THE FINAL REPORT

ผลของสารสกัดจากดาวเรืองและ GA_3 ต่อการงอก การดูดน้ำและกิจกรรมอะไมเลสของเมล็ดผักโขม

Effects of *Tagetes erecta* L. leaf extract and exogenous gibberellic acid (GA_3) on seed germination, seed imbibition and α-amylase activity induction of *Amaranthus spp.*

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Project title: Effects of *Tagetes erecta* L. leaf extract and exogenous gibberellic acid (GA_3) on seed germination, seed imbibition and α-amylase activity induction of *Amaranthus spp.*

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ABSTRACT

Marigold leaf (*Tagetes erecta* L.) was extracted with 75% ethanol in water and evaporated ethanol to obtain crude ethanol extract. The sticky residue was diluted with distilled water, resulting in aqueous solution which was hydrolized by acidified to pH 3 with 6 N HCl. The filtrate was extracted with ethyl acetate three times. The ethyl acetate solutions were combined, dried over anhydrous $MgSO_4$, and then evaporated to obtain the ethyl acetate soluble hydrolyzed fraction (Hy fraction). The Hy fraction was mixed with adjuvant at the ratio of 30 : 70 to give of 30% active ingredient (a.i.) in soluble concentrate formulation (SC). The SC formulation was bioassayed on germination, seedling growth, seed imbibition and α-amylase activities against 3 species of amaranth at the concentrations of 250 - 8000 ppm. The results showed that natural herbicide from Marigold leaf in SC formulation at 1000 ppm inhibited germination and seedling growth completely of *Amaranthus spinosus* L. and *Amaranthus gracilis* Desf. At the higher concentration 2500 ppm had completely inhibit germination and seedling growth of *Amaranthus tricolor* L. Then, further studies were extended to explore the effects of the SC formulation from marigold leaf on seed imbibition and α-amylase activities of *A. tricolor, A. spinosus* and *A. gracilis* seeds. The results indicated that marigold leaf extract in SC formulation decrease in seed imbibition and

α-amylase activities of 3 above species of amaranth seeds in comparison with controls. By these results, marigold leaf extract in SC formulation had significant allelopathic effects against *A. tricolor, A. spinosus* and *A. gracilis*. Hence the use of leaf extract in SC formulation from *T. erecta* as a potential natural herbicide for weed control might be possible. In this study, exogenous GA_3 was used for induction of seed germination through seed imbibition and α-amylase activity induction however it had insignificant effects against *T. erecta* leaf extract in soluble concentrate formulation on *A. tricolor, A. spinosus* and *A. gracilis.* Thus, the present results support the hypothesis that allelochemical mechanism of *T. erecta* leaf extract and exogenous GA_3on *Amaranthus spp.* seed germination are dependent.

Keywords: *Tagetes erecta* L., *Amaranthus spp.,* allelopathy, seed germination, α-amylase activity

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CHAPTER 1

INTRODUCTION

1.1 The problem and its background

Marigold (*Tagetes erecta* L.) is in *Tagetes* genus belonging to the family *Asteraceae*; comprises about 56 species distributed around the world. It is demonstrated with the high allelopathic activity in the bioassay was fractionated by simple partitioning procedure which separated the component into gross chemicals classes. Allelopathy has been introduced as a viable option for alternative weed management under sustainable agriculture (Fujii, 2001; Singh et al., 2003; Hong et al., 2003) recently. It can play a beneficial role in various sustainable weed management tools such as cover cropping (Isik et al., 2009; Campiglia et al., 2010), soil surface mulching (Aladesanwa and Adigun, 2008) and soil incorporation (Chon et al., 2005; Kobayashi et al., 2008). Establishing that allelopathic activity is actually present in extracts of many higher plants and many plant organs can be accomplished with bioassays under laboratory conditions. Almost studies show that allelopathic activity may inhibit the seeds germination by inhibiting the induction of α-amylase activity (Kato-Noguchi H, Macías FA (2005), Meksawat S, Pornprom T (2010), Laosinwattana C, Boonleom C, Teerarak M, Thitavasanta S, Charoenying P (2010), etc) whereas gibberellins plays an important role in many essential plant growth and development processes, including seed germination, stem elongation, leaf expansion and reproductive development (Razem et al., 2006, Olszewski et al.2002). The stimulative action of applied gibberellins (GA) on seed germination has reported for a large number of species in the past three decades. Applied GA often replaces the need for environmental stimuli like specific temperature pretreatment or light. Therefore, it has been suggested that GAs are essential intermediates in the stimulation of germination. Some researchers have implied that GA stimulates seed germination via α-amylase synthesis. Water uptake and α-amylase (EC 3.2.1.1) activity is consistently linked with seed germination process. Seeds begin to germinate after imbibition of an adequate moisture level and become metabolically active. Continuous seed imbibition activates the stored hydrolytic enzymes and stimulates synthesis of new enzymes (Chong et al., 2002).

The bioassay chosen for studying mode of action of these natural compounds is an important consideration to evaluate of allelopathy as well as gibberellins. One of them is amaranth that is a C_4 plant noted by its ability to tolerate stressful conditions and produce highly nutritious seeds. The relation of regulation of allelopathic activity in the bioassay and GA is poorly understood. Therefore this present study was carried out to evaluate the effects of allelopathic potential of different concentrations from marigold leaf extract (*Tagetes erecta* L.) and exogenous gibberellic acid (GA_3) through bioassays of seed germination and α-amylase activity induction of 3 species of amaranth (*Amaranthus spinosus* L., *Amaranthus tricolor* L. and *Amaranthus gracilis* Desf.) and understand interaction mechanisms of allelopathic potential from marigold leaf extracts as well as determine effective concentration that have inhibition effects.

1.2 The purpose of the research project

To investigate the allelopathic effects of crude leaf extract from *Tagetes erecta* L. and exogenous GA_3 on seed germination, seed imbibition and α-amylase activity induction of *Amaranthus spinosus* L., *Amaranthus tricolor* L. and *Amaranthus gracillis* Desf.

1.3 The scope of the research project

1. Determine extract concentration of *Tagetes erecta* L. the most effects inhibition on seed germination, seed imbibition and α-amylase activity induction.

2. Determine combination of exogenous GA_3 concentration and extract concentration of *Tagetes erecta* L. on seed germination, seed imbibition and α-amylase activity induction.

3. Determine exogenous GA_3 concentration is the most effective of seed germination, seed imbibition and α-amylase activity induction.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction of marigold

Mexican marigold (*Tagetes erecta* L.) is in *Tagetes* genus belonging to the family *Asteraceae;* comprises about 56 species distributed around the world. It was almost studied as an antibiotic and antioxidant plant. P. O. Owino (1992) studied effects of marigold leaf extract and captafol on fungal parasitism of root knot nematode egg-kenyan isolates. Olabiyi, T. I. and E. E. A. Oyedunmade (2007) studied on marigold as interplant with cowpea for the control of nematode pests (*Meloidogyne spp.*, *Pratylenchus spp.*, and *Helicotylenchus spp.)*. Besides Nandita Dasgupta and et al. (2012) studied on the antibacterial effect of marigold leaf extract at room temperature against 10 gram positive and 6 gram negative. The maximum antibacterial effect of Mexican Marigold leaf extract among micro- organism was obtained for Acinetobacter and Propioni bacterium acne. The results suggest that species of Mexican marigold can be useful in developing drugs for diseases like dermatitis, acne, skin races and also can be developed as antiseptic. Moreover antioxidant activity of marigold essential oil was researched by Martha Perez Gutierrez, Heliodoro Hernandez Luna & Sergio Hernandez Garrido (2006). The essential oil from flowers of marigold was evaluated for antioxidant activity in vitro using diphenyl-1-picrylhydrazyl (DPPH), thiocyanate, β-carotene bleaching, free radical scavenging activity and oxidation of deoxyribose assay. The GC-MS analysis of the oil has resulted in the identification of 18 components; β-caryophyllene, limonene, methyleugenol, (E)-ocimene, piperetone, piperitenone and terpinolene were the main components. It is demonstrated with the high allelopathic activity in the bioassay.

2.2. Seed development, structure and general mechanisms of germination

To elucidate the mechanisms of seed germination, it is essential to analyze the structure of seeds common to many different species and also to recognize characteristics of seeds, such as the chemical and physical properties of the testa, which vary considerably among species. The proper-ties of the testa have been analyzed in greater detail in the next section. The two major components of seed, the embryo and the endosperm and their interaction are presented here. Seed is generally defined as a mature ovule. In angiosperm seeds, the embryo and the endosperm start to differentiate after double fertilization in the ovule. A pollen sperm nucleus penetrates into the ovule through one of the synergic cells and fuses with an egg cell nucleus forming a diploid embryo (Higashiyama et al. 2003). The embryo develops into globular, heart; torpedo and walking-stick stage sand eventually exhibits a mature morphology (Bewley et al.2000). The triploid endosperm, derived from the fusion between another pollen sperm nucleus and two central nuclei, also differentiates during embryogenesis as does the testa, which is derived from maternal integuments. Analyzing the genetic origin of seed tissues is important for interpreting and applying results from molecular and genetic studies. Investigations using Arabidopsis mutants indicated that the development of the testa and endosperm can occur indepen-dently of embryogenesis (Ohad et al. 1996, Chaudhury et al.1997, Kiyosue et al. 1999). However, it is also possible that there are significant interactions and communication between these tissues during seed development (Hiroyuki Nonogaki (2006). Before considering dormancy, which imposes a block to the completion of germination, it is appropriate first to consider the processes that comprise germination. Germination commences with the uptake of water by the dry seed-imbibition and is completed when a part of the embryo, usually the radicle, extends to penetrate the structures that surround it imbibition and the resumption of metabolism. Uptake of water by a mature dry seed is triphasic with a rapid initial uptake followed by a plateau phase. A further increase in water uptake occurs only after germination is completed, as the embryonic axes elongate because dormancy seeds do not complete germination. The influx of water into the cells of dry seeds during phase I results in temporary structural perturbations, particularly to membranes, which lead to an immediate and rapid leakage of solutes and low molecular weight metabolites into the surrounding imbibition solution. This is symptomatic of a transition of the membrane phospholipid components from the gel phase achieved during maturation drying to the normal, hydrated liquid-crystalline state (Crowe, 1992). Within a short time of rehydration, the membranes return to their more stable configuration, at which time solute leakage is curtailed. How repair to desiccation and rehydration induced damage to membranes and organelles is achieved is unknown. However, during the imbibition of cotton seeds, the amount of N-acetylphosphatidyl ethanol amine, a phospholipid with membrane-stabilizing properties, increases, as does that of the corresponding synthase. These molecules may be involved in maintaining or enhancing membrane integrity (Sandoval et al., 1995). Upon imbibition, the quiescent dry seed rapidly resumes metabolic activity. The structures and enzymes necessary for this initial resumption of metabolic activity are generally assumed to be present within the dry seed, having survived, at least partially intact, the desiccation phase that terminates seed maturation. Reintroduction of water during imbibition is sufficient for metabolic activities to resume, with turn over or replacement of components occurring over several hours as full metabolic status is achieved. One of the first changes upon imbibition is the resumption of respiratory activity, which can be detected within minutes. After a steep initial increase in oxygen consumption, the rate declines until the radicle penetrates the surrounding (J. Derek Bewley, 1997).

About pattern of seed germination, Miller B. McDonald and et al., studied in 2006. Prior to germination, seeds are in a "maintenance" phase that is often characterized as dormancy being imposed by ABA, metabolic blocks or some other agent hindering the transition to germination. At some point, the seed becomes sensitive to the presence of "trigger" agents. A "trigger" agent such as light or temperature alterations shift the balance of inhibitors to favor promoters such as gibberellins. A "trigger" agent can be defined as a factor that elicits germination but whose continued presence is not required throughout germination. In contrast, a "germination" agent must be present throughout the germination process. An example is gibberellic acid. The major sequence of events leading to germination is imbibition, enzyme activation, initiation of embryo growth, rupture of the seed coat and emergence of the seedling. The early stages of imbibition or water uptake into a dry seed represent a crucial period for seed germination. It is the first key event that moves the seed from a dry, quiescent, dormant organism to the resumption of embryo growth. Thus, any consideration of seed germination physiology and its resultant impact on stand establishment should focus initially on water uptake. The extent to which water imbibition occurs is dependent on three factors: (1) composition of the seed, (2) seed coat permeability, and (3) water availability.

2.3. Related researches

Many authors illustrated some allelochemicals may inhibit the germination of bioassays by inhibited imbibition and α-amylase activity in bioassay seeds (itchgrass (*Rottboellia cochinchinensis*), *Suregada multiflorum*, *Aglaia odorata* granules, *Melia Azedarach* L., *Echinochloa crus-galli* and *Phaseolus lathyroides* L.) during germination such as: Kato-Noguchi H, Macias FA (2005); Meksawat S, Pornprom T (2010); Laosinwattana C, Boonleom C, Teerarak M, Thitavasanta S, Charoenying P (2010) etc… However *T. erecta* has not studied about weed control through inhibit the weed germination yet. It still remains unclearly how are modulated during seed germination by its inhibition whereas GAs plays crucial roles in aspects of plant growth and development, including seed germination, stem elongation, leaf expansion, trichome development, and flower and fruit development (Olszewskiet al., 2002).

For exogenous GA_3 studies, exogenous GA_3, relationship of GA_3 induced germination and amylase and GAs gene expression were mentioned by many findings and author groups. Mambouh M, Nematt Alla, Nemat L (1998) investigated efficacy of exogenous GA_3 and herbicide safener and protection. The research investigated contents of gibberellic acid (GA_3), glutathione (γ-L-glutamyl-Lcysteinyl glycine, GSH) and protein, activities of α-amylase (EC 3.2.1.1) and glutathione S-transferase (GST, EC 2.5.1.18) as well as metolachlor residues in shoots of 6-d old maize seedlings during an 8-d period following treatment with metolachlor, either alone or combined with GA_3, naphthalic anhydride (NA) or flurazole (FL). Externally applied GA_3 relieved this effect while NA and FL relatively raised the enzyme activity but still remained below control levels. Significant increases of GSH content were induced by metolachlor or its combinations; the magnitude of the increase was more pronounced with FL. GST activity was significantly enhanced by metolachlor; the effect was not influenced by GA_3, being augmented by the presence of NA and multiplied by FL. The results indicate that GA_3 compensates the loss of the endogenous GA_3 content and of α-amylase activity while FL, and to some extent NA, stimulated the detoxification rate of metolachlor by enhancing GSH content and GST activity.

In 2002, the α-amylase induction in endosperm during rice seed germination is caused by gibberellin synthesized in epithelium was studied by Miyuki Kaneko, Hironori Itoh, Miyako Ueguchi-Tanaka, Motoyuki Ashikariand, Makoto Matsuoka.

Bozena Bia lecka et al. (2009) studied on effect of ethephon and gibberellins A_3 on *Amaranthus caudatus* seed germination and α- and β-amylase activity under salinity stress. This study assessed the effects of different doses of ethephon and gibberellin A_3 on germination and α- and β-amylase activity in *A. caudatus* seeds exposed to different levels of salt stress. Jan Ke, pczynski, Pawel Sznigir (2013) investigated about response of *Amaranthus retroflexus* L. seeds to gibberellic acid, ethylene and abscisic acid depending on duration of stratrification and burial. The results indicated that *A. retroflexus* seed dormancy can be released either by stratification or by autumn–winter burial. The response to GA_3 and ethylene increased with increasing time of stratification. The presence of GA_3 and ethephon during stratification may stimulate germination at 35°C. Thus, both GA_3 and ethylene can partially substitute the requirement for stratification or autumn–winter burial. Both hormones may also stimulate germination of secondary dormant seeds, exhumed in September. Endogenous GAn, ethylene and ABA may be involved in the control of dormancy state and germination of *A. retroflexus*. It is possible that releasing dormancy by stratification or partial burial is associated with changes in ABA/GA and ethylene balance and/or sensitivity to these hormones.

CHAPTER 3

RESEARCH METHODOLOGY

3.1. Test plant preparation

The healthy seeds of *Amaranthus spinosus* L., *Amaranthus tricolor* L. and *Amaranthus graciliS* Desf. were hand collected from experimental field at King Mongkut's Institute of Technology Ladkrabang and paddy fields in the Ladkrabang district, Bangkok, Thailand. Their seeds were removed from pods by lightly shaking in collection bags to release seeds. Seeds of these species were examined and seeds with damaged seed coats were discarded.

3.2. Crude marigold leaf extracts preparation

Marigold plant was grown at the experimental field at King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. The mature and healthy leaves of *T. erecta* were collected at 50 days after planting, then cleaned from soil immediately with running tap water, dried-up in a hot-air oven at 45°C for 5 days and ground into powder (100 mesh) in an electrical blender. According to method of Laosinwattana et al., the crude extracts were prepared from *T. erecta* leaf powder by extraction with 75% ethanol in water for 48 hours at room temperature and repeatedly extracted 3 times, followed by filtration through three layers of cheese clothes to remove deris.

After filtration using Whatman No. I filter paper, the filtrates was combined and evaporated in the rotary evaporator at 45°C, leaving a sticky residue (crude ethanol fraction; OR fraction). This residue was then diluted with 500 mL of distilled water and stirred vigorously on a magnetic stirrer at 45°C for 20 min, resulting in an aqueous solution which was acidified to pH 3 by 6N HCl. The filtrate was extracted with ethyl acetate three times. The ethyl acetate solutions was combined, dried over $MgSO_4$ and then evaporated to obtain the ethyl acetate soluble hydrolyzed fraction (Hy fraction) and the remains of the aqueous phase was discarded (Figure 3.1). The Hy fraction was mixed with adjuvant at the ratio of 30 : 70 to give of 30% active ingredient (a.i.) in soluble concentrate formulation (SC). The inhibitory activities from each fraction were prepared by dissolved crude of each fraction to contain different concentrations.

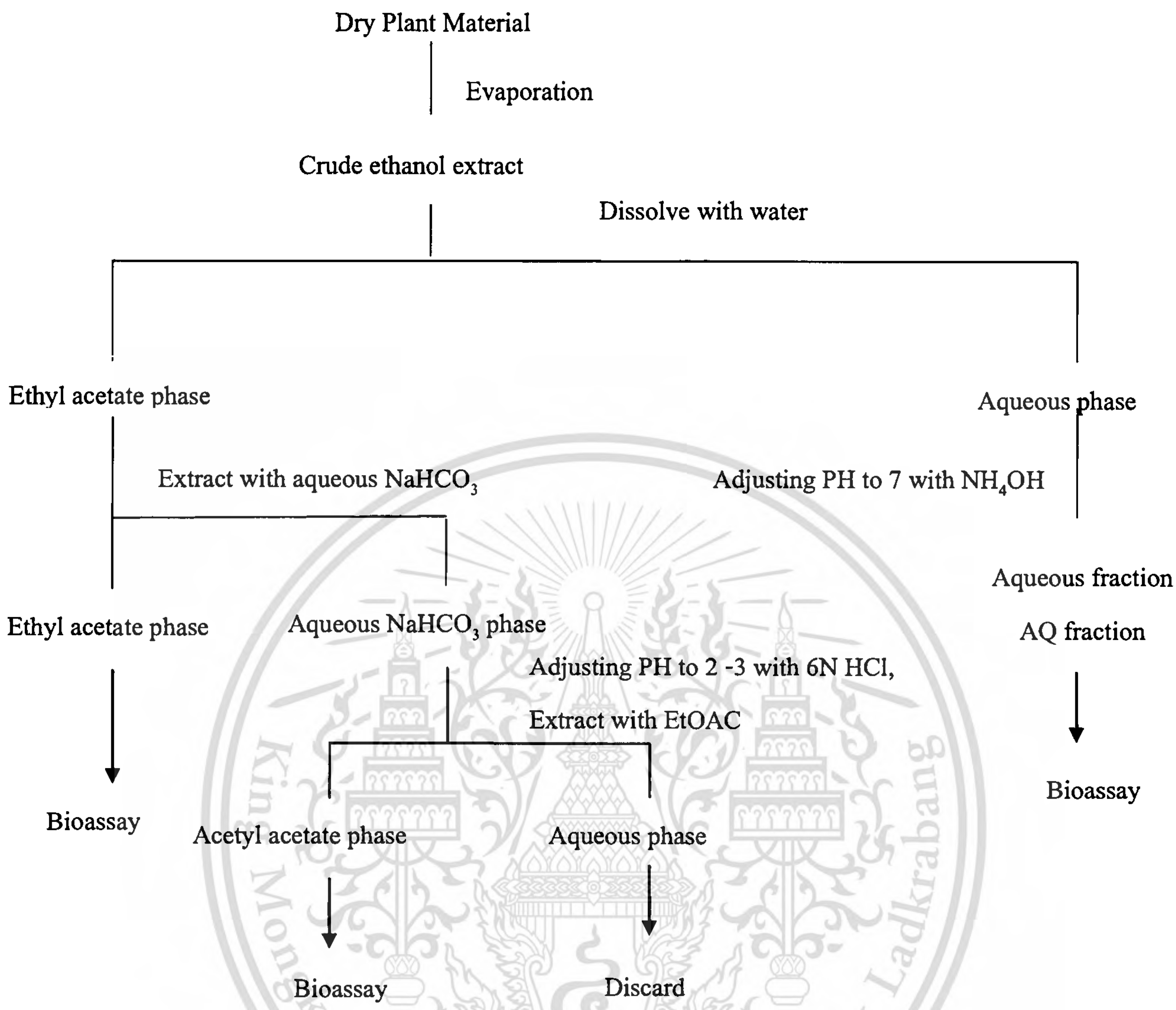

Figure 3.1. Flow chart for extraction and partially separation of active compounds from marigold dried leaves

3.3. Effects of different concentrations of *T. erecta*, exogenous GA_3 and the combination of *T. erecta* and exogenous GA_3 on seed germination, seed imbibition and α-amylase activity induction of *A. tricolor*, *A. spinosus* and *A. gracilis*.

The crude solution from *T. erecta* was diluted with distilled water to 250 ppm, 500 ppm, 750 ppm, 1000 ppm, 2000 ppm, 4000 ppm, 8000 ppm. 5 ml of each concentration was added to each petri dish (9 cm in diameter) containing 2 layers of germination paper and then 20 healthy seeds of test plant were placed on the germination paper as per treatment.

Exogenous GA_3 (Gibberellic acid) (Eastman organic chemicals Rochestor, N.Y, USA.) stock solution was prepared and stored at 5°C. Then stock solution was diluted with distilled water to contain different concentrations (0.1 ppm, 0.2 ppm, 0.4 ppm and 0.8 ppm).

Control: Distilled water

Treatment 2: 250 ppm

Treatment 3: 500 ppm

Treatment 4: 750 ppm

Treatment 5: 1000 ppm

Treatment 6: 2000 ppm

Treatment 7: 4000 ppm

Treatment 8: 8000 ppm

After completed above experiment, the best result was used for next to treatment and its sign was Concentration*

The combination of *T. erecta* and exogenous GA_3 concentration was carried out about Concentration * + different concentrations of exogenous GA_3.

5 ml of above concentration combination (Concentration* + different concentrations of exogenous GA_3) was added to each petri dish (9 cm in diameter) containing 2 layers of germination paper

Treatment 9: Concentration* + 0.1 ppm GA_3

Treatment 10: Concentration* + 0.2 ppm GA_3

Treatment 11: Concentration* + 0.4 ppm GA_3

Treatment 12: Concentration* + 0.8 ppm GA_3

The control was only received distilled water. Each treatment had 3 - 4 replications in a completely randomized design (CRD).

All petri dishes were placed at a growth chamber with condition (cool white 840 Climacell 707, Munich, Germany) at 25-32°C, 12h dark/ light photoperiod light intensity of 100 μmol $m^{-2}s^{-1}$, and relative humidity of 80%. After 7 days, germination percent (%) (SG), shoot length (SL) and root length (RL) were observed and recorded in all treatments.

The inhibition percentage relative to control was calculated from following equation:

SG, SL or RL (% of control) = (Sample extracts/ Control) x 100

Seed imbibition and assay of α-amylase activity induction:

Measurement of seed imbibition was performed using method of Turk and Tawaha (2003). Four replicates of 100 healthy seeds of *A.spinosus*, *A. tricolor* and *A. gracilic* were weighed and recorded as original seed weight (W_1). These seeds were separately germinated in 5 ml of each treatment, distilled water as control (according to above treatments). Seed weights were recorded as final seed weight (W_2) for each concentration and exposure time. Seed imbibition percentage was calculated from following equation:

$$\text{Seed imbibition (\%)} = [(W_2 - W_1)/ W_1] \times 100$$

Measurement of activity of α-amylase weas performed by following the method of Bernfield (1955) and Sadasivam and Manickam (1996). After measuring water uptake, seeds (100 seeds for one determination) was homogenized with 4 mL ice-cold solution of 0.1 M $CaCl_2$ and centrifuged at 10,000 rpm for 10 min at 25°C. Supernatant was used as enzyme extract. The α - amylase was then assayed by measuring rate of generation of reducing sugars from soluble starch. The reaction medium (2 ml) contained 1 ml of 1% soluble starch in acetate buffer solution at pH 5.5. The assay medium was incubated for 15 min at 37°C. The reaction was terminated by addition of 1 ml DNS reagent (40 mM 3,5 dinitrosalicylic acid, 0.4 N NaOH and 1M K-Na tartrate), and immediately heated in a boiling water bath for 5 min. The mixture was cooled under running tap water. The intensity of colour was measured as 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 sample was calculated from standard curve and expressed as μmol maltose/min/g (FW).

3.4. Data Analysis/ Statistical analysis

Each treatment consists of four replications in completely randomized design (CRD). Analysis of variance was calculated for all data and comparisons between treatments will be made at probability level $p \leq 0.05$ using Tukey's test.

3.5. Duration and location of research work

The research was carried out to study during 1 year (9/ 2013 – 9/ 2014) in laboratory and greenhouse experiments, Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok10520, Thailand.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Experiment 1. Effects of different concentrations of *T. erecta*, exogenous GA_3 and the combination of *T. erecta* and exogenous GA_3 on seed germination, seed imbibition and α-amylase activity induction of *A. tricolor.*

4.1.1. Effects of different concentrations of *T. erecta* on inhibition of seed germination and seedling growth of *A. tricolor.*

The results show that *T. erecta* leaf extract in soluble concentrate formulation had significant allelopathic effects against *A. tricolor* (Table 4.1). At 1000 ppm dose, germination of seed of *A. tricolor* was inhibited by 17.5%. Its shoot and root length inhibition were 32.83 and 31.52%, respectively. By increasing the dose of application at 2500, 4000 and 8000 ppm, the inhibition magnitude was complete. In general, there are no significant differences in the inhibitory effect between on root length and shoot length. These results indicate that *T. erecta* leaf extract contains some inhibitory principles upon inhibited germination and seedling growth. However, the nature of inhibitory principles contained in *T. erecta* leaf crude extract is unknown. Thus, further studies were carried out to evaluate and understand interaction mechanisms of allelopathic potential about inhibition on seed imbibition and α-amylase activities of *A. tricolor* seeds.

Table 4.1. Effects of different concentrations of *T. erecta* on inhibition of seed germination and seedling growth of *A. tricolor*.

Concentration (ppm)	Inhibition (% of control)		
	Seed germination	Shoot length	Root length
1000	17.5b	32.83b	31.52b
2500	100a	100a	100a
4000	100a	100a	100a
8000	100a	100a	100a

Means followed by the same letter in a column do not differ significantly by Turkey's Studentized Range Test (p=0.05)

4.1.2 Effects of different concentrations of *T. erecta* on inhibition of seed imbibition and α-amylase activity induction of *A. tricolor*

Data that further shows the differences in the percentage of seed imbibition between control and concentration application of natural herbicide from *T. erecta* leaf extract in soluble concentrate formulation at different imbibition periods presented in Table 4.2 and Figure 4.1. The percentages of imbibition in the control seeds exhibited a marked increase by prolonging the imbibition periods and had the highest seed imbibition at all the imbibition time whereas the percentage of imbibition in treated seeds increased slightly by prolonging the imbibition period. After imbibiton time of 12 hours, the percentage of seed imbibition inhibited remarkable according to the increasing the dose of application during the whole experiment.

Many findings indicate that between the imbibition and α-amylase activities induction had closed relation (Kato-Noguchi H, Macias FA. 2005, Derek J. Bewley. 1997, Montinee Teerarak, Chamroon Laosinwattana. 2012). Thus, further studies were carried out to evaluate about inhibition on α-amylase activities of *A. tricolor* seeds. The activities of α-amylase of *A. tricolor* seeds were also investigated and the results are shown in Table 4.3 and Figure 4.2. Under the same extract concentration, α-amylase activity increased by prolonging the imbibition period, especially in control. For all treatment conditions, no significant differences in α-amylase activity after 6 hours imbibition time were observed. After imbibition time of 12 hours and 24 hours an increased concentration of natural herbicide from crude marigold leaf extract in soluble concentrate formulation inhibited induction α-amylase activity.

Table 4.2. Effects of different concentrations of *T. erecta* on inhibition of seed imbibition of *A. tricolor*

Concentration (ppm)	Seed imbibitions (%)		
	6 hours	12 hours	24 hours
Distilled water	28.14a	39.42a	66.51a
1000	27.75a	35.92b	40.97b
2000	27.97a	33.84bc	38.9b
4000	26.34a	36.18c	37.74b
8000	23.9a	32.96d	36.35b

Means followed by the same letter in a column do not differ significantly by Turkey's Studentized Range Test (p=0.05)

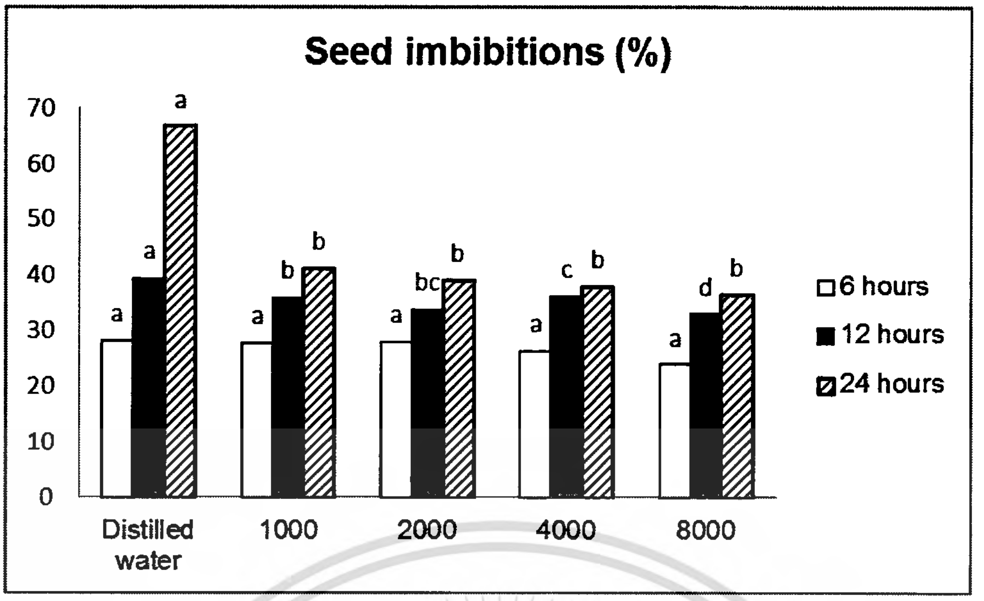

Figure 4.1. Effects of different concentrations of *T. erecta* on inhibition of seed imbibition of *A. tricolor*. Means followed by the same letter are do not differ significantly from each another by the Turkey's Studentized Range Test (p=0.05)

Table 4.3. Effects of different concentrations of *T. erecta* on α-amylase activity induction of *A. tricolor*

Concentration	α-Amylase activity (μmol maltose min^{-1} g^{-1}FW)		
	6 hours	12 hours	24 hours
Distilled water	5.85a	10.91a	14.32a
1000 ppm	5.91a	9.2ab	12.61a
2000 ppm	6.21a	8.07abc	12.45ab
4000 ppm	6.23a	7.71bc	8.71bc
8000 ppm	5.57a	7.38c	6.26c

Means followed by the same letter in a column do not differ significantly by Turkey's Studentized Range Test (p=0.05)

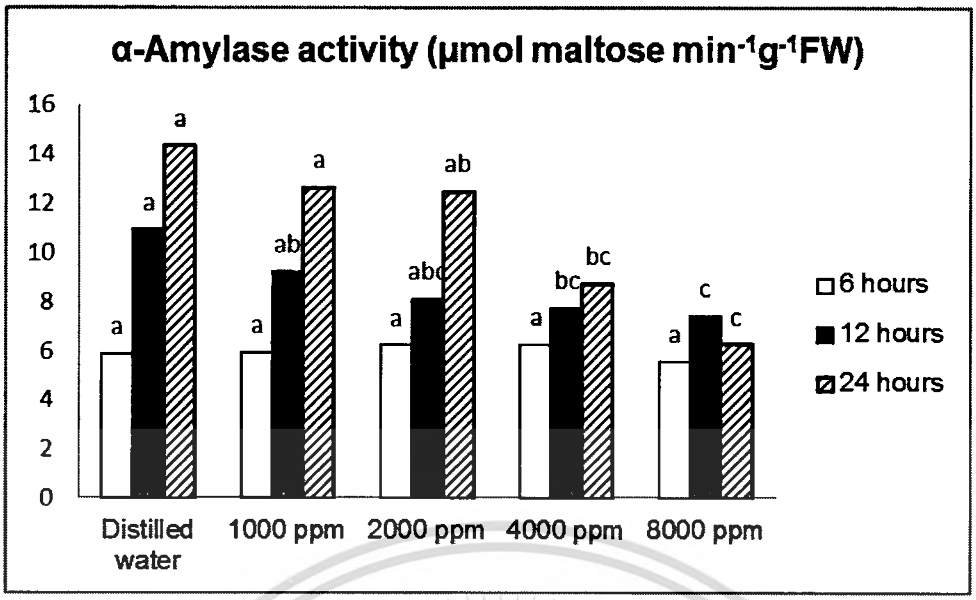

Figure 4.2. Effects of different concentrations of *T. erecta* on α-amylase activity induction of *A. tricolor*. Means followed by the same letter are do not differ significantly from each another by the Turkey's Studentized Range Test (p=0.05)

4.1.3. Effects of *T. erecta* and exogenous GA_3 on inhibition of seed germination of *A. tricolor*

As shown in table 4.4, seed inhibition on *A. tricolor* (% of control) after treated with combination of *T. erecta* leaf extract considered as natural herbicide and exogenous GA_3 was slightly decreased throughout the entire experiment with respect to untreated exogenous GA_3 (2500 ppm *T. erecta* leaf extract). This decrease was most pronounced with applied 0.01 ppm GA_3 and the inhibition of seed germination, shoot length and root length reached about 57.50%; 82.11% and 97.29%, respectively. However by increasing the dose of applied GA_3 at 0.1 ppm, the inhibition increased remarkably and inhibition of shoot length and root length at all treatment was still more than 90%. Hence this result suggests that these applied GA_3 had insignificant to prevent and relieve the herbicide effect on *A. tricolor*.

Table 4.4. Effects of *T. erecta* and exogenous GA_3 on inhibition of seed germination of *A. tricolor*

	Concentration (ppm)	Seed inhibition (% of control)		
		Seed germination	Shoot length	Root length
Treat. 2	1000 ppm Crude extract	12.50d	36.01b	71.44bc
Treat. 3	1000 ppm crude extract + 0.005 ppm GA_3	18.75d	44.50b	79.26b
Treat. 4	1000 ppm crude extract + 0.01 ppm GA_3	11.25d	25.46b	63.33c
Treat. 5	1000 ppm crude extract + 0.1 ppm GA_3	8.75d	44.04b	74.65bc
Treat. 6	2500 ppm Crude extract	100a	100a	100a
Treat. 7	2500 ppm crude extract + 0.005 ppm GA_3	71.25bc	97.71a	97.80a
Treat. 8	2500 ppm crude extract + 0.01 ppm GA_3	57.50c	82.11a	97.29a
Treat. 9	2500 ppm crude extract + 0.1 ppm GA_3	83.75ab	93.81a	98.40a

Means followed by the same letter in a column do not differ significantly by Turkey's Studentized Range Test (p=0.05)

4.1.4. Effects of *T. erecta* and exogenous GA_3 on seed imbibtion and α-amylase activity induction of *A. tricolor*

Gibbereilic acid is known to break dormancy of several types of seeds: (a) light-promoted seeds, such as Grand Rapids lettuce seed (*Lactuca sativa* L. var. Grand Rapids); (b) light inhibited seeds, such as the seed of the honey-bee plant (*Phacelia tanacetifolia* Benth; (c) seeds requiring stratification (storage at low temperatures in a moist condition), such as the hazel nut (*Corylus avellana* L.); (d) seeds requiring after-ripening (storage at room temperature in dry condition), such as the wild oat (*Avena fatua* L.). In this research, exogenous GA_3 was used for induction of seed germination by α-amylase activity after completed inhibition because of applied 2500 ppm *T. erecta* leaf extract. The results in table 4.5 and 4.6 show that exogenous GA_3 had insignificant effects against *T. erecta* leaf extract in soluble concentrate formulation on *A. tricolor* entire experimental period (after 6 an 12 hours imbibition) by both of seed imbibition and α-amylase activity induction. After 6 hours imbibition, seed imbibition and α-Amylase activity was 24 – 26% and 5 – 6 μmol maltose $min^{-1}g^{-1}$FW at all of treatments, respectively. So on after 12 hours imbibition, seed imbibition and α-Amylase activity was about 32 -36% and 9 - 11 μmol maltose $min^{-1}g^{-1}$FW, respectively. Therefore this result suggests that exogenous GA_3 had insignificant effects against *T. erecta* leaf extract in soluble concentrate formulation on *A. tricolor.*

Table 4.5. Effects of *T. erecta* and exogenous GA_3 on seed imbibtion and α-amylase activity induction of *A. tricolor* (after 6 hours imbibition)

		Seed imbibitions (%)	α-Amylase activity (μmol maltose $min^{-1}g^{-1}FW$)
Control	Distilled water	24.70a	5.84a
Treat. 2	2500 ppm Crude extract	24.99a	5.45a
Treat. 7	2500 ppm crude extract + 0.005 ppm GA_3	25.67a	6.14a
Treat. 8	2500 ppm crude extract + 0.01 ppm GA_3	26.03a	6.23a
Treat. 9	2500 ppm crude extract + 0.1 ppm GA_3	24.38a	5.69a

Means followed by the same letter in a column do not differ significantly by Turkey's Studentized Range Test (p=0.05)

Table 4.6. Effects of *T. erecta* and exogenous GA_3 on seed imbibtion and α-amylase activity induction of *A. tricolor* (after 12 hours imbibition)

	Concentration	Seed imbibitions (%)	α-Amylase activity (μmol maltose $min^{-1}g^{-1}FW$)
Control	Distilled water	36.56a	11.48a
Treat. 2	2500 ppm Crude extract	32.28a	9.42b
Treat. 3	2500 ppm crude extract + 0.005 ppm GA_3	32.36a	9.16b
Treat. 4	2500 ppm crude extract + 0.01 ppm GA_3	34.06a	9.53b
Treat. 5	2500 ppm crude extract + 0.1 ppm GA_3	33.31a	8.58b

Means followed by the same letter in a column do not differ significantly by Turkey's Studentized Range Test (p=0.05)

4.2. Experiment 2. Effects of different concentrations of *T. erecta*, exogenous GA_3 and the combination of *T. erecta* and exogenous GA_3 on seed germination, seed imbibition and α-amylase activity induction of *A. spinosus*

4.2.1. Effects of different concentrations of *T. erecta* on inhibition of seed germination and seedling growth of *A. spinosus*

The table and figure below show the inhibition of seed germination and seedling growth of *A. spinosus* by *T. erecta* leaf extract in soluble concentrate formulation. At 1000 – 8000 ppm dose, seed germination of *A. spinosus* was inhibited completely whereas there is no inhibition of shoot length and root length at control. Hence *T. erecta* leaf extract contains some inhibitory principles upon inhibited germination and seedling growth. Thus, further studies were carried out to evaluate and understand interaction mechanisms of allelopathic potential about inhibition on seed imbibition and α-amylase activities of *A. spinosus* seeds.

Table 4.7. Effects of different concentrations of *T. erecta* on inhibition of seed germination and seedling growth of *A. spinosus*

Concentration (ppm)	Inhibition (% of control)		
	Seed germination	Shoot length	Root length
Distilled water	12.50b	0.00b	0.00b
1000	100.00a	100.00a	100.00a
2000	100.00a	100.00a	100.00a
4000	100.00a	100.00a	100.00a
8000	100.00a	100.00a	100.00a

Means followed by the same letter in a column do not differ significantly by Turkey's Studentized Range Test (p=0.05)

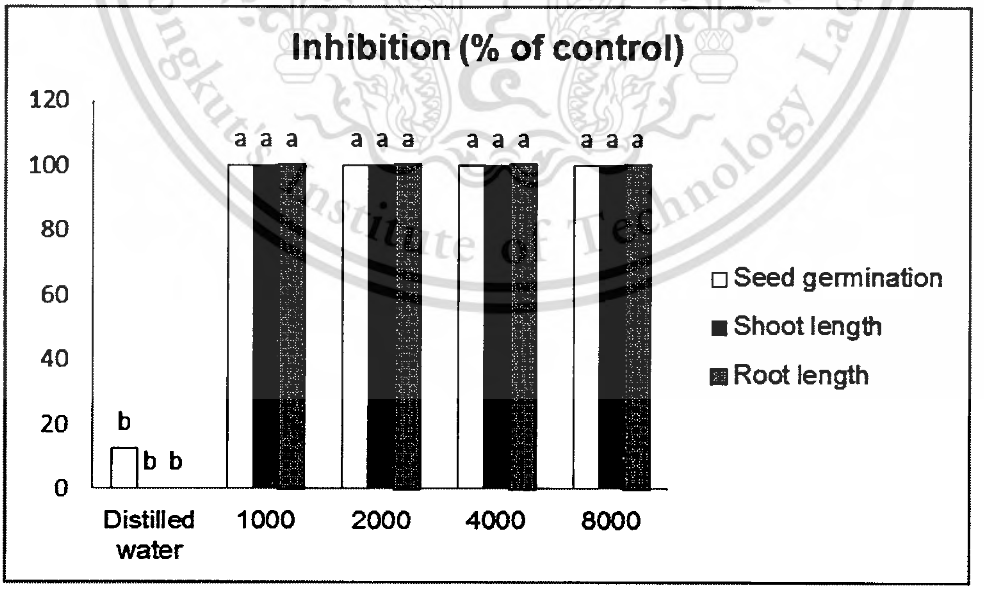

Figure 4.3. Effects of different concentrations of *T. erecta* on inhibition of seed germination and seedling growth of *A. spinosus*. Means followed by the same letter are do not differ significantly from each another by the Turkey's Studentized Range Test (p=0.05)

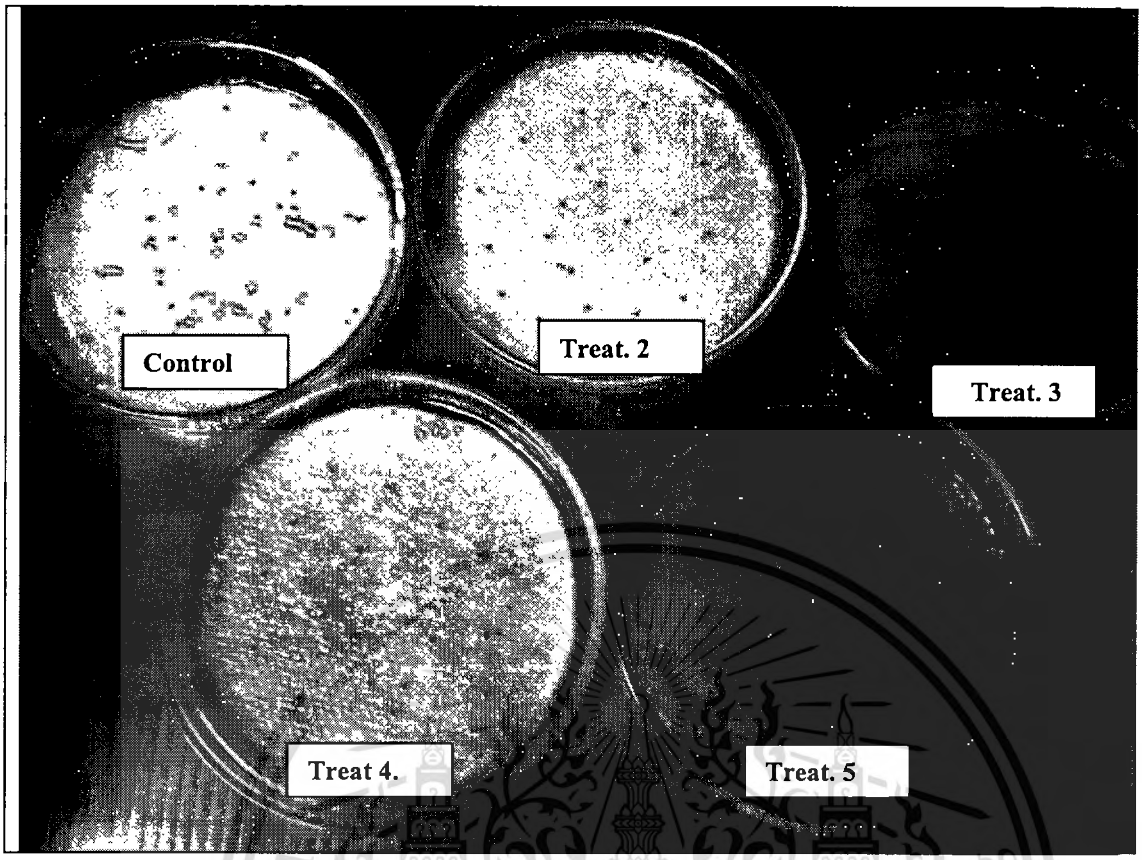

Figure 4.4. Effects of different concentrations of *T. erecta* on inhibition of seed germination and seedling growth of *A. spinosus*

4.2.2. Effects of different concentrations of *T. erecta* on seed imbibition and α-amylase activity induction of *A. spinosus*

Data below shows the differences in the percentage of seed imbibition between control and concentration application of *T. erecta* leaf extract in soluble concentrate formulation at different imbibition periods. The percentages of imbibition in the control seeds markedly increased by prolonging the imbibition periods and had the highest seed imbibition at all the imbibition time whereas the percentage of imbibition in treated seeds increased slightly by prolonging the imbibition period. After imbibiton time of 12 hours, 24 hours and 36 hours, the percentage of seed imbibition inhibited significantly upon the increasing the dose of application during the entire experiment. After imbibiton time of 12 hours, 24 hours and 36 hours, seed imbibition inhibited from 8.14%, 14.38% and 105.64% in comparison with the control.

The activities of α-amylase of *A. spinosus* seeds were also investigated and the results are shown in below data. Under the same concentration of *T. erecta* leaf extract, α-amylase activity increased by prolonging the imbibition period, especially in control. After imbibition time of 12, 24 and 36 hours, increased concentration of *T. erecta* leaf extract in soluble concentrate formulation inhibited remarkably induction α-amylase activities were 9.4, 22.8 and 41.46 µmol maltose $min^{-1}g^{-1}$FW, respectively. By these results, the mechanisms of allelopathic potential of *T. erecta* leaf extract inhibited seed imbibition and α-amylase activities at high concentration on *A. spinosus*.

Table 4.8. Effects of different concentrations of *T. erecta* on seed imbibition of *A. spinosus*

Concentration (ppm)	Seed imbibitions (%)		
	12 hours	24 hours	36 hours
Distilled water	23.95a	38.68a	130.42a
1000	19.59a	30.49b	33.95b
2000	23.58a	30.68b	29.74bc
4000	18.73a	24.50b	29.26bc
8000	15.81a	24.30b	24.78c

Means followed by the same letter in a column do not differ significantly by Turkey's Studentized Range Test (p=0.05)

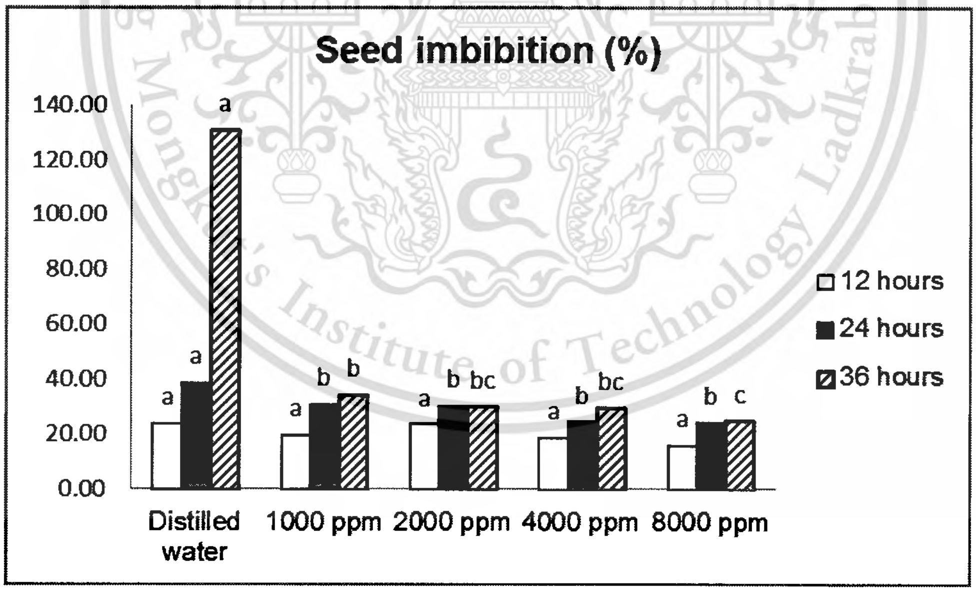

Figure 4.5. Effects of different concentrations of *T. erecta* on seed imbibition of *A. spinosus*. Means followed by the same letter are do not differ significantly from each another by the Turkey's Studentized Range Test (p=0.05)

Table 4.9. Effects of different concentrations of *T. erecta* on α-amylase activity induction of *A. spinosus*

Concentration (ppm)	α-Amylase activity (μmol maltose $min^{-1}g^{-1}FW$)		
	12 hours	24 hours	36 hours
Distilled water	17.76a	26.18a	46.43a
1000	15.34ab	19.32b	20.50b
2000	12.98b	14.83b	21.07b
4000	9.85c	9.47c	14.29bc
8000	8.36c	3.38c	4.97c

Means followed by the same letter in a column do not differ significantly by Turkey's Studentized Range Test (p=0.05)

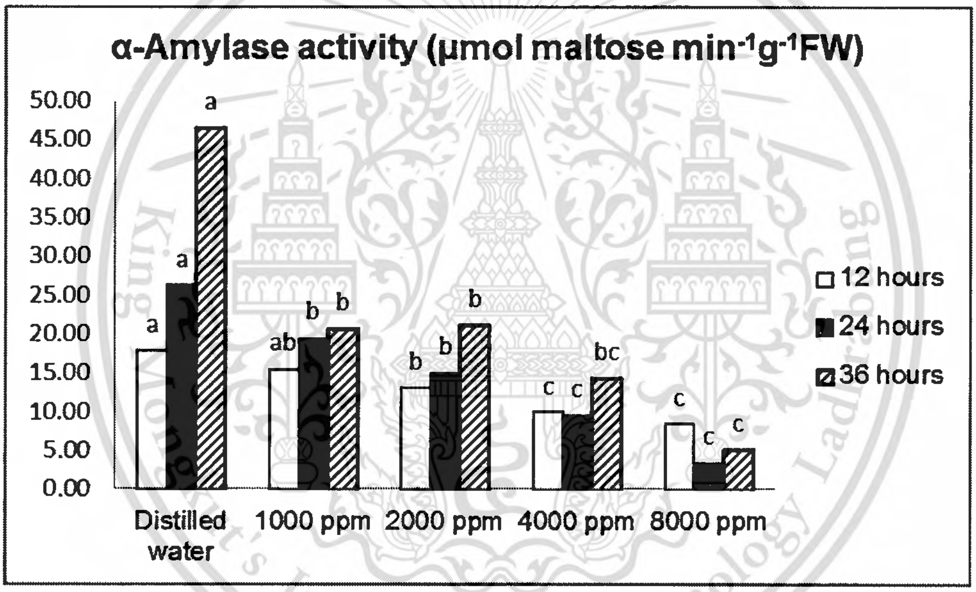

Figure 4.6. Effects of different concentrations of *T. erecta* on α-amylase activity induction of *A. spinosus*. Means followed by the same letter are do not differ significantly from each another by the Turkey's Studentized Range Test (p=0.05)

4.2.3. Effects of *T. erecta* and exogenous GA_3 on inhibition of seed germination and seedling growth of *A. spinosus*

As shown in below table and figure, in comparison of control, *A. spinosus* seeds had insignificant on seed germination and seedling growth after applied exogenous GA_3 (from 0.1 – 0.8 ppm). Seed inhibition of *A. spinosus* (% of control) after treated with concentration combination of *T. erecta* leaf extract considered as natural herbicide and exogenous GA_3 was slightly decreased in whole experiment in comparison with untreated exogenous GA_3 (1000 ppm *T. erecta* leaf extract). This decrease was most pronounced with applied 0.1 ppm GA_3 and the inhibition of seed germination, shoot length and root length were 68.75%; 80.30 % and 95.39%, respectively. However by increasing the dose of applied GA_3 at 0.2 to 0.8 ppm, the inhibition increased. In addition, entire experiment shoot length and root length still had a remarkable inhibition (more than 80%). These applied GA_3 had insignificant to prevent and relieve the herbicide effect on *A. spinosus.*

Table 4.10. Effects of *T. erecta* and exogenous GA_3 on inhibition of seed germination and seedling growth of *A. spinosus*

	Concentration	Inhibition (% of control)		
		Seed germination	Shoot length	Root length
Treat. 2	1000 ppm Crude extract	100.00a	100.00a	100.00a
Treat. 3	0.1 ppm GA_3	2.50c	-6.06c	-3.95c
Treat. 4	0.2 ppm GA_3	0.00c	18.18b	19.74b
Treat. 5	0.4 ppm GA_3	2.50c	15.15b	5.26c
Treat. 6	0.8 ppm GA_3	3.75c	9.09b	15.79b
Treat. 7	1000 ppm crude extract + 0.1 ppm GA_3	68.75b	80.30a	95.39a
Treat. 8	1000 ppm crude extract + 0.2 ppm GA_3	88.75ab	87.88a	100.00a
Treat. 9	1000 ppm crude extract + 0.4 ppm GA_3	85.00ab	87.88a	100.00a
Treat. 10	1000 ppm crude extract + 0.8 ppm GA_3	98.75a	100.00a	100.00a

Means followed by the same letter in a column do not differ significantly by Turkey's Studentized Range Test (p=0.05)

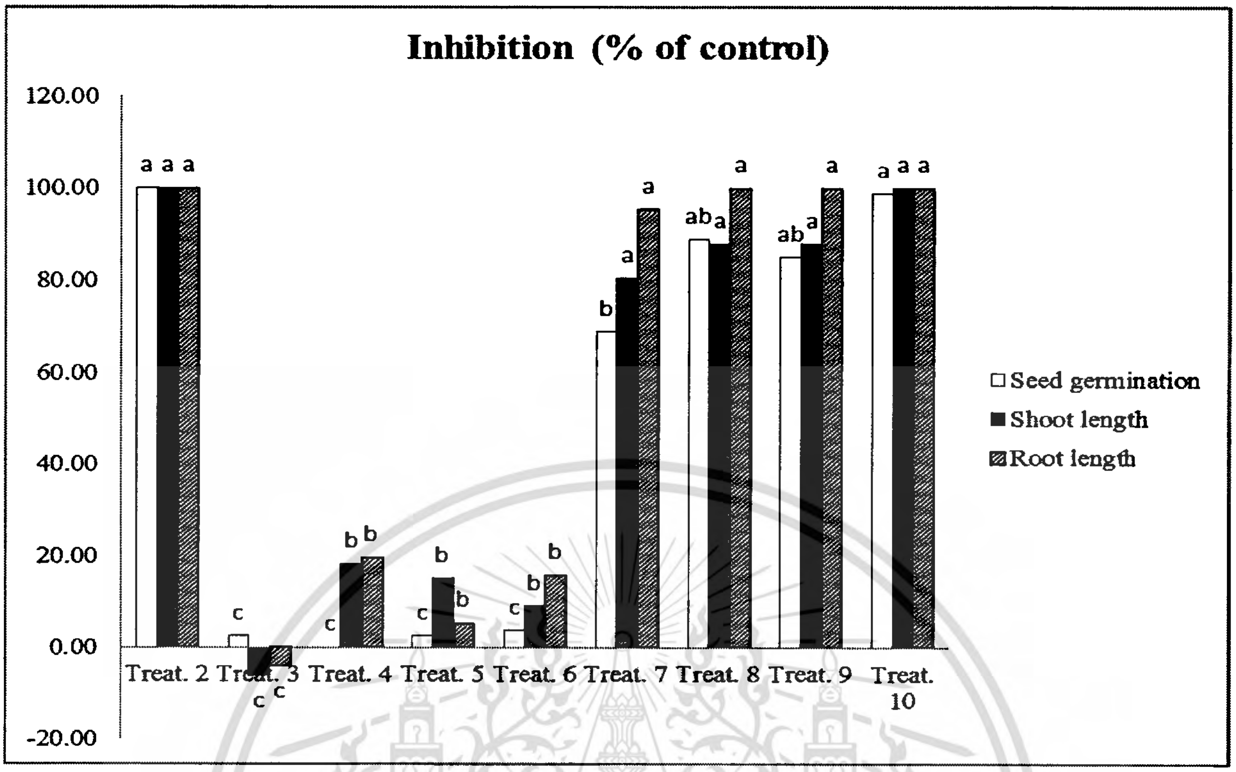

Figure 4.7. Effects of *T. erecta* and exogenous GA_3 on inhibition of seed germination and seedling growth of *A spinosus*. Means followed by the same letter are do not differ significantly from each another by the Turkey's Studentized Range Test (p=0.05)

4.2.4. Effects of *T. erecta* and exogenous GA_3 on seed imbibition and α-Amylase activity induction of *A. spinosus*

Exogenous GA_3 was used for induction of seed germination by α-amylase activity after completed inhibition because of applied 2500 ppm *T. erecta* leaf extract in this study. The results in the Table 4.11 and the Figure 4.8 show that exogenous GA_3 had insignificant effects against *T. erecta* leaf extract in soluble concentrate formulation on *A. spinosus* entire experimental period (after 12 hours imbibition) by both of seed imbibition and α-amylase activity induction. Seed imbibition and α-amylase activity in all treatments in whole experiment were about 20 - 23% and 9 - 12 μmol maltose $min^{-1}g^{-1}FW$, respectively. Even though in comparison with control, treatments were applied with only exogenous GA_3 had a marked indifferences of seed imbibition and α-amylase activity induction.

Therefore this result suggests that exogenous GA_3 had insignificant effects against *T. erecta* leaf extract in soluble concentrate formulation on *A. spinosus*.

Table 4.11. Effects of *T. erecta* and exogenous GA_3 on seed imbibition and α-Amylase activity induction of *A. spinosus* (after 12 hours imbibition)

	Concentration	Seed imbibitions (%)	α-Amylase activity (μmol maltose $min^{-1}g^{-1}$FW)
Control	Distilled water	23.32a	12.26ab
Treat. 2	1000 ppm Crude extract	20.37a	8.78dc
Treat. 3	0.1 ppm GA_3	24.26a	13.06a
Treat. 4	0.2 ppm GA_3	23.20a	12.79a
Treat. 5	0.4 ppm GA_3	23.77a	12.54a
Treat. 6	0.8 ppm GA_3	21.06a	11.85abc
Treat. 7	1000 ppm crude extract + 0.1 ppm GA_3	20.12a	9.16bdc
Treat. 8	1000 ppm crude extract + 0.2 ppm GA_3	19.27a	8.98bdc
Treat. 9	1000 ppm crude extract + 0.4 ppm GA_3	21.17a	8.54d
Treat. 10	1000 ppm crude extract + 0.8 ppm GA_3	20.14a	8.95dc

Means followed by the same letter in a column do not differ significantly by Turkey's Studentized Range Test (p=0.05)

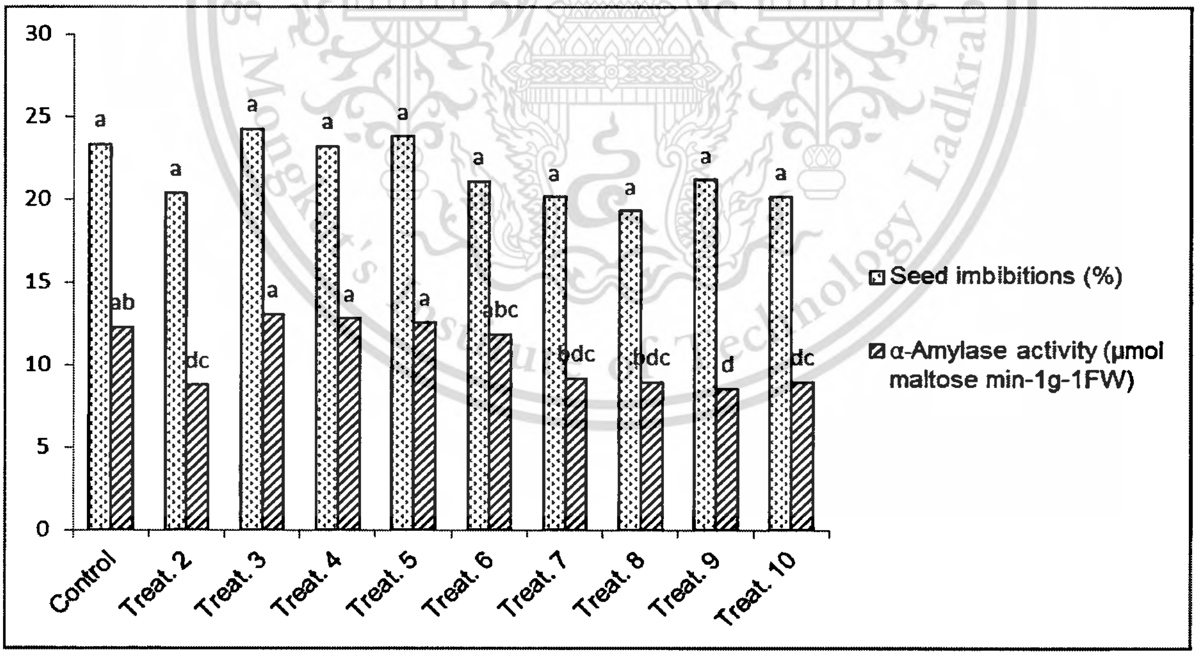

Figure 4.8. Effects of *T. erecta* in soluble concentrate formulation (SC) and exogenous GA_3 on seed imbibtion and α-amylase activity induction of *A. spinosus* (after 12 hours imbibition). Means followed by the same letter are do not differ significantly from each another by the Turkey's Studentized Range Test (p=0.05)

4.3. Experiment 3. Effects of different concentrations of *T. erecta*, exogenous GA_3 and the combination of *T. erecta* and exogenous GA_3 on seed germination, seed imbibition and α-amylase activity induction of *A. gracilis*

4.3.1. Effects of different concentrations of *T. erecta* on inhibition of seed germination and seedling growth of *A. gracilis*

The Table 4.12 and the Figure 4.9 show that *T. erecta* leaf extract in soluble concentrate formulation had significant allelopathic effects against *A. gracilis* (Table 4.12 and Figure 4.9). At 250 ppm dose, germination of seed of *A. gracilis* was inhibited by 68.33%. Its shoot and root length inhibition were 24.14 and 3.81%, respectively. By increasing the dose of application at 750 ppm, germination of seed of *A. gracilis* was inhibited by 88.33% whereas its shoot and root length inhibition were 96.55 and 95.24%, respectively. At reached concentrations of 1000 – 8000 ppm, the inhibition magnitude was complete. This suggests that *T. erecta* leaf extract contains some inhibitory principles upon inhibited germination and seedling growth. Hence next experiments were carried out to evaluate and understand interaction mechanisms of allelopathic potential about inhibition on seed imbibition and α-amylase activities.

Table 4.12. Effects of different concentrations of *T. erecta* on inhibition of seed germination and seedling growth of *A. gracilis*

Concentration (ppm)	Inhibition (% of control)		
	Seed germination	Shoot length	Root length
250	68.33c	24.14b	3.81b
500	85.83b	87.93a	88.57a
750	88.33b	96.55a	95.24a
1000	100.00a	100.00a	100.00a
2000	100.00a	100.00a	100.00a
4000	100.00a	100.00a	100.00a
8000	100.00a	100.00a	100.00a

Means followed by the same letter in a column do not differ significantly by Turkey's Studentized Range Test (p=0.05)

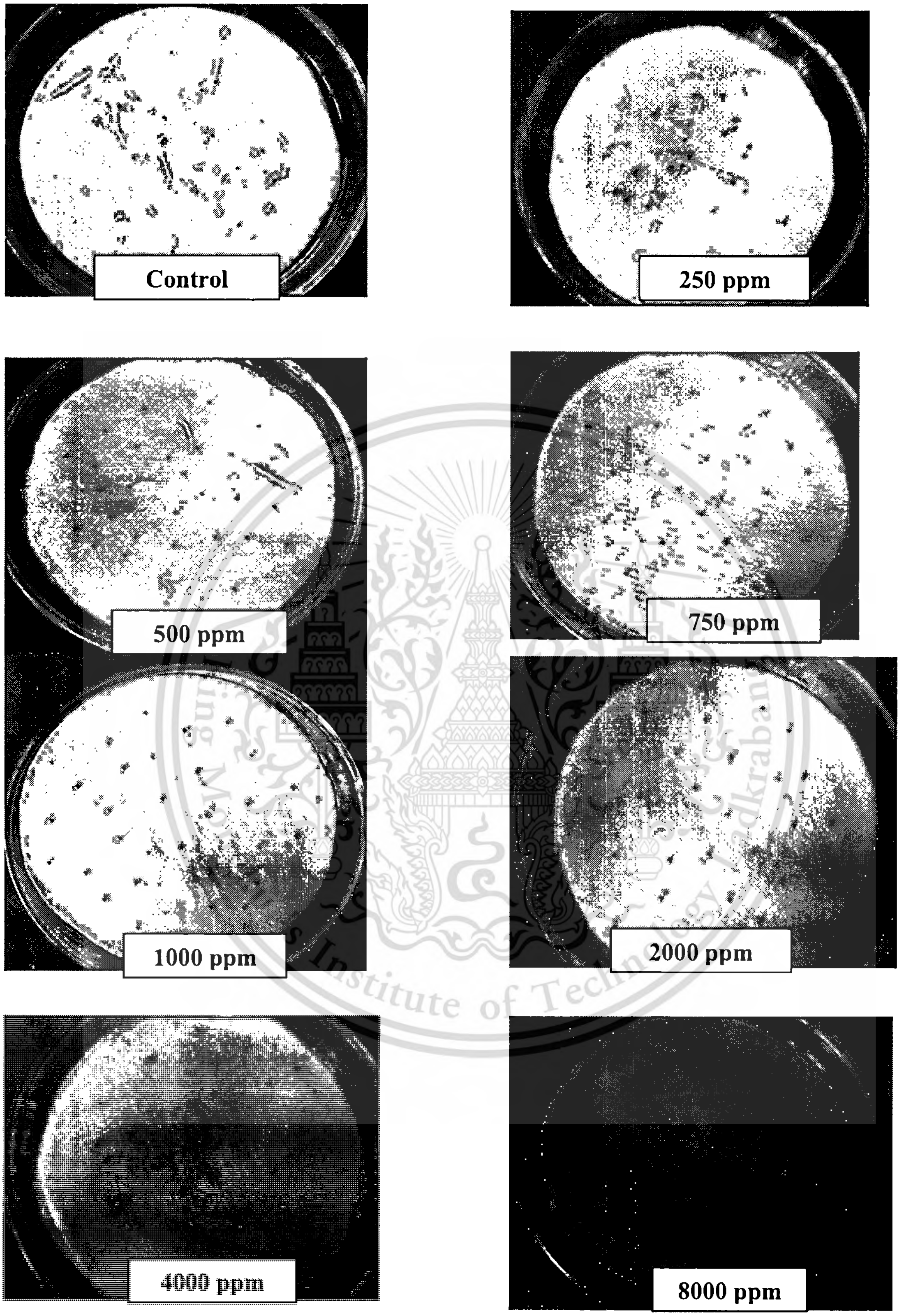

Figure 4.9. Effects of different concentrations of *T. erecta* on inhibition of seed germination and seedling growth of *A. gracilis*

4.3.2. Effects of different concentrations of *T. erecta* on seed imbibition and α-amylase activity induction of *A. gracilis*

The Table 4.13 and the Figure 4.10 show the differences in the percentage of seed imbibition between control and concentration application of *T. erecta* leaf extract in soluble concentrate formulation at different imbibition periods. By prolonging the imbibition periods, the percentages of imbibition in the control seeds markedly increased and had the highest seed imbibition at all the imbibition time whereas the percentage of imbibition in treated seeds increased slightly by prolonging the imbibition period. After imbibition time of 12 hours, 24 hours and 36 hours, the percentage of seed imbibition inhibited significantly according to the increasing the dose of application during the entire experiment. During above imbibition time, seed imbibition significantly inhibited from 1.83%, 10.15% and 10.01%, respectively in comparison with the control.

The activities of α-amylase of *A. gracilis* seeds were also investigated and the results are shown in the Table 4.14 and the Figure 4.11. Under the same concentration of *T. erecta* leaf extract, α-amylase activity increased by prolonging the imbibition period, especially in control. After imbibition time of 12, 24 and 36 hours, by increased concentration of *T. erecta* leaf extract in soluble concentrate formulation reached to 1000 ppm, an inhibited induction α-amylase activities were 2.05, 10.34 and 6.39 μmol maltose $min^{-1}g^{-1}$FW, respectively in comparison with the control. By going on these results, the mechanisms of allelopathic potential of *T. erecta* leaf extract inhibited seed imbibition and α-amylase activities at high concentration on *A. gracilis*.

Table 4.13. Effects of different concentrations of *T. erecta* on seed imbibition of *A. gracilis*

Concentration (ppm)	Seed imbibitions (%)		
	12 hours	24 hours	36 hours
Distilled water	24.74a	32.83a	33.83a
250	27.55a	28.77ab	28.00ab
500	26.49a	27.37abc	26.40ab
750	22.03a	25.94bc	25.93ab
1000	22.91a	22.68c	23.82b

Means followed by the same letter in a column do not differ significantly by Turkey's Studentized Range Test (p=0.05)

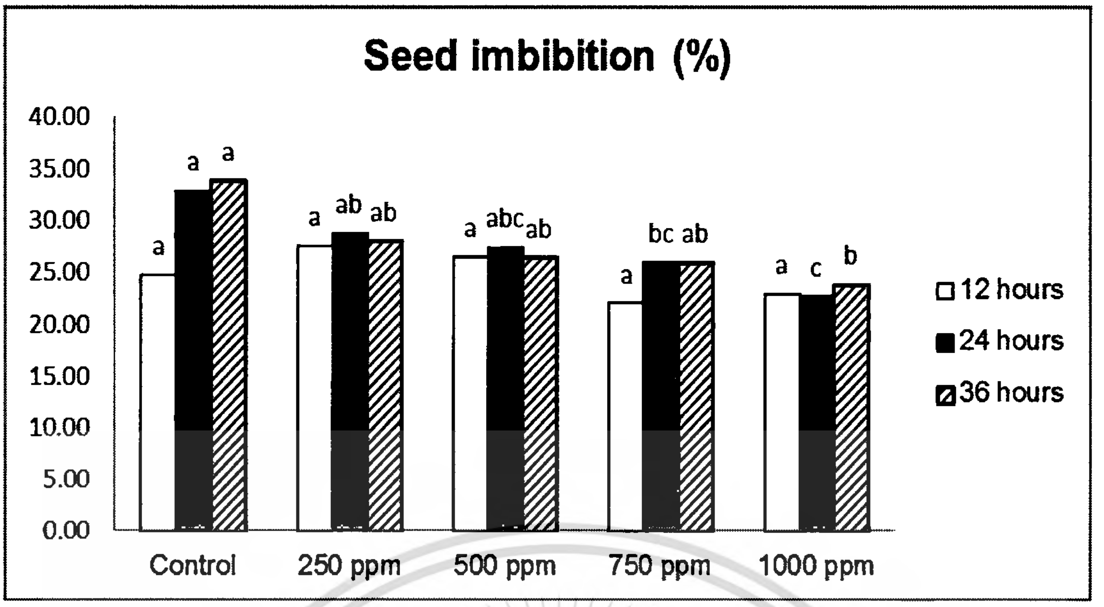

Figure 4.10. Effects of different concentrations of *T. erecta* on seed imbibition of *A. gracilis*. Means followed by the same letter are do not differ significantly from each another by the Turkey's Studentized Range Test (p=0.05)

Table 4.14. Effects of different concentrations of *T. erecta* on α-amylase activity induction of *A.gracilis*

Concentration (ppm)	α-Amylase activity (μmol maltose min^{-1} g^{-1} FW)		
	12 hours	24 hours	36 hours
Distilled water	11.95a	19.56a	12.64a
250	10.06a	15.70ab	13.43a
500	9.60a	13.99ab	7.62b
750	10.08a	9.94b	7.91b
1000	9.90a	9.22b	6.25b

Means followed by the same letter in a column do not differ significantly by Turkey's Studentized Range Test (p=0.05)

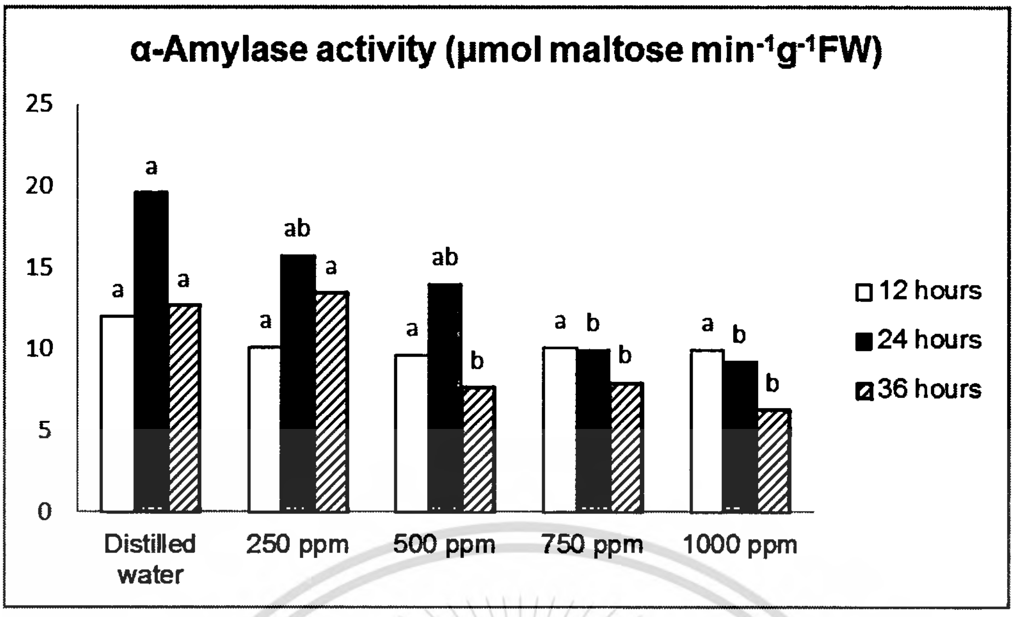

Figure 4.11. Effects of different concentrations of *T. erecta* on α-amylase activity induction of *A. gracilis*. Means followed by the same letter are do not differ significantly from each another by the Turkey's Studentized Range Test (p=0.05)

4.3.3. Effects of *T. erecta* and exogenous GA_3 on inhibition of seed germination and seedling growth of *A. gracilis*

As shown in the Table 4.15 and the Figure 4.12, *A. gracilis* seed after treated with concentration combination of *T. erecta* leaf extract and exogenous GA_3 still inhibited completely seed germination and seedling growth throughout the entire experiment (100% inhibition of control) whereas *A. gracilis* seed was applied with only exogenous GA_3 had insignificant promotion seedling growth in comparison of control. Hence this result suggests that these applied exogenous GA_3 had insignificant to prevent and relieve the *T. erecta* leaf extract effect on *A. gracilis*.

Table 4.15. Effects of *T. erecta* and exogenous GA_3 on inhibition of seed germination and seedling growth of *A. gracilis*

	Concentration	Inhibition (% of control)		
		Seed germination	Shoot length	Root length
Treat. 2	2000 ppm crude extract	100.00a	100.00a	100.00a
Treat. 3	0.1 ppm GA_3	67.50b	10.71b	-12.50b
Treat. 4	0.2 ppm GA_3	45.83c	-10.71b	-1.56b
Treat. 5	0.4 ppm GA_3	72.50b	21.43b	-9.38b
Treat. 6	0.8 ppm GA_3	56.67bc	14.29b	-12.50b
Treat. 7	1000 ppm crude extract + 0.1 ppm GA_3	100.00a	100.00a	100.00a
Treat. 8	1000 ppm crude extract + 0.2 ppm GA_3	100.00a	100.00a	100.00a
Treat. 9	1000 ppm crude extract + 0.4 ppm GA_3	100.00a	100.00a	100.00a
Treat. 10	1000 ppm crude extract + 0.8 ppm GA_3	100.00a	100.00a	100.00a

Means followed by the same letter in a column do not differ significantly by Turkey's Studentized Range Test (p=0.05)

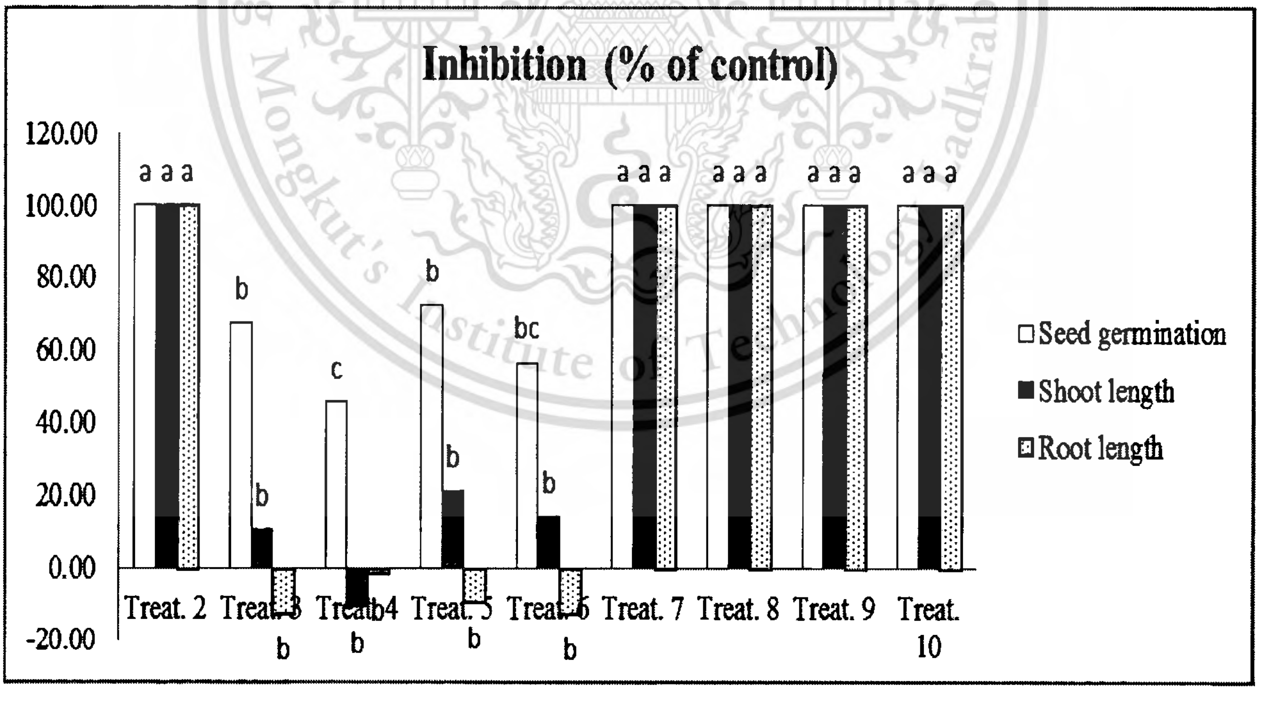

Figure 4.12. Effects of *T. erecta* and exogenous GA_3 on inhibition of seed germination and seedling growth of *A. gracilis*. Means followed by the same letter are do not differ significantly from each another by the Turkey's Studentized Range Test (p=0.05)

4.4. Discussions

Comparisons of different concentrations of *T. erecta* on *Amaranthus spp.* (*A. tricolor*, *A. spinosus* and *A. gracilis*)

The below figures show allelopathic potential of *T. erecta* leaf extract had significantly different inhibition on difference of amaranth species. The inhibitory effects of seed germination and seedling growth on *A. tricolor* were less than those on *A. spinosus* and *A. gracilis*. At dose of 1000 ppm *T. erecta* leaf extract in soluble concentration formulation inhibition of control was complete whereas the inhibition on *A. tricolor* was 2500 ppm *T. erecta* leaf extract.

The figures also show the effects of different concentrations of *T. erecta* on inhibition of seed imbibition and α- amylase activity induction had the most significant in *A. gracilis* afterward *A. spinosus*.

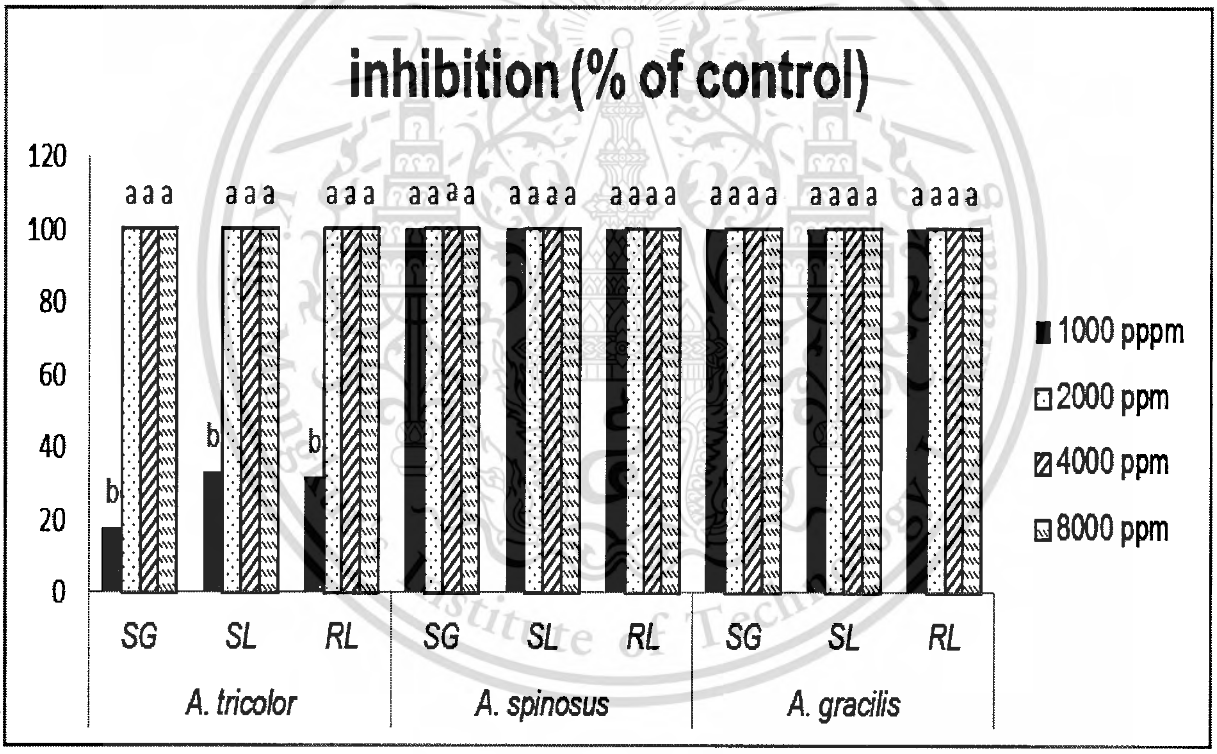

Figure 4.13. Effect of different concentrations of *T. erecta* on inhibition of seed germination and seedling growth. Means followed by the same letter are do not differ significantly from each another by the Turkey's Studentized Range Test (p=0.05)

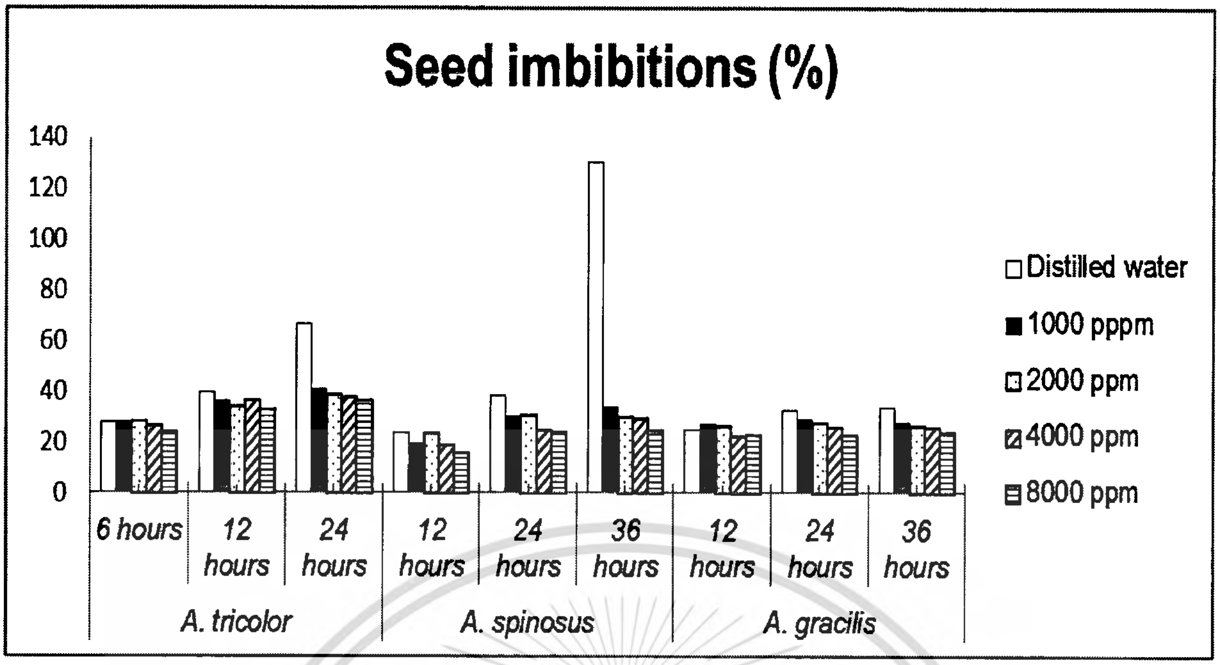

Figure 4.14. Effect of different concentrations of *T. erecta* on inhibition of seed imbibition. Means followed by the same letter are do not differ significantly from each another by the Turkey's Studentized Range Test (p=0.05)

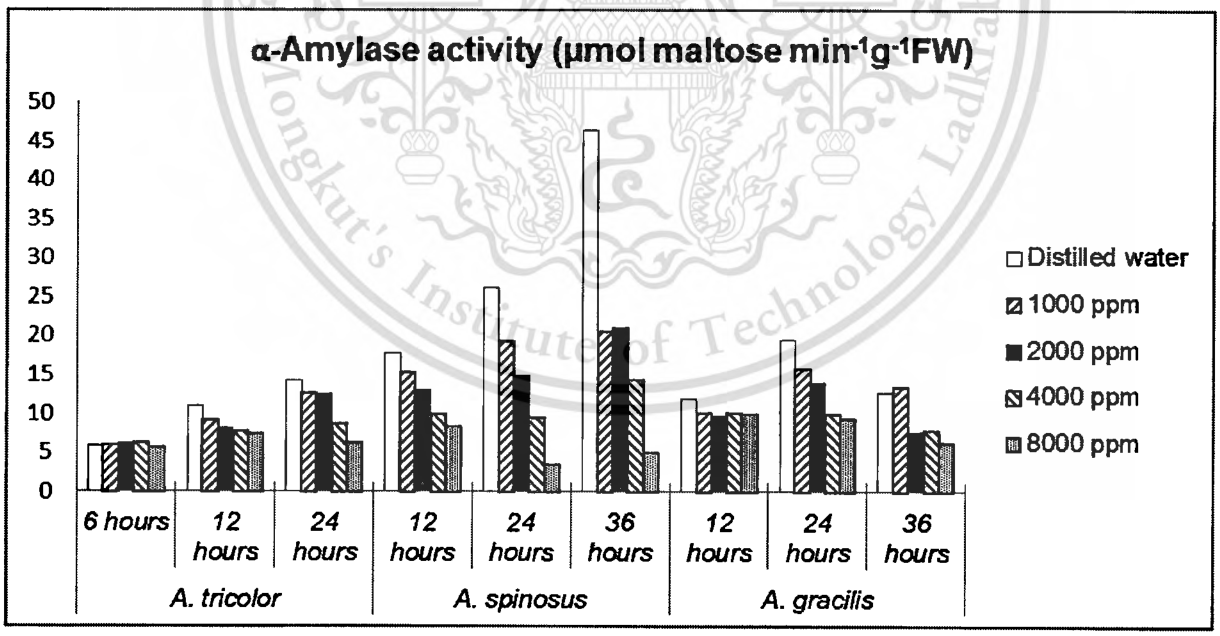

Figure 4.15. Effect of different concentrations of *T. erecta* on α- amylase activity induction. Means followed by the same letter are do not differ significantly from each another by the Turkey's Studentized Range Test (p=0.05)

In this study, allelopathic inhibition was accordance with other studies (Laosinwattana et al., 2010; Lin et al. (2006); Han et al. (2008); (Chong et al., 2002). The findings showed the inhibitory effects of *Saururaceae* (*Houttuynia cordata* Thunb.) varied with weed indicator species evaluated; the aqueous extracts from young leaves of *M. azeradach* inhibited *E. crus-galli* seed germination; ginger aqueous extracts inhibited imbibition in seeds of chive and soybean; seed was inhibited the imbibition because of limited in specific enzymes required for metabolism of reserved food and hence exhibited poor seed germination; the α-amylase activity catalyzes endosperm starch hydrolysis and transformation into soluble sugars and hence its utilization for providing energy during seed germination; mechanism of aqueous extract from young leaf stage of *M. azeradach* may be associated with inhibition of activity of α-amylase. It was shown that α-amylase activity was inhibited by presence of allelochemicals. Besides Kato-Noguchi and Macıas (2005) also had reported that lettuce (*Lactuca sativa* L. cv. Grand Rapids) seeds treated by 6-methoxy-2- benzoxazolinone (MBOA) reduced activity of α-amylase during seed germination.

CHAPTER 5

CONCLUSIONS AND RECOMENDATIONS

5.1 Conclusions

The crude extract from *T. erecta* had significant allelopathic effects against 3 species of amaranth. It completely inhibited on seed germination and seedling growth of *A. spinosus* and *A. gracilis* at the concentration of 1000 ppm whereas 2500 ppm on *A. tricolor*. The mechanisms of allelopathic potential of *T. erecta* crude extract are inhibition on seed imbibition and α-amylase activities at high concentration on amaranth. Hence the use of crude extract from *T. erecta* as a potential natural herbicide for weed control might be possible.

Exogenous GA_3 was used for induction of seed germination by seed imbibition and α-amylase activity induction however it had insignificant effects against *T. erecta* leaf extract in soluble concentrate formulation on *A. tricolor, A. spinosus* and *A. gracilis.* Therefore this suggests that mechanism of *Tagetes erecta* L. leaf extract on *Amaranthus spp.* through seed germination is not affected by exogenous GA_3.

5.2. Recomendations

5.2.1. Carry out further studies about molecular and cytogenetic mechanisms of *T. erecta* leaf extract effects on *A. tricolor, A. spinosus* and *A. gracilis*.

5.2.2. Carry out studies about effects of *T. erecta* leaf extract on seed germination and seedling growth of 3 above amaranth species in green house.

5.2.3. Carry out studies about *T. erecta* leaf extract effects on other bioassays.

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