ha). The visual injury level according and plant survival was determined at 5 and 14 days after spraying. The dried shoot biomass was collected at 14 days after treatment with oven-drying at 80 °C.

3. Efficacy of natural herbicide on different weeds and crops

This experiment aimed to study the dose-response of natural herbicide on different age of weeds and crops by foliar application. Seeds of barnyardgrass, fingergrass (*Chlrosis barbata* Sw.), wiregrass (*Eleusine indica* (L.) Gaertn.), rice (*Oryza sativa* L.), corn (*Zea may* L.), mungbean (*Vigna radiata* L.), chinese kale (*Brassica alboglabra*) hairy begger's tick (*Biden pilosa* L.), white head (*Eclipta*

prostrata L.), coat buttons (*Tridax procumbens* L.), sensitive plant (*Mimosa pudica* L.), wild poinsettia (*Euphorbia hetterophylla* L.), redweed (*Melochia corchorifolia* L.) and horse purslane (*Trianthema portulacastrum* L.) were harvested from agricultural field of Naresuan university, Thailand and dried under sunlight for 48 hours and kept for 15°C. Before use, the seeds were check germination percentage that had least 70%. The seeds were planted by seeds in the soil prepared as same as the experiment 3.1 in 9 cm-diameter square plastic pots. The seedlings were allowed to grow under a glass house condition. At 1 week after sowing, plants were thinned to 3 - 5 plants per pots depended on plant species. Until 1 to 4 weeks after sowing, individual plants were foliar spray by different concentrations of natural herbicides (1%, 2%, 4% and 8% ai.) at the spray volume of 1,000 L/ha. The visual injury level according to the experiment 3.1 was collected at 7 days after treatment. The dried shoot biomass was collected at 7 days after treatment with oven-drying at 55–60 °C. Four pots were maintained as replicates for each treatment in a completely randomized design.

Data analysis

Data were subjected to analysis of variance (ANOVA) with sums of squared partitioned to reflect trial effects and means were separated via Tukey's studentized range test at 95% level of probability.

CHAPTER VI

RESULTS AND DISSCUSSIONS

Screening allelopathic potential of essential oils

Effects of 18 essential oils on seed germination and seedling growth of test weeds by Petri dish test

Seed germination: Results shown in Table 2 demonstrate that the phytotoxic effects of 18 essential oils on seed germination were different depending on the essential oil's sources and doses. The dose of 10 μL had a more inhibitory effect than 5 μL. Lemongrass and citronella grass oils completely inhibited (100% inhibition) seed germination of slender amaranth at both doses (5 μL and 10 μL). Pine oil also completely inhibited at 10 μL only. Anise oil and black pepper oil at 10 μL inhibited seed germination of slender amaranth being 83.18% and 71.03% respectively. The other oils that showed greater than 40% inhibition were wintergreen oil (48.6%), ajowan oil (44.86%), tangerine oil (43.93%), kaffir lime oil and bay oil (40.19%). Only lemongrass essential oil showed complete inhibition of seed germination of barnyard grass at 10 μL, while citronella grass oil, pine oil and black pepper oil inhibited 81.58%, 60.53% and 42.11% respectively. Tangerine oil, kaffir lime oil and bay oil had the lowest inhibitory effect (>10%) on barnyard grass while bush tea oil and cajeput oil had the lowest (12.5%) on slender amaranth.

Shoot length: The complete inhibition (100%) on shoot elongation was caused by the complete inhibition of seed germination. The results of shoot elongation were similar to the results of seed germination. The higher dose (10 μL) had a more inhibitory effect than the low dose (5 μL). Citronella grass showed over 50% inhibition of shoot elongation (59.47%) of barnyard grass. While, black pepper and anise also showed over 50% on slender amaranth (61.11% equally). Bay oil and ajowan oil had the lowest inhibitory effect (>10%) on shoot elongation of barnyard grass, while bush tea oil had the lowest on slender amaranth (Table 4). At 5 μL, pummelo oil and lime showed a stimulatory effect on shoot elongation of barnyard

grass. Holy basil oil, hairy basil oil, white sage oil, tangerine oil, kaffir lime oil, lime oil, bay oil and ajowan oil also showed a stimulatory effect on slender amaranth.

Table 3 Effect of 18 essential oils at different doses on seed germination (%inhibition) of barnyard grass and slender amaranth

Essential oils	Barnyard grass		Slender amaranth	
	5 µL	10 µL	5 µL	10 µL
Lemongrass	45.61a	100.00a	100.00a	100.00a
Citronella grass	40.35ab	81.58b	100.00a	100.00a
Bush tea	14.04def	15.79f	8.41de	12.15f
Sweet basil	4.39fgh	10.53f	4.67de	20.56ef
Holy basil	1.75h	16.67ef	8.41de	20.56ef
Hairy basil	5.26fgh	14.91f	5.61de	26.17e
White Sage	4.39fgh	28.95e	3.74de	24.30ef
Tangerine	9.65efgh	9.65f	7.48de	43.93c
Kaffir lime	4.39fgh	9.65f	12.15de	40.19cd
Pummelo	4.39fgh	10.53f	2.80e	24.30ef
Lime	7.02efgh	12.28f	11.21de	28.97de
Anise	30.70bc	47.37d	25.23bc	83.18b
Pine	21.05cd	60.53c	37.38b	100.00a
Cajeput	3.51gh	10.53f	9.35de	12.15f
Bay	13.16defg	8.77f	8.41de	40.19cd
Black pepper	3.51gh	42.11d	15.89cd	71.03b
Wintergreen	4.39fgh	11.40f	13.08cde	48.60c
Ajowan	16.67de	17.54ef	7.48de	44.86c
MSD.	9.95	12.90	12.91	13.63

Mean values indicated by the same letter in a column did not differ significantly at 95% level using Tukey's studentized range test. MSD: minimum significant difference.

Root length: The results of root elongation were similar to the results of the seed germination and shoot elongation tests. Citronella grass and white sage oil showed over 50% inhibition of root elongation (79.51% and 51.71% respectively) of barnyard grass. Anise oil, black pepper oil and ajowan oil showed over 50% inhibition on slender amaranth (86.62%, 71.34% and 54.78% respectively). Bush tea oil, holy basil oil, kaffir lime oil and lime oil showed a stimulatory effect on root elongation of barnyard grass at 5 µL. However, there was no essential oils that had any stimulatory effect on root elongation of slender amaranth (Table 5).

18 essential oils were shown to have different inhibitory effects depending on the species from which the oils were extraction. These observations agree with earlier studies documenting the inhibitory effect of essential oils from aromatic plants. For example, essential oils from 32 aromatic plants extracted by hydro distillation were bioassayed on seed germination and seedling growth of wheat (*Triticum aestivum* L.), black mustard [Brassica nigra (L.) Koch] and palmer amaranth (*Amaranthus palmeri* S. Watson). Essential oil from *Origanum syriacum*, *Micromeria fruticosa* and *Cymbopogon citratus* showed the interesting inhibitory effect (Dudai, et al., 1999). De Almeida, et al. (2010) reported that essential oil from thyme (*Thymus vulgaris* L.), lemon balm (*Melissa officinalis* L.), vervain (*Verbena officinalis* L.) and caraway (*Carum carvi* L.) had more phytotoxic effect on germination and growth of *Raphanus sativus*, *Lactuca sativa* and *Lepidium sativum* than hyssop (*Hyssopus officinalis* L.), lavender (*Lavandula angustifolia* Mill.), marjoram (*Majorana hortensis* L.), basil (*Ocimum basilicum* L.), oregano (*Origanum vulgare* L.), sage (*Salvia officinalis* L.), caraway (*Carum carvi* L.), fennel (*Foeniculum vulgare* Mill.), anise (*Pimpinella anisum* L. Essential oils extracted by *Achillea gypsicola* Hub-Mor. and *Achillea biebersteinii* Afan. at 1 mg/mL soluted in 1% of DMSO-water solution completely inhibited the germination of *Amaranthus retroflexus* L., *Cirsium arvense* L. (Scop.) and *Lactuca serriola* L., and inhibited over 50% of *Chenopodium album* L., and *Rumex crispus* L. (Kordali, et al., 2009). Kaur, et al. (2010) reported that *Artemisia scoparia* Waldst et Kit. at 25 µL reduced germination of *Achyranthes aspera*, *Cassia occidentalis*, *Parthenium hysterophorus*, *Echinochloa crus-galli*, and *Ageratum conyzoides* by 31-74%. (Mabrouk, et al., 2013) reported that essential oils were obtained by hydrodistillation of different parts (flower heads, leaves, stems and roots)

of *Conyza sumatrensis* (Retz.) plants at 10 µL/mL inhibited the hypocotyl growth of *Raphanus sativus* varied from 28.6 to 90.1% and radicle from 42.3 to 96.2%.

Table 4 Effect of 18 essential oils at different doses on shoot elongation (%inhibition) of barnyard grass and slender amaranth

Essential oils	Barnyard grass		Slender amaranth	
	5 µL	10 µL	5 µL	10 µL
Lemongrass	20.00*a	100.00a	100.00a	100.00a
Citronella grass	11.58abc	59.47b	100.00a	100.00a
Bush tea	0.00cde	33.16c	13.89bcd	2.78e
Sweet basil	7.37bc	27.89c	0.00bcde	25.00cd
Holy basil	8.42abc	31.05c	-5.56cde	36.11bc
Hairy basil	2.63bcd	31.05c	-5.56cde	33.33bc
White Sage	11.05abc	36.32c	-16.67de	30.56cd
Tangerine	6.32bc	15.79ef	-19.44e	27.78cd
Kaffir lime	1.58cde	16.84de	-13.89de	30.56cd
Pummelo	-6.84de	5.79fg	11.11bcde	19.44cd
Lime	-10.00e	27.37cd	-5.56cde	22.22cd
Anise	11.05abc	34.74c	19.44bc	61.11b
Pine	11.58abc	35.79c	30.56b	100.00a
Cajeput	2.11cde	12.11efg	0.00bcde	27.78cd
Bay	5.26bcd	2.11g	-8.33cde	33.33bc
Black pepper	14.74ab	31.58c	8.33bcde	61.11b
Wintergreen	7.37bc	14.21ef	0.00bcde	33.33bc
Ajowan	2.63bcd	5.26fg	-2.78cde	36.11bc
MSD.	12.15	10.75	31.81	29.11

Mean values indicated by the same letter in a column did not differ significantly at 95% level using Tukey's studentized range test. MSD: minimum significant difference.

Table 5 Effect of 18 essential oils at different doses on root elongation (%inhibition) of barnyard grass and slender amaranth

Essential oils	Barnyard grass		Slender amaranth	
	5 µL	10 µL	5 µL	10 µL
Lemongrass	62.44a	100.00a	100.00a	100.00a
Citronella grass	49.27b	79.51b	100.00a	100.00a
Bush tea	-5.37ghi	39.51de	6.37g	43.95efg
Sweet basil	2.93efg	27.80fg	11.46fg	35.67g
Holy basil	-1.95fghi	28.78fg	5.10g	40.76efg
Hairy basil	1.46efgh	31.22efg	12.10efg	43.95efg
White Sage	4.39efg	51.71c	21.02de	35.03g
Tangerine	1.46efgh	11.22i	25.48d	37.58fg
Kaffir lime	-4.39ghi	15.12hi	18.47def	40.76efg
Pummelo	-10.24i	-8.78i	16.56def	35.67g
Lime	-8.78hi	23.90gh	15.92ef	36.31fg
Anise	17.56cd	36.10def	49.04c	86.62b
Pine	24.39c	35.12def	63.06b	100.00a
Cajeput	8.78de	28.29fg	11.46fg	49.04de
Bay	10.73de	37.07def	12.10efg	46.50def
Black pepper	15.61cd	41.46d	63.06b	71.34c
Wintergreen	11.71de	33.17defg	9.55fg	41.40efg
Ajowan	8.29def	39.02de	6.37g	54.78d
MSD.	10.28	10.08	9.34	10.6

Mean values indicated by the same letter in a column did not differ significantly at 95% level using Tukey's studentized range test. MSD: minimum significant difference.

Figure 16 Effect of 18 essential oils on seed germination and seedling growth of barnyard grass at the concentration of 10 µL/Petri dish

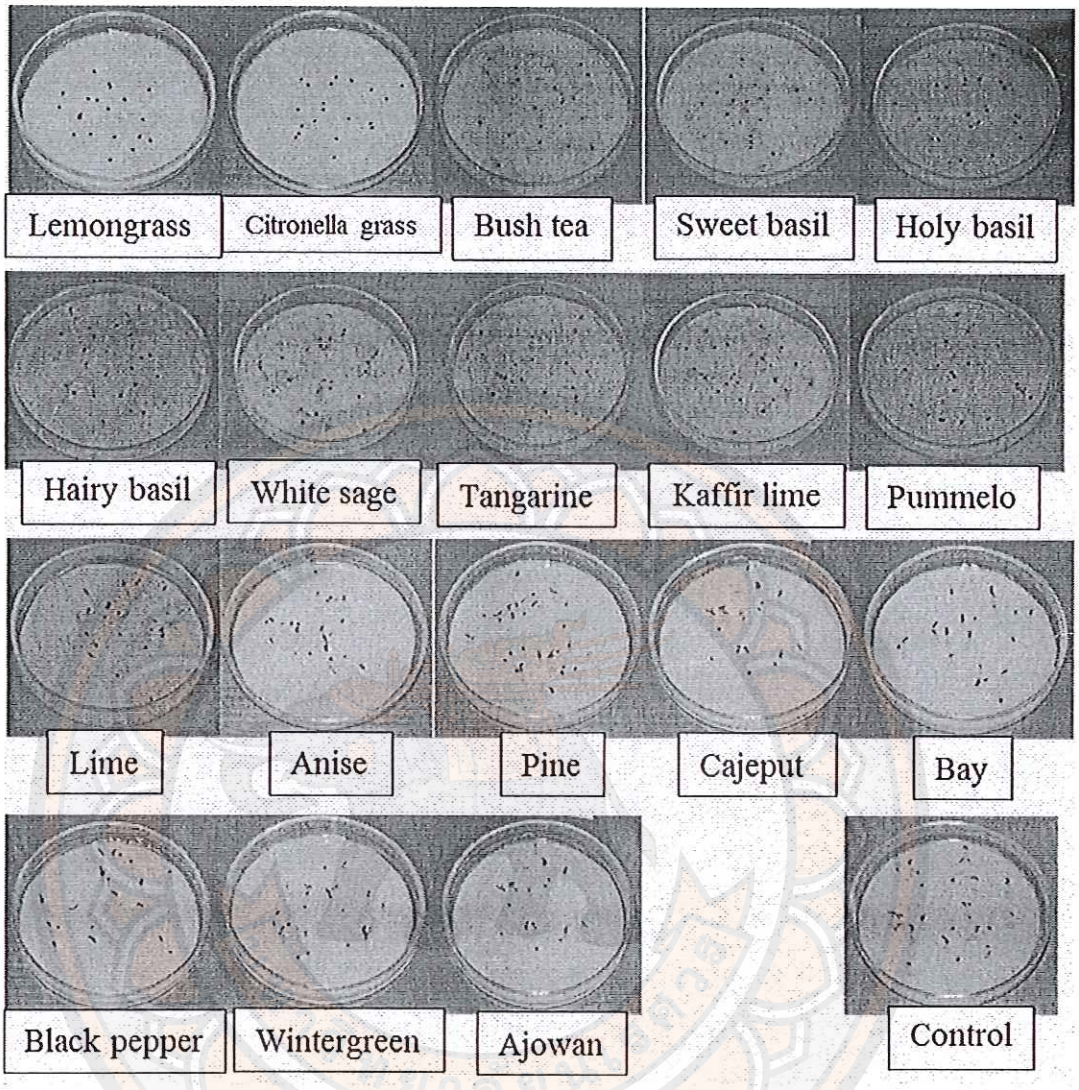


Figure 17 Effect of 18 essential oils on seed germination and seedling growth of slender amaranth at the concentration of 10 μL/Petri dish

The mechanisms by which essential oils inhibit seed germination and seedling growth remain unknown. However, there have been studied that reported that essential oils and their constituents inhibit cell division and interfere with DNA synthesis in growing root tips and meristems, respectively (Romagni, et al., 2000; Nishida, et al., 2005). However, a mechanism involving enzyme activity during seed germination was explained into a result of study on the effect of lemongrass essential oil on α-amylase activity during seed germination.

Phytotoxic effect of 18 essential oils by foliar application on barnyard grass and slender amaranth

The next experiment was for screening and selecting the best essential oils by foliar application. The results were similar to the results of Petri dish test. The level of injury of both weed species depended on the essential oil's source and the concentration of the oils. The Tween20 at the concentration of 5,000 ppm had no effect on the injury level of both weed species (Figure 18). Lemongrass essential oil showed the most phytotoxic effect on barnyard grass and slender amaranth followed by anise oil and citronella grass oil, respectively, with visual injury levels observed in 1 - 5 levels (Figure 18 A). At 4%, lemongrass essential oil completely killed (level 9) slender amaranth and nearly killed barnyard grass (level 8.5). There was little injury from holy basil oil, hairy basil, white sage oil, pine oil, black pepper oil and ajowan oil. Tangerine oil, kaffir lime oil, lime oil and pummel oil seemed to have no an injury effect on barnyard grass at both concentrations and caused little injury on slender amaranth at 4%. Barnyard grass seemed more sensitive than slender amaranth, except to tangerine oil, kaffir lime oil, lime oil and pummel oil. This different effect are probably to the different chemical compositions of the essential oils. The main constituent of lemongrass essential oil are citral (aldehydes geranial + neral), myrcene and geraniol (Chisowa, et al., 1998; Kasali, et al., 2001; Bassolé, et al., 2011). The major constituents of citronella grass essential oil are citronella and geraniol (Mahalwal and Ali, 2003). The main constituent of citrus families is limonene.

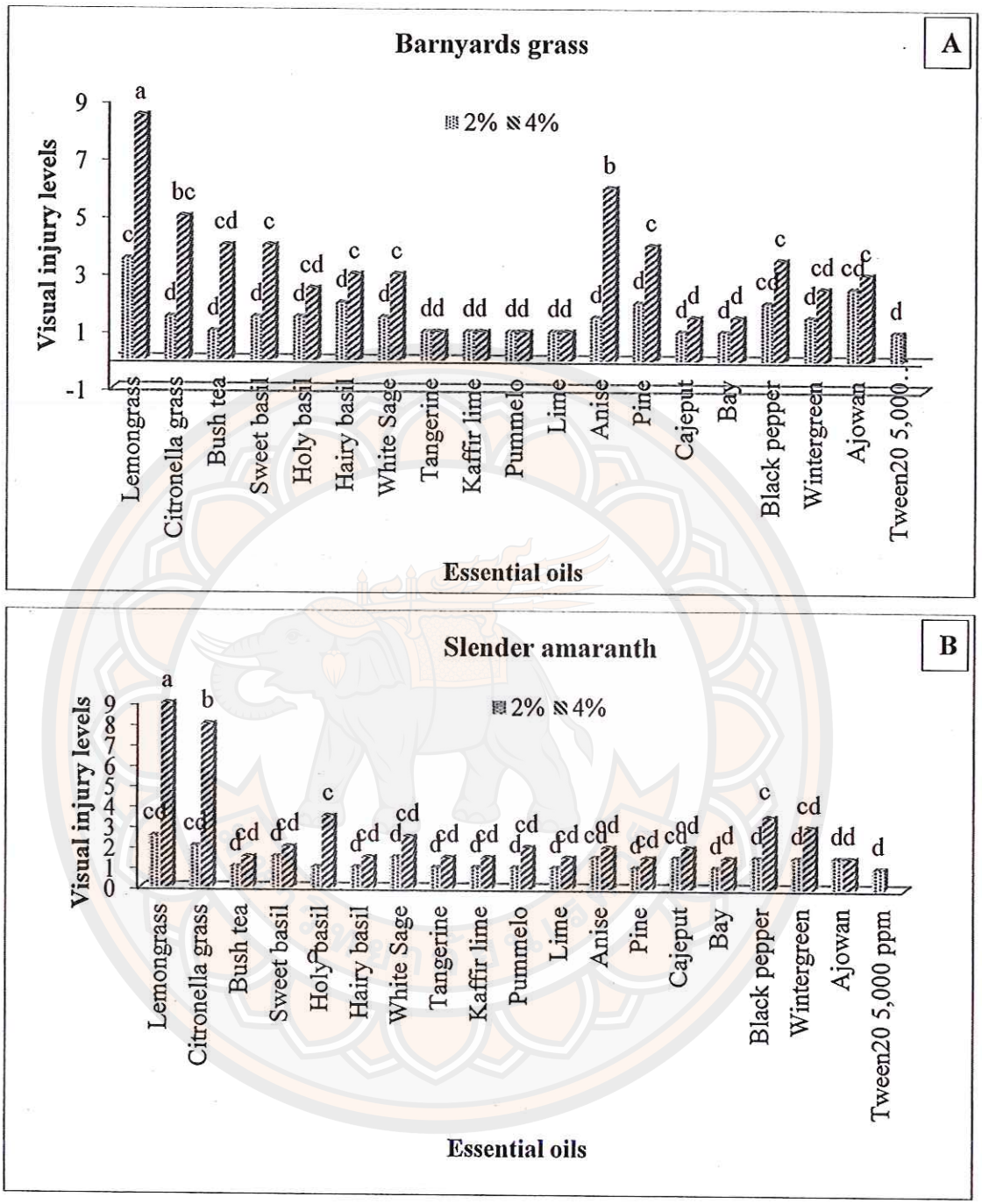


Figure 18 Visual injury levels of test weeds treated by foliar application by 18 essential oil at 7 days after treatment

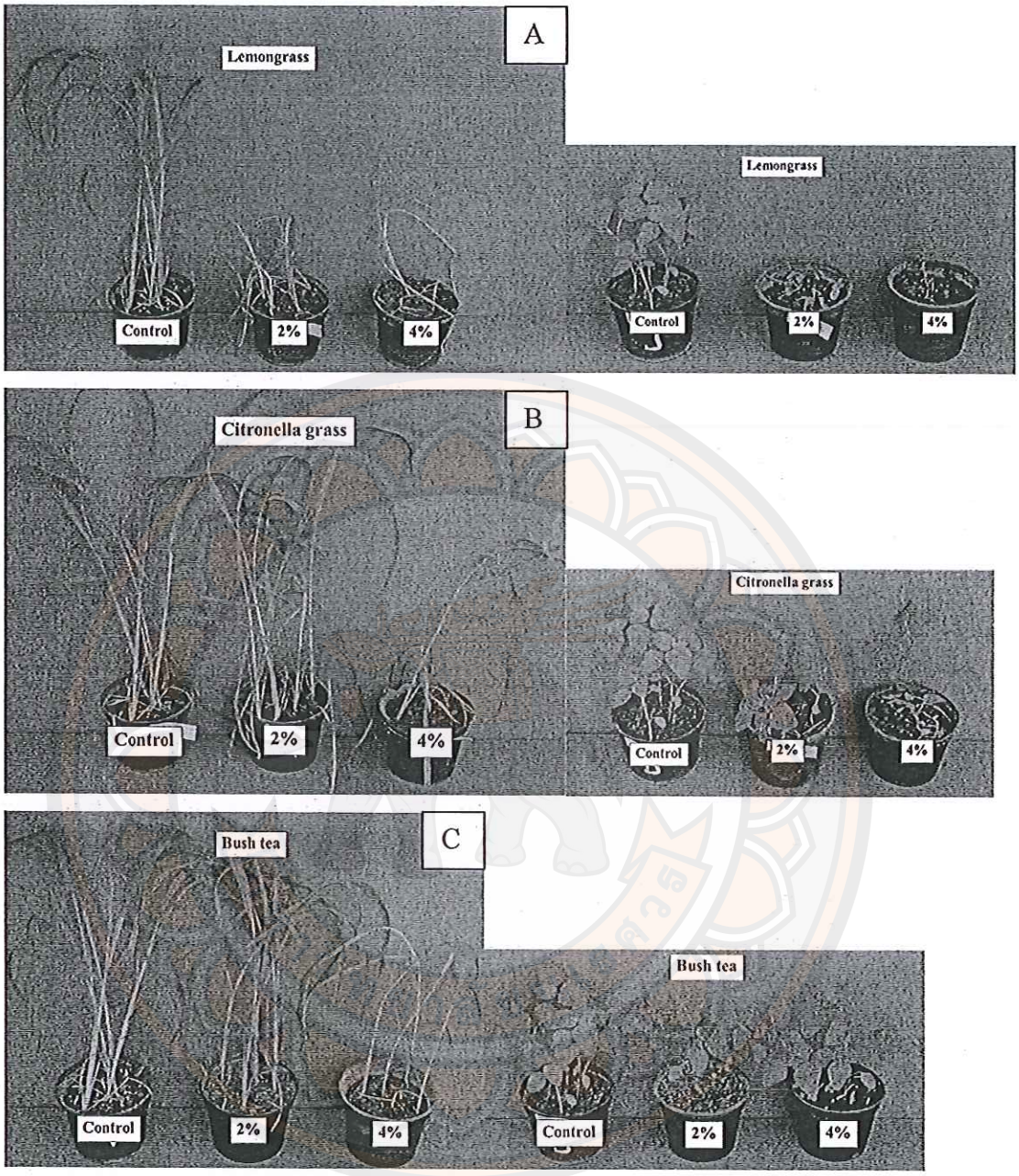


Figure 19 Effect of lemongrass essential oil (A), citronella grass oil (B) and bush tea oil (C) on barnyardgrass (right) and slender amaranth (left) by foliar application at 7 days after treatment

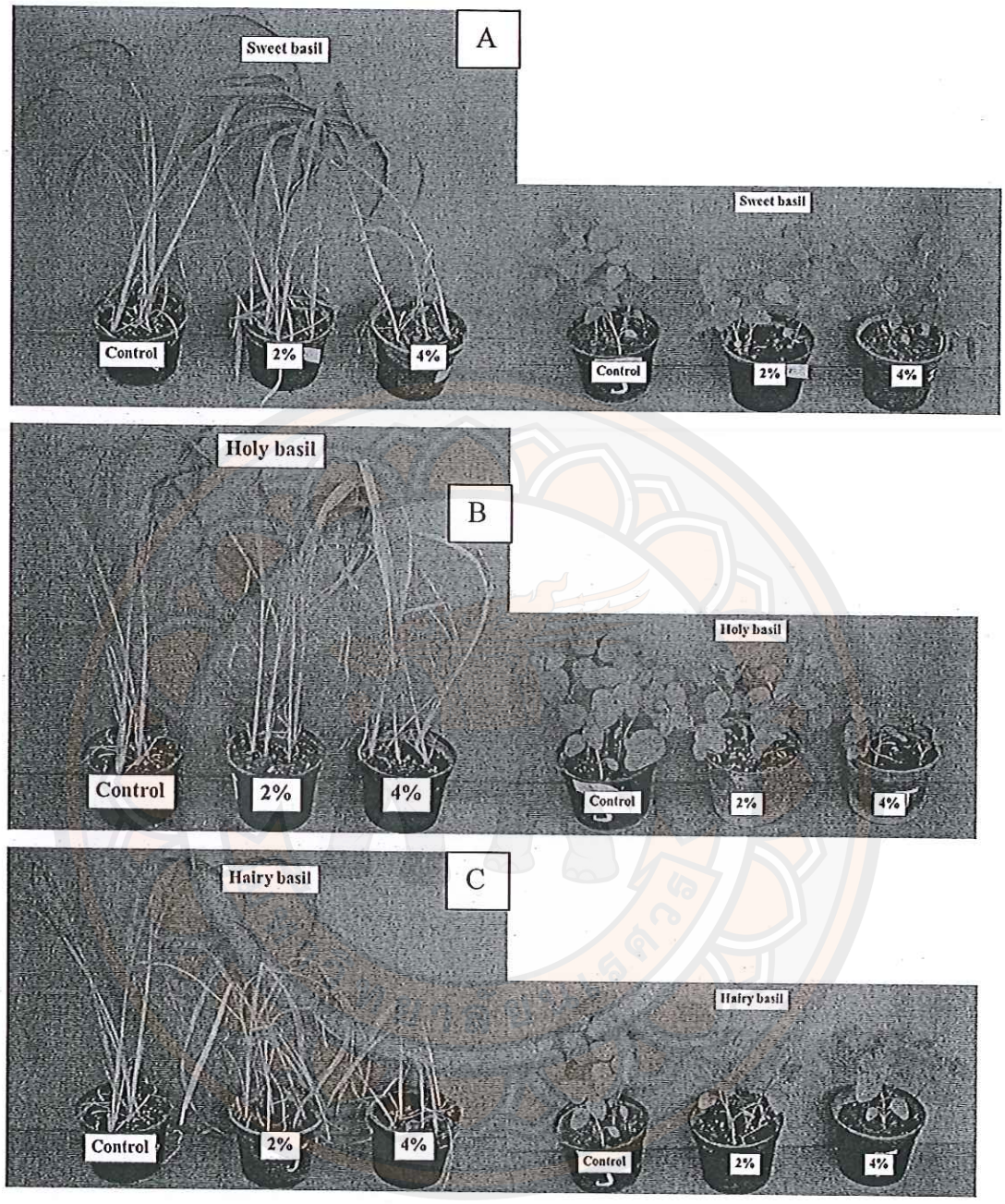


Figure 20 Effect of sweet basil oil (A), holy basil oil grass oil (B) and hairy basil oil (C) on barnyardgrass (right) and slender amaranth (left) by foliar application at 7 days after treatment

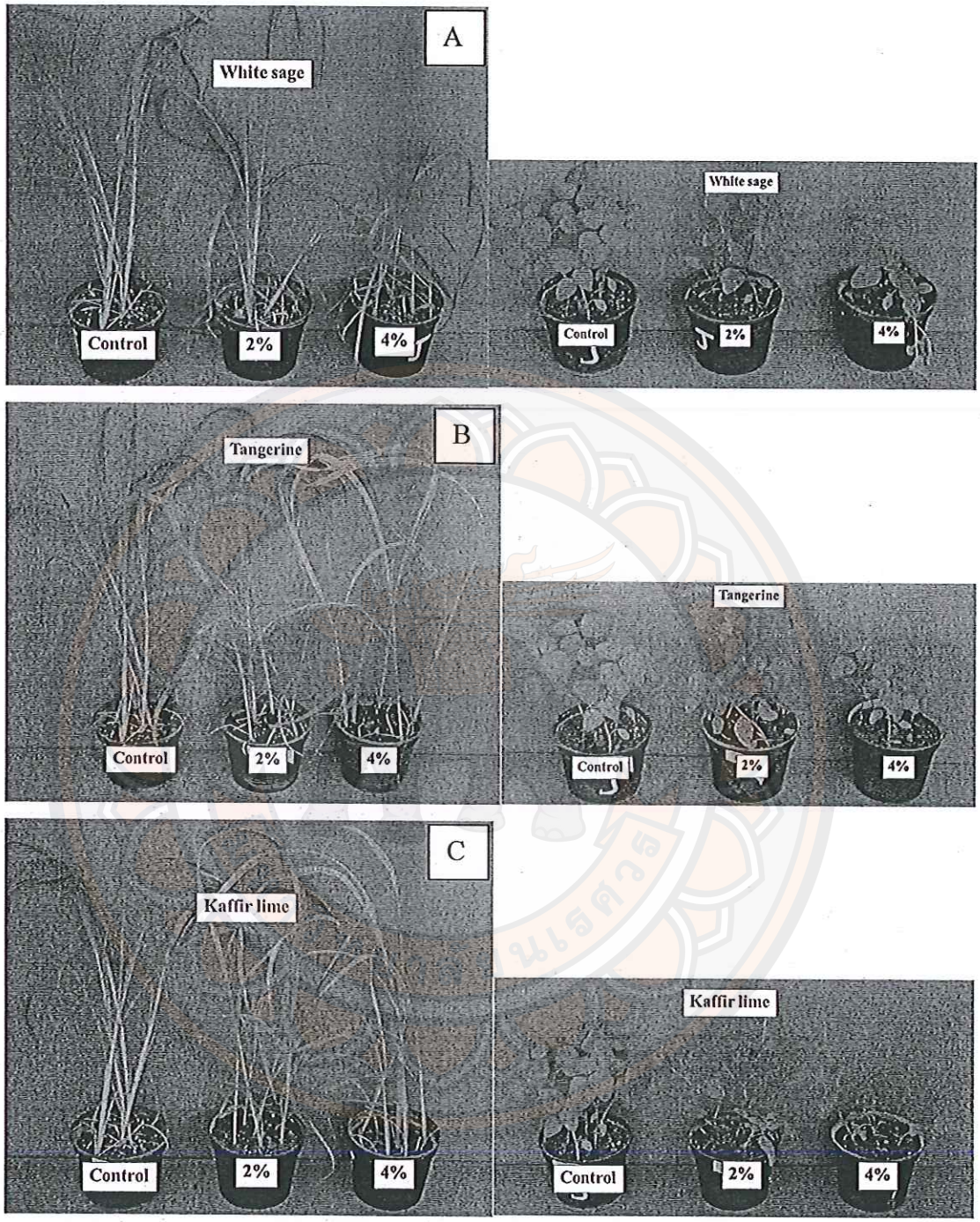


Figure 21 Effect of white sage oil (A), tangerine oil (B) and kaffir lime oil (C) on barnyardgrass (right) and slender amaranth (left) by foliar application at 7 days after treatment

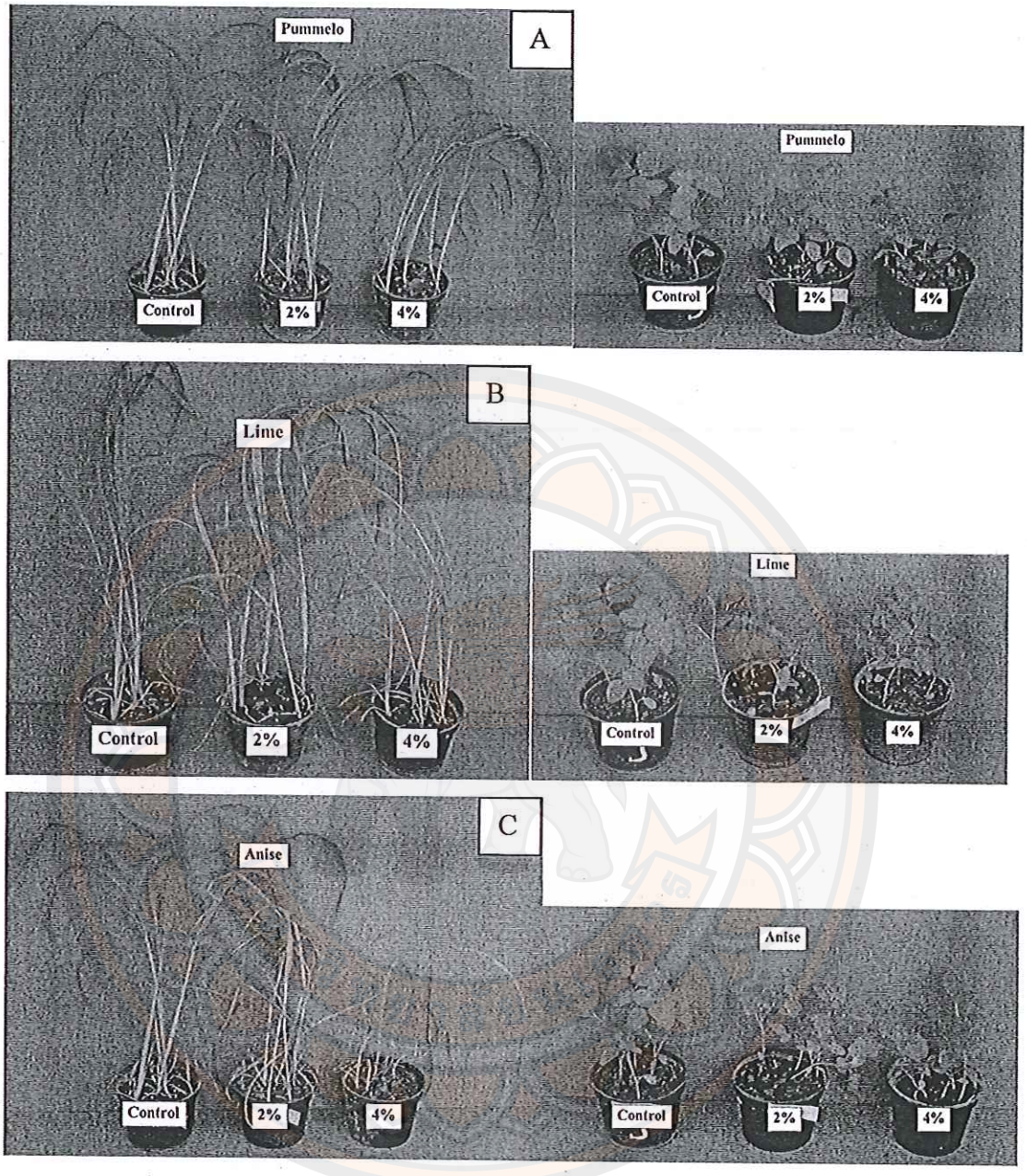


Figure 22 Effect of pummelo oil (A), lime oil (B) and anise oil (C) on barnyardgrass (right) and slender amaranth (left) by foliar application at 7 days after treatment

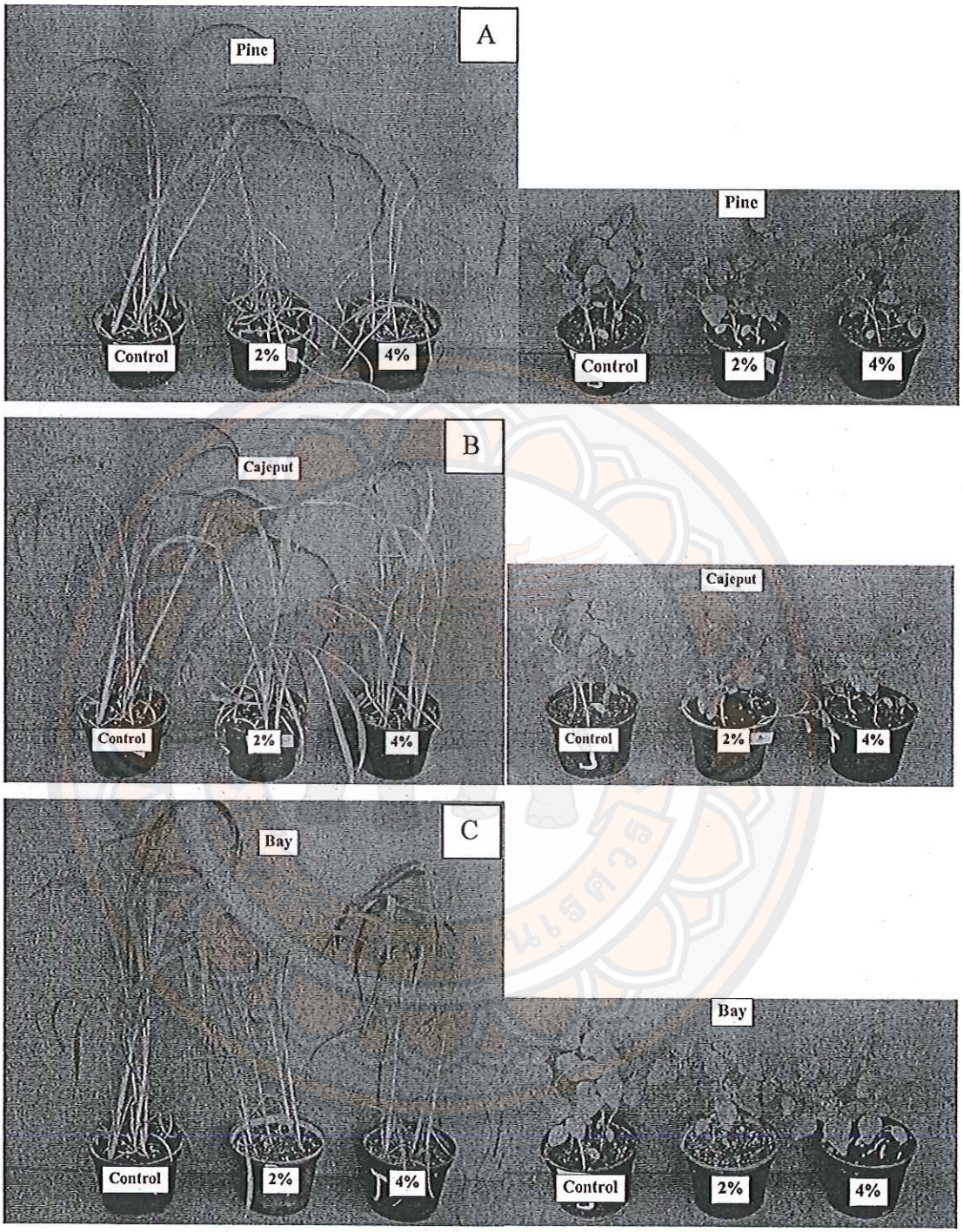


Figure 23 Effect of pine oil (A), cajeput oil (B) and bay oil (C) on barnyardgrass (right) and slender amaranth (left) by foliar application at 7 days after treatment

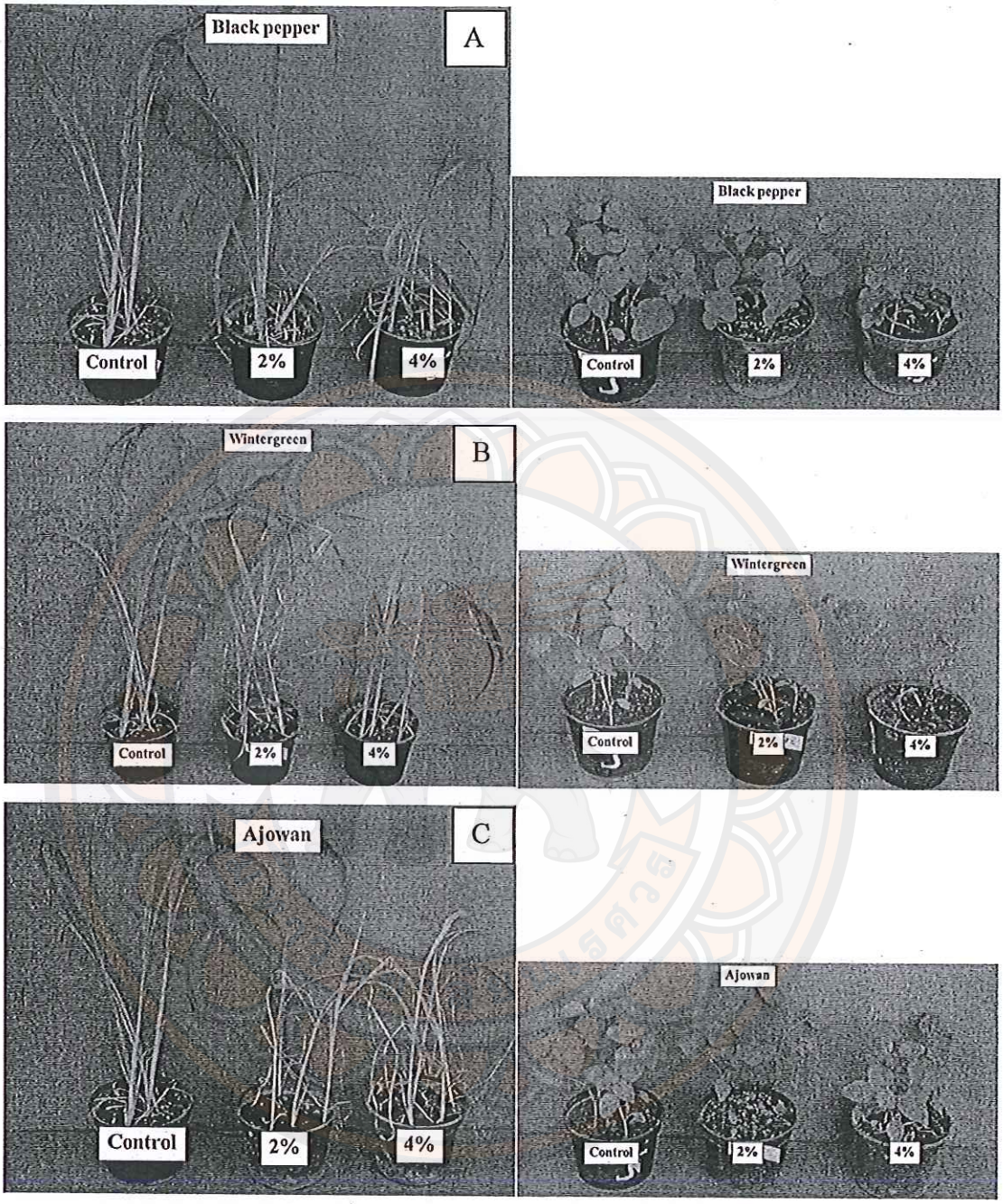


Figure 24 Effect of black pepper oil (A), wintergreen oil (B) and ajowan oil (C) on barnyardgrass (right) and slender amaranth (left) by foliar application at 7 days after treatment

Analysis of lemongrass (C. citratus) essential oil by GC-MS

According to the results of experiment 3.1, it has been that essential oil from lemongrass (Cymbopogon citratus) has the most phytotoxic effect both by Petri dish test in the laboratory and by foliar application in glasshouse conditions. Thus, lemongrass was selected for this experiment and for the next study. In order to identify the chemical constituents of lemongrass essential oil, the oil was obtained by hydro distillation by boiling of lemongrass fresh leaves, which gave an oil yield of 0.6% of the fresh weight of the leaf material. The chromatogram of the lemongrass essential oil, obtained by gas chromatography coupled with mass spectrometry, indicated monoterpene citral as a mixture of the sterioisomers geranial (41.94%) and neral (34.06%) as well as β-myrcene (10.39%), Z-β-ocimene (0.22%) and geraniol (4.63%) as its main compounds (Table 5).

This result suggested that citral is the major component (>70%) of lemongrass essential oil, similar to the results indicated earlier reports (Mahanta, et al., 2007; Negrelle and Gomes, 2007; Bassolé, et al., 2011; Vazirian, et al., 2012). However, there have been oil yield and minor component differences reported (Mahanta, et al., 2007; Bassolé, et al., 2011). Genetic diversity, habitat and/or agronomic treatment probably were the factors influencing these differences (Mahanta, et al., 2007).

Table 6 Relative percentage peak areas of six most abundant detected in lemongrass essential oil

No	RI[a]	Constituents	Percentage
1	921	2,6 Dimethyloctane	1.35
2	1156	β-Myrcene	10.39
3	1228	Z-β-ocimene	0.22
4	1680	Neral	34.06
5	1730	Geranial	41.94
6	1797	Geraniol	4.63

[a]Elution order and retention indices on a Carbowax column

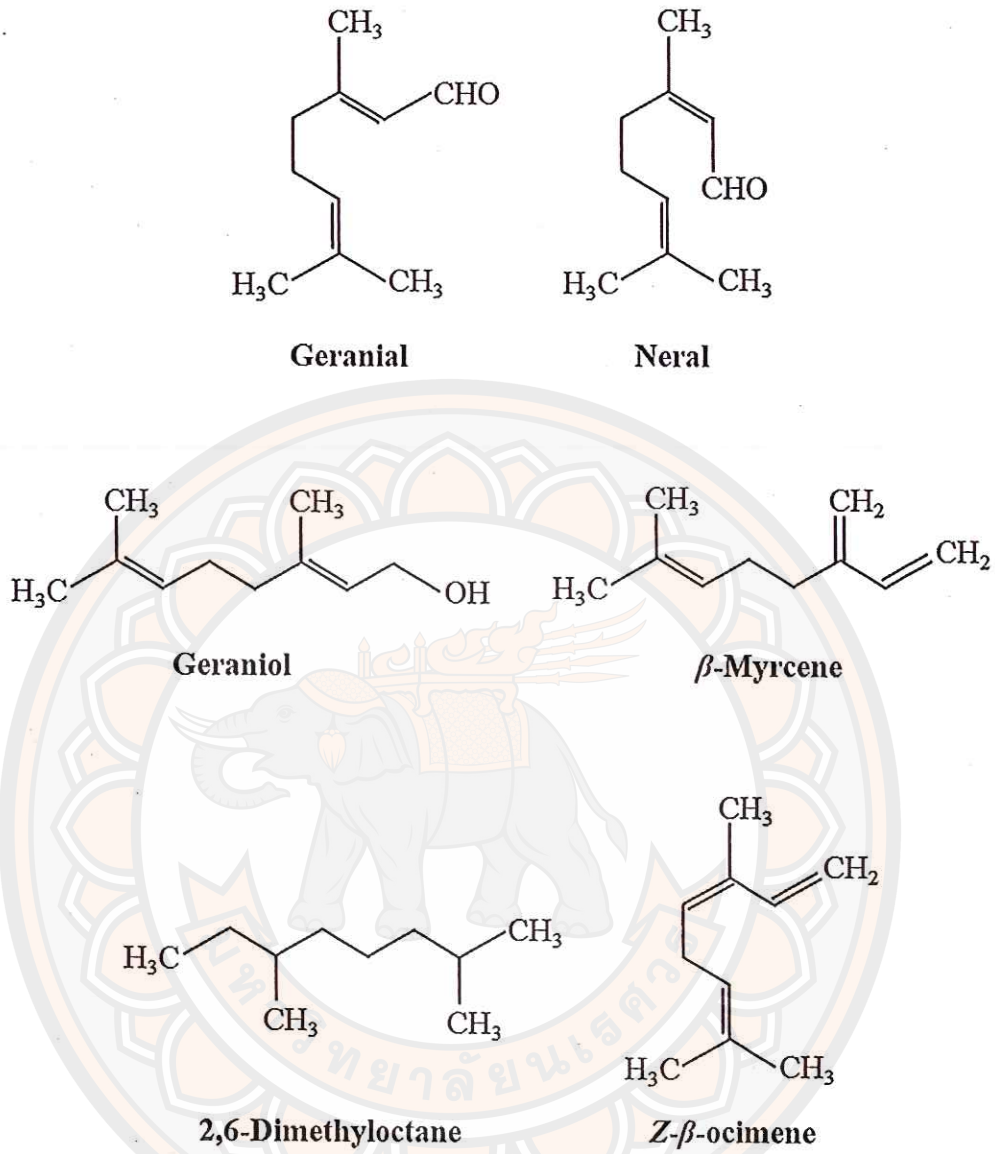


Figure 25 Structures of major constituents of lemongrass essential oil

Comparative allelopathic effect of lemongrass essential oil and its major constituents against weeds by bioassay test

Petri dish test

Over 90% of the major constituents of lemongrass essential oil have the combination of citral, geraniol and myrcene; citral about 75%, myrcene 10% and geraniol 5%. Thus, in the Petri dish test the lemongrass essential oil at 1, 2, 4 and 8 µL/Petri dish was compared with citral at 0.75, 1.5, 3 and 6 µL/Petri dish, while myrcene was 0.1, 0.2, 0.4 and 0.8 µL/Petri dish, and geraniol was 0.05, 0.1, 0.2 and 0.4 µL/Petri dish. Since the micropipette cannot be used for the treatments lower 0.2 µL/Petri dish, the treatment of myrcene at 0.1 µL, and geraniol at 0.05 and 0.1 µL could not be done. The result in seed germination and seedling growth of barnyard grass and slender amaranth shows that citral showed the major activity of lemongrass essential oil. Lemongrass essential oil at 4 and 8 µL/Petri dish showed the inhibition of barnyard grass germination by 57.50% and 100%, respectively. While, citral at equal doses (3 and 6 µL/Petri dish) was by 45% and 100%, respectively (Figure 26). In addition, the result in slender amaranth was similar to barnyard grass but slightly less (Figure 26 and Figure 27). Myrcene and geraniol at any dose showed not-significant effect on both germination and seedling growth of barnyard grass and slender amaranth when compared with the control (Table 7).

Table 7 Effect of lemongrass essential oil and it's major constituents (citral, myrcene and geraniol) at different concentrations on seed germination and seedling growth by Petri dish test

Treatments		Barnyard grass			Slender amaranth		
		Germination (%)	Shoot length (cm)	Root length (cm)	Germination (%)	Shoot length (cm)	Root length (cm)
Control		95.00a	4.50a	5.40a	90.00a	1.10a	4.85a
Lemongrass	1 µL	95.00a	4.80a	5.20a	20.00b	0.30b	0.50b
essential oil	2 µL	92.50a	4.35a	5.10a	0.00c	0.00c	0.00c
	4 µL	42.50b	3.25b	2.25b	0.00c	0.00c	0.00c
	8 µL	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c
Citral	0.75 µL	90.00a	4.45a	5.25a	35.00b	0.35b	0.65b
	1.5 µL	92.50a	4.20a	4.80a	0.00c	0.00c	0.00c
	3 µL	55.00b	3.40b	2.50b	0.00c	0.00c	0.00c
	6 µL	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c
Myrcene	0.2 µL	95.00a	4.85a	5.20a	88.50a	1.05a	4.85a
	0.4 µL	90.00a	4.95a	5.35a	90.00a	1.00a	5.15a
	0.8 µL	92.50a	5.10a	4.85a	88.50a	1.10a	5.00a
Geraniol	0.2 µL	88.50a	5.15a	5.20a	92.50a	1.15a	4.50a
	0.4 µL	92.50a	4.90a	5.35a	90.00a	1.10a	4.85a
MSD.		8.85	0.65	0.55	9.50	0.2	0.55

Mean values indicated by the same letter in a column did not differ significantly at 95% level using Tukey's studentized range test. MSD: minimum significant difference.

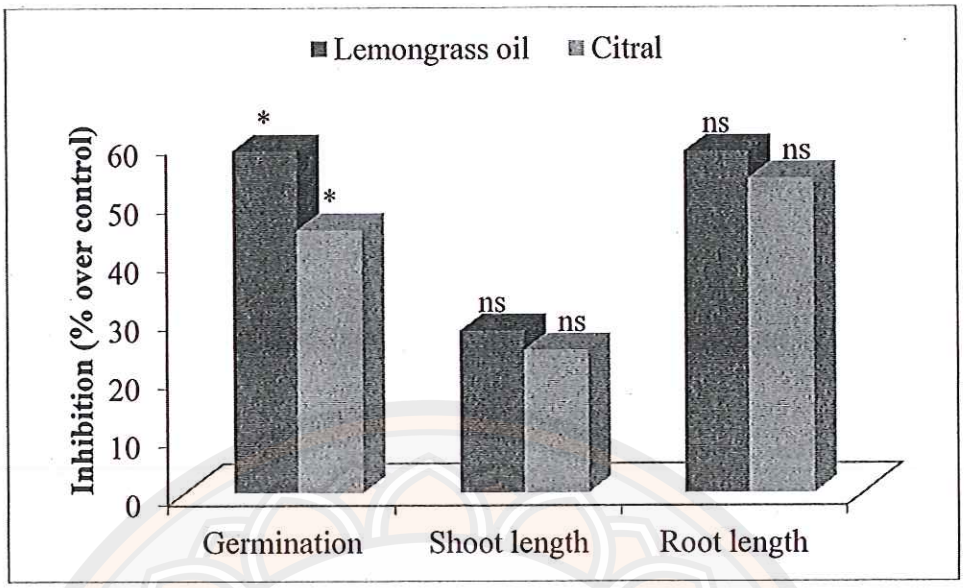


Figure 26 Comparison of lemongrass essential oil at 4 µL and citral at 3 µL (75% of lemongrass essential oil) on germination and seedling growth of barnyard grass

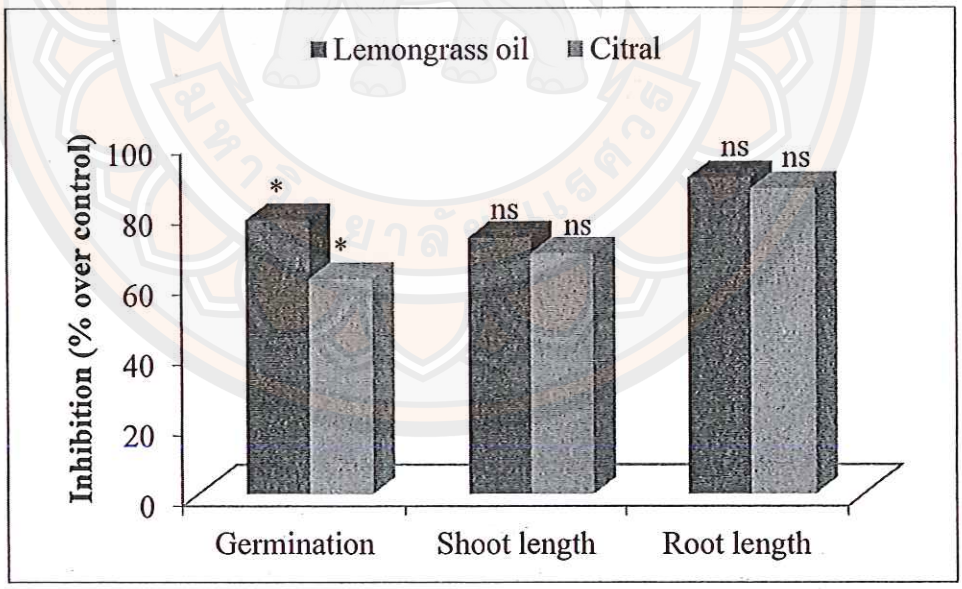


Figure 27 Comparison of lemongrass essential oil at 1 µL and citral at 0.75 µL (75% of lemongrass essential oil) on germination and seedling growth of slender amaranth

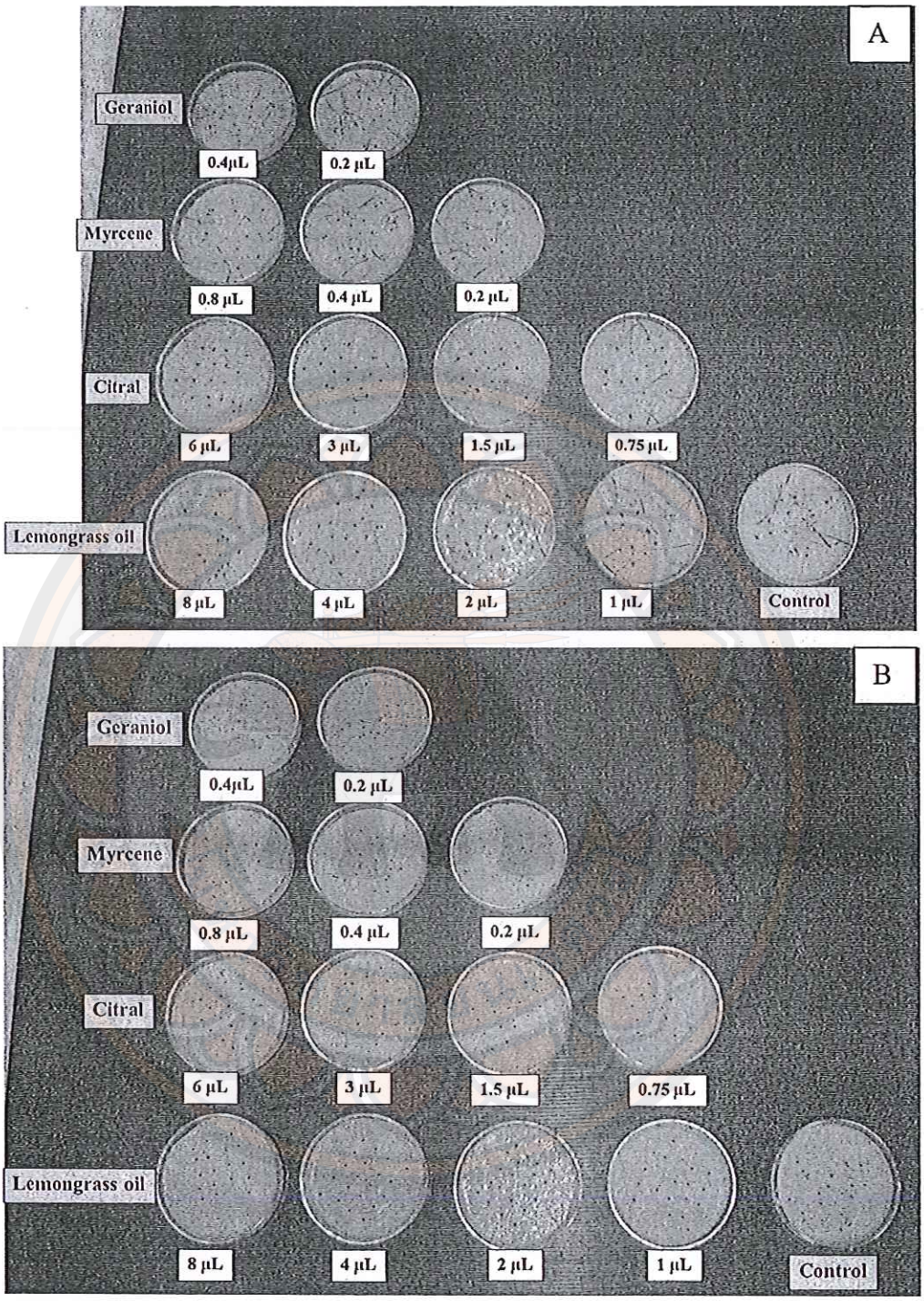


Figure 28 Effect of lemongrass essential oil, citral, myrcene and geraniol at different doses on seed germination and seedling growth of barnyard grass (A) and slender amaranth (B) at 7 days after treatment

Phytotoxic effect by foliar application

In a whole plant assay by foliar application, lemongrass essential oil at the concentrations of 2%, 4% and 6% ai were spray-applied on the 3-week-old barnyard grass and slender amaranth. At the equal rate of lemongrass essential oil, citral was applied at the concentrations of 0.75%, 1.5% and 3% ai, while myrcene at 0.2%, 0.4% and 0.6%, and geraniol at 0.1%, 0.2% and 0.3%, respectively. The result was similar to the Petri dish test. Citral showed the major activity on plant injury. Lemongrass essential oil at the concentration of 2%, 4% and 6% showed visual injury levels at 3, 8.5 and 9 respectively, while citral at 1.5%, 3% and 4.5% showed 2, 8 and 9, respectively (Table 8). The others, myrcene and geraniol, at all concentrations caused no injury. Thus, the active ingredient for herbicidal activity of lemongrass essential oil was citral.

Citral, scientific name: 3,7-dimethyl-2,6-octadienal, is a linear monoterpene with two geometric isomers - geranial (*trans*-citral or citral A) and neral (*cis*-citral or citral B) (Djordjevic, et al., 2008; Chaimovitsh, et al., 2010; Graña, et al., 2013). Citral presents a volatile component which is found in several different species such as *Cymbopogon citratus*, *Lippia alba* and *Melissa officinalis* (Quintans Jr, et al., 2011; Graña, et al., 2013). Citral is used for cosmetics, in food and in medical (Ben-Yehoshua and Ofir, 2010; Kamdem, et al., 2011; Modak and Mukhopadhaya, 2011; Quintans Jr, et al., 2011; Graña, et al., 2013). As well, it is used in agricultural industries as a bactericide, fungicide, insecticide and nematicide (da Silva, et al., 2008; Guimarães, et al., 2011; Singh, et al., 2011). Phytotoxic of citral on plants has been rarely studied. Citral is the main component of lemongrass essential oil about 70 – 80%. My results showed that the inhibitory effect on germination and seedling growth of citral in laboratory conditions, and the visual injury level in glasshouse condition was nearly with lemongrass essential oil when compared with equal rate (Figure 29). However, citral was slightly less effective than lemongrass essential oil, probably because of the synergistic effect of citral with other components in lemongrass essential oil.

Table 8 Effect of lemongrass essential oil, citral, myrcene and geraniol at different concentrations on visual injury level and dry weight of barnyard grass and slender amaranth

Treatments	Visual injury level		Dry weight (g)	
	Barnyard grass	Slender amaranth	Barnyard grass	Slender amaranth
Control	-	-	5.82a	1.84a
Lemongrass essential oil				
2%	3d	2c	5.41a	1.68a
4%	6c	9a	1.45b	0.82b
6%	9a	9a	-	-
Citral				
1.5%	2.5d	2c	5.54a	1.76a
3%	8b	9a	1.56b	0.93b
4.5%	9a	9a	-	-
Myrcene				
0.2%	1e	1d	5.74a	1.77a
0.4%	1e	1d	5.78a	1.74a
0.6%	1e	1d	5.82a	1.78a
Geraniol				
0.1%	1e	1d	5.87a	1.82a
0.2%	1e	1d	5.82a	1.74a
0.3%	1e	1d	5.84a	1.80a

Mean values indicated by the same letter in a column did not differ significantly at 95% level using Tukey's studentized range test.

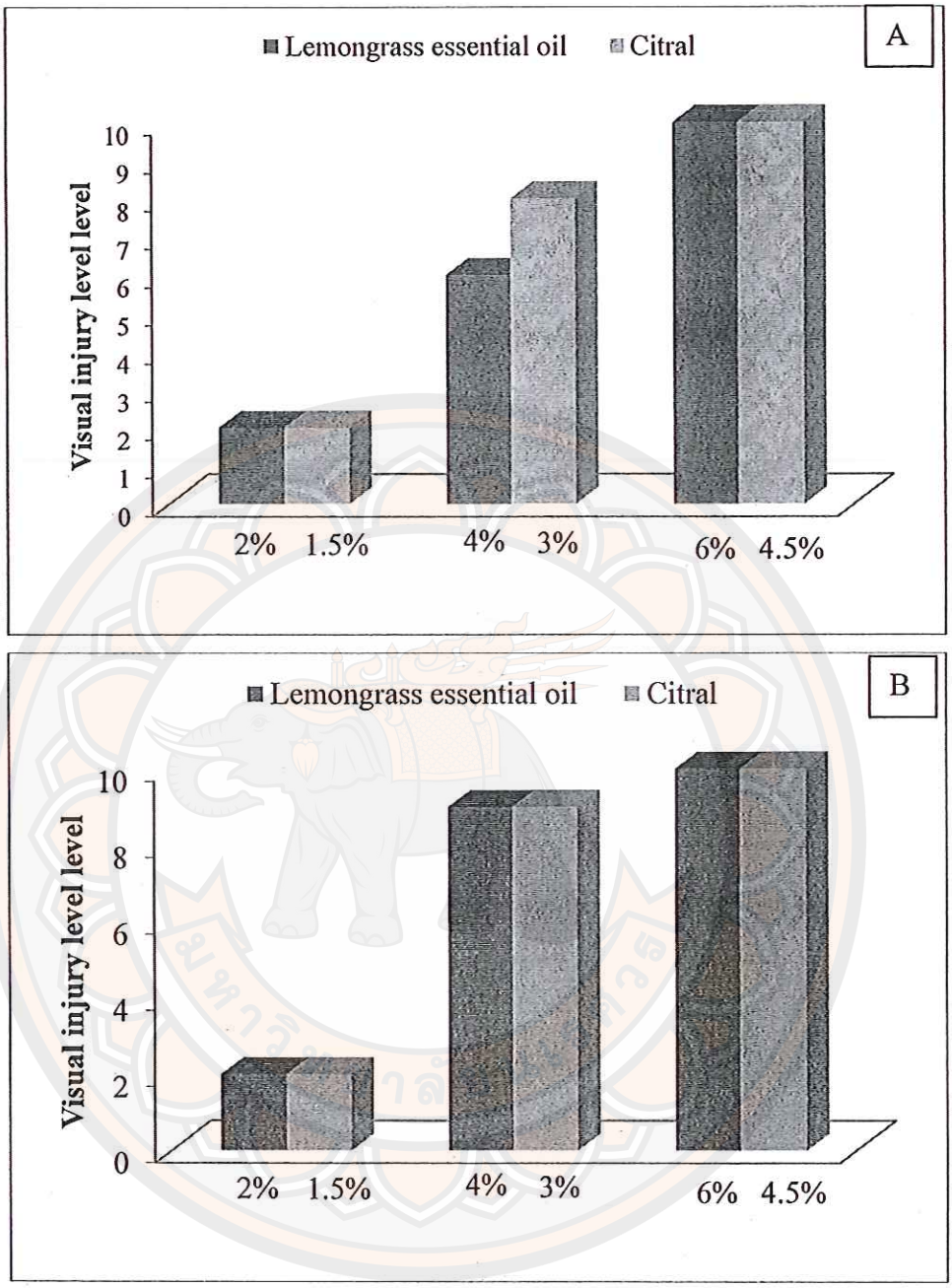


Figure 29 Comparison of lemongrass essential oil and citral at equal concentrations (2, 4 and 6% of lemongrass essential oil and 1.5, 3 and 4.5% of citral) on visual injury levels of barnyard grass (A) and slender amaranth (B)

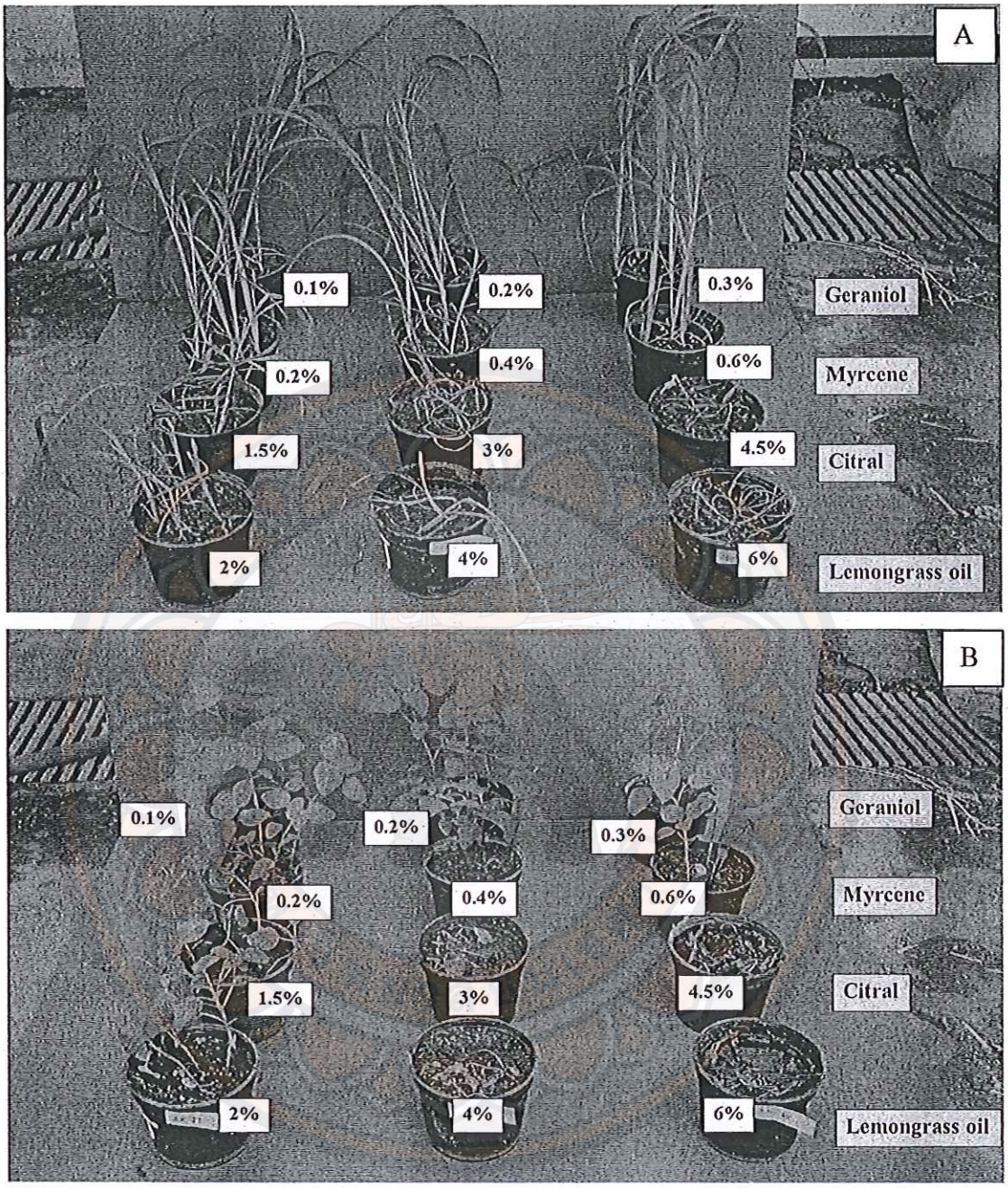


Figure 30 Effect of lemongrass essential oil, citral, myrcene and geraniol at different doses on seed germination and seedling growth of barnyard grass at 7 days after treatment

Effect of lemongrass essential oil on α-amylase activity during seed germination

As indicated by the results of experiment 3.1 and 3.3, lemongrass essential oil affected on the inhibition of seed germination and seedling growth of barnyard grass. To understand the mechanisms inhibiting seed germination, α-amylase activity was analyzed. The result showed a decrease in the induction of α-amylase activity. Changes of α-amylase activity in barnyard grass seeds after treatment are shown in Figure 31. The α-amylase activity of seeds increased in the concentration with germination, where radicals of barnyard grass seeds emerged almost at 36 h after sowing. Lemongrass essential oil initially decreased the induction of α-amylase activity within 12 h after treatment, and the inhibitory effect was greater with greater oil concentrations. At 36 h, the inhibitions of activities in seeds treated with oil were 20.55%, 39.59%, 49.51% and 57.45% at concentrations of 1, 2, 4 and 8 µL/Petri dish, respectively.

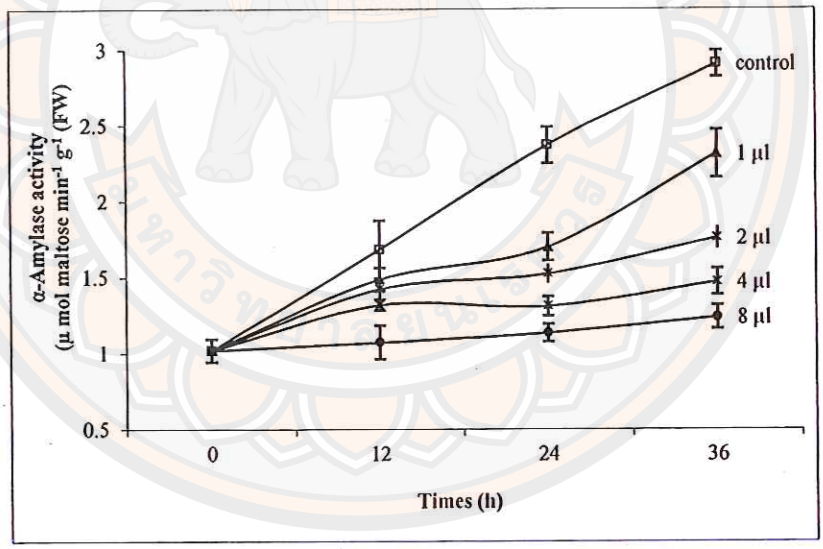


Figure 31 Changes in α-amylase activity of barnyard grass seeds. The seeds were treated with lemongrass essential oil in the dark at 25 oC

The study correlated with herbicidal activity of essential oils with their α-amylase inhibitory effect. The study correlated herbicidal activity of essential oils with their α-amylase inhibitory effect. The effect of essential oil from *Teucrium polium* has been attributed to decreased activities of α-amylases (Bigham, et al., 2010). Nevertheless, essential oil from wood of *Cedrus libani* has been reported to exhibit α-amylase inhibitory activity (Loizzo, et al., 2007). Furthermore, some plant extracts and allelochemicals have been documented as inhibiting amylase activity. For example, ethyl acetate fraction of *Aglaia odorata* Lour. extract at 1,000 – 8,000 ppm reduced seed imbibition and α-amylase activity of barnyard grass seeds (Teerarak, et al., 2012). Zhang, et al. (2011) reported that root exudates from ginseng inhibited seed germination and α-amylase activity of both ginseng and American ginseng. (Kato-Noguchi and Macías, 2005) reported that 6-methoxy-2-benzoxazolinone (MBOA), allelochemical commonly found in root exudates from some families, at concentrations ranging from 0.03 to 3 mmol/L inhibited α-amylase activity of lettuce (*Lactuca sativa* L.). 2,4-dihydroxy-7-methoxy - 2H - 1,4 - benzoxazin - 3 (4H)- one (DIMBOA), 2,4 – dihydroxy - 2H - 1,4 -benzoxazin-3(4H)-one (DIBOA) and benzoxazolin-2(3H)-one (BOA) also inhibited the induction of α-amylase activity in barley seeds. They also gibberellin-induced α-amylase activity in de-embryonated barley seeds (Kato-Noguchi, 2008).

Lemongrass essential oil that reduced amylase activity indicates that the mechanism on inhibiting seed germination probably involves the inhibition of enzyme activity. For germination, plant seeds produce metabolic energy and biosynthetic precursor by its respiratory metabolism. Carbohydrates and soluble sugars are supplied to its respiratory metabolism. However, because of limitations of soluble sugars, starches accumulated in seeds are the main reserve carbohydrates. Therefore, α-amylase has been determined as playing a major role in the degradation of reserve carbohydrates to soluble sugars that produce metabolic energy and biosynthetic precursor. (Perata, et al., 1997; Kato-Noguchi and Macías, 2005). The induction of α-amylase was not only on seed germination but also subsequent seedling growth until photosynthesis sufficient to support growth (Beck and Ziegler, 1989; Kato-Noguchi, 2008). For this reason, essential oil's inhibition of inducing α-amylase activity may decrease seed germination rates and seedlings growth in barnyard grass.

Physiological mechanisms on weed injury by foliar application

Spray-application of lemongrass essential oil on barnyard grass has been shown to result in visual injury. The symptoms ranged from chlorosis to necrosis to even complete wilting of barnyard grass. These observations indicated that lemongrass essential oil induces severe injuries in barnyard grass upon contact. The symptoms of the injury level were initially seen at 1 hour after spray application. The injury level increased over the 7 days after spraying. Then, the seedlings recovered, at 2.5%. Increasing the concentrations showed an increase in the injury level of the barnyard grass. The lemongrass essential oil nearly completely killed the barnyard grass seedlings at the concentration of 10% while it was seemed to be non-effective at 1.25%.

Figure 32 Whole plant response of 4-week-old barnyard grass by foliar application with different concentration of lemongrass essential oil at 3 days after spraying

Such observations parallel many earlier studies. For example, eucalypt oil at 8% and 10% completely damaged 4-week-old seedlings of *C. occidentalis* and caused 80% damage of *E. crus-galli* (Batish, et al., 2004). Clove oil (*Syzygium aromaticum* (L.) Merr. & L. M. Perry) at 2.5% concentration reduced seeding growth of common lambsquarters (*Chenopodium album* L.) and redroot pigweed (*Amaranthus retroflexus*

L.) 91% and 99%, respectively (Bainard, et al., 2006). Essential oil from *Artemisia scoparia* Waldst et Kit. at 6% concentration completely killed 6-week-old *E. crus-galli* and caused 60 - 80% injury of *Achyranthes aspera*, *Cassia occidentalis*, *Parthenium hysterophorus* and *Ageratum conyzoides* (Kaur, et al., 2010).

Chlorophyll and carotenoid contents

Treated leaves were analyzed for pigment composition at 6 h after spraying. The results showed a decrease in both total chlorophyll and carotenoid contents, depending on Lemongrass essential oil concentration (Table 8). Increasing essential oil concentration decreased chlorophyll a, chlorophyll b and carotenoid contents. Contents of chlorophyll a were reduced by 47.73%, 29.19%, 21.55% and 12.06% at concentrations of 1.25%, 2.5%, 5% and 10% of the control, respectively. Likewise, chlorophyll b was reduced by 47.79%, 33.89%, 24.73% and 16.16%, and carotenoid by 48.23%, 26.01%, 20.13% and 10.58%, respectively. The decrease in chlorophyll a, and b and carotenoid content slightly varied (Table 9). Chlorophyll a decreased more than chlorophyll b, which led to a reduction in the chlorophyll a/b ratio while the reduction in carotenoid content was similar to that of chlorophyll a.

A reduction in chlorophylls and carotenoid contents in response to lemongrass essential oil suggests its negative impact on photosynthesis. The observed effects were similar to the effects of *Eucalyptus citriodora* and *Artemisia scoparia* essential oils that reduced chlorophylls content and thus affected photosynthetic activity (Batish, et al., 2004; Singh, et al., 2005; Kaur, et al., 2010). Chlorophyll a, b and carotenoid are specific major pigments in plants detected in the chloroplasts that capture light energy (Scott, 2008). However, whether the reduction in chlorophyll content is due to a decrease in de novo biosynthesis of chlorophylls or its enhanced degradation is unknown. Nevertheless, results showed a decline in carotenoid content. This may cause a result of carotenoid deficiency-induced photooxidation of the chlorophylls (Dankov, et al., 2009).

Table 9 Effect of foliar-applied of Lemongrass essential oil on chlorophyll a, chlorophyll b and carotenoids content of E. crus-galli leaf at 6 h after treatment

Concentration (% oil)	Chlorophyll a (mg/g DW)	Chlorophyll b (mg/g DW)	Carotenoid (mg/g DW)	Chlorophyll a/b ratio
0 (control)	43.37 ± 10.24 a (100%)	15.25 ± 3.19 a (100%)	9.66 ± 2.36 a (100%)	2.84 ± 0.11 a
1.25	20.70 ± 7.31 b (47.73%)	7.29 ± 2.36 b (47.79%)	4.99 ± 1.50 b (48.23%)	2.82 ± 0.14 a
2.5	12.66 ± 3.72 bc (29.19%)	5.17 ± 1.69 bc (33.89%)	2.60 ± 0.81 bc (26.01%)	2.47 ± 0.19 b
5	9.34 ± 1.18 bc (21.55%)	3.77 ± 0.45 bc (24.73%)	1.94 ± 0.27 bc (20.13%)	2.48 ± 0.03 b
10	5.23 ± 0.88 c (12.06%)	2.47 ± 0.30 c (16.16%)	1.02 ± 0.21 c (10.58%)	2.11 ± 0.12 c

Data are expressed as means+ S.E.

Mean with same letters in column is not significantly different at $P < 0.05$.

Values in the parentheses are percentage over control.

Relative electrolyte leakage

An obvious electrolyte leakage difference was showed different concentrations of essential oil. Electrolyze leakage initially changed at 30 min after exposure. Changes of electrical conductivity values at different concentrations and times are shown in Figure 33. However, there was no significant difference in treatment of 1.25% and 2.5% compared with the control. At 10% essential oil concentration, the electrolyte leakage obviously increased over times.

The increase in electrolyte leakage indicates that lemongrass essential oil disrupts the membrane integrity resulting in increased permeability and thus enhanced solute leakage. The observations made in the present study are in agreement with earlier reports the essential oils and their constituent terpenes inhibiting plant growth through electrolyte leakage (Singh, et al., 2005; Kaur, et al., 2010).

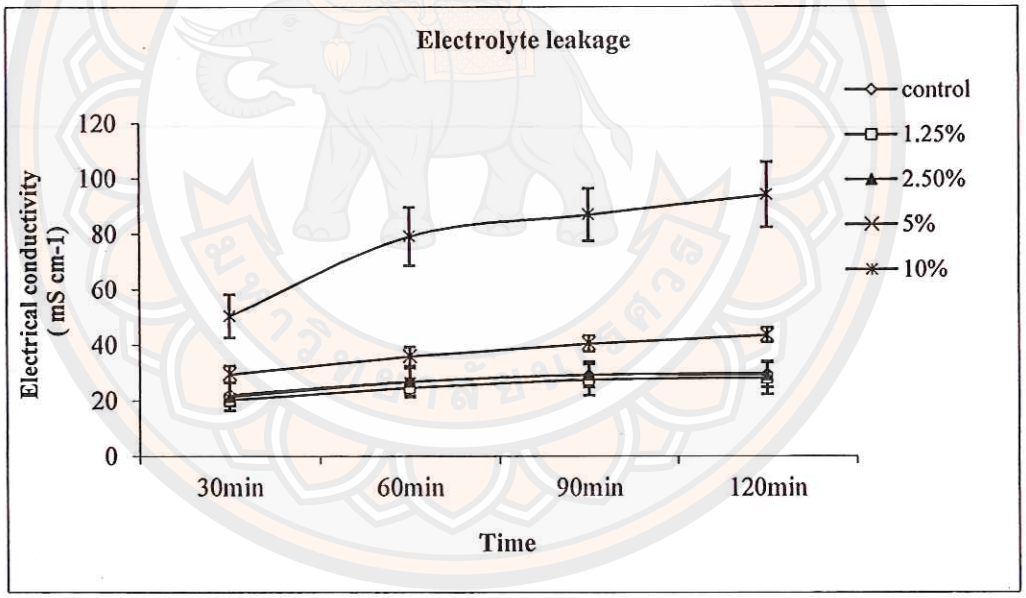


Figure 33 Change of electrolyte leakage from barnyard grass leaf disks treated with different concentration of lemongrass essential oil

Lipid peroxidation

The results of TBARS content in sprayed leaves of 4-week-old barnyard grass are shown in Figure 34. The amount of TBARS increased with increased essential oil concentration. At concentrations of 0 (control), 1.25%, 2.5%, 5% and 10%, the TBARS content was 6.12, 10.91, 21.02, 23.90 and 38.27 nmol/g FW, respectively.

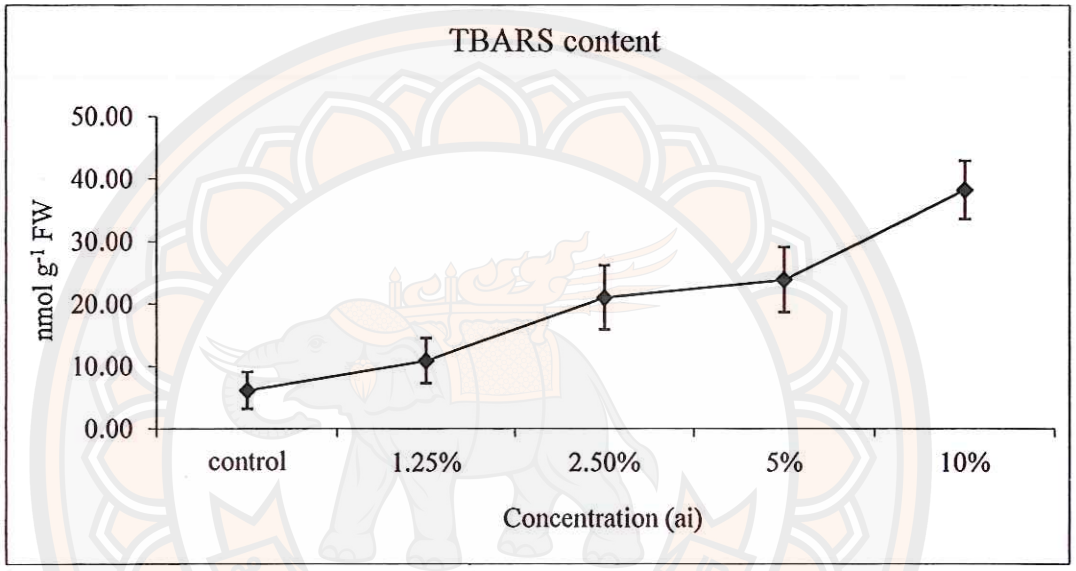


Figure 34 Effect of *C. citratus* oil on TBARS in barnyard grass plants after foliar application for 6 h

The change of membrane permeability in turn affects other physiological and biochemical processes linked to membrane functioning. Thus, lipid peroxidation was measured. Lipid peroxidation refers to the oxidative degradation of lipids. The TBARS assay is the most commonly used test for malondialdehyde (MDA) content, the end-products of lipid peroxidation (Marnett, 1999). As fatty acids and other lipids are known as structural components of membranes, it is reasonable to suppose that membrane degradation could result in free lipids within the cytoplasm of targeted cells. Then, the free lipids within the cytoplasm could be the target of an oxidative action (Scrivanti, et al., 2003). Results demonstrated that the amount of TBARS increased with increasing concentrations of *C. citratus* essential oil (Figure 33). This

suggests that lipid peroxidation is occurring. Some studies have reported that volatile oil from several allelopathic plants and their monoterpenes caused accumulation of H_2O_2 in some plants (Singh, et al., 2006; Singh, et al., 2009; Mutlu, et al., 2011). Accumulation of H_2O_2 in weed plants further enhance lipid peroxidation, thus increasing oxidative stress, and leading to disruption of cell metabolic activities. In addition, it has been suggested that the effect of the essential oils is to disrupt the permeability of the cell membrane structure of weeds. Such a phenomenon is due to the penetration of monoterpenes through the cell wall and cell membrane, or causes a leakage of cellular potassium that inhibits glucose-dependent respiration (Mutlu, et al., 2011).

Glasshouse evaluation of herbicidal formulation based on lemongrass essential oil

Pre and post emergence efficacy in the glasshouse

On the pre-emergence test, the formulation was been sprayed on the soil surface at 1 day after planted. On the post-emergence test, the formulation at different concentrations was foliar-sprayed on 2 week-old test plants. NHL had no effected on pre-emergence at all concentrations. The emergence and shoot biomass of crops and weeds was not significant when compared with control (Table 10). The result of post-emergence effects of NHL was more striking. Even the highest concentration (8%) killed all tested plants except corn and mungbean. At 4% ai slender amaranth and radish were also completely killed, while other species had severe injury levels between 1 – 5 levels (Table 11). There was little injury on all test plants at 2%. There was no-significant effect on all test plants at 1% ai. Radish seemed to be the most sensitive species to NHL.

The effects of NHL that had been shown to have greater herbicidal activity on post-emergence than on pre-emergence was caused by soil factors, such as being adsorbed by soil solids and metabolized by chemical and biological reactions during the movement in soil (Kobayashi, 2004). These factors did not appear on foliar application.

The strong post-emergence effect of NHL suggested that its herbicidal effects should be tested on different weeds and crops to understand its selectivity and activity on different stages of plants.

Table 10 Emergence and biomass of six plant species 14 days after pre-emergence spray with NHL

Parameters	Species	Dose (kg active ingredient/ha)				
		0	1	2	4	8
Seedling emergence (as % of seeds planted)	Corn	100a	100a	100a	100a	100a
	Mungbean	90a	90a	90a	90a	90a
	Radish	100a	100a	95a	90a	90a
	Slender amaranth	90a	90a	85a	85a	80a
	Barnyardgrass	90a	90a	90a	85a	85a
	Fingergrass	85a	80a	80a	80a	80a
Shoot biomass (g)	Corn	0.89a	0.88a	0.86a	0.84a	0.78a
	Mungbean	1.22a	1.28a	1.24a	1.15b	1.08b
	Radish	0.56a	0.54a	0.57a	0.53a	0.51a
	Slender amaranth	0.78a	0.77a	0.75a	0.73a	0.68a
	Barnyardgrass	1.45a	1.56a	1.53a	1.49a	1.39a
	Fingergrass	1.12a	1.09a	0.97a	0.95a	0.92a

Means for a particular species followed by a different lowercase letter differ at $P \leq 0.05$ in Tukey's studentized range test.

Table 11 Plant injury level (expressed as percentage of seedling initially present at time of spraying) of six plant species 7 days after post-emergence spray with NHL

Parameters	Species	Concentrations (% active ingredient)				
		0	1	2	4	8
Injury level*	Corn	-	0d	1c	3b	7a
	Mungbean	-	0d	1c	3b	7a
	Radish	-	0c	5b	9a	9a
	Slender amaranth	-	0c	3b	9a	9a
	Barnyardgrass	-	0c	1c	6b	9a
	Fingergrass	-	0c	2c	8b	9a

Means for a particular species followed by a different lowercase letter differ at $P \leq 0.05$ in Tukey's studentized range test.

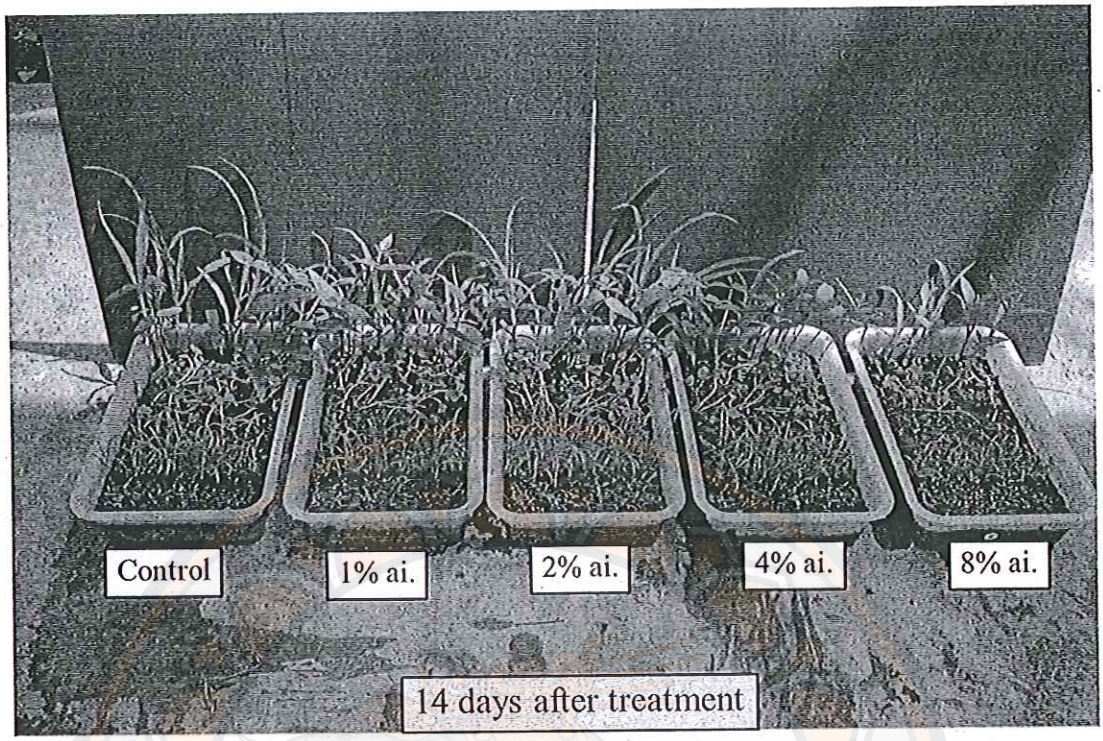


Figure 35 Pre-emergence effects of natural herbicide based on lemongrass essential oil (NHL) shown 7 days after application to seeds planted in soil. (Application rates (left to right) are 0 (control), 1, 2, 4, and 8 % ai by spray volume at 1,000 L/ha. The plant species, as shown by the control, are (front to rear of flats) finger grass, barnyardgrass, slender amaranth, radish, mungbean and corn)

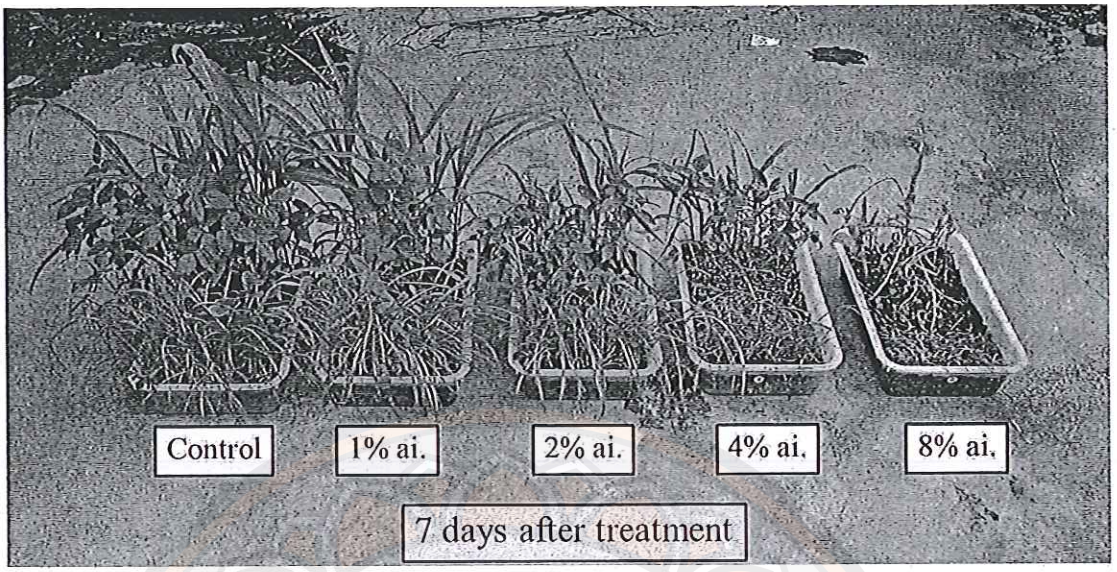


Figure 36 Post-emergence effects of NHL shown 7 days after spraying on emerged seedlings. (Application rates (left to right) are 0 (control), 1, 2, 4, and 8 % ai by spray volume at 1,000 L/ha. The plant species, as shown by the control, are (front to rear of flats) finger grass, barnyardgrass, slender amaranth, radish, mungbean and corn)

Effects of natural herbicide based on lemongrass essential oil on different weeds and crops

A survey of glasshouse-grown crops and weeds for post-emergence sensitivity to NHL at the concentrations of 1%, 2%, 4% and 8% ai was tested by foliar application at the sprayed volume on 1,000 L/ha on 1, 2, 3 and 4 week-old plants of 4 crops and 10 weeds. The results showed that the herbicidal effect of NHL depended on weed species, stage of plants and the concentrations of NHL. At 8% ai NHL completely killed 1, 2 and 3 week-old plants to all species except mungbean, sensitive plant, wild pointsettia and white head (Figure 39 - 64). The 4-week-old samples of all plants were severely injured but they subsequently recovered. There was no significant effect at 1% ai on all plants. Table 12, shows a wide range of susceptibility of 2-week-old plants. At 8% ai, all plants were completely killed except sensitive plant that showed severe injury (8 levels) but subsequently recover. At 4% ai coat buttons and horse purslane were completely killed, while others showed severe injury (6-8) except mungbean and sensitive plant which showed moderate injury (4 and 5 levels, respectively). Coat buttons seem to be the most sensitive to NHL because it was severely injured at 2% ai (8 levels), while other plants showed only slight injury (1 – 3 levels). At 1% no injury was noted on corn, mungbean, barnyardgrass, wiregrass, hairy begger's tick, white head, coat buttons and horse purslane, while the others showed slight injury (1 – 2 levels).

Table 12 Species sensitivity survey 7 days after post-emergence spray with foliar NHL on 2-week-old of 4 crops and 10 weeds

Species	Concentrations (% active ingredient)			
	1	2	4	8
Corn	0d	2c	7b	9a
Rice	1d	3c	7b	9a
Mungbean	0c	1c	4b	9a
Chinese kale	2c	3c	7b	9a
Barnyardgrass	0c	2b	8a	9a
Fingergrass	1d	4c	7b	9a
Wiregrass	0d	4c	6b	9a
Hairy begger's tick	0d	3c	8b	9a
White head	0d	2c	6b	9a
Coat buttons	0c	7b	9a	9a
Sensitive plant	1d	3c	5b	7a
Wild pointsettia	1d	4c	7b	9a
Redweed	1d	4c	9b	9a
Horse purslane	0b	0b	9a	9a

Means for a particular species as same row followed by a different lowercase letter differ at $P \leq 0.05$ in Tukey's studentized range test.

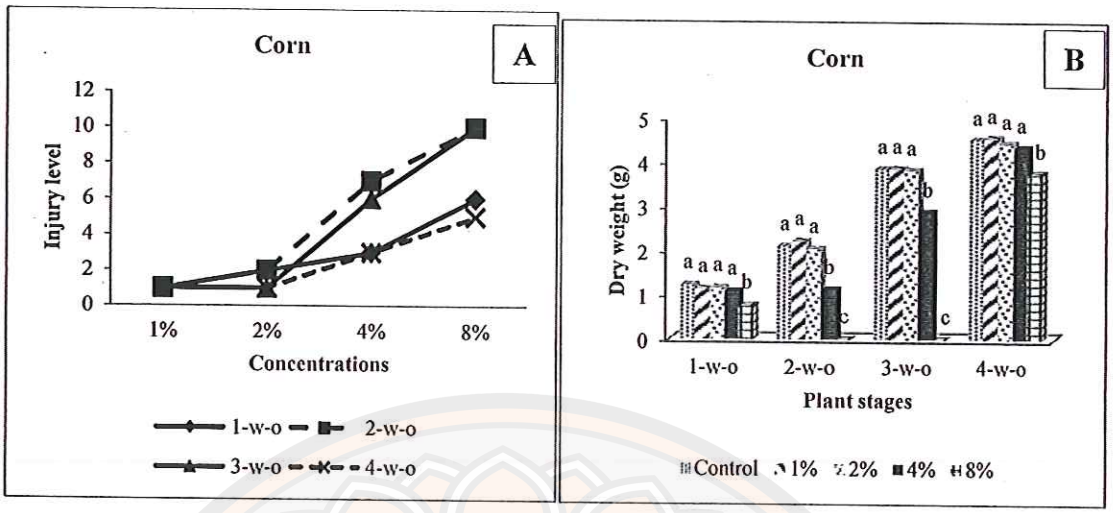


Figure 37 Effect of lemongrass essential oil formulation at 1, 2, 4 and 8% ai on injury levels (a) and dry weight (b) of corn by foliar application at 7 days after treatment

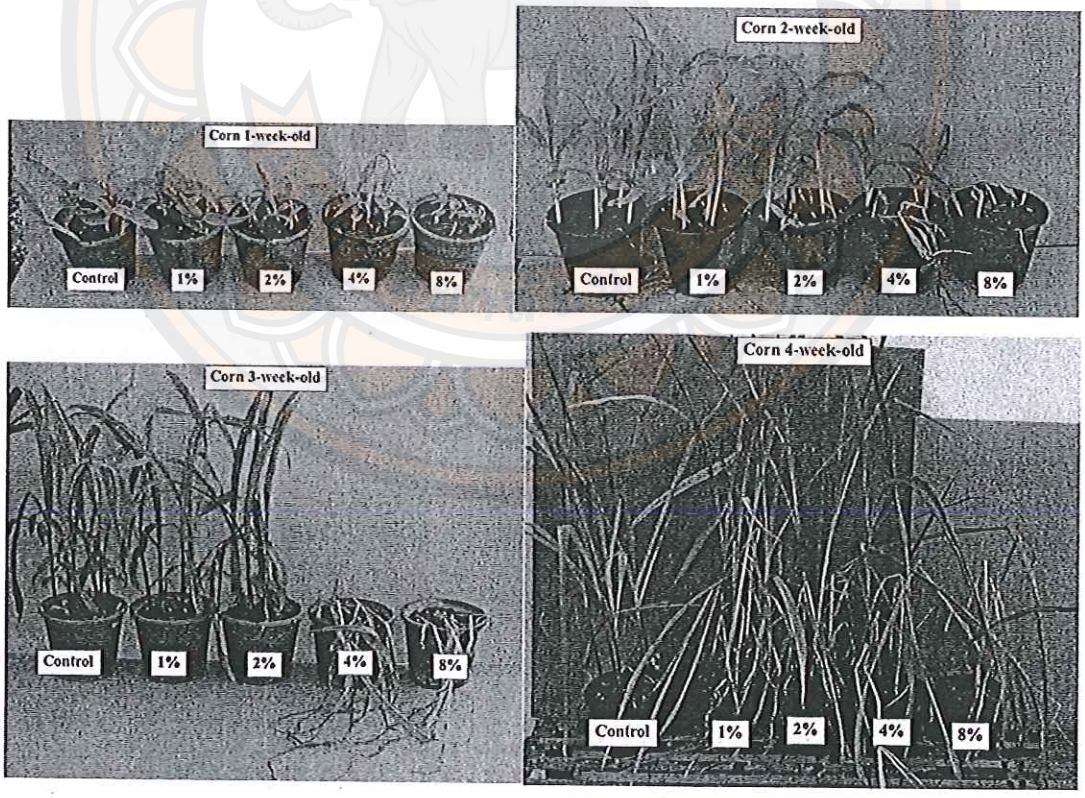


Figure 38 Foliar application effects of NHL at different concentrations shown 7 days after treatment on different stages of corn

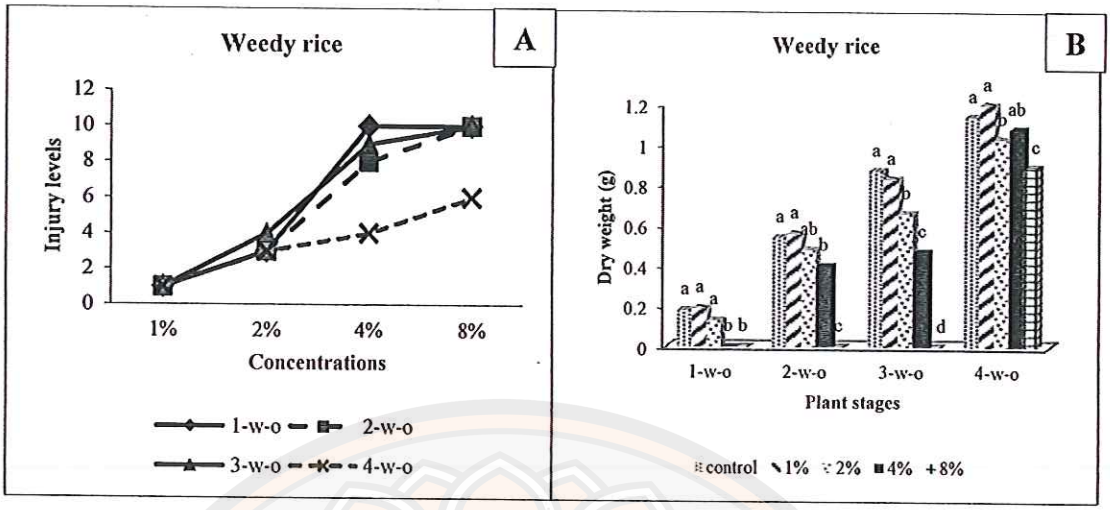


Figure 39 Effect of lemongrass essential oil formulation at 1, 2, 4 and 8% ai on injury levels (a) and dry weight (b) of rice by foliar application at 7 days after treatment

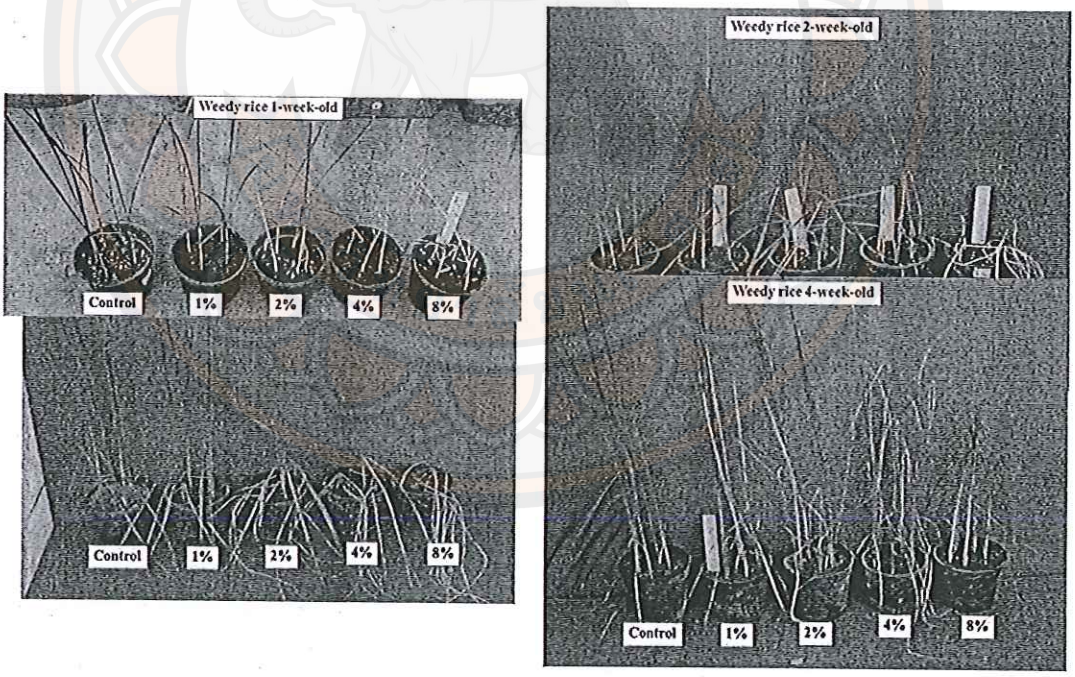


Figure 40 Foliar application effects of NHL at different concentrations shown 7 days after treatment on different stages of rice

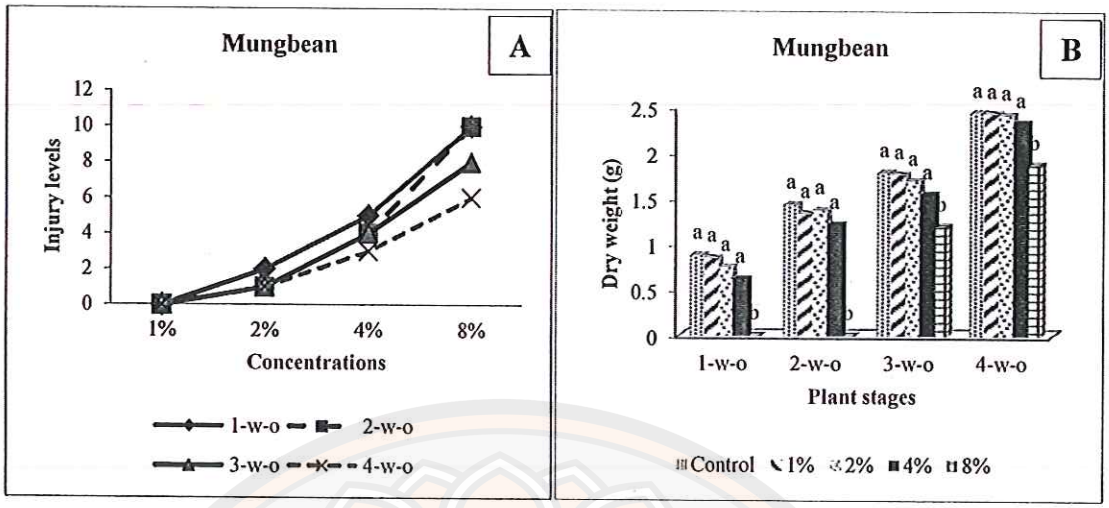


Figure 41 Effect of lemongrass essential oil formulation at 1, 2, 4 and 8% ai on injury levels (a) and dry weight (b) of mungbean by foliar application at 7 days after treatment

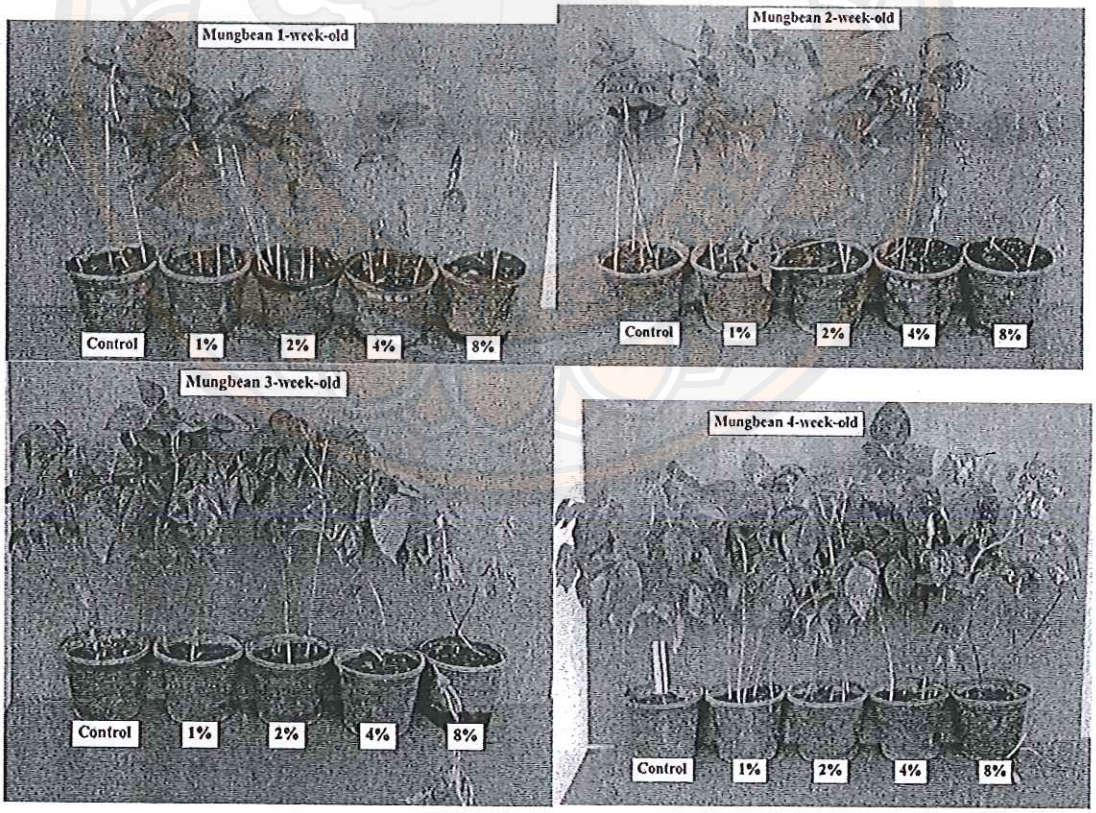


Figure 42 Foliar application effects of NHL at different concentrations shown 7 days after treatment on different stages of mungbean

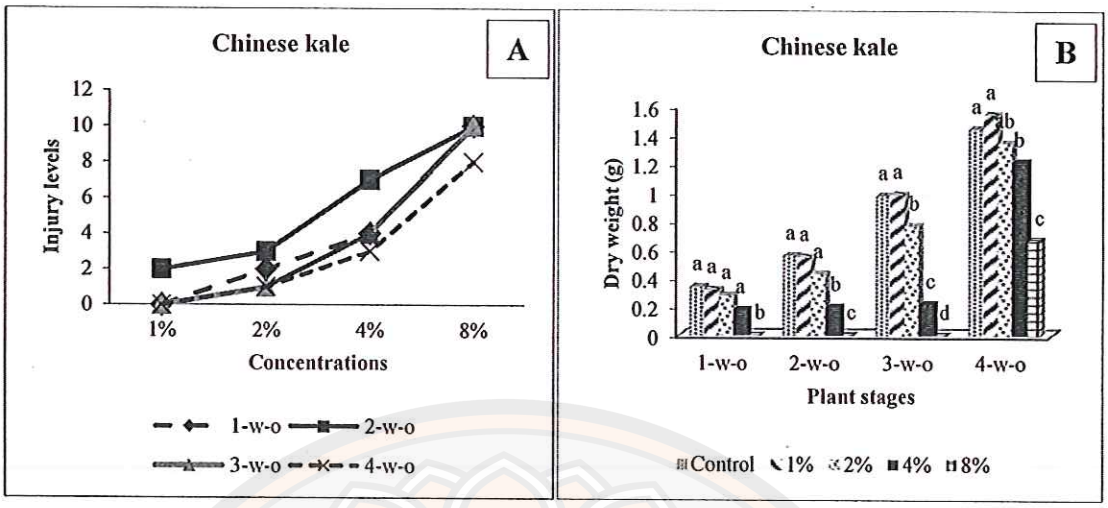


Figure 43 Effect of lemongrass essential oil formulation at 1, 2, 4 and 8% ai on injury levels (a) and dry weight (b) of chinese kale by foliar application at 7 days after treatment

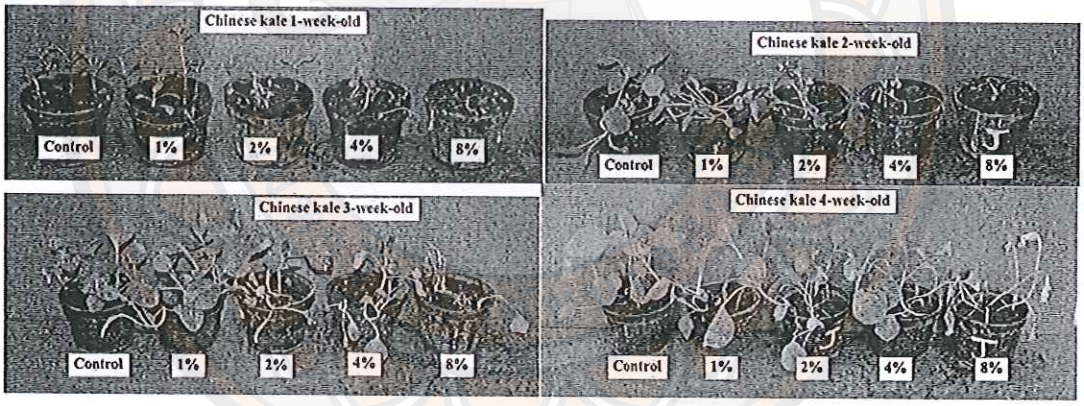


Figure 44 Foliar application effects of NHL at different concentrations shown 7 days after treatment on different stages of chinese kale

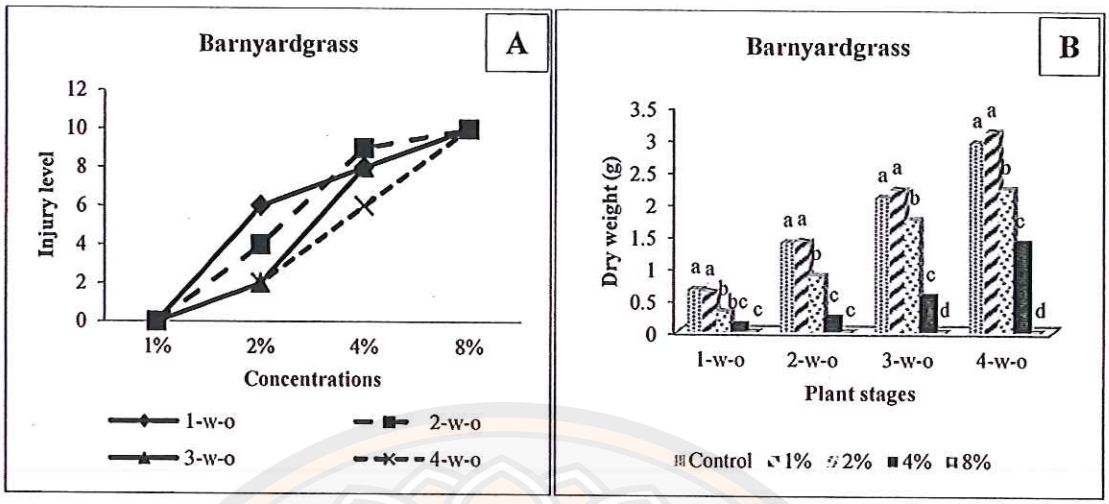


Figure 45 Effect of lemongrass essential oil formulation at 1, 2, 4 and 8% ai on injury levels (a) and dry weight (b) of barnyardgrass by foliar application at 7 days after treatment

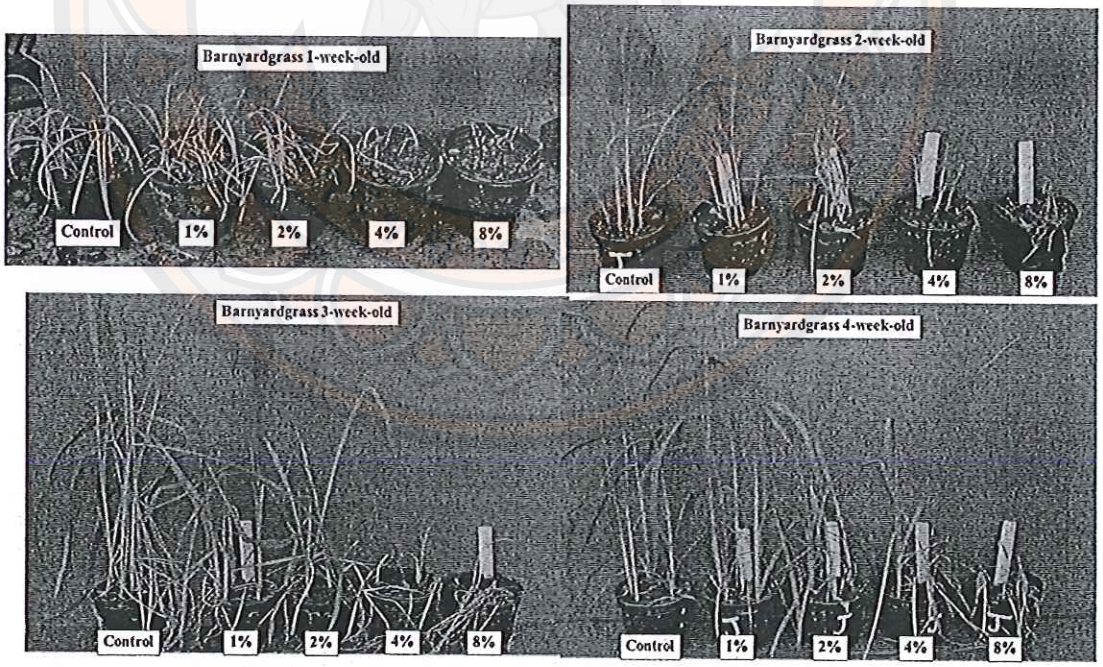


Figure 46 Foliar application effects of NHL at different concentrations shown 7 days after treatment on different stages of barnyardgrass

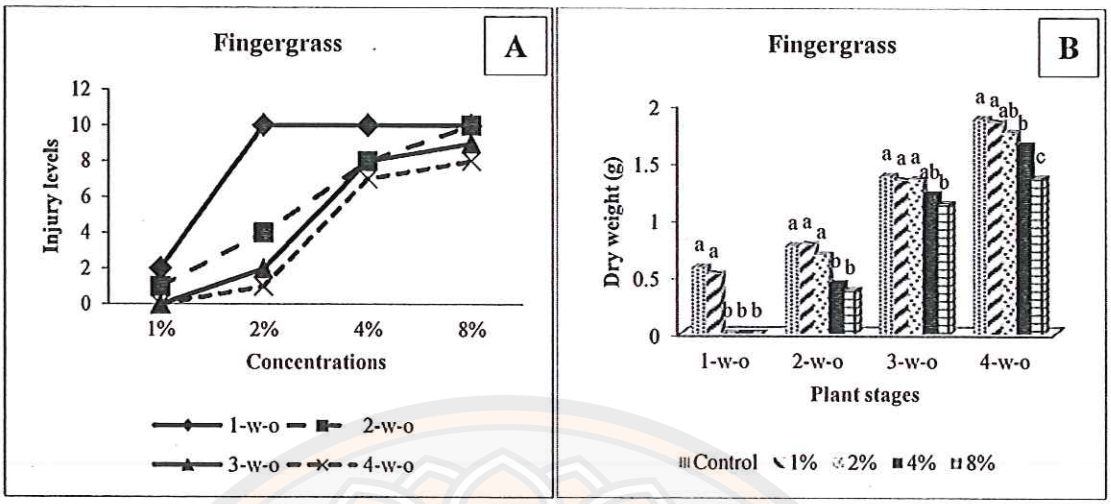


Figure 47 Effect of lemongrass essential oil formulation at 1, 2, 4 and 8% ai on injury levels (a) and dry weight (b) of fingergrass by foliar application at 7 days after treatment

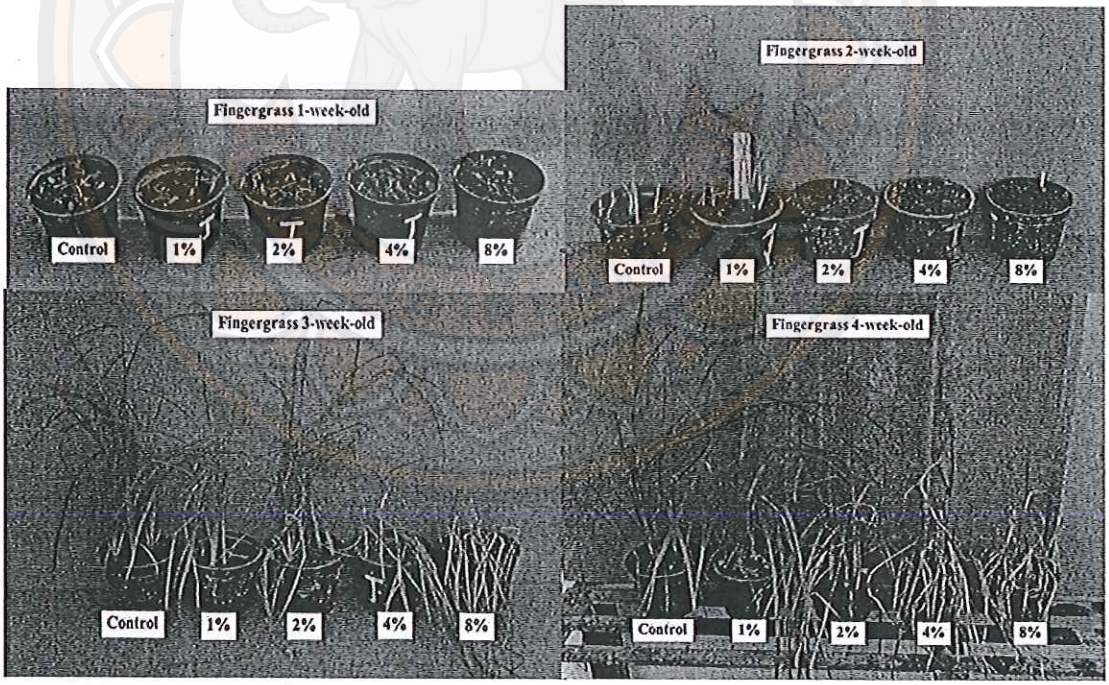


Figure 48 Foliar application effects of NHL at different concentrations shown 7 days after treatment on different stages of fingergrass

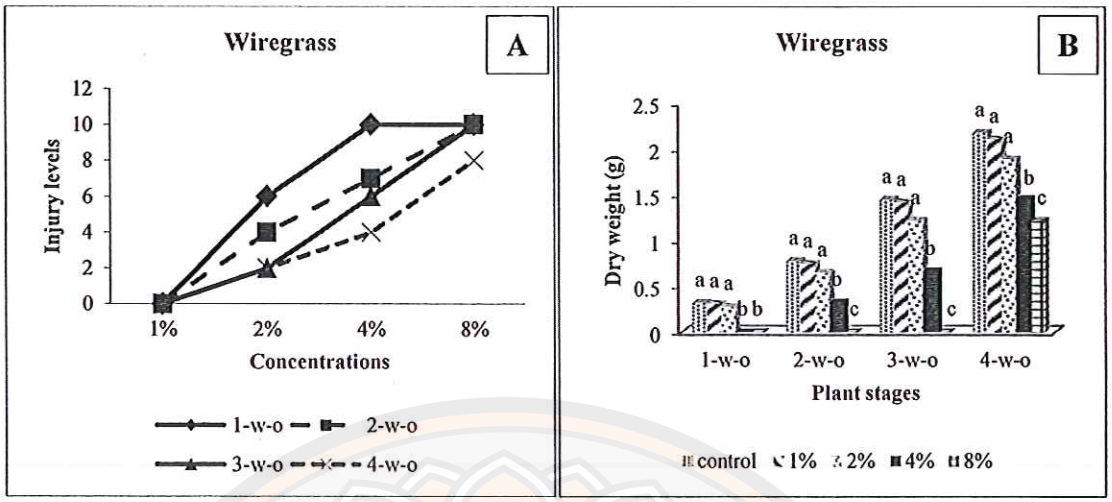


Figure 49 Effect of lemongrass essential oil formulation at 1, 2, 4 and 8% ai on injury levels (a) and dry weight (b) of wiregrass by foliar application at 7 days after treatment

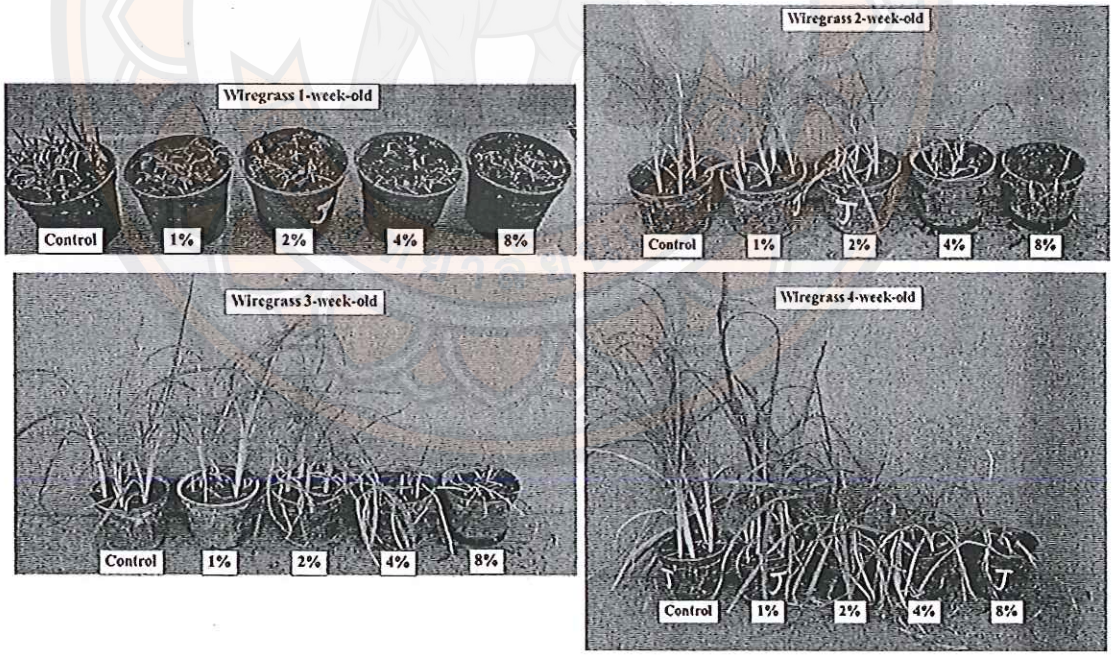


Figure 50 Foliar application effects of NHL at different concentrations shown 7 days after treatment on different stages of wiregrass

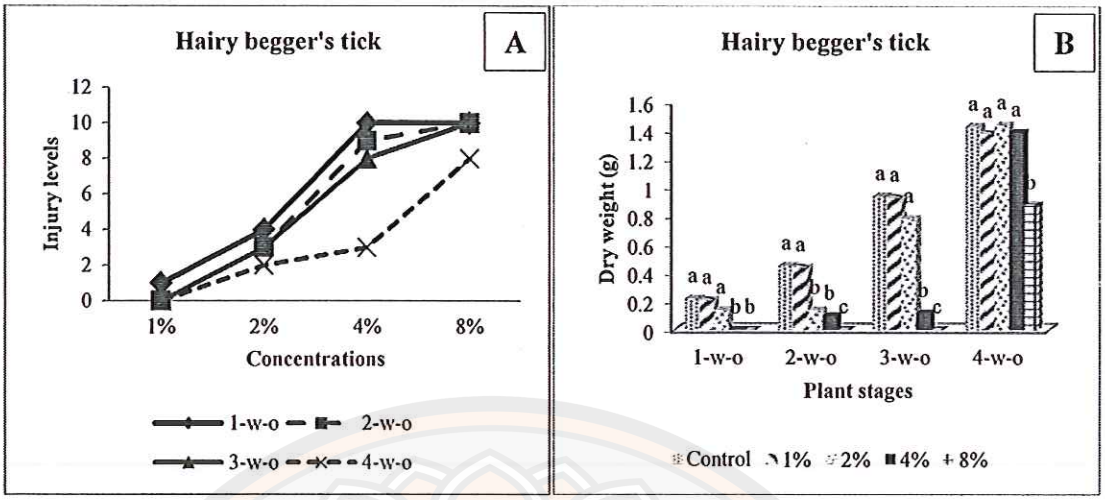


Figure 51 Effect of lemongrass essential oil formulation at 1, 2, 4 and 8% ai on injury levels (a) and dry weight (b) of hairy begger's tick by foliar application at 7 days after treatment

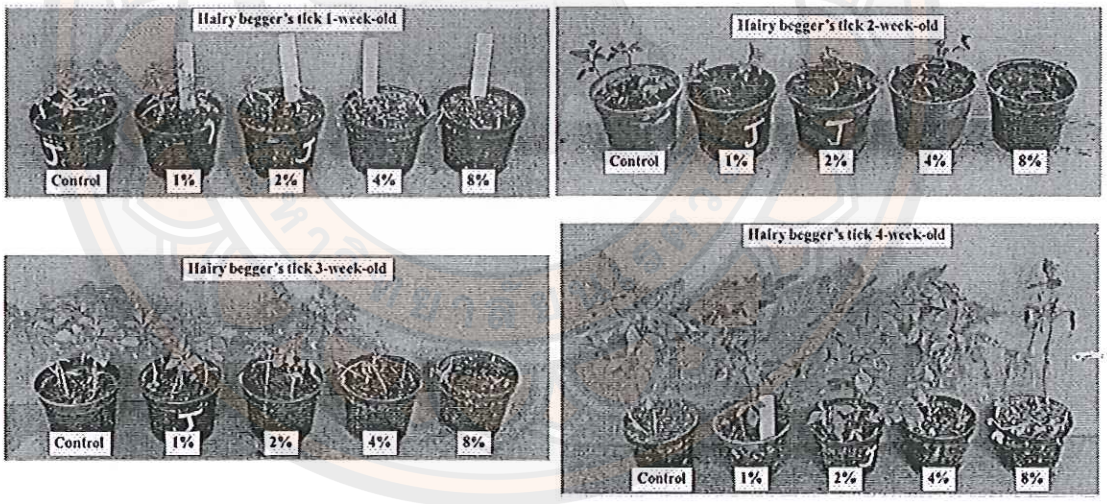


Figure 52 Foliar application effects of NHL at different concentrations shown 7 days after treatment on different stages of hairy begger's tick

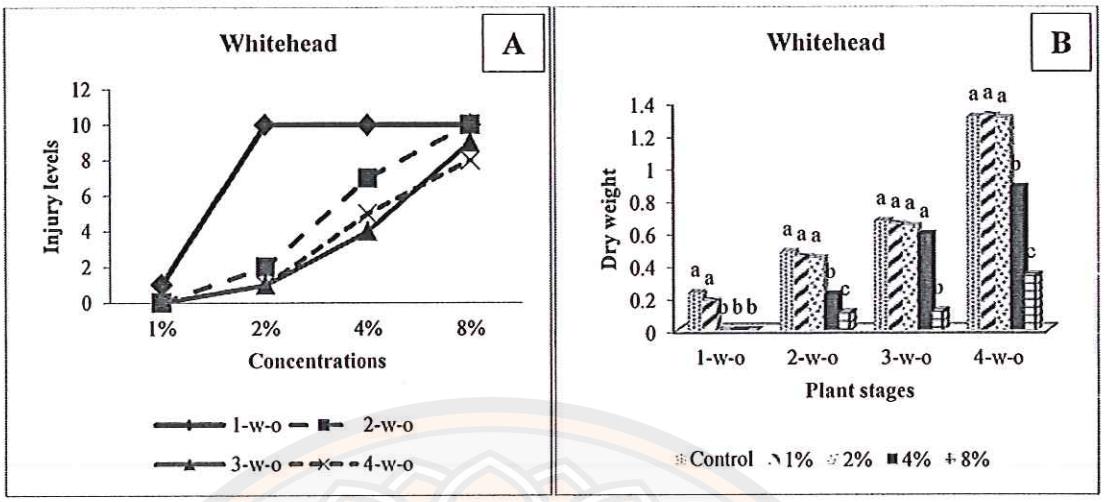


Figure 53 Effect of lemongrass essential oil formulation at 1, 2, 4 and 8% ai on injury levels (a) and dry weight (b) of white head by foliar application at 7 days after treatment

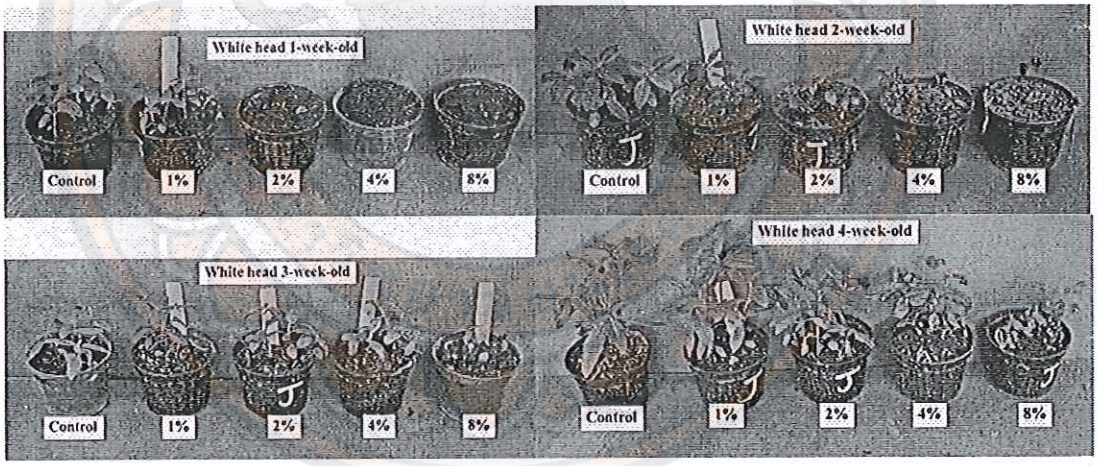


Figure 54 Foliar application effects of NHL at different concentrations shown 7 days after treatment on different stages of white head

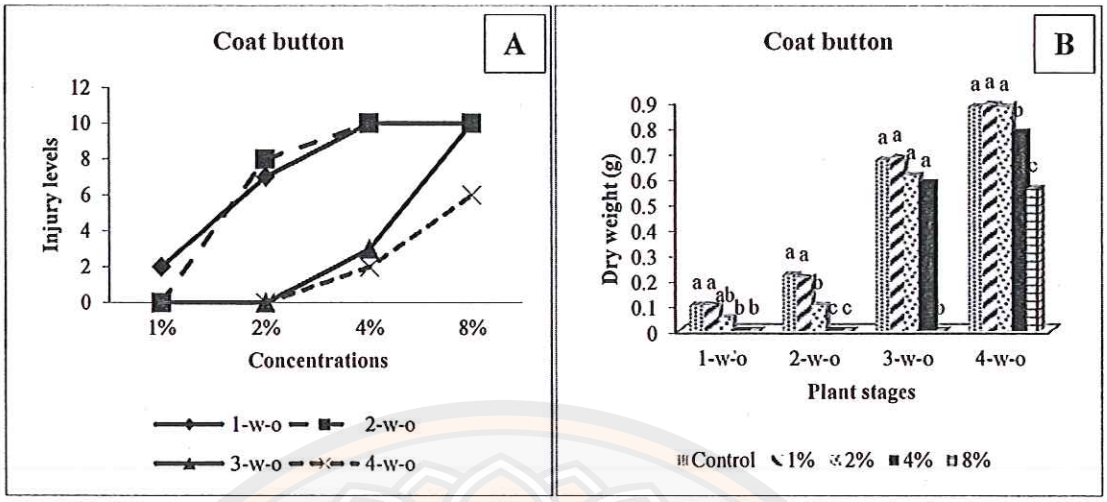


Figure 55 Effect of lemongrass essential oil formulation at 1, 2, 4 and 8% ai on injury levels (a) and dry weight (b) of coat button by foliar application at 7 days after treatment

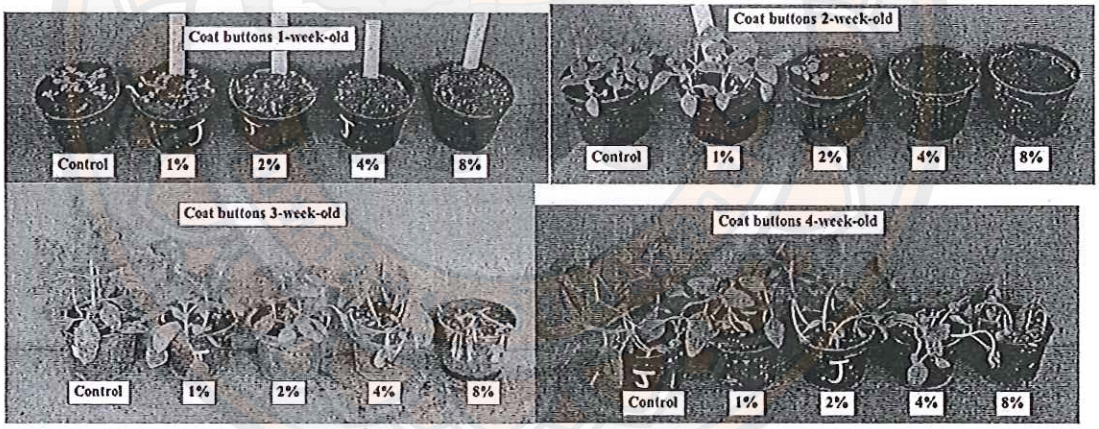


Figure 56 Foliar application effects of NHL at different concentrations shown 7 days after treatment on different stages of coat button

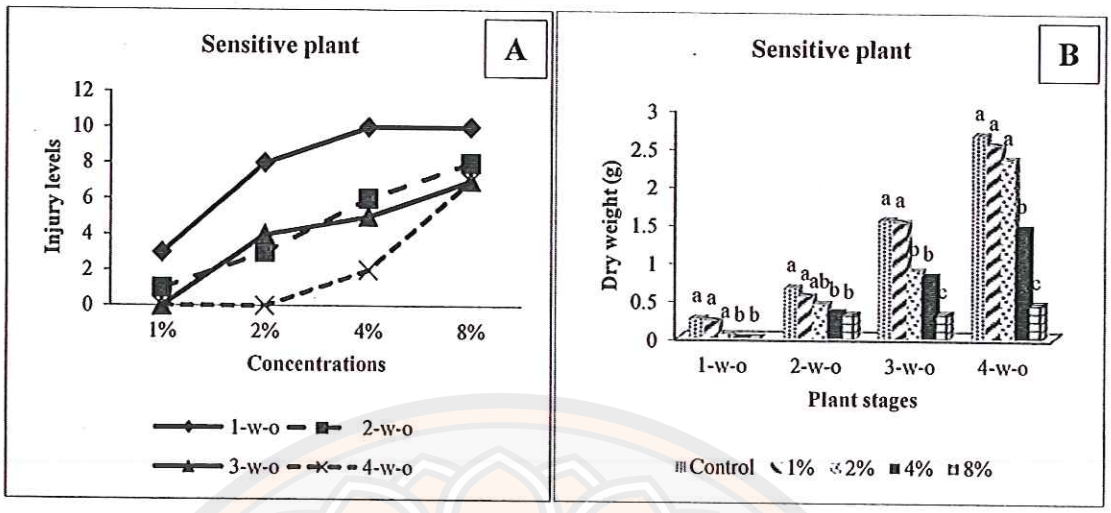


Figure 57 Effect of lemongrass essential oil formulation at 1, 2, 4 and 8% ai on injury levels (a) and dry weight (b) of sensitive plant by foliar application at 7 days after treatment

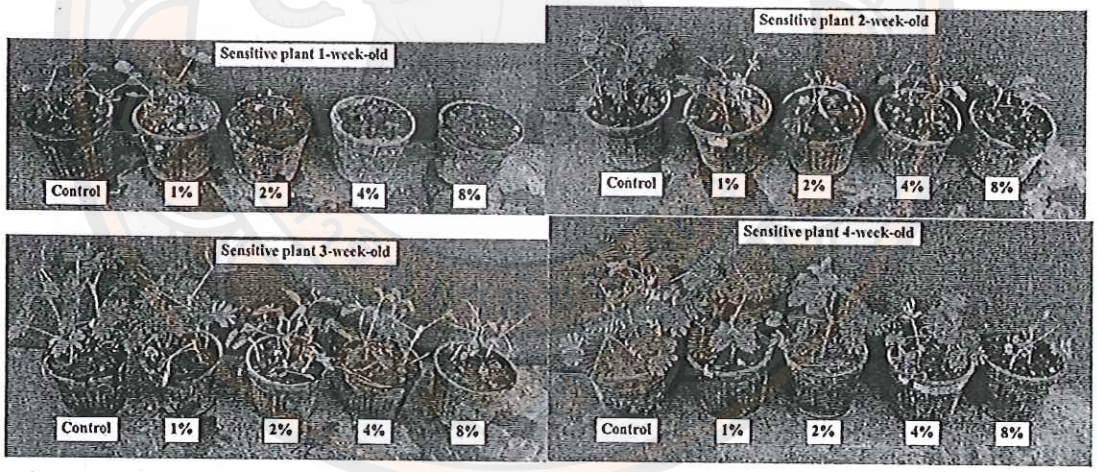


Figure 58 Foliar application effects of NHL at different concentrations shown 7 days after treatment on different stages of sensitive plant

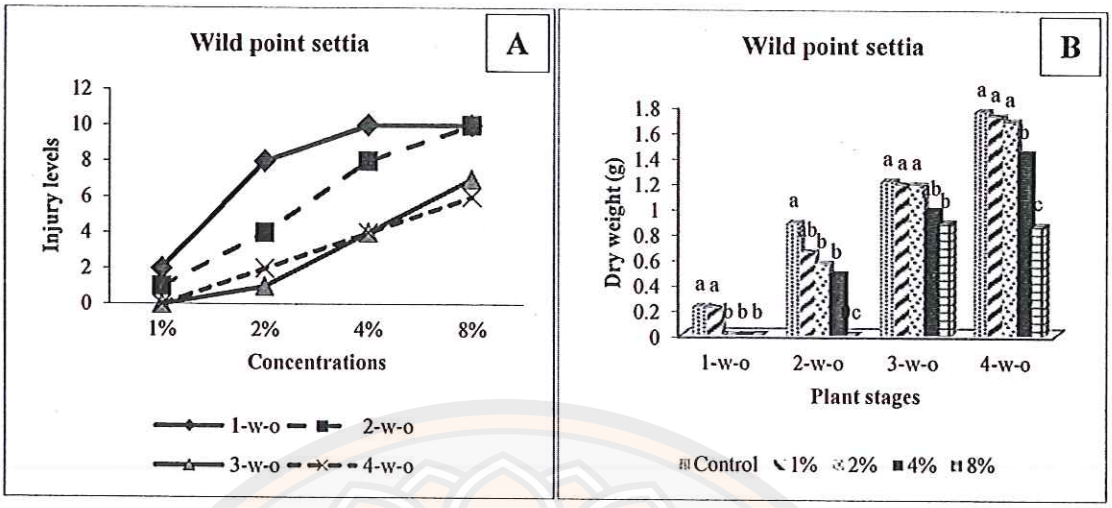


Figure 59 Effect of lemongrass essential oil formulation at 1, 2, 4 and 8% ai on injury levels (a) and dry weight (b) of wild pointsettia by foliar application at 7 days after treatment

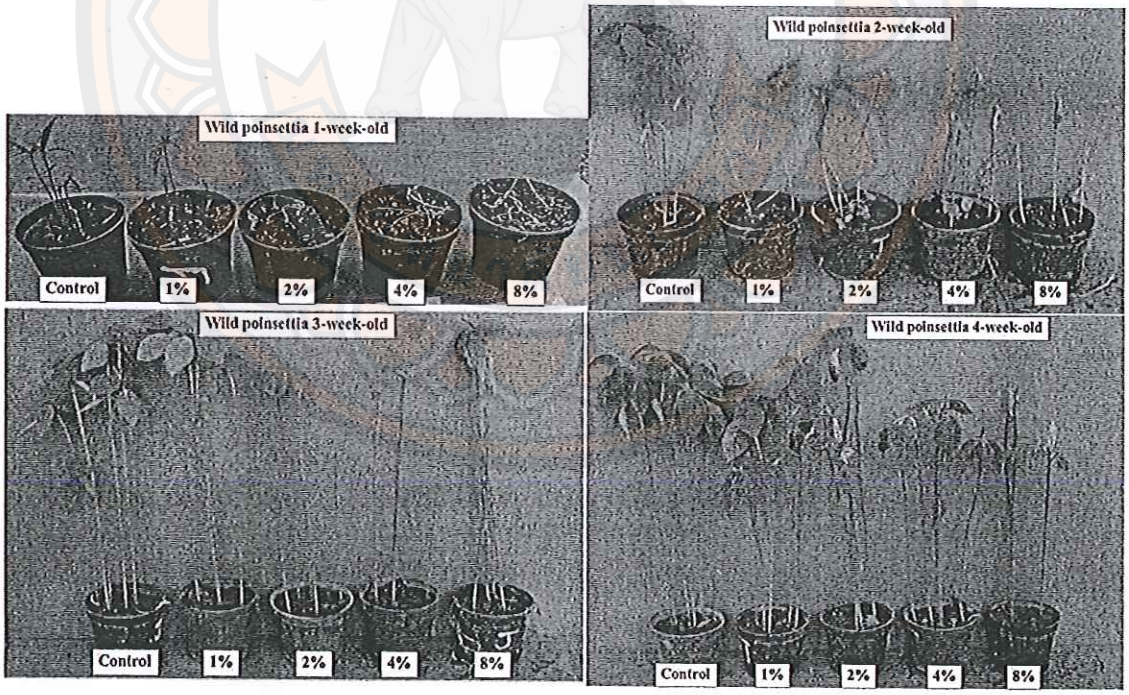


Figure 60 Foliar application effects of NHL at different concentrations shown 7 days after treatment on different stages of wild pointsettia

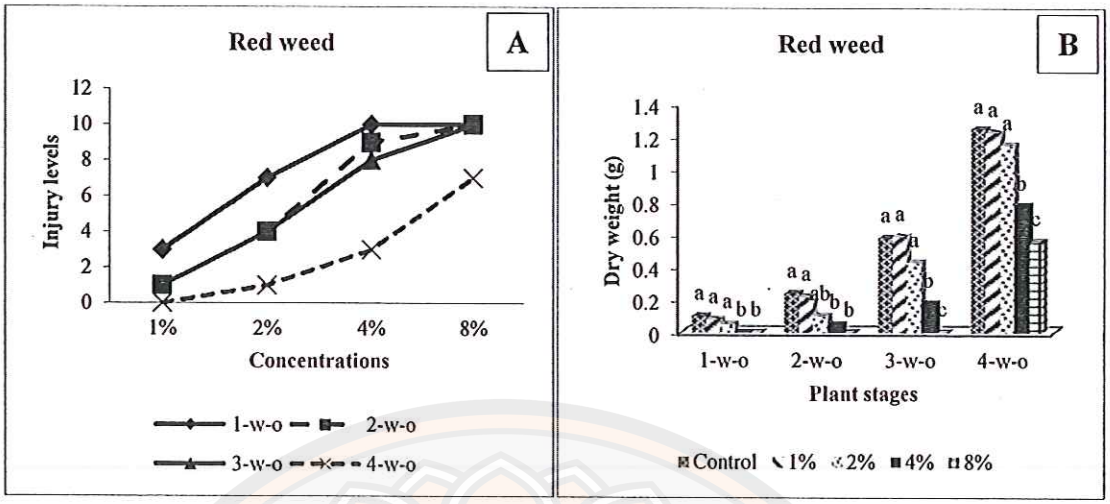


Figure 61 Effect of lemongrass essential oil formulation at 1, 2, 4 and 8% ai on injury levels (a) and dry weight (b) of redweed by foliar application at 7 days after treatment

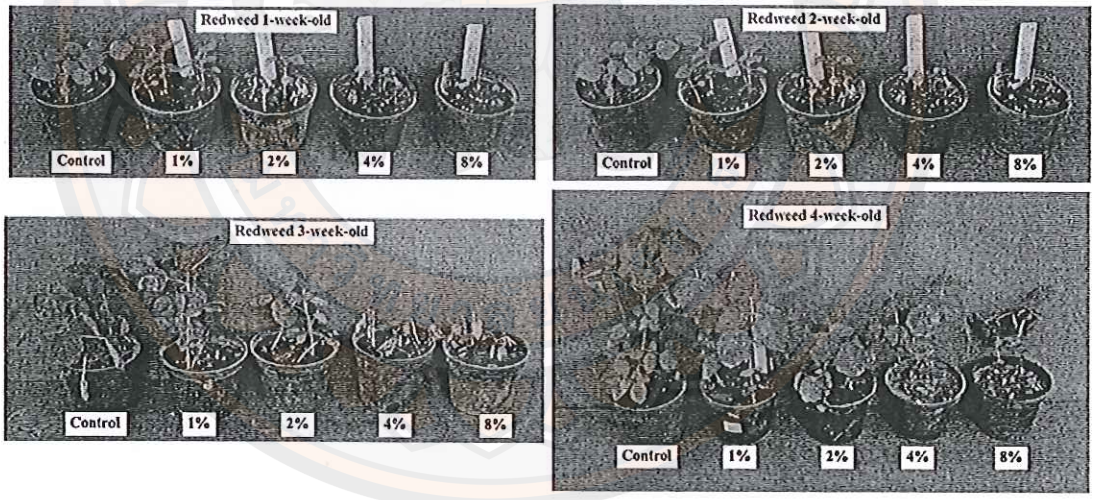


Figure 62 Foliar application effects of NHL at different concentrations shown 7 days after treatment on different stages of redweed

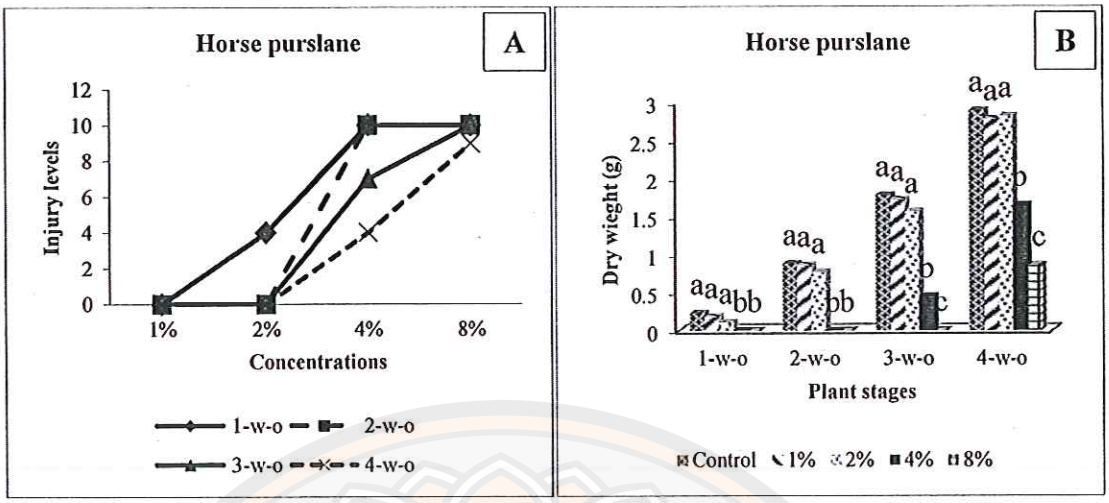


Figure 63 Effect of lemongrass essential oil formulation at 1, 2, 4 and 8% ai on injury levels (a) and dry weight (b) of horse purslane by foliar application at 7 days after treatment

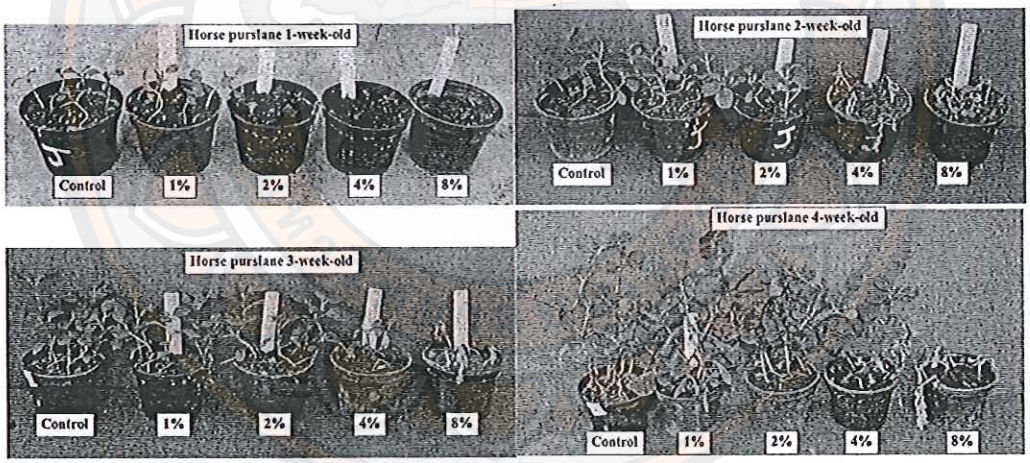


Figure 64 Foliar application effects of NHL at different concentrations shown 7 days after treatment on different stages of horse purslane

CHAPTER V

CONCLUSION

Screening allelopathic potential of essential oils

In order to screen and select the essential oil for developing a natural herbicide, the allelopathic effects of 18 essential oils extracted by steam distillation or purchased commercially had been tested by Petri-dish test and foliar application. The essential oil from lemongrass (*Cymbogopon citratus* (DC.) Stapf) showed the highest inhibitory activity on seed germination and seedling growth of tested plants, and also showed the highest injury effect by foliar application on the tested plants. The next most effective essential oils were citronella grass and pine oil respectively. Thus, we selected the lemongrass essential oil to study its major constituents and herbicide properties in next our subsequent.

Analysis of lemongrass (*C. citratus*) essential oil by GC-MS

Lemongrass (*C. citratus*) essential oil was analyzed by Gas Chomatography – Mass Spectrometry (GC-MS). It was found that a major constituent was geranial (41.94%) and neral (34.06%) followed by β-myrcene (10.39%), geraniol (4.63%) and Z-β-ocimene (0.22%). However, it is known that geranial and neral is the sterioisomers of citral. Thus, it can be concluded that citral is the main constituent of lemongrass essential oil.

Comparative allelopathic effect of lemongrass essential oil and its major constituents against weeds by bioassay test

Each of the major constituents of lemongrass essential oil, include citral, myrcene, and geraniol, were compared with lemongrass essential oil by applying them at the equal rates in order to understand the effects of constituent of each of these lemongrass essential oil. It was showed that citral has the highest inhibitory activity. However, it seems that the effect lemongrass essential oil is caused by the mixing of

compounds rather than any single compound. Thus, whole lemongrass essential oil was selected to use as herbicide.

Effect of lemongrass essential oil on amylase activity during seed germination

Lemongrass essential oil had shown the greatest inhibition on seed germination and seedling growth of test weeds. Therefore, the mechanism during seed germination and seedling was studied. α-Amylase activity was investigated on barnyard grass seeds that were selected as the model weed. Lemongrass essential oil decreased α-amylase induction by causing the inhibition on seed germination and seedling growth.

Physiological mechanisms on weed injury by foliar application

Lemongrass essential oil was tested by foliar application on the barnyard grass, a model plant. They showed wilt and necrosis symptoms until they completely die. Thus, the mechanisms involved with these effects on chlorophylls content, electrolyzed leakage and lipid peroxidation were studied. Total chlorophylls content was decreased in treated leaves of barnyard grass. As well, the the electrical conductivity value was increased in the treated leaves which caused electrolyze leakage. The amount of TBARs was also increased in treated leaves which caused the occurrence of lipid peroxidation. Thus, it was concluded that lemongrass essential oil damaged the plant tissues, especially in the cell walls and cell membranes of the leaf, which caused the disturbance of lipids and then electrolyze leakage occurred. Another effect of the penetration of lemongrass essential oil in plant cells was damaged chlorophylls which caused abnormal photosynthesis.

Glasshouse evaluation of herbicidal formulation based on lemongrass essential oil

The natural herbicide based on lemongrass essential oil (NHL) was prepared by the mixing of lemongrass essential oil at 60 % w/w, white oil at 15% w/w, coconut diethanolamide at 15% w/w, Tween20 at 5% w/w, sodium lauryl sulfate bat1.25% w/w and water at 3.75% w/w thus giving 60% active ingredient (ai).

NHL was tested as to pre and post emergence effects by foliar application on various stages of weeds and crops.

According to these experimental fundings. It can be concluded that;

1. NHL is a post-emergence herbicide when applied on the leaves and rarely has an effect in soil.
2. NHL is a non-selective herbicide.
3. NHL is a contact herbicide.
4. NHL is more effective on young plants (7-14 day-old) than on mature plants.

NHL is recommended for controling weeds at the concentrations of 4 – 8% ai by foliar application and by directed application using a spray shield for protecting crops at 7 – 14 days after planting or 2 – 3 leaf stages.

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