EFFICACY OF *Ficus* HYDROSEEDING FOR FOREST RESTORATION IN AN ABANDONED LIMESTONE QUARRY

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MASTER OF SCIENCE
IN BIOLOGY

GRADUATE SCHOOL
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Thesis Title Efficacy of *Ficus* Hydroseeding for Forest Restoration in an Abandoned Limestone Quarry

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ASTRACT

Reforestation of limestone quarries is more challenging compared to other degraded sites, due to rocky conditions cliff and the lack of top soil. Trees species of the genus *Ficus* can survive well under various conditions; from riparian zones to dry cliff faces. Therefore, this genus may have high potential to survive in the harsh conditions of limestone mines substrates. Hence, this study tested a hydroseeding technique to germinate seeds of *Ficus* spp. in a limestone quarry of Siam Cement (Lampang). The seeds of *Ficus benjamina*, *F. hispida* and *F. semicordata* were tested with a hydrogel, comprised of sodium carboxymethyl cellulose (NaCMC) mixed, in various proportions, with agar or corn starch.

NaCMC (1% w/v) was used as a base component (0, 25, 50, 75 and 100% v/v) with added agar (0.45% w/v) or corn starch (5% w/v) to investigate the gels' chemical and physical properties. The pH of the hydrogel mixes did not vary significantly (pH 5.82 to 6.08, ANOVA, $p < 0.05$). Corn starch significantly increased hydrogel viscosity ($p < 0.05$), whilst adding agar significantly reduced it ($p < 0.05$). Increased NaCMC content increased both the equilibrium water content and the swelling ratio. Moreover, adding corn starch had better results on equilibrium water content, swelling ratio and water vapor permeability (WVP) than agar, in the same proportions. Agar with NaCMC had insignificant WVPs, but WVPs increased with increasing proportion of corn starch ($p < 0.05$). At high relative humidity, film blending with NaCMC and agar had higher moisture sorption than film with corn starch and equilibrium moisture contents (Mo) from a GAB model were ranged from 0.573 to 0.998 g water/g dry film.

High NaCMC content inhibited germination of *Ficus* spp. seeds. Per cent germination differed significantly among the gel formulae ($p < 0.05$). Germination in control was highest on the 10th day.

Hydroseeding was tested in the nursery. A syringe was used to deliver exactly 50 seeds, mixed with 50 ml of hydrogel, into mine substrate spread on germination trays. Per cent germination, median length of dormancy (MLD) and per cent survival did not differ significantly among treatments ($p < 0.05$). However, differences among species were significant ($p < 0.05$). *F. benjamina* had the lowest germination percentage, MLD and survival percentage, whilst *F. hispida* and *F. semicordata* had high values.

Hydrogels of 50:50 NaCMC with agar or corn starch, were tested for *Ficus* hydroseeding on limestone mine slopes, at the beginning of rainy season in 2013. All treatments, including the control, resulted in very low germination and survival percentage. In conclusion, hydrogel was not an effective as a way to establish *Ficus* seedlings on mines.

หัวข้อวิทยานิพนธ์ ประสิทธิภาพของไฮโดรซีดดิงในพืชสกุล *Ficus* เพื่อการฟื้นฟูป่าในเหมืองหินปูนที่สิ้นสุดกิจกรรม

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บทคัดย่อ

การปลูกป่าทดแทนในพื้นที่เหมืองหินปูนนั้นเป็นสิ่งที่กระทำได้ยากเนื่องจากเหมืองหินปูนเป็นบริเวณที่ถูกทำลายจนเหลือแต่บริเวณหน้าผาหินที่ปราศจากหน้าดินปกคลุม พืชในสกุลมะเดื่อและไทร (*Ficus* spp.) สามารถพบเห็นได้ในหลายหลายพื้นที่ตั้งแต่บริเวณริมน้ำไปจนถึงพื้นที่สูงชันและพืชในสกุลนี้สามารถอยู่รอดได้ในสภาพของหินปูน การศึกษานี้จึงมุ่งเน้นเพื่อใช้พืชสกุลมะเดื่อและไทรร่วมกับเทคนิคไฮโดรซีดดิงในพื้นที่ของเหมืองปูนบริษัทปูนซีเมนต์ไทยลำปาง จำกัด โดยเมล็ดของ *Ficus benjamina, F. hispida* และ *F. semicordata* ถูกนำมาทดสอบกับไฮโดรเจลที่มี Sodium Carboxymethyl Cellulose (NaCMC) ผสมกับแป้งข้าวโพดหรือวุ้นในหลายอัตราส่วน

ไฮโดรเจลที่มีส่วนผสมของ NaCMC (0.1% w/v) ตั้งแต่สัดส่วน 0, 25, 50, 75 และ 100% v/v และถูกแทนด้วยสัดส่วนของวุ้น (0.45% w/v) หรือแป้งข้าวโพด (5% w/v) เพื่อทดสอบคุณสมบัติทางเคมีและกายภาพ พบว่าค่าความเป็นกรดด่างของไฮโดรเจลไม่แตกต่างทางสถิติ (pH 5.82 ถึง 6.08, ANOVA, $p < 0.05$) โดยไฮโดรเจลที่มีส่วนผสมของแป้งข้าวโพดจะมีค่าความหนืดมากที่สุดและแตกต่างทางสถิติอย่างมีนัยสำคัญ ($p < 0.05$) แต่ไฮโดรเจลที่มีส่วนผสมของวุ้นมากขึ้นจะทำให้ความหนืดลดลงอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) อัตราส่วนของ NaCMC ที่สูงขึ้นจะเพิ่มปริมาณน้ำในไฮโดรเจลและสัดส่วนการดูดซึมน้ำกลับ อีกทั้งการเพิ่มอัตราส่วนของแป้งข้าวโพดยังทำให้ปริมาณน้ำ สัดส่วนการดูดซึมน้ำกลับและการแพร่ผ่านของไอน้ำผ่านไฮโดรเจลมีค่ามากกว่าไฮโดรเจลที่มีวุ้นในอัตราส่วนที่เท่ากัน ส่วนผสมระหว่าง NaCMC กับวุ้นไม่มีความแตกต่างของการแพร่ผ่านของไอน้ำผ่านไฮโดรเจล หากเพิ่มอัตราส่วนของแป้งข้าวโพดทำให้การแพร่ผ่านของไอน้ำเพิ่มขึ้นอย่าง

มีนัยสำคัญทางสถิติ ($p < 0.05$) อีกทั้งยังพบว่าในสภาวะความชื้นสัมพัทธ์สูง ไฮโดรเจลที่มี NaCMC และวุ้นจะมีค่าการดูดความชื้นมากกว่าแป้งข้าวโพดและ GAB model สามารถคำนวณค่าปริมาณความชื้นที่จุดสมดุล (Mo) ซึ่งมีค่าอยู่ระหว่าง 0.573 ถึง 0.998 g/g

การทดสอบการงอกของมะเดื่อและไทรในจานเพาะเลี้ยงเนื้อเยื่อพบว่าอัตราส่วนของ NaCMC ที่สูงจะยับยั้งการงอก โดยค่าร้อยละการงอกในไฮโดรเจลแต่ละชนิดมีความแตกต่างทางสถิติอย่างมีนัยสำคัญ ($p < 0.05$) โดยชุดควบคุมซึ่งไม่ใช้ไฮโดรเจลจะมีค่าร้อยละการงอกสูงที่สุดในการทดลองวันที่ 10

การทดลองเทคนิคไฮโดรซีดดิงในเรือนเพาะชำจะใช้หลอดฉีดยาสำหรับบรรจุเมล็ดมะเดื่อและไทรร่วมกับไฮโดรเจลแต่ละชนิด ทำการฉีดพ่นส่วนผสมทั้งหมดไปยังผิวหน้าของดินที่เก็บตัวอย่างมาจากเหมืองหินปูน ผลของชนิดไฮโดรเจลไม่มีความแตกต่างทางสถิติในค่าร้อยละของการงอก ค่ากลางการพักตัวของเมล็ดหรือ MLD และร้อยละของการอยู่รอด ($p < 0.05$) แต่พบว่าชนิดของมะเดื่อและไทรส่งผลต่อความแตกต่างทางสถิติอย่างมีนัยสำคัญ ($p < 0.05$) ต่อค่าร้อยละการงอก MLD และร้อยละการอยู่รอด (*F. benjamina* ต่ำกว่า *F. hispida* และ *F. semicordata*)

ไฮโดรเจลส่วนผสมระหว่าง NaCMC กับวุ้นหรือแป้ง (50:50) ถูกคัดเลือกเพื่อนำมาทดสอบด้วยเทคนิคไฮโดรซีดดิงกับเมล็ดมะเดื่อและไทรในพื้นที่ลาดเอียงของเหมืองหินปูน โดยเริ่มทำการทดลองที่ช่วงเริ่มต้นฤดูฝนในปี 2556 พบว่าร้อยละของการงอกและการอยู่รอดในทุกชุดการทดลองมีค่าต่ำ ดังนั้นไฮโดรเจลที่ใช้ในการทดลองจึงยังไม่มีประสิทธิภาพเพื่อใช้กับเมล็ดพืชสกุลมะเดื่อและไทรในเทคนิคไฮโดรซีดดิง

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LIST OF ABBREVIATIONS

ASTM	American Society for Testing and Materials
GAB	Guggenheim-Anderson-de Boer
GLM	Generalised Linear Model
IUCN	The World Conservation Union
Me	Equilibrium Moisture Content
MLD	Median Length of Dormancy
NaCMC	Sodium Carboxymethyl Cellulose
PVC	Polyvinyl Chloride
RCBD	Randomized Complete Block Design
RFD	Thailand Royal Forest Department
RH	Relative Humidity
SCG	Siam Cement group
Seedling	The tree and treelet plants since have true leaves, height <1 meter
SEM	Scanning Electron Microscopy
WVP	Water Vapor Permeability
WVTR	Water Vapor Transmission Rate
WWF	The World Wide Fund for Nature

CHAPTER 1

Introduction

The growing population of humans is having a major impact on earth's biosphere including forests which provide natural resources and regulate the environment. Tropical forest contains many biological supports that also included food, fuel-wood, medical plants and other resources for human life, such as water, clean air and soil (Lamb and Gilmour, 2003).

Thailand is a country which has tropical rain forest covered and forest represent the most importance as natural resources. Thailand still has the environmental problems, as loss of natural forest by overexploitation for development. Deforestation in Thailand is the one of the main causes of forest area loss due to plant and animal species destruction then becomes to the crisis in the environmental issue. Therefore, over-exploitation of forest resources, such as illegal logging, shifting cultivation, hunting and collection, were the main causes of deforestation then the remaining of natural forest might be low (Elliott *et al*., 2006).

Deforestation continues in Thailand, the remnants of forest in Thailand were exploited for over many pass decades, before the logging ban since 1989. Thailand's forest area diminished from 53.33 percent of the total land area in 1961 to 25.28 percent in 1998 and increasing up to 33.44 percent in 2008 (RFD, 2012). In recent years, deforestation in Thailand was gradually decrease by promoted responsibilities in the increased number of forest area to protect the remaining forest and establish as many human-made forests as possible.

Many countries in tropical regions have been concerned with forest land destruction and degradation for long times, it has only been able to protect forests minimally through forest acts such as a reforestation. Forest restoration is very importance for increase tree over in deforested land. Its goals are biodiversity recovery and environmental protection by measured in terms of increased biological diversity, biomass, primary productivity, soil organic matter and water resource, as well as the return of wild life species and keystone species of the target ecosystem (Elliott *et al.*, 2000). Current forest restoration methods usually involve planting tree saplings as planting stock.

Direct seeding provides an alternative to tree planting as a restoration method. It is relatively inexpensive, because there are no nursery and planting costs. Furthermore, direct seeding reduces transportation costs because it is easier to transport seeds to restoration sites compared with heavy saplings in containers full of media making the technique particularly suitable for less accessible sites (Schirmer and Field, 1999). Direct seeding is suitable for small or large areas, where natural regeneration or conventional tree planting cannot be applied and should be used at an early stage in succession. However, direct seeding may not be suitable for upland sands, or dry areas, and on steep slopes because the seeds are easily washed away (Duryea, 1992).

Hydroseeding involves mixing seed, fiber mulch and fertilizer together in a mixing tank. This mixture is then sprayed through a large hose and nozzle over the prepared seedbed (Strauss, 2009). It is an adaptation of the direct seeding method and is capable of quickly establishing herbaceous vegetation to highly degraded sites. Hydroseeding can be applied on rough, inaccessible and steep areas, where traditional techniques cannot be used. It protects seeds temporarily from desiccation because the emulsions used can maintain soil humidity by retarding evaporation (Department of Transportation, 2003). The material use for hydroseeding is known as hydrogel. Hydrogels are sticky-hard, crystal-like polymers that they can hold and reabsorb water very well. Their constituents may include water, seeds, soil, and mulch fibers, to form a slurry. The exact mixture depends on the purpose. In theory, soaked hydrogel releases water slowly to plant roots to prevent or delay water stress. High seed germination of woody plants requires good quality seed, a suitable surface, as well as high humidity, which can be provided by the hydrogel, and which increases plant survival rates by 20% (Rakow, 2013). Hydroseeding is therefore one way to re-establish plants on inaccessible areas, especially on arid sites, by promoting germination and plant establishment.

Increasing human demands for building materials has resulted in expansion of the quarrying industry. Open cast quarries destroy both natural forest and topography. Lack of environmental management contributes to unnecessarily high losses of wildlife habitats (Eurogypsum, 2013). The impact of mining must be minimized and restoration planning is therefore essential to restore wildlife habitats after mine closure both within and around the mining site. However open cast mines represent the most severe form of forest degradation since there is no top soil, no sources of natural regeneration. In many cases there may be no surrounded forest and no seed dispersing fauna. Mining site is classified at stage 5 degradation where has poor soil and no tree establishment. Plantations at mine must begin with soil improvement such as establishing green mulch, addition of fertilizers or micro-organisms in soil. In addition, planting nurse trees can help the forest restoration success by create soil fertility before planting the native forest tree species (FORRU, 2008). Therefore, restoring forests activity before abandoned a mined land is a challenge.

The study presented here was carried out at a limestone quarry operated by Siam Cement (Lampang). The company is legally obligated to restore the site to its original condition (deciduous dipterocarp forest) and recovery biodiversity. These ultimate goals include recovery of soil fertility and acceleration of ecological succession. Thus, recovery of the vegetation in mining site as the ground cover is one urgent task within reclamation of post mining process (SCG Cement and WWF Thailand, 2009).

It is very hard to restore forest mining sites because few of the tree species of the indigenous forest ecosystem can survive well and become established on the highly compacted and nutrient poor mine substrate. There is no soil development. Burger and Zipper (2002) stated that mine substrates are generally less fertile than natural soils because of low quantities of nutrients and organic matter. Plants selected to restore forest vegetation on mine sites must survive and growth well on rocky substrates, with low soil nutrients and water, extreme soil pH values and steep slopes. Some grasses and leguminous herbs and shrubs can tolerate such conditions, forest restoration necessitates the establishment of less tolerant tree species, typical of the original indigenous forest ecosystem type.

Ficus spp. have been suggested as suitable for mine rehabilitation since they often light living pioneer species, which produce small seeds in vast quantities (<2mm) (Shanahan, 2000). The genus includes several species which are known to survive well on limestone crags and have high potential to grow well on natural limestone crags and so should be suitable for limestone mine restoration (Elliott *et al*, 2013a). Furthermore, Pandey *et al*. (2005) reported that *Ficus* species is vital in forest ecosystem by support food for wildlife and included function as keystone tree species when other food resources in forest are deficiency.

The figs they produce attract seed-dispersing animals at a young age (1-6 years after planting) (Elliott *et al*., 2006) which enhances natural seed dispersal mechanisms and thus could bring about rapid biodiversity recovery at a mine site still surrounded by natural forest, which maintains populations of seed dispersers, such as bulbuls and primates. Since fig seeds are tiny, they are ideal for testing the hydroseeding method using a tree species.

Hydrodeeding is usually used for establishing grasses and swards of mixed herb species. This project explores the potential use of hydroseeding to establish *Ficus* spp. trees on a mine site and it is the first attempt to use it for establishing tree species by sowing the seed on Limestone Quarry at Siam Cement (Lampang). The Project tested the potential of hydroseeding might be successful to initiate forest restoration by enhancing the germination of germination of *Ficus* spp. seeds in limestone cracks (similar to nature). Hence, the seeds of *Ficus benjamina*, *F. hispida* and *F. semicordata* were tested, with 2 original biopolymers with new hydrogel formulae, containing sodium carboxymethyl cellulose (NaCMC), blended with various amounts of either agar or corn starch.

The properties of the new hydrogels were determined, particularly their moisture properties, drying, swelling, water vapour permeability and sorption isotherm properties. Physical properties of hydrogel may affect *Ficus* spp. establishment and a suitable hydrogel can be chosen to use as hydroseeding substrate in the very harsh conditions of limestone mines. Therefore, testing of fig tree establishment, by various types of hydrogel, may identify a suitable hydrogel, that may promote the use of *Ficus* tree species to restoration forest to limestone mines.

Hypothesis

Hydrogels enable *Ficus* spp. seeds to germinate, survive and seedlings to become established on open cast limestone mines.

Objectives

1. To determine the physical properties of the hydrogel and how those properties affect its effectiveness.
2. To test the effectiveness of the hydrogel in the nursery (using mine substrate) and at the mine itself.
3. To develop a hydrogel, which enables germination and early seedling development of *Ficus benjamina, F. hispida* and *F. semicordata* on limestone mine substrates.

CHAPTER 2

Literature Review

2.1 Definitive of restoration

Degradation can be addressed in a variety of ways and at a range of scales. While site-level interventions are important, they need to be coordinated with effective planning across landscapes (Lamb and Gilmour, 2003). The process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed is called ecological restoration. It involves bringing back the original, normal functioning, and structure of the original ecosystem, including the provision of ecological services and processes. The term "restoration" means to restore land to a former original state, by returning the system to a sustainable level of productivity, such as reversing or ameliorating soil erosion and salinization problems. It has several forms:

- Rehabilitation is the action of restoring a thing to a previous condition or status.
- Remediation is the act of remedying a process rather than the endpoint reached.
- Reclamation is a term used for making land fit for use or bringing it back to a proper state. Here there is no implication of returning to an original state but rather to a useful one.

Restoration also aims to return a system to a sustainable level of productivity. It is not easy to achieve completely. Many approaches have been offered particularly potential and demonstrate its effectiveness to restoration (Foundation for Ecological Security, 2008).

2.2 Definition and approaches to forest restoration

Tropical forests have already been lost or substantially degraded. If nothing is done to reverse this trend, most of the rest area may disappear in the next few decades (Burton and Macdonald, 2011). The obvious solution is reforestation, but this is a broad term which means re-establishing any type of tree cover, including plantations of exotic species, agro-forestry and so on.

Forest restoration is directing and accelerating ecological succession towards an indigenous forest ecosystem of the maximum biomass, structural complexity, biodiversity and ecological functioning that are self-sustainable within prevailing climatic and soil limitations. Forest restoration may include passive protection of remnant vegetation or more active interventions to accelerate natural regeneration, as well as planting trees and/or sowing seeds of species that are representative of the target ecosystem (Elliott *et al*., 2013b).

There are 5 effective tools to restore forest ecosystems (FORRU, 2008):

1) Protection
2) Accelerated (or assisted) natural regeneration (ANR)
3) The framework species method
4) Maximum diversity methods
5) Nurse crops or foster ecosystems.

Table 2.1 Various reforestation approaches and their merits (FORRU, 2008)

Reforestation Approach	Costs	Biodiversity	Time for Development	Research Input Required
Commercial monoculture plantation	High	Low	Fast	Low
Monoculture of commercial nurse trees	High	Low to medium	Fast	Low
ANR without enrichment planting	Low	Low to medium	Slow to medium	Low
ANR with enrichment planting	Low to medium	Medium	Medium	Low to medium
Framework species method	Medium to high	Medium	Medium	High
High-density planting of forest trees	High	High	Fast	High

2.2.1 Framework Species Method (FORRU, 2008)

The framework species method can restore forest regeneration and recover biodiversity by planting the smallest number of trees of forest tree species, whilst attracting seed dispersal animal. This method does not need to plant all of the tree species that can combines with various techniques to enhance natural regeneration from a single planting tree.

The method is acceptable for using in the framework tree species, refined on based information sources and results of nursery experiments or field trials. This method involves planting trees to shade out weeds by spreading their crowns. The candidate trees or framework species may also have nectar-rich flowers, fleshy fruits at a young age for support food to animal and attract seed disperser to achieve rapid tree species recruitment in restoration plots. Moreover, the framework species method involves planting with forest tree species that are high survival rates in deforested sites.

2.2.2 Nurse plantation approach (FORRU, 2008)

Nurse plantation approach is establishing plantations of highly resilient tree species to improve the soil and modify the site conditions that can improve restoration practices for subsequent biodiversity recovering. The planting of nurse trees create better conditions for rapidly re-establishing. Nurse trees have closed canopy and high litter fall to create cooler, shadier and more humid conditions in soil. This should lead to the accumulation of humus and soil nutrients for the subsequent seed germination of less tolerant tree species or new seedling establishment.

Nurse plantations improve both the physical structure and the fertility of soils, without the need for the expensive physical soil treatments by fast-growing pioneer species which are tolerant in many harsh conditions. Therefore, native tree species are preferred such as legumes (Family Leguminosae) that can rapidly improve soil nutrient by the nitrogen-fixing capability. Furthermore, native fig tree species (*Ficus* spp.) is also listed to good nurse plantation species as well as other useful framework tree species. The roots of fig trees are capable of invading and breaking apart compacted soils and even rocks on the most degraded of sites.

2.3 Direct seeding

2.3.1 Direct seeding overview

Direct seeding is a regeneration method of sowing seeds directly, which can establish trees in the field. The laborious task of nursery plants and transplanting seedlings to the planting site is omitted. Direct seeding offers various ways to have maximum germination or ability to rapidly increase forest area but need to concern about optimal time for sowing the seed in field conditions (Ochsner, 2011). Where natural regeneration may be impossible, because of the lack of trees as a seed source and where conventional tree planting is impractical, direct seeding can be applied. This study views direct seeding as a form of artificial regeneration for re-establishing forest cover. In the past, direct seeding was commonly used by farmers for crop plants. Seeds are directly sown into the soil, with or without pretreatments and/or soil cultivation (Duryea, 1992).

The main advantage of this technique, over planting seedlings, is significantly lower costs, due to the reduced time and labor required (Schirmer and Field, 1999). Direct seeding can be applied over large areas by broadcasting seeds from the air by airplanes or helicopters. This technique is fastest and has the most accurate and complete coverage. Techniques for medium to large area include seed-sprayed machine from helicopter and even hand-sowing (Figure 2.1).

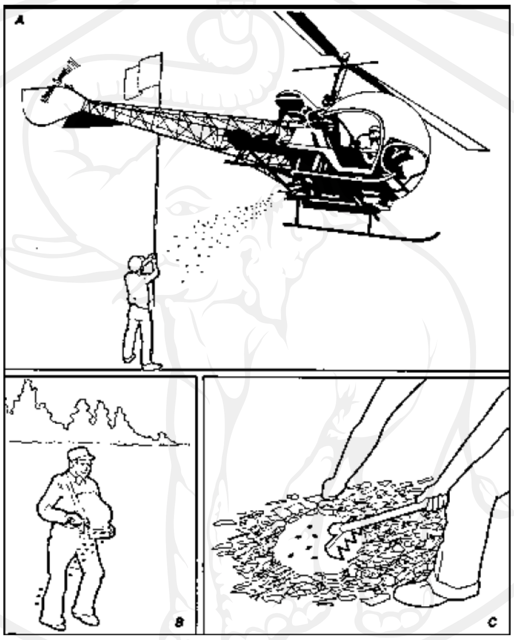


Figure 2.1 Methods used to sow seed (Duryea, 1992)

2.3.2 Advantages of direct seeding (Duryea, 1992)

1) Low cost, because a tree nursery is not needed and transportation of planting materials are cheaper, compared to planting seedlings
2) Seedling root development adapts immediately to the local environment of the planting site
3) Transplanting is not needed
4) Can be applied to different topographical situations such as hilltop, mudflat, swamp
5) Essential for some species that are not suitable for nursery production

6) Can be spread across the landscape easily, including sites that might be difficult to reach when carrying baskets of seedlings.

2.3.3 Disadvantage of direct seeding (Duryea, 1992)

1) Requires huge amounts of seed
2) Losses due to weeds and insect during are high
3) Tree density is difficult to control.

2.3.4 Planting by direct seeding method

Direct seeding can have a potential advantage over conventional planting, so this value can improve wildlife habitat. Moreover, using local collected seed can conserve genetic pool within plant population and improve values of re-established indigenous plant (Georges River catchment, 2004). Seedlings growing from direct seeding have better root development than for nursery-raised saplings, because the roots are not constrained within a container (Woods and Elliott, 2004). Moreover, growth rates are often faster than for nursery-raised saplings (Tunjai, 2005).

Burns (2005) reported that direct seeding of native trees and shrubs is now widely used to reforestation. On inaccessible sites, many factors must be considered and can affect to plant establishment, such the variety of seed quality, soil conditions, as well as soil water content and some seed will be lost by seed predation, some will fail to germinate under field conditions and some seedlings will die after germination (Ochsner, 2011).

Using direct seeding to establish native plants has attracted the attention of researchers in many countries. In Thailand, Wood and Elliott (2004) studied native tree species that could be used for direct seeding to accelerate forest restoration in Doi Suthep-Pui National Park, while applying seed treatments and different sowing practices in an abandon agricultural field.

2.4 Hydroseeding

2.4.1 Hydroseeding overview

Hydroseeding is a viable approach for re-establishing vegetation (U.S. Army Corps of Engineers, 2009). Hydroseeding uses a water-based medium, to which seeds are added, before spraying onto the planting site. The slurry is stored in a tank on a trailer or truck, which can be several hundred feet long. A pump then sprays the slurry through a nozzle. Alternatively humans can apply the slurry from a back pack (Figure 2.2). Hydroseeding is possible to reach rough or inaccessible areas, including areas of high slope, where traditional techniques cannot be used.

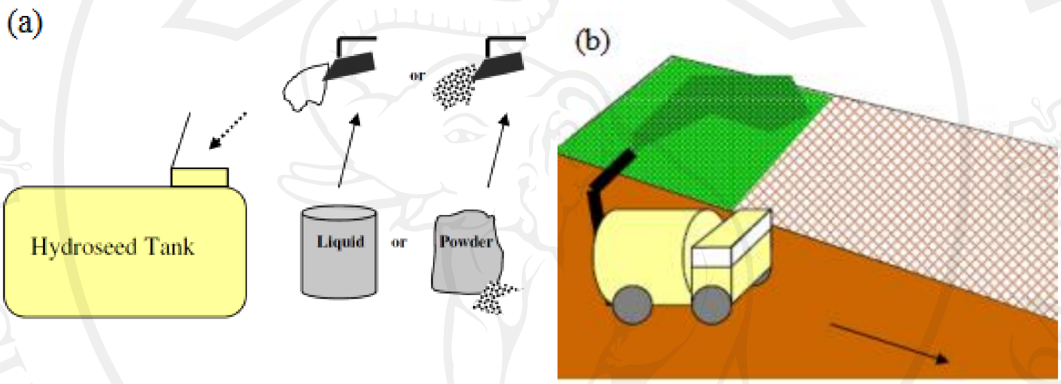


Figure 2.2 Material in hydroseeding (a) and its application by machine (b)

The function of the slurry is to provide a medium for the seeds that retains moisture, while allowing gaseous exchange and light transmission. Components can include:

1) A mulch composed of organic fibers that will degrade in several months
2) A binding solution polymer chain linkages
3) A tackifier powder or hydrogel to bind the mulch to the soil and absorb water into the slurry
4) Fertilizers to guaranteed nutrient supply (listed in total nitrogen (N), phosphorus (P) and potassium (K))
5) A soil component or soil micro-organisms to improve soil structure, aeration, infiltration, and drainage.

Not all of these components may be added to all pre-packaged hydroseeding products from suppliers. Hydroseeding treatments prevent soil erosion and create rapid vegetation cover. Hydroseeding, after fire damages vegetation, is becoming common practice**.** Selecting the components of the slurry must take into account the final viscosity of the product, especially where being applied on steep slopes. Hence, cross linked network in polymers influence on water swelling that is also importance by contain water supply (Figure 2.3).

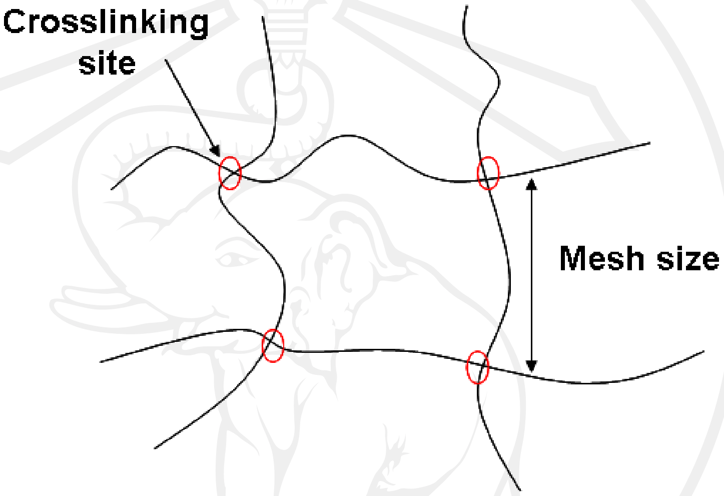


Figure 2.3 Cross linked networks between polymer chains (Sannino *et al*., 2009)

In agriculture, hydroseeding also improves soil physical properties (Ekebafe *et al*., 2011);

1) Increasing water holding capacity
2) Increasing water use efficiency
3) Enhancing soil permeability and infiltration rates
4) Reducing irrigation frequency
5) Reducing compaction tendency
6) Reducing erosion and water run-off
7) Increasing plant performance.

Furthermore, Sannino *et al*. (2009) described the distribution of hydrogel among soil particles (Figure 2.4).

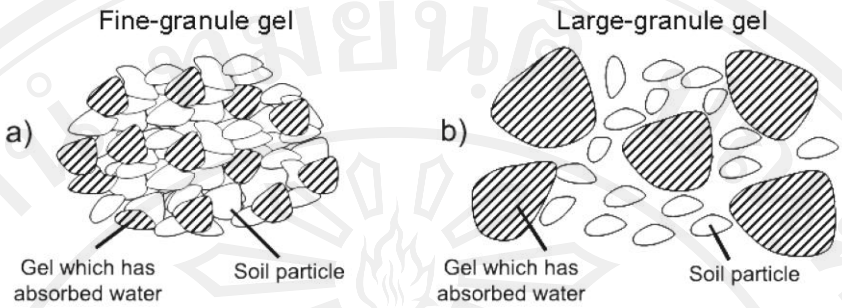


Figure 2.4 Fine-granule gel substrate in soil (a) and large-granule gel in soil (b)

2.4.2 Advantages (Strauss, 2009)

1) An effective method for applying seed on hills prone to erosion and windy sites, where seed can be washed away. It also works well on flat sites.
2) For grass, seed is mixed with water and the mulch holds the moisture so, hydroseeded lawns come up quickly.
3) An commercial hydrogel company can give instruction for costumer to use the suitable hydrogel.

2.4.3 Disadvantage (Strauss, 2009)

1) Hydroseeded lawns require the same care and watering as young seedlings.
2) It is necessary to supply consistent moisture to young seedlings.

2.4.4 Hydroseeding applications

Sannino *et al*. (2009) experimented with hydrogels for tomato cultivation. After no watering, hydrogel conserved water in the soil. The highest soil humidity was achieved with the maximum content of hydrogel (1% of the soil). Hydrogel can hold water in soil and performs as soil capability for retain more water by irrigation. Furthermore the hydrogel is totally digested approximately 6 months in the soil.

Biopolymers provide more sustainable and environmentally friendly products which are photodegradable or biodegradable and nontoxic. Sojka *et al*. (2005) stated that biopolymer could be one major component that may possibly develop to use in soil application so that using of polysaccharides can be the one of suitable polymers in hydrogel component. Several natural polymers including polysaccharides can be used to make hydrogels because they mimic a suitable environment for seed germination. Hydrogels are regarded as ‘intelligent’ materials, because they can respond to external stimuli by either shrinking or swelling in their hydrophilic bonds and can be add some compositional polysaccharides to enhance the water absorption (Rathna *et al.*, 1996).

Hydrophilic side chain in several natural polymers involved by adding with cellulose, starch, chitosan, gelatin and etc. (Zohuriaan-Mehr and Kabiri, 2008). One promising material for hydrogels is a highly water soluble polysaccharide, made from sodium carboxymethyl cellulose (NaCMC). The material is biodegradable and has a wide range of applications, because its low cost. NaCMC is used in hydrogels because it is highly water absorbent (Nnadi and Brave, 2000; Sannino *et al*., 2009). It is also used as a viscosity modifier or thickener and to stabilize emulsions in various products. It solidifies to a gel, when cooled, and is easier to prepare than other hydrogel materials (Li *et al*., 2008). The hydrogel-forming of NaCMC has water absorption when immersed in a compatible solvent by cross links between side chains such as OH, NH_2, COOH, SO_3H and etc. Therefore, an improvement in swelling capacity of hydrogel probably be due to building more hydrophilic chains or hydration functional groups such as in the chains of OH and $COOCH_3Na$ (Hezaveh and Muhamad, 2012). Ongoing using with NaCMC and polysaccharides could established their potential to a modifying hydrogel and enhancing moisture properties.

Starch is a polysaccharide, which consists of the linear D-glucan amylose and the highly branched amylopectin. An amylose with many hydroxyl groups can absorb water (Corn Refiners Association, 2006). Agar is a water-soluble, gel-forming polysaccharide from algae, members in rhodophyta (Praiboon *et al.*, 2006). Agar is a polysaccharides and the main structure has repetitive units, to form amylose or amylopectin. About physical properties of the agar, when it form in gel can be held some content of water which can be used in vegetable tissue culture media. In parallel, agar is one of the most potential ingredients for use as a food additive, biotechnology uses, cell and tissue culture or as supporter for electrophoresis or chromatography (Armise´n and Galatas, 2000).

Nnadi and Brave (2000) reported that NaCMC, with starch, is an absorbent biopolymer and can able to absorb amounts of water and can be used as sponge to retain soil moisture. They tested combinations of various starches in the hydro slurry such as cassava, corn, potato and yam starch. The potato starch performed the best of water retention, with 73%, while corn gave 56%. Furthermore, the result of a planting radish plants by using absorbent polymers, composed of CMC looked more healthier and higher weight than control whereas planting without absorbent polymer showed signs of dehydration during lack of irrigation. These results proved that absorbent polymers from CMC and starch could be added to soil and can reabsorb water into its dried form.

2.4.5 Revegetation by hydroseeding

Hydroseeding is commonly used for leguminous herbs and several grass and grass-like species. Seeds are usually sown on bare soil, to promote rapid seedling establishment (Hoag *et al*., 2011). Below are examples and case studies that explore the use of hydroseeding for re-vegetation.

Gonzalez-Alday *et al*. (2008) developed hydroseeding to re-vegetate the steep slopes of a coal mine in Spain, using six grasses species and four legumes. The hydroseeding slurry also contained soluble chemical fertilizers (N, P and K). *Festuca* spp., *Lolium perenne* and legume, *Trifolium repens* contributed most to vegetation cover using this technique.

De Ona *et al.* (2011) tested a slurry, containing mulch (cereal straw) and fertilizer (quick-release N, P and K) and sewage sludge, with seeds sprayed at a density of 35 g/m^2 (species not mentioned). They concluded that sewage sludge improved hydroseeding success, because of high levels of the principal plant nutrients, N and P, and organic material. Slurry with sewage sludge reduced erosion and increased vegetation cover more effectively than when sludge was not added.

De Frank (2007) described a hydroplanting technique suitable for native an Hawaiian groundcover species, *Fimbristylis cymosa*. This study evaluated the establishment success of three techniques; hand sowing, hydroseeding and hydro planting. Hydroseeded treatments showed the highest plant density after two months, followed by hand sowing and hydro planting. Hydroseeded plots also exhibited the highest per cent of visual ground cover over six months.

In Thailand, the limestone quarry of Thung Song, Nakhon Si Thammarat completely restored an area after mining, by first improving the drainage pattern. The area was graded to a slope of 32 degrees and grass was re-established by hydroseeding (Figure 2.5). A mixture of grass seed, mulch, fertilizer, binding agent, and commercial hydroseeding product were applied by high pressure pumping into a slope surface. In first year, the result was successful when determined by ground cover and not found soil erosion due to the average annual rainfall was relatively high (SCG Cement and WWF Thailand, 2009). In addition, an alternative approach to restoration in limestone mining site was developed, using the hydroseeding in the post-mining landscape.

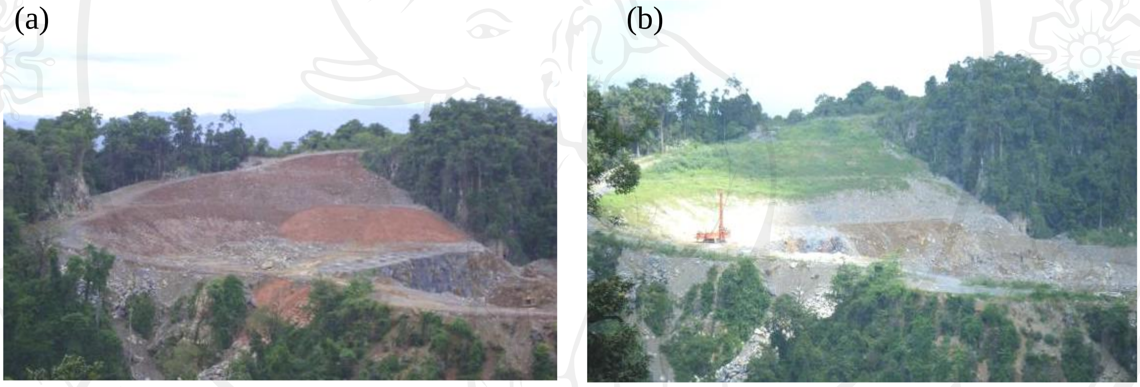


Figure 2.5 Before hydroseeding of grass seeds (a) and new seedlings establishment (b)

2.5 Limestone mine condition

Opencast mine sites provide probably the most extreme degradation site. Open cast limestone mines have a huge environmental impact due to removal of vegetation and top soil. Furthermore, they leave behind steep slopes with unstable and roughly graded surface. Soil or spoil is highly alkaline due to high levels of carbonates. Consequently, major macronutrients, N, P and K, are often deficient. Substrates have sandy textures that cannot hold as much water or nutrients in finer textured substrates can. Planting on such mines first requires the application of large amounts of fertilizer before plant establishment can begin. Moreover, pioneer species such as leguminous trees, shrubs, pine and hardwood species can survive and also grow well under mine soil conditions (Sheoran *et al.*, 2010).

2.6 Potential of using *Ficus* spp. to restore forest in limestone mine

Revegetation, after mining, can accelerate plant recovery that can be done by planting seedlings and direct seeding, as described earlier. Although, poor sowing technique or harsh conditions can limit seed germination and increase mortality of new seedlings. Moreover, poor species selection can also contribute to failure of tree planting at limestone mines.

Jim (1998) assessed the characteristics of tree species that establish well in vertical habitats. Of thirty tree species that established in stone walls, 88% were in the family moraceae (Mulberry family) including 6 *Ficus* spp.

Some *Ficus* species thrive on limestone with roots capable of invading and breaking apart compacted soils and even rocky surface. So, fig trees should be excellent for restoring mine sites, provided that the planting stock is of the highest quality. They also make good nurse plantation species. Suitable species include *F. auriculata*, *F. benjamin*a, *F. callosa*, *F. capillipes*, *F. fistulosa*, *F. glaberrima*, *F. heteropleura*, *F. hirta*, *F. hispida*, *F. microcarpa*, *F. racemosa*, *F. rumphii*, *F. semicordata*, *F. variegata* and *F. virens* (Elliott *et al.*, 2013a).

Moreover, *Ficus* spp. produce very tiny seeds (Figure 2.6), which are effectively dispersed by birds in their droppings and which are capable of germinating in rocky crevices. Wenny (2001) explained that fig seeds are dispersed widely by many bird and mammal species.

Consequently, *Ficus* spp. grow well on cliffs and rocky surfaces, where moisture and nutrients are scarce and plants are exposed to wind and heat. Moreover, *Ficus coronulata* and *F. hispida* were included in a list of species suitable for direct seeding on mine sites (Burns, 2005).

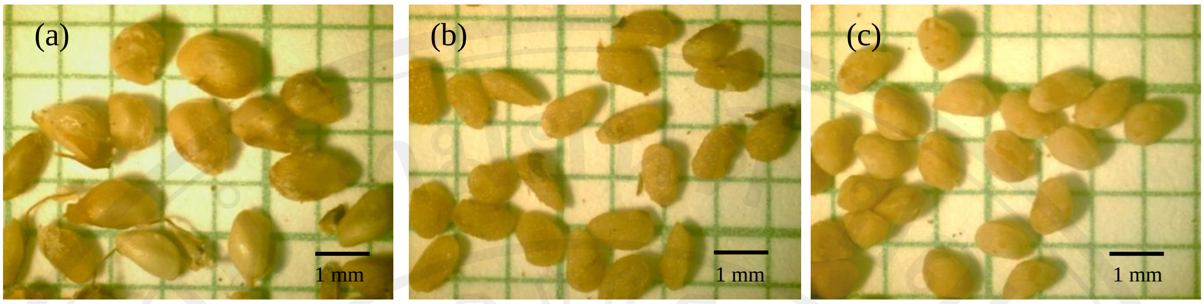


Figure 2.6 Seeds of *Ficus benjamina* (a), *F. hispida* (b) and *F. semicordata* (c)

Furthermore, local *Ficus* species, suitable for limestone revegetation, were reported by Indian researchers. Sood and Bhimta (2011) reported that *Ficus auriculata, F. bengalensis, F. carica, F. elastica, F. glomerata and F. religiosa* are commonly found in areas surrounding cement mines. Consequently *F. religiosa and F. glomerata* were planted with good results at Darlaghat, India. Moreover, *F. religiosa* and *F. bengalensis* were chosen to plant by direct seeding in limestone mine because they could accelerate succession in mine spoils and enhance natural seed dispersal by provide a food for fruit-eating animal so that planting *Ficus* spp. are very helpful on restoration program (Pandey *et al*., 2005).

Kuaraksa and Elliott (2012) stated that the most efficient method of producing *Ficus* spp. seedlings (*Ficus auriculata*, *F. fulva*, *F. hispida*, *F. oligodon*, *F. semicordata*, and *F. variegate*) was from seed; propagation from cuttings was much less successful. Moreover, direct seeding of *Ficus* spp. was not successful in field trials because seedling survival of all species tested was very low. The highest mortality for all species occurred during the first rainy season (>90% of mortality rate by 1 month after germination). However, using *Ficus* spp. for direct seeding could become a very cost effective technique because *Ficus* spp. grew rapidly and without nursery costs.

2.7 Study site

2.7.1 Location

Siam Cement's limestone quarry at Lampang has an annual production capacity of 2.1 million tons. The mine is located between Chae Hom district and Muang Lampang at 18^{o}N 29$^{/}$E - 18^{o}N 37$^{/}$ E and 300-400 m above the average of sea level. An innovative process of quarrying, called semi-open cut mining, is being used to preserve the appearance of the limestone mountain.

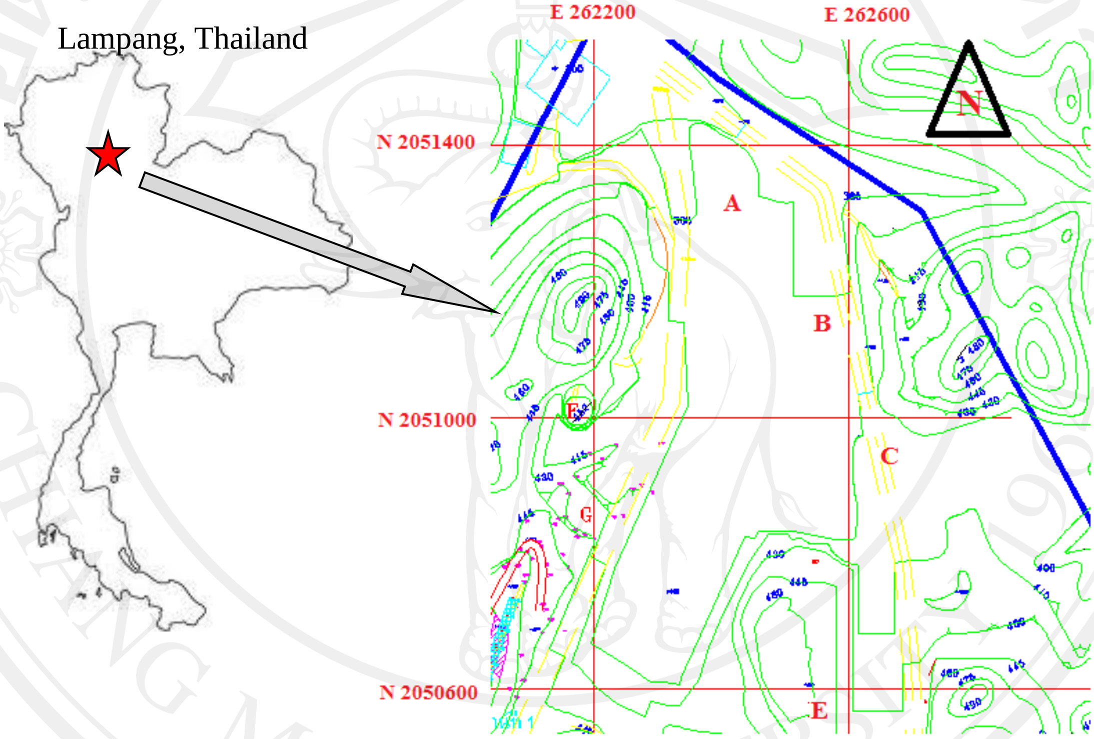


Figure 2.7 Study site at Limestone Quarry at Siam Cement (Lampang) Co., Ltd

2.7.2 Vegetation type

The biodiversity of the mine was studied in 2009. Eighty six vascular plant species were recorded, representing 34 families and 78 genera, including *Afzelia xylocarpa*, and *Dalbergia cochinchinensis*, ranked as endangered and vulnerable tree species, respectively, by the IUCN (1998). The mining area covers 8,224 rai and more than 85% of total area was covered by various forest types;

1) Dry Dipterocarp Forest 2.42%
2) Mixed Deciduous Forest 74.08%
3) Bamboo Forest 9.78%
4) Grassland 0.07%.

2.7.3 Reclamation and environmental management

The reclamation in mine land by restoration is an important part that can provide the opportunity to re-establish native plant. The mine has been divided into 3 zones;

1) Buffer zone, restrict explosions and transportation to the quarry area, minimize dust and noise
2) Conservation zone, natural forest
3) Mining site in the exposed area, clearance of mining site began by cutting small hills to create a flat level area between the mountains then continuously reclaim by forestation. The outer shell of the limestone mountain remains intact to delicate area of natural forest. The inner crust is excavated but the shell is left wide enough to retain a stable shape that will not be a later cave. This makes it easier to fill soil and start to re-establish plant before abandon (SCG Cement and WWF Thailand, 2009).

CHAPTER 3

Methodology

3.2 Development of hydrogel

3.2.1 Liquid hydrogel preparation

1) Hydrogel combination of NaCMC with agar

NaCMC solution (1 g % in water) was blended by tap water and stirred for 10 minutes. Agar (0.45 g % in water) was added in boiled water for 30 minutes. The gradient of NaCMC; 100% (A1), 75% (A2), 50% (A3), 25% (A4) to 0% (A5) was mixed with agar solution instead by stirring. Then, the hydrogel combinations were included 0.75 g % of NPK fertilizer (30-20-10) (Table 3.1).

2) Combination with NaCMC/corn starch

NaCMC solution (1 g % in water) was blended by tap water and stirred for 10 minutes. Corn starch solution (5 g % in water) was added in boiled water for 30 minutes. The gradient of NaCMC; 75% (B1), 50% (B2), 25% (B3) to 0% (B4) was mixed with corn starch solution instead by stirring. Then, the hydrogel combinations were included 0.75 g % of NPK fertilizer (30-20-10) (Table 3.1).

Table 3.1 Composition of hydrogel slurry

Treatment	Hydrogel composition (in 100 ml) with 0.75g% of fertilizer (30-20-10)		
	Corn Starch 5%	Agar 0.45%	CMC 1%
A1	0	0	100
A2	0	25	75
A3	0	50	50
A4	0	75	25
A5	0	100	0
B1	25	0	75
B2	50	0	50
B3	75	0	25
B4	100	0	0

3.2.2 Hydrogel film formation

Films were formed by combinations of hydrogel, NaCMC blended with agar or corn starch, following by liquid hydrogel preparation (3.2.1). A 100 ml of hydrogel was poured onto clean and dry Petri dishes and allowed to dry at 30^{o}C for 12-16 hours. Then, thin films were peeled off and kept in desiccator chambers which were contained silica gel beads. The thickness of film was measured by micrometer and an acceptable film for experiment must had thickness approximately 0.1mm.

3.3 Determination of the quantitative properties of liquid hydrogel

3.3.1 pH and viscosity

The pH of hydrogels (A1-A5 and B1-B4) was measured with a pH meter in tri-replicate and viscosity was measured with a Brookfield viscosity meter also in tri-replicate.

3.3.2 Drying of hydrogel

A 100 g of hydrogel treatment (A1-A5 and B1-B4) was kept separately at temperatures of 25 °C, 30 °C and 35 °C, 30 ± 5% of relative humidity with tri-replicate. Then, record weight remaining every 2, 4, 8, 14, 24, 38 and 52 hours. Weight percentage was calculated by an equation below

$$\text{Weight (\%)} = \frac{W_t}{W_0} \times 100$$

Where W_0 is initial weight (g) and W_t is weight after time ‘t’ respective (g), maximum 52 hours.

3.4 Determination of moisture properties in hydrogel film

3.4.1 Water content

Films were put in distilled water at a room temperature, 22 ± 3.0 °C. The tissue paper and filter paper were used for absorb exceed water on surface of soaked film then the weight of film was recorded. After that, soaked hydrogel was returned to distilled water and weighed every 1 minute to constant weight. Water content was determined using the equation below

$$\text{Water content (\%)} = \frac{(W_s - W_d)}{W_s} \times 100$$

Where Wd is the weight of dry film (g), Ws is constant weight (g).

3.4.2 Swelling ratio

Swelling ratio can indicate the absorbance ability and can be tested following the step of water content (3.4.1). Swelling ratio was calculated according to the equation below

$$\text{Swelling Ratio} = \frac{(W_s - W_d)}{W_d} \text{ (g Liquid/g Film)}$$

Where Wd is the weight of dry film (g), Ws is constant weight (g).

3.4.3 Water vapor permeability, WVP

Film samples were kept in a desiccator for 3 days at 22 ± 1°C to eliminate the initial moisture content inside hydrogel film by added silica gel beads inside the desiccator. The WVP of the film was determined in triplicate, according to ASTM (1996). Each film sample was sealed over a circular of aluminum cup (0.00332 m^2) and 50 g of silica gel was placed inside the cup. Then, samples were stored in a desiccator chamber at 25 ± 1°C, which contained distilled water. Every day, samples were weighed at the same time, for 5 days, at room temperature. WVPs were calculated as follows

$$\text{WVTR} = \frac{\Delta W}{A \times \Delta t} \quad (g/hr.m^2)$$

$$\text{WVP} = \frac{\text{WVTR} \times L}{\Delta P} \quad (g.mm/day.m^2.Pa)$$

Where $\Delta W/\Delta t$ is the amount of water gain (g/day), A is the test area (m^2), L is the film thickness (mm), ΔP is the partial vapor pressure difference between both sides of film (Pa).

3.4.4 Determination of water sorption isotherms

Hydrogel film samples (15 x 90 mm) were kept in a desiccator, containing silica gel beads, for 3 days at 22 ± 1°C, to eliminate the initial moisture content. Film specimens were placed in other desiccators at 25 ± 1°C, that had been contained each saturated salt solution ($MgCl_2$, $Mg(NO_3)_2$, NaCl and KCl with the relative humidity 33%, 54%, 75% and 85%, respectively). Weight gain of films was determined daily, until the equilibrium weight was attained. The equilibrium moisture contents (Me) of the films were determined. After finishing the measuring equilibrium weight, hydrogel samples were put to the hot air oven at 105°C for 3 hours, for measure the dry weight. Me were calculated as following equation

$$\text{Me} = \frac{We - Wd}{Wd} \quad (g/g)$$

Where We is the equilibrium weight of the films (g), Wd is the dry weight of the films (g).

3.4.5 GAB (Guggenheim-Anderson-de Boer) model

The GAB model can determine physical adsorption that can predict the equilibrium moisture content at a specific water activity. GAB parameter makes it possible to derive the materials sorption behavior of moisture. GAB method follows the experimental of water sorption isotherm and its data can be calculated to GAB model as following equation

$$M = \frac{M_0\, C k a_w}{(1-a_w)[1+(C-1)k\, a_w]}$$

Where M = equilibrium moisture content on a dry basis, M_0 = GAB monolayer moisture content, C = Guggenheim constant, k = factor correcting properties of the multiplayer molecules corresponding to the bulk liquid and a_w = water activity. This model was solved by using nonlinear regression analysis, with the least sum of squares method, to obtain values M_0, C, and k by Statistica Version 11, Copyright 1984-2012.

3.4.6 Film surface with scanning electron microscopy

Scanning electron microscopy (TM3000, Hitachi, Japan) was used for the study of film surface characteristics. SEM analysis was begun after the hydrogel film was dried and completely cut. The film was put in a vacuum chamber for 4 hours and coated with pure carbon. The surface of film was photographed with the SEM under electric energy (15 kV) then image was adjusted to fine resolution and could be zoomed into 2000 times.

3.5 Selection of *Ficus* spp. seeds

Ficus benjamina, *F. hispida* and *F. semicordata* were chosen for this thesis work, because all are known to grow well on limestone (Elliot *et al.*, 2013a). Seeds for all experiments were extracted from ripe figs from mature *Ficus* spp. trees in Doi Suthep - Pui National Park and Chiang Mai city, *F. benjamina* (N=4), *F. hispida* (N=5) and *F. semicordata* (N=3). The fig pulp was sieved through a mosquito net in the water, except for *F. benjamina*, for which the seeds were separated under a stereomicroscope. All of the seeds were spread on paper to dry and stored under dry conditions by silica gel bead container.

3.6 Experimental design by hydroseeding

Hydrogels for hydroseeding were used for testing the seed germination of *Ficus benjamina*, *F. hispida* and *F. semicordata* and their early seedling growth data.

3.6.1 Germination tests in Petri dish

Preliminary experiments in Petri dishes tested the potential use of hydrogel and established that the hydrogels tested were non-toxic to the seeds. 50 ml of each hydrogel (A1-A5 and B1-B4) was spread out evenly in a Petri dish, to make a layer 0.8-10 mm thick. Then 50 seeds of each 3 *Ficus* spp. were surface sterilized with 0.75% Clorox (Sodium hypochlorite solution) and evenly dipped into the surface of the hydrogel and control treatment (water with 0.75g % of fertilizer). The dishes were cultured at 25^{o}C and 2000 Lux in light intensity, for 12 hours per day. Seed germination was determined by counting the number of seeds showing emergence of the radicle.

3.6.2 Hydroseeding in the nursery

1) Collection of soil substrates

The nursery experiment was test with mine soil. Mine substrates contain scatter rock which were collected at field plots at positioned along the slopes, zone B. Soil substrates were divided into 2 types following the field experimental plot, from site 1 and 2. Moreover, the substrates were analyzed by the Soil Science Laboratory, Faculty of Agriculture, Chiang Mai University.

2) Germination and seedling performance

The nursery experiment was begun at the beginning of August, 2012 and finished in February, 2013. Fig seeds in hydrogel were applied to the mine substrates at the center of baskets, 15x30x10 cm, filled up mine substrate, in the nursery at Biology Department, Chiang Mai University.

The fig seeds were sown by syringe injection. Fifty ml of hydrogel slurry (A1-A5 and B1-B4) was used to deliver exactly 50 seeds and control was used water with 0.75% fertilizer to deliver seeds.

The experimental design was a randomized complete block design (RCBD) for each species, totally 9 treatments + 1 control, on soil from site 1 and 2. The seed trays were placed in 2 nurseries by equal replication and hand-watered every day. After that, the number of visible seedlings was recorded every day, until no new germination events were recorded for a month. Air temperature and light intensity inside nursery were recorded.

The parameters used to indicate the effectiveness of hydroseeding were germination per cent, median length of dormancy (MLD) and number of alive seedlings remaining alive at 4th week after last germination occur. Differences among treatments in germinated seeds were determined by generalize linear model (GLM).

3.6.3 Hydroseeding at the limestone mine

The field experiment was set up at beginning of rainy season in July, 2013. The experimental design was RCBD for 3 *Ficus* spp. with 2 sites (block) at Zone B on mine slopes. The experiment tested the effectiveness of the two hydrogel formulae with the best nursery results A3 and B2, along with 1 control (fig seeds sown without hydrogel). The fig seeds approximately 10,000 seeds in each species were mixed into 1.5 litter of slurry hydrogel and spread on each plot (1x1 m), 3 replications, by using 1 m of PVC water pistol.

The experiment was conducted from rainy to the winter season, July-November of 2013. Data collected included number of visible germinants, MLD and seedling survival up to November 2013. Moreover, air temperature and light intensity in the limestone mine were recorded and rain fall data was collected from the Thai Meteological Department.

3.7 Statistical analysis

A Generalized Linear Model (GLM) was used to identify result from parameter by ANOVA with R program (2.12.1).

CHAPTER 4

Results

4.1 Hydrogel properties

The various hydrogel formulae were slightly acidic with pH's ranging from 5.82 to 6.08. The 100% agar (A5) and corn starch (B4) gels had lower pH's than the NaCMC mixes and pH increased as the content of NaCMC in the hydrogels was increased. However, differences in pH among the hydrogel treatments were not statistically significant (ANOVA, $p < 0.05$, Figure 4.1).

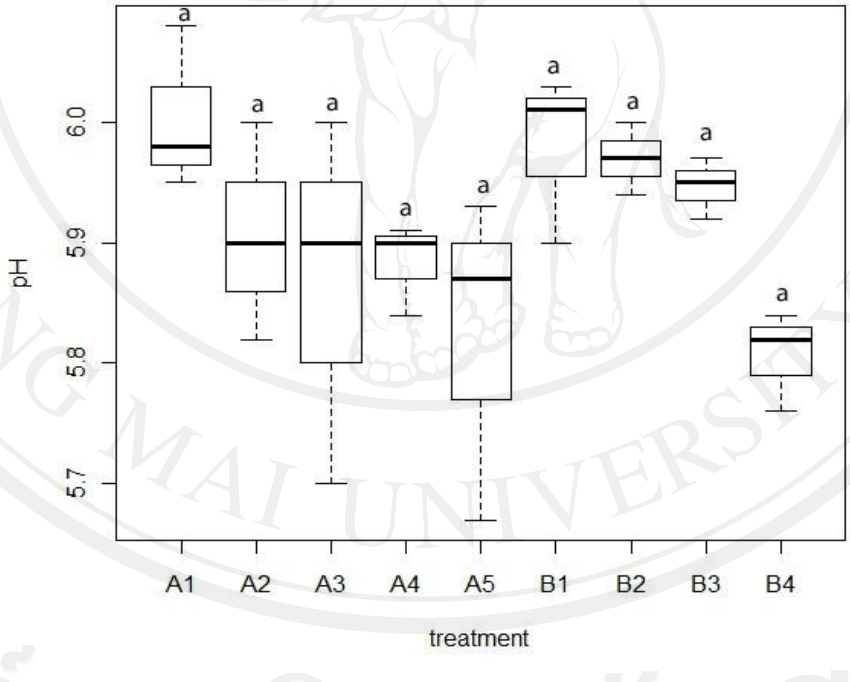


Figure 4.1 pH of hydrogel
(A1-A5 NaCMC + increasing agar content; B1-B4 NaCMC + increasing corn starch content)

All hydrogels had much higher viscosity than water, equal 0 centipoise (cP). Increasing corn starch in the gel formulae (B1 to B4) significantly increased viscosity (ANOVA, $p < 0.05$), whilst adding agar (A2 to A5) reduced it (Figure 4.2).

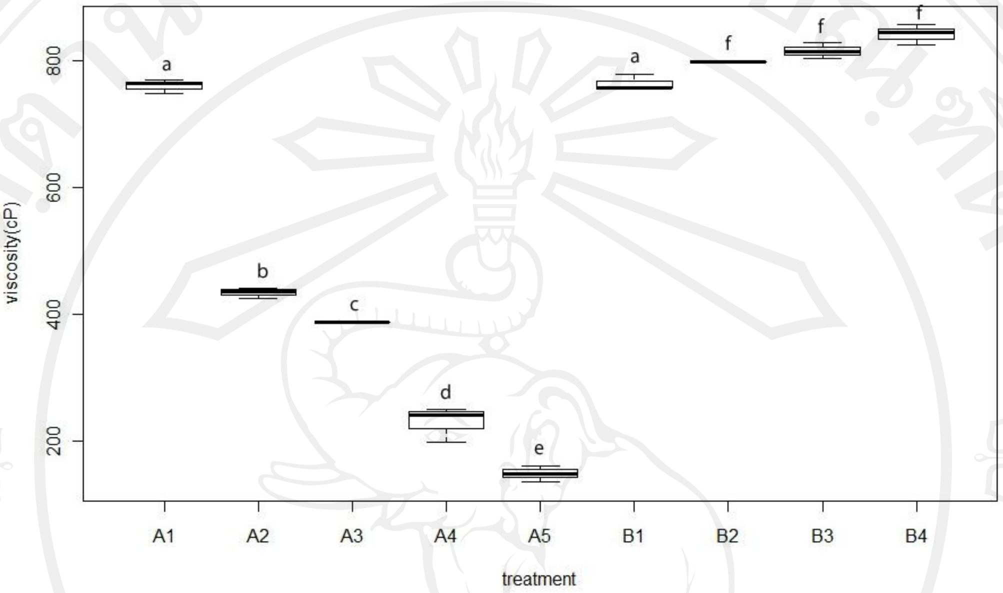


Figure 4.2 Viscosity of hydrogel

The rate at which the hydrogels dried out was measured as per cent weight remaining under controlled conditions. Not surprisingly, the drying rate increased as the temperature increased from 25 ^{o}C to 30 ^{o}C and 35^{o}C (Table 4.1 and Figure 4.3).

At 25^{o}C and 30 ^{o}C, differences in drying among gel formulae were statistically insignificant over 52 hours (ANOVA, $p < 0.05$). From 0-24 hours, every treatments of hydrogel continuously reduced on their weight which had statistical significance ($p < 0.05$). After 24 hours, hydrogel weight did not decline further ($p < 0.05$).

At 35^{o}C, differences in weight loss among the hydrogel formulae were not significant over 52 hours. From 0-14 hours, every treatments of hydrogel continuously reduced on their weight and differ significantly ($p < 0.05$). After 14 hours, hydrogel weight did not decline any further ($p < 0.05$).

Table 4.1 Estimated drying trends from regression line

Treatment	Rate of drying from hydrogel					
	25°C at 38 hours		30°C at 38 hours		35°C at 24 hours	
	Slope	R^2	Slope	R^2	Slope	R^2
A1	-16.233	0.8246	-18.328	0.9662	-21.309	0.9576
A2	-16.166	0.8438	-18.225	0.9478	-21.598	0.9661
A3	-16.135	0.8403	-16.797	0.9625	-21.353	0.9598
A4	-16.944	0.8391	-18.479	0.9631	-21.375	0.9712
A5	-17.003	0.8422	-18.965	0.9521	-21.062	0.9717
B1	-16.554	0.7892	-17.388	0.9742	-21.035	0.9684
B2	-16.948	0.8702	-18.658	0.9605	-20.911	0.9745
B3	-16.378	0.7878	-16.484	0.9686	-21.105	0.9599
B4	-15.006	0.7872	-15.534	0.9494	-20.199	0.9574

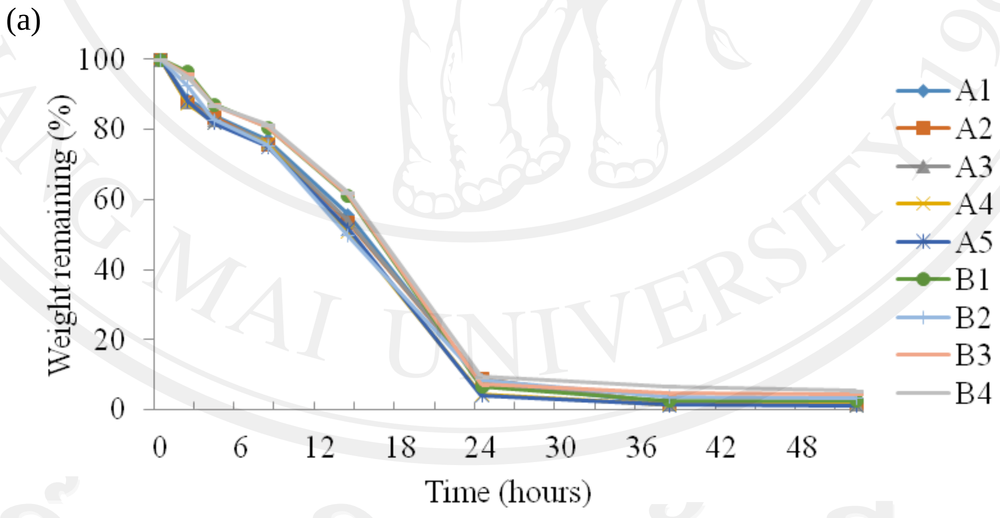


Figure 4.3 Percentage of hydrogel's weight remaining at 25 °C (a), 30 ± 5% of relative humidity

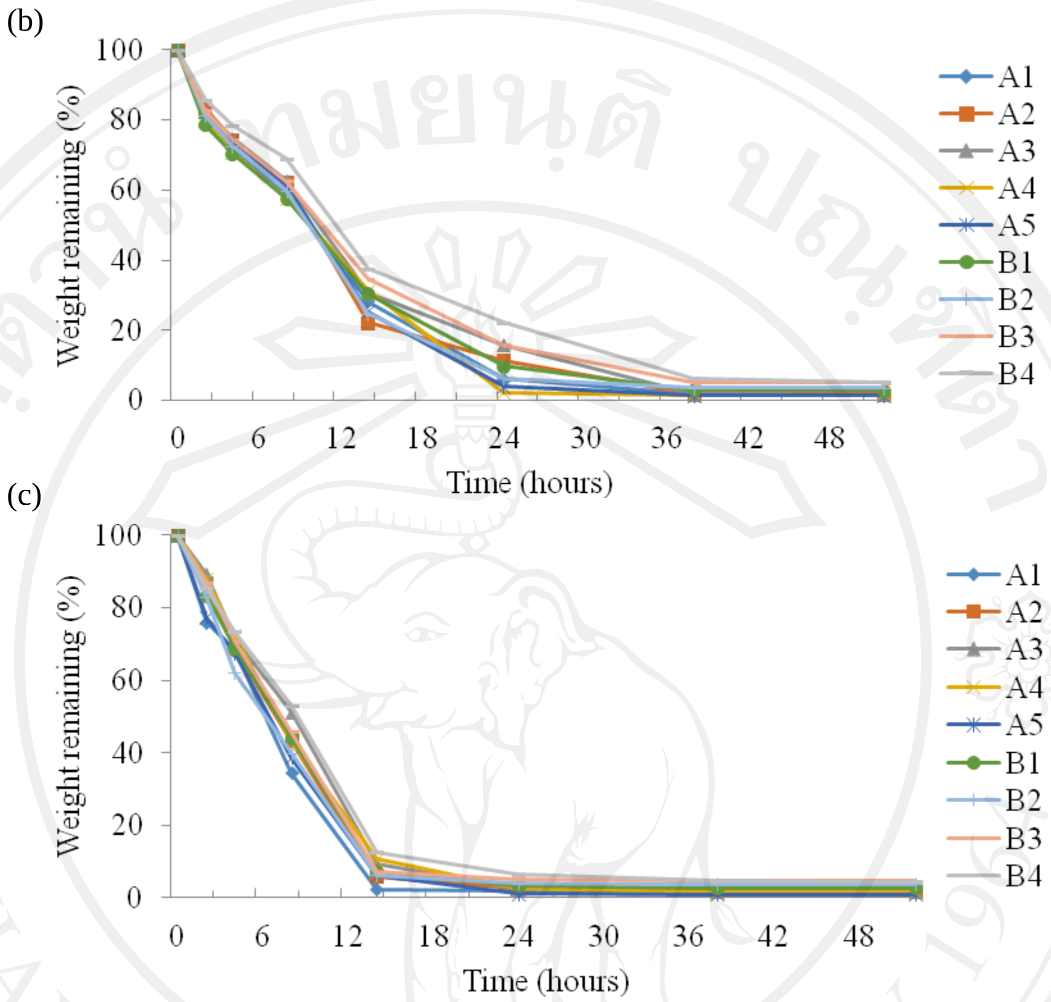


Figure 4.3 (cont.) Percentage of hydrogel's weight remaining at 30 °C (b) and 35°C (c), 30 ± 5% of relative humidity

4.2 Moisture properties in hydrogel film

The amount of water that could be held by each hydrogel formula differed significantly (ANOVA, $p < 0.05$). Water-holding capacity declined with declining NaCMC content and the agar-based formulae (A2-A5) held less water than the starch-based formulae (B1-B4) (Figure 4.4).

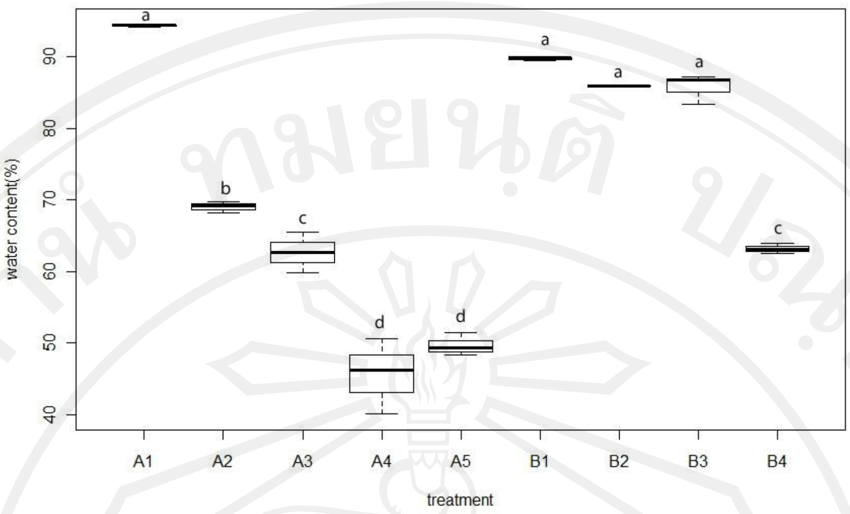


Figure 4.4 Water content among agar and corn starch based films with NaCMC

When comparing swelling ratio, the highest value (g Liquid/g Film) was achieved with 100% NaCMC (A1). Swelling ratio differed significantly among the formulae (ANOVA, p < 0.05). It declined with declining NaCMC content; with the starch-based formulae having higher swelling ratios than the agar-based ones (Figure 4.5).

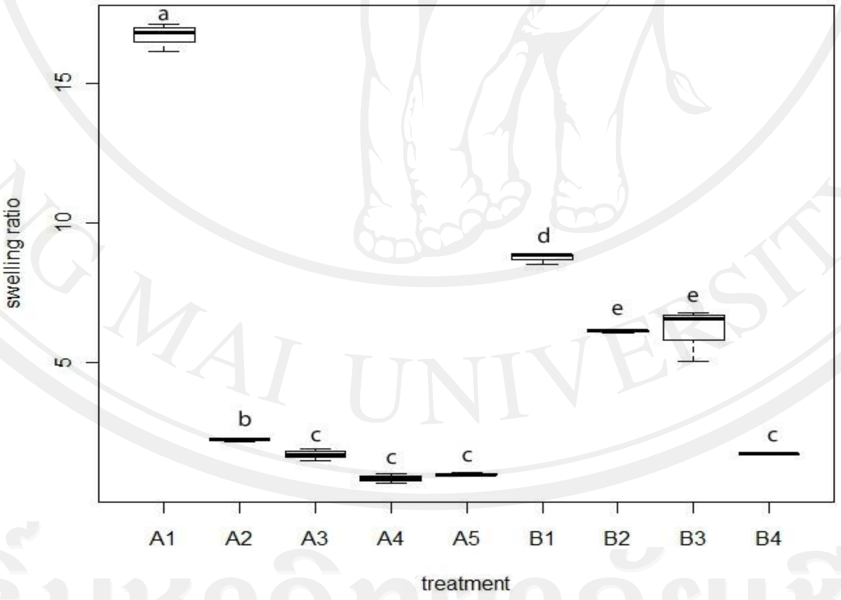


Figure 4.5 Swelling ratio among agar and corn starch based films with NaCMC

The test of water vapor permeability, WVP, found that increasing agar content had no effect on WVP (ANOVA, p < 0.05). In contrast, adding starch significantly increased WVP (p < 0.05) quantitatively (Figure 4.6).

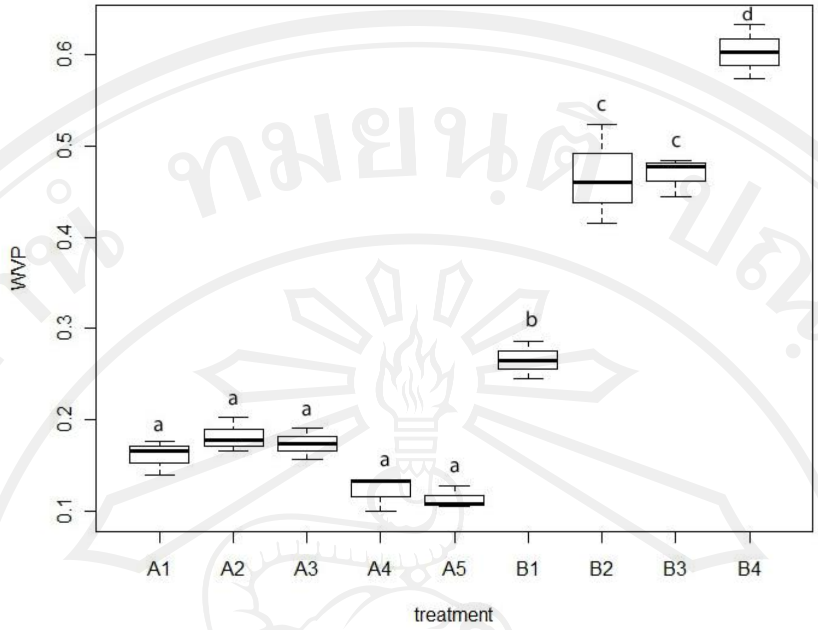


Figure 4.6 WVPs of agar and corn starch based films with various amounts of NaCMC, 25 ± 1°C

The moisture sorption isotherm for agar and corn starch blended with NaCMC showed equilibrium moisture contents or Me (Figure 4.7). The moisture sorption isotherm was determined at each equilibrium weight, slowly increased to stable (± 0.01g/day). When the weight reached a plateau point seemed to be equilibrated moisture contents. In this case, lower relative humidity (RH) took equilibrium weight longer than high RH. Especially, for films stored at RH 84.3% reached at equilibrium within 7 days whereas for 10 days at RH 33.8% (data not show).

Increasing RH increased Me, so that can indicated that gained weight from water sorption through the hydrogel. When placed in RH above than 33%, agar and corn starch hydrogel had significant difference on Me (ANOVA, $p < 0.05$).

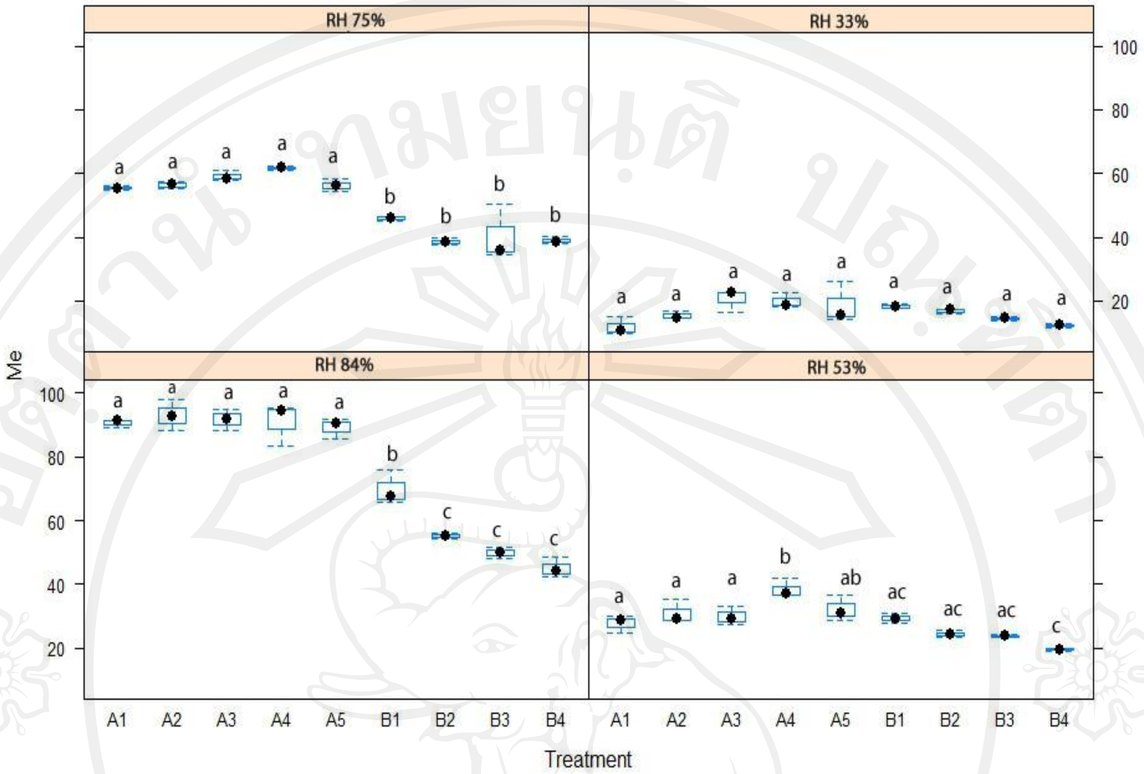


Figure 4.7 Moisture sorption isotherms of NaCMC with agar and corn starch based films at 25 ± 1°C

At 33% of RH, Me among gel formula were statistically insignificant differences (ANOVA, $p < 0.05$).

At RH 53%, 75% and 84%, Me differed significantly among the hydrogel formulae ($p < 0.05$). A1-A5, NaCMC content + agar-based formulae, had more Me than the starch-based formulae (B1-B4). Me increased sharply for every gel formula when RH was high (Figure 4.7).

The sorption isotherm data were determined by the GAB (Guggenheim-Anderson-de Boer) model and the constants are presented in Table 4.2. These values indicated the maximum amount of water that could be adsorbed in a single layer per gram of dry film. They are therefore indicators of moisture absorbed by each monolayer (Balakrishnan *et al.*, 2005). GAB monolayer moisture contents (Mo) were in the range of 0.573-0.998 g water/g dry film, respectively. Scanning electron microscopy (SEM) revealed that pure NaCMC, pure agar and NaCMCM + agar had a rough with stretch marks throughout surfaces. In contrast, pure corn starch and NaCMC + corn starch had a smoother (Figure 4.8).

Table 4.2 Sorption isotherm model constants of agar and corn starch based film with NaCMC at 25 ± 1°C.

Treatment	Value in GAB			
	Mo	k	C	R-square
A1	0.984	2.610	16.588	0.999
A2	0.998	6.434	15.126	0.999
A3	0.994	22.065	15.042	1.000
A4	0.923	5.953	21.016	0.998
A5	0.987	15.431	15.148	0.999
B1	0.958	1.927	13.345	0.998
B2	0.900	3.574	11.800	0.999
B3	0.753	3.011	21.729	1.000
B4	0.573	0.993	49.254	0.997

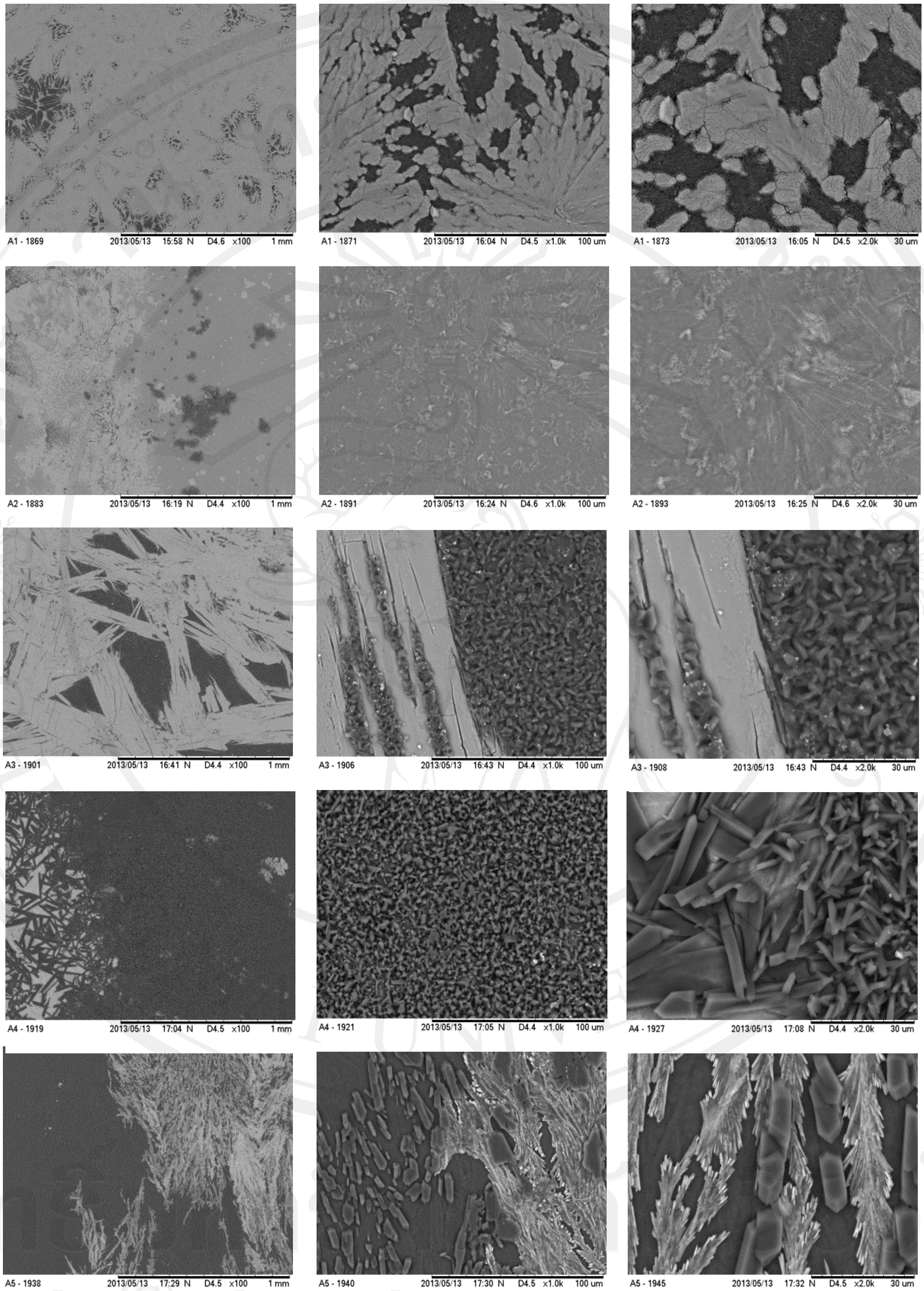


Figure 4.8 SEM micrographs of NaCMC + agar; A1 to A5, from left to right are 100x, 1000X and 2000X, respectively

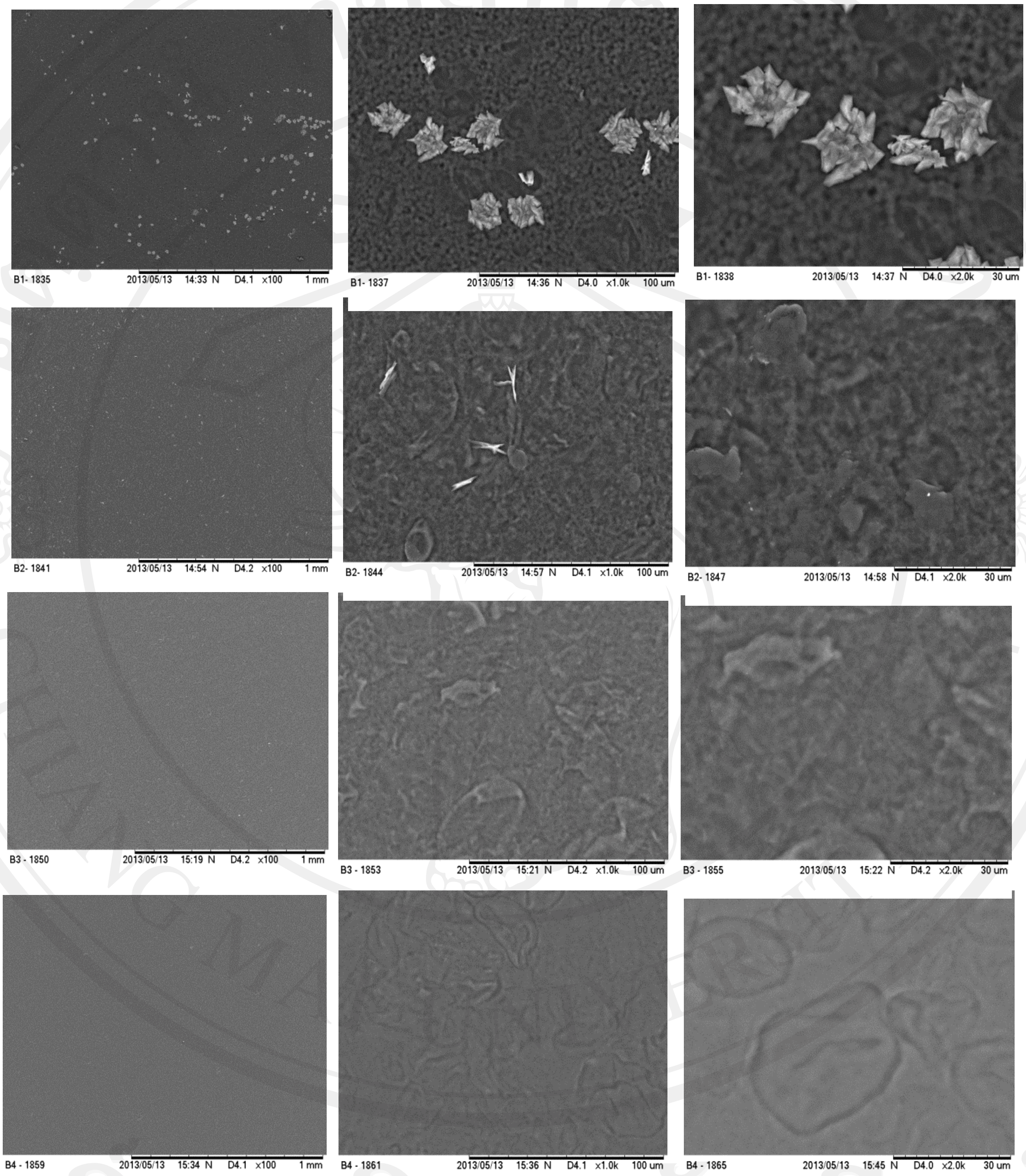


Figure 4.8 (cont.) SEM of NaCMC + corn starch; B1 to B4, from left to right are 100x, 1000X and 2000X

4.3 Preliminary germination tests of *Ficus* spp. in Petri dishes

Preliminary tests in Petri dishes revealed significant differences among the gel formulae in their effects on seed germination (ANOVA, $p < 0.05$) for all of the 3 *Ficus* spp. tested. A5, 100% agar and B4, 100% corn starch resulted highest per cent germination. A1, 100% NaCMC, A2, B1 and B2 resulted in the lowest per cent seed germination. Treatments A3, A4 and B3 had lower proportions of NaCMC and consequently moderate germination percentages (Figure 4.9). Even within each *Ficus* species, germination rates still differed consistently and significantly among the gel formulae ($p < 0.05$).

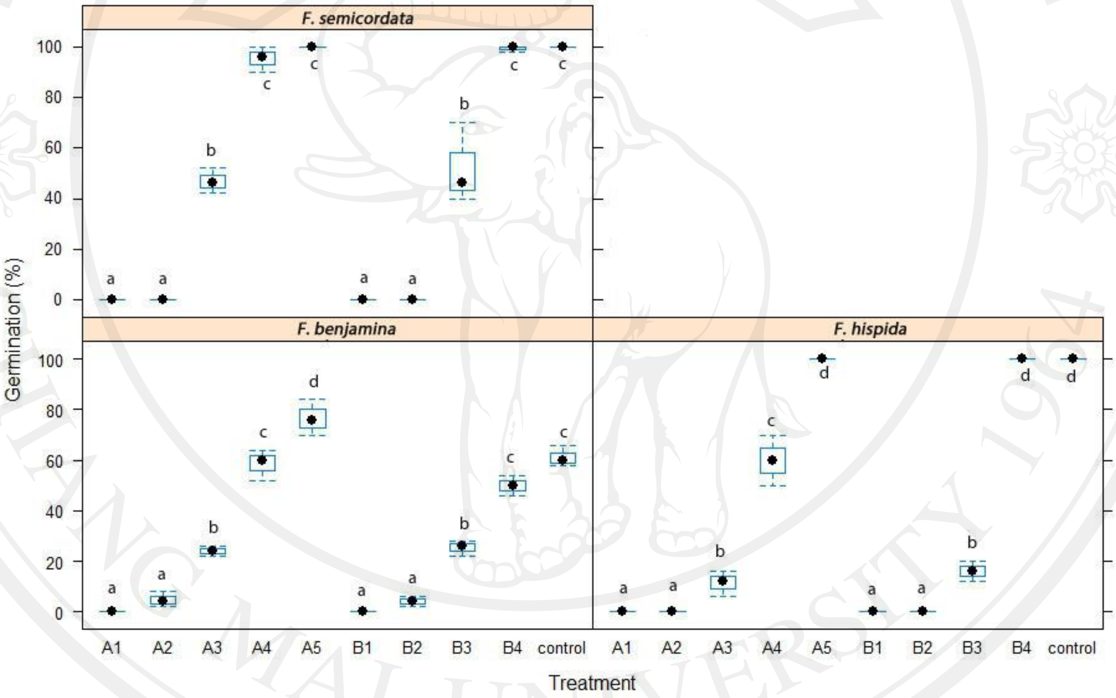


Figure 4.9 Per cent germination of seedlings in each species and treatment after 10 days

In *F. benjamina*, formulae A5 and B4 resulted the highest per cent germination; equal to that of the control, followed by A4, the group A3, B3 and the group A1, A2, B1, B2.

In *F. hispida*, A5 and B4 resulted in the highest per cent germination; similar to that of the control, followed by A4, the group A3, B3 and the group A1, A2 and B1. No seeds germinated in the B2 formula.

In *F. semicordata*, the group of formulae A4, A5 and B4 and the control resulted in the highest per cent germination, followed by the group A3, B3 and the group A1, A2, B1. Formula B2 did not support any germination.

4.4 Hydroseeding in the nursery

The limestone mine substrate was moderately alkaline (pH 8) with a clay/sandy loam texture. Nutrient contents are shown in Table 4.3. In the nursery the mean ambient temperature of the experiments with *F. benjamina*, *F. hispida* and *F. semicordata* was 25.78 ^{o}C ± 1.51, 29.45 ^{o}C ± 2.24 and 26.32 ^{o}C ± 1.13, respectively. Moreover, mean light intensity values were 3,000.00 Lux ± 338.62, 2,968.75 Lux ± 270.10 and 2,653.85 Lux ± 490.94, for *F. benjamina*, *F. hispida* and *F. semicordata* respectively.

In general, germination did not differ significantly among the gel treatments or substrate types (ANOVA, $p < 0.05$). Moreover, there was no a significant interaction between substrates and hydrogel treatments ($p < 0.05$) (Figure 4.10 and 4.11). In contrast germination differed significantly among the 3 *Ficus* species *F. benjamina* (11.46a % ± 9.01), *F. hispida* (22.44b % ± 13.12) and *F. semicordata* (21.97b % ± 20.74) ($p < 0.05$) (Figure 4.12).

Table 4.3 Soil analysis report

Sample	pH	Organic Matter (g/100g)	Nitrogen (g/100g)	Phosporus (mg/kg)	Potassium (mg/kg)	CEC (cmol/kg)	Sand (%)	Silt (%)	Clay (%)	Texture
Site 1	7.98	0.59	0.68	9.58	34.80	10.90	41.70	20.70	37.60	Clay loam
Site 2	8.09	0.84	0.72	0.60	31.49	5.87	59.50	23.10	17.40	Sandy loam

Data from Soil Science Laboratory, Division of Soil Science and Conservation, Faculty of Agriculture, Chiang Mai University

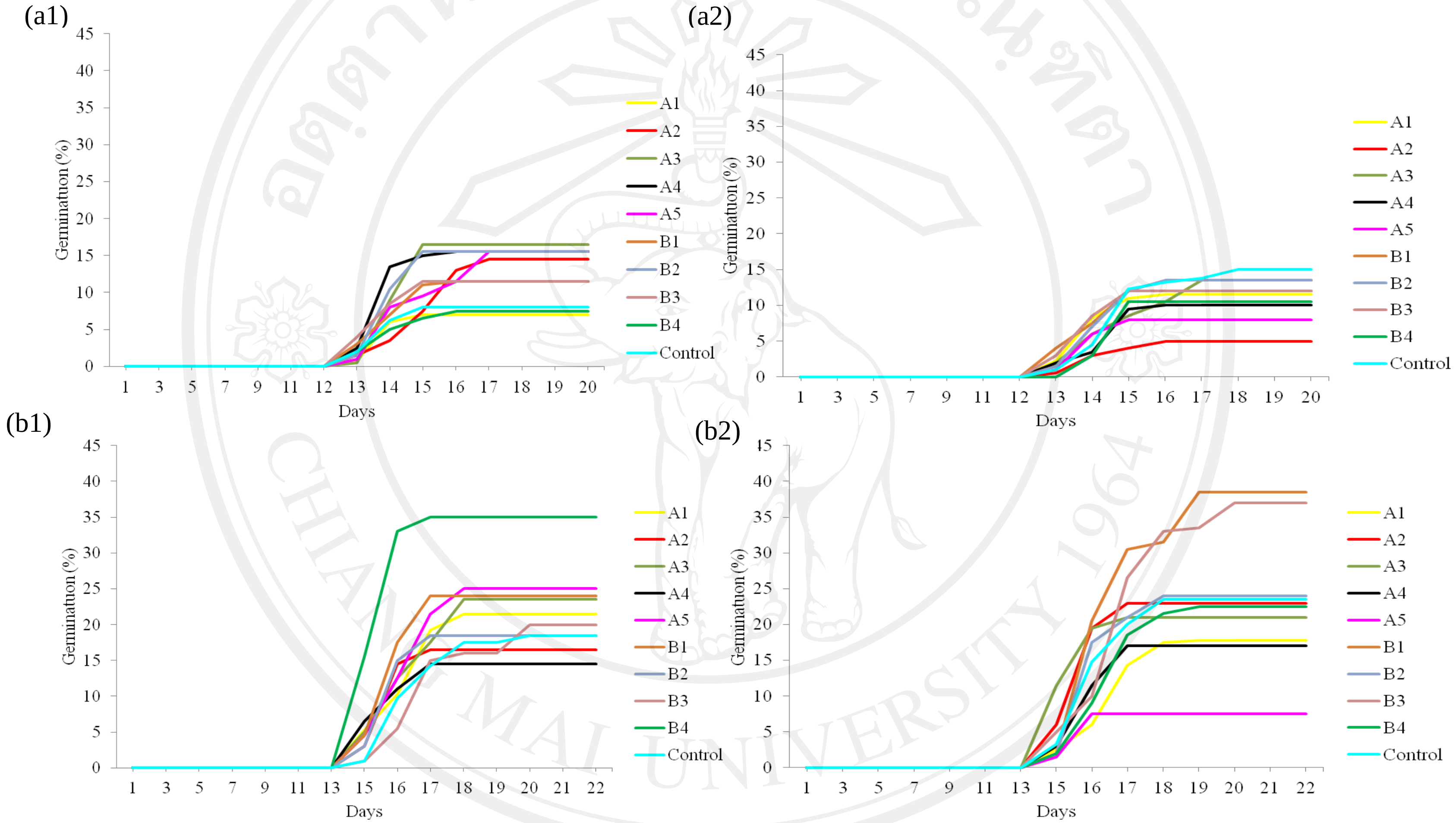
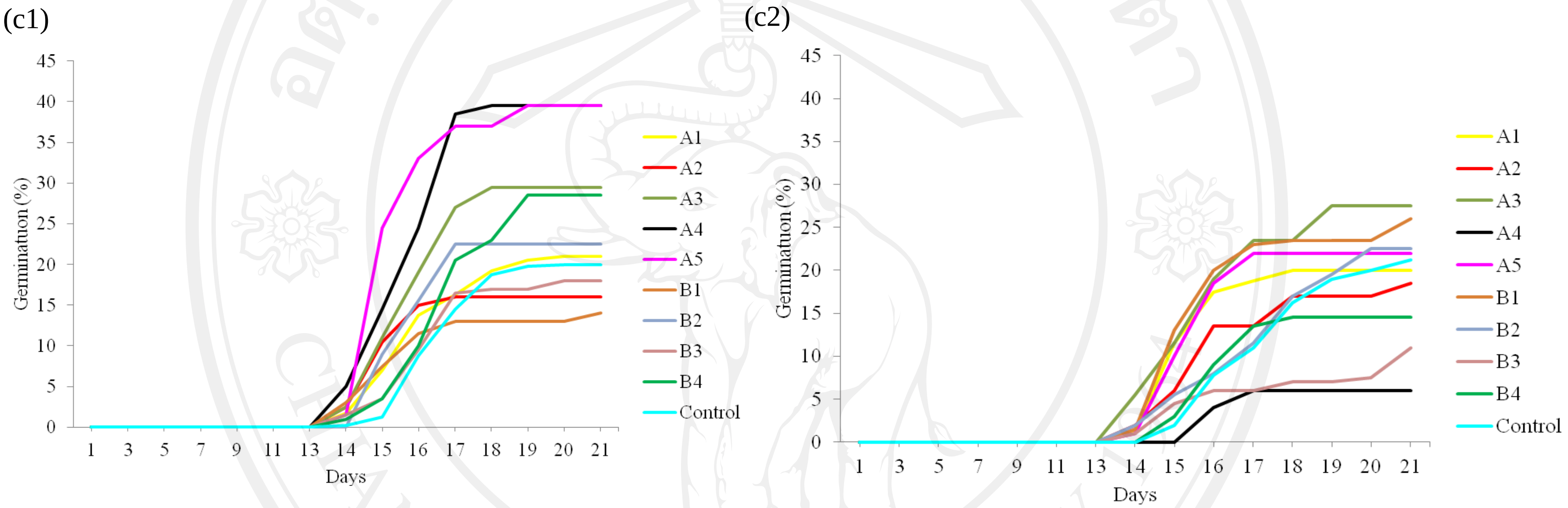


Figure 4.10 Per cent germination (mean of 8 replicates) of *F. benjamina* (a) *and F. hispida* (b) in each soil (block), soil from site 1 (1) and site 2 (2)


(c1)

(c2)

Figure 4.10 (cont.) Per cent germination (mean of 8 replicates) of *F. semicordata* (c) in each soil (block), soil from site 1 (1) and site 2 (2)

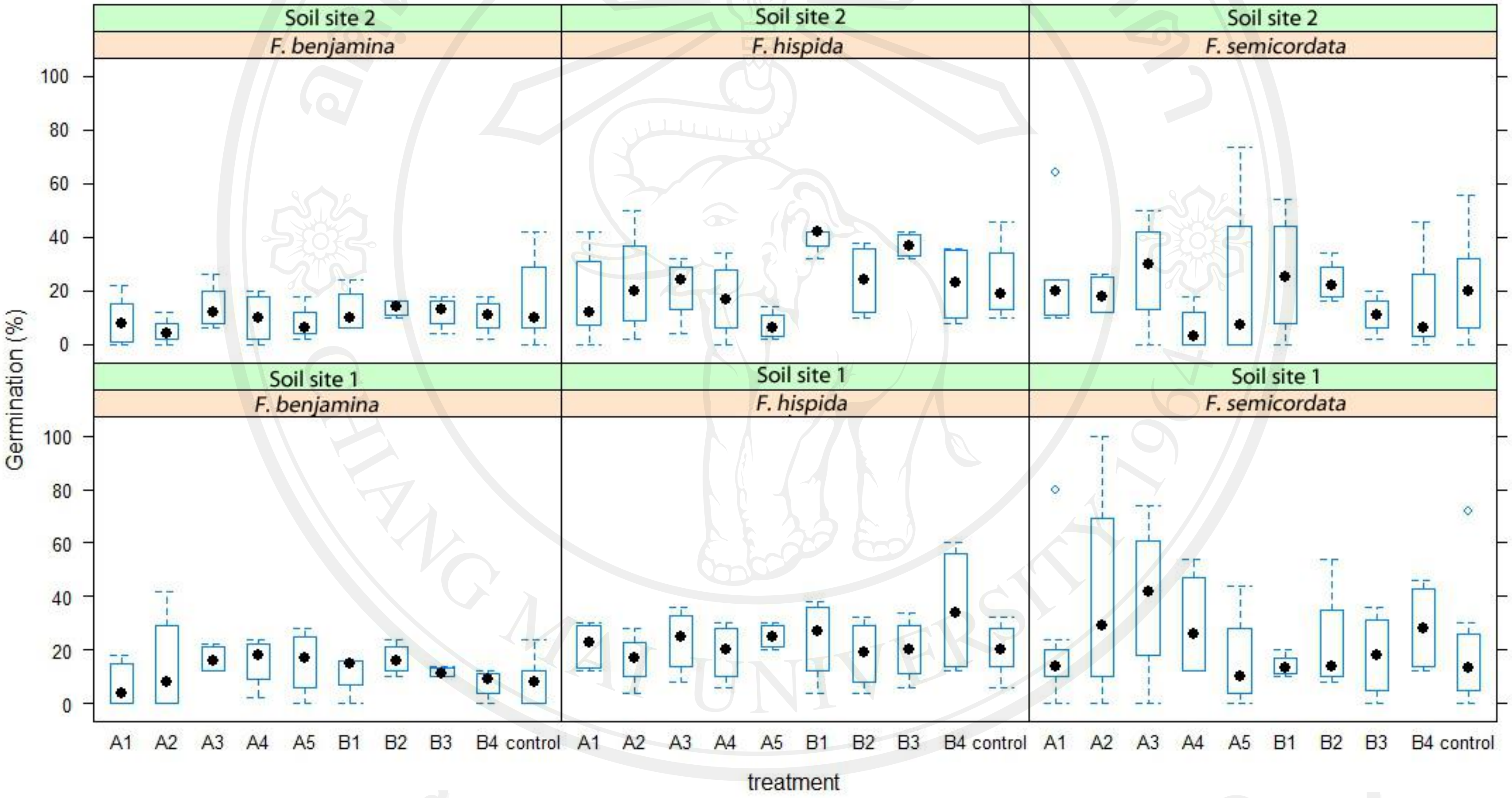


Figure 4.11 Per cent germination with various treatments in nursery

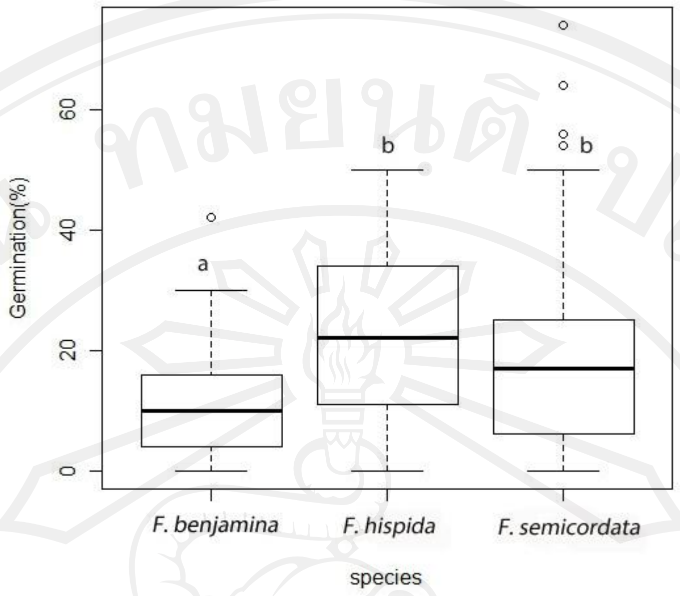


Figure 4.12 Per cent germination of *Ficus* spp. in nursery

Median length of dormancy (MLD) did not differ significantly among the hydrogel treatments and substrate types (ANOVA, $p < 0.05$) in figure 4.13. Moreover, there was no a significant interaction between substrate type and hydrogel treatment ($p < 0.05$). In contrast, MLD did differ significantly among the 3 *Ficus* species tested ($p < 0.05$). *F. benjamina* (14.18a days ± 1.22), *F. hispida* (16.20b days ± 1.02) and *F. semicordata* (16.60b days ± 1.69) (Figure 4.14).

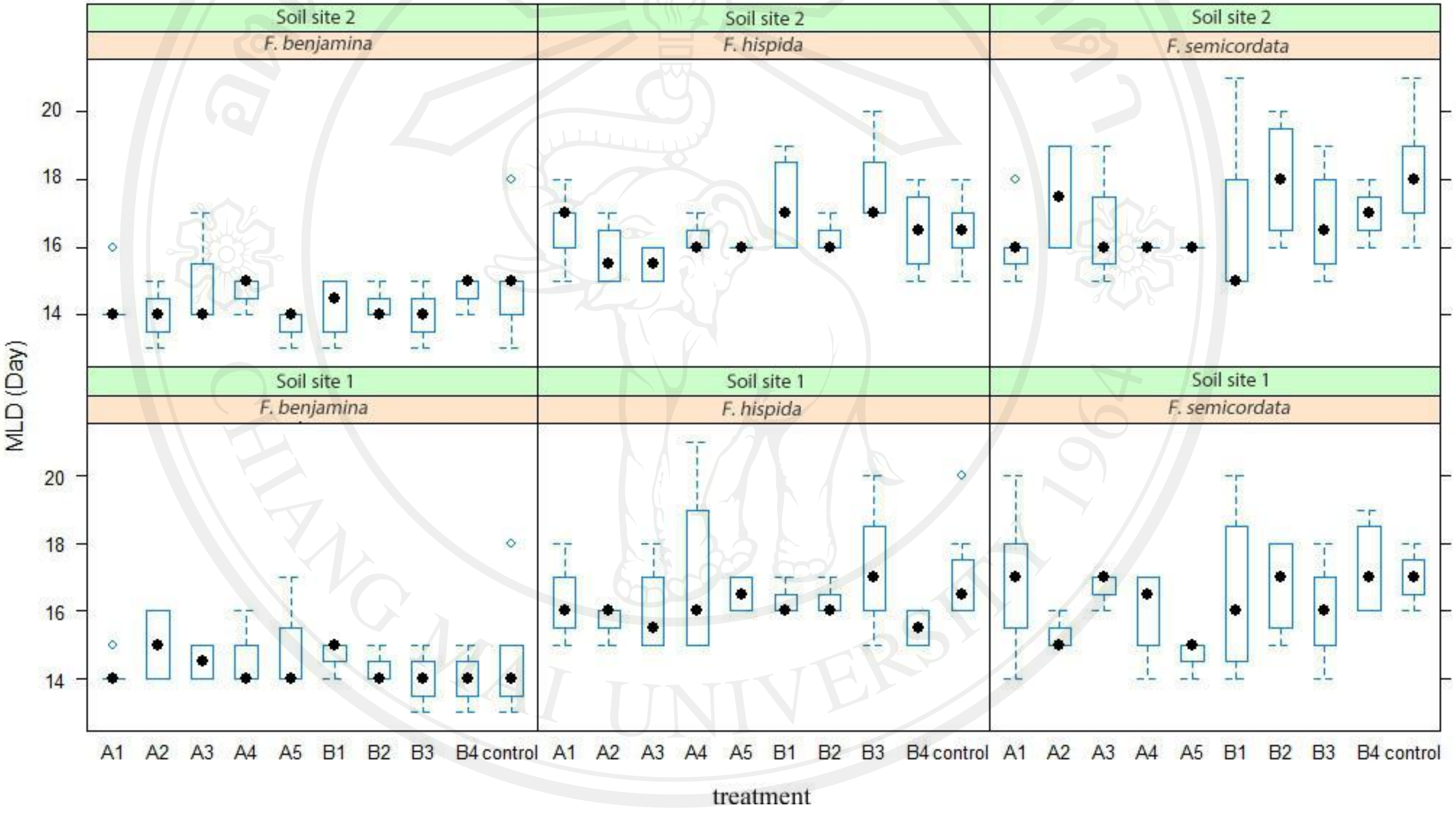


Figure 4.13 MLD with various treatments in nursery

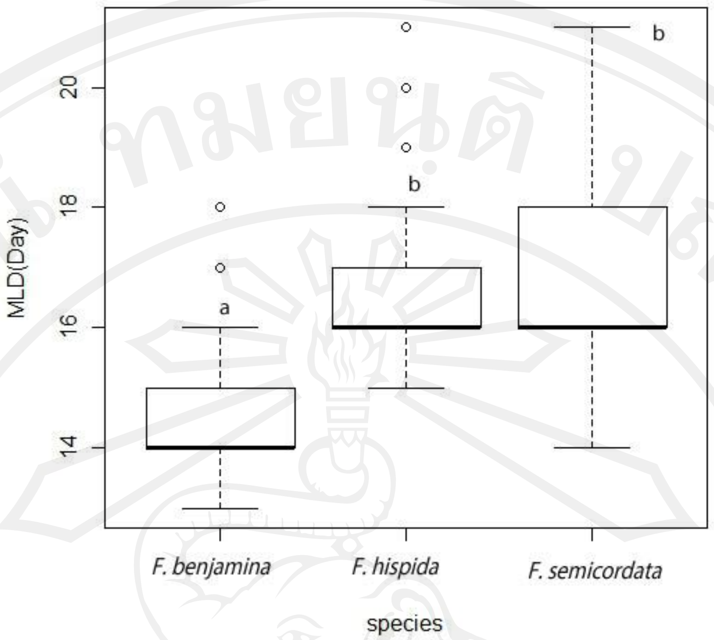


Figure 4.14 MLD of *Ficus* spp. in nursery

Survival of *Ficus* spp. seedlings in nursery were determined 4 weeks after the last seed had germinated (Figure 4.15). Neither the hydrogel treatments nor the substrate type had any significant effect on mean survival (ANOVA, $p < 0.05$). There was no interaction between substrate type and hydrogel treatment ($p < 0.05$). However mean survival did differ significantly among the 3 species tested ($p < 0.05$): *F. benjamina* (10.47a % ± 8.50), *F. hispida* (19.63b % ± 17.24) and *F. semicordata* (75.60c % ± 12.14) (Figure 4.16).

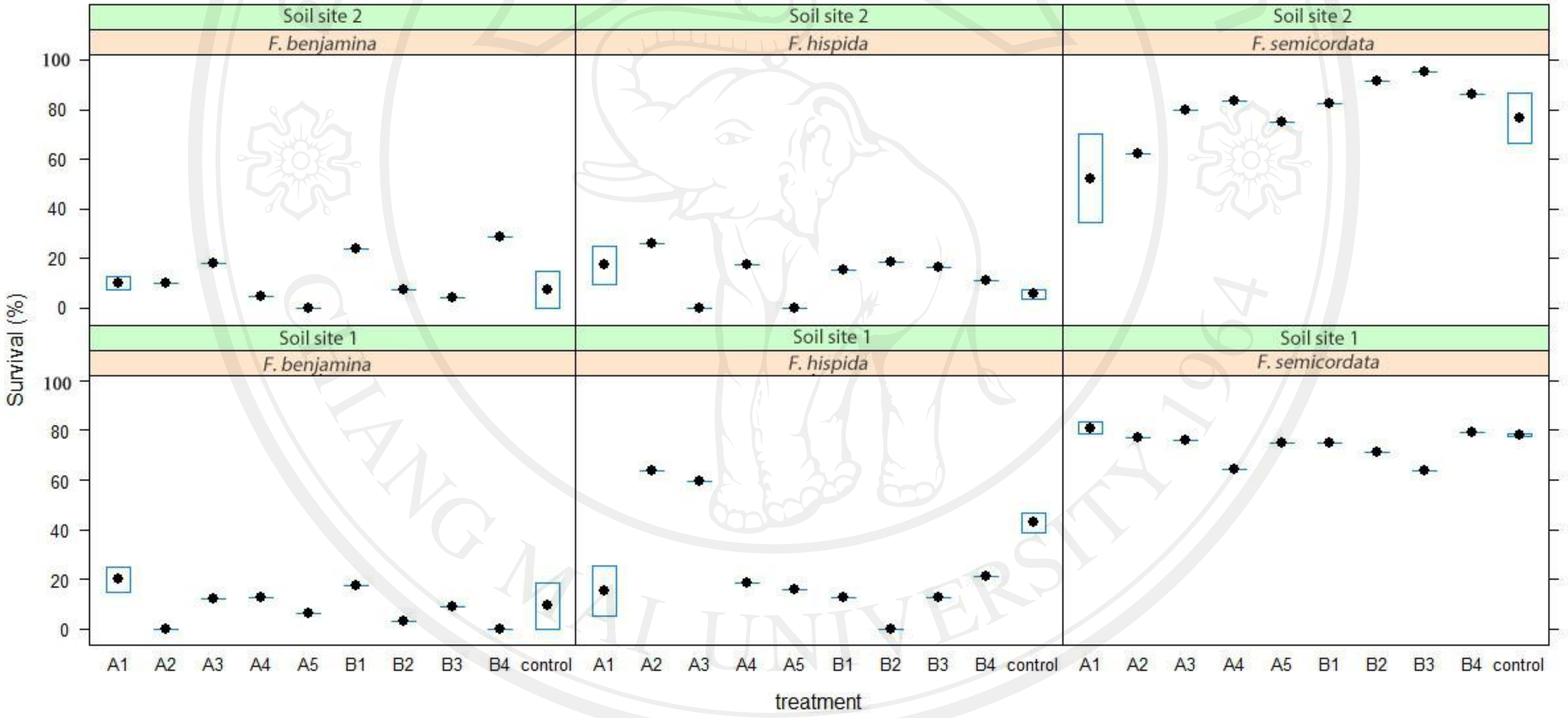


Figure 4.15 Per cent survival with various treatments in nursery

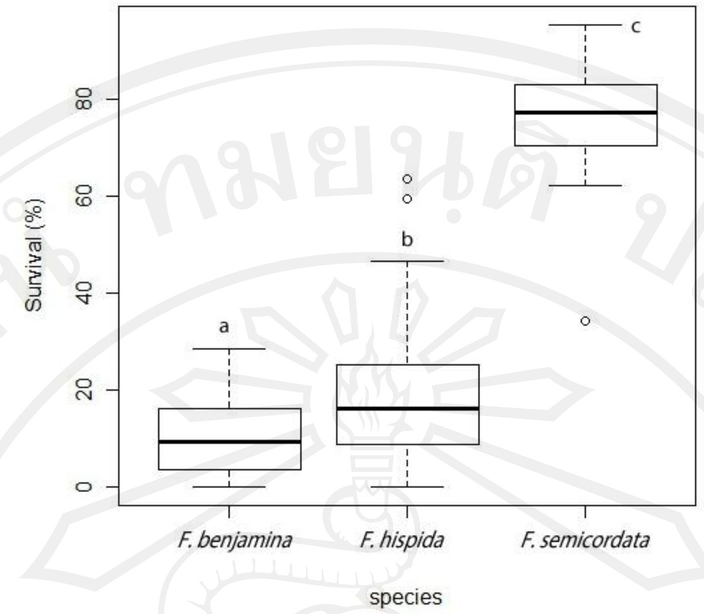


Figure 4.16 Per cent survival of *Ficus* spp. in nursery

4.5 Hydroseeding at the limestone mine

The ground flora recorded in study sites were *Urochloa repens*, *Vigna mun*go, *Phyllanthus amarus*, *Stylosanthes sundaica*, *Eupatorium odoratum*, *Sporobolus indicus*, *Vernonia divergens*, *Merremia vitifolia*, *Tridax procumbens*, *Pennisetum polystachyum* and *Dioscorea* sp.

Data collection of rain fall from Thai Meteological Department was used to represent daily rain fall at limestone mine (Figure 4.17).

At site 1, the average temperature was 33.20 ^{o}C ± 2.05 (N=8) and mean of light intensity was 54829 Lux ± 9931 (N=24).

At site 2, the average temperature was 32.60 ^{o}C ± 1.94 (N=8) and mean of light intensity was 54558 Lux ± 10035 (N=24).

Seedling establishment was investigated at intervals time that related on the MLD from germination in nursery. The first data collections were begun after 10 days in study site and continued until no new seedling occur (Figure 4.18).

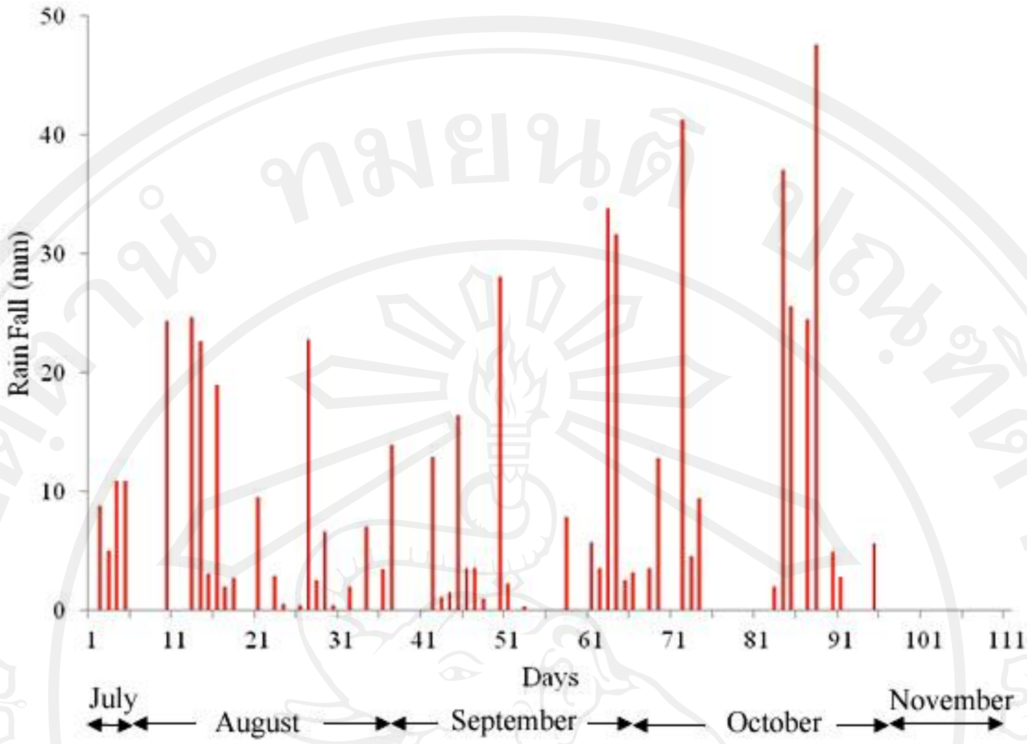


Figure 4.17 Daily rain fall at limestone mine,

from 26th July to 13th November, 2013 (Thai Meteological Department)

Differences in germination among hydrogel treatments were not significant (ANOVA, $p < 0.05$). There were no interactions between site and hydrogel treatments for all three of the tested species, *F. benjamina*, *F. hispida* and *F. semicordata* ($p < 0.05$). Difference in germination percentage among species were also insignificant ($p < 0.05$, Figure 4.18 and 4.19).

In contrast, germination differed significantly between the two study sites ($p < 0.05$), with site 2 supporting much higher germination (0.089b %) than site 1 (0.014a %) (Figure 4.20).

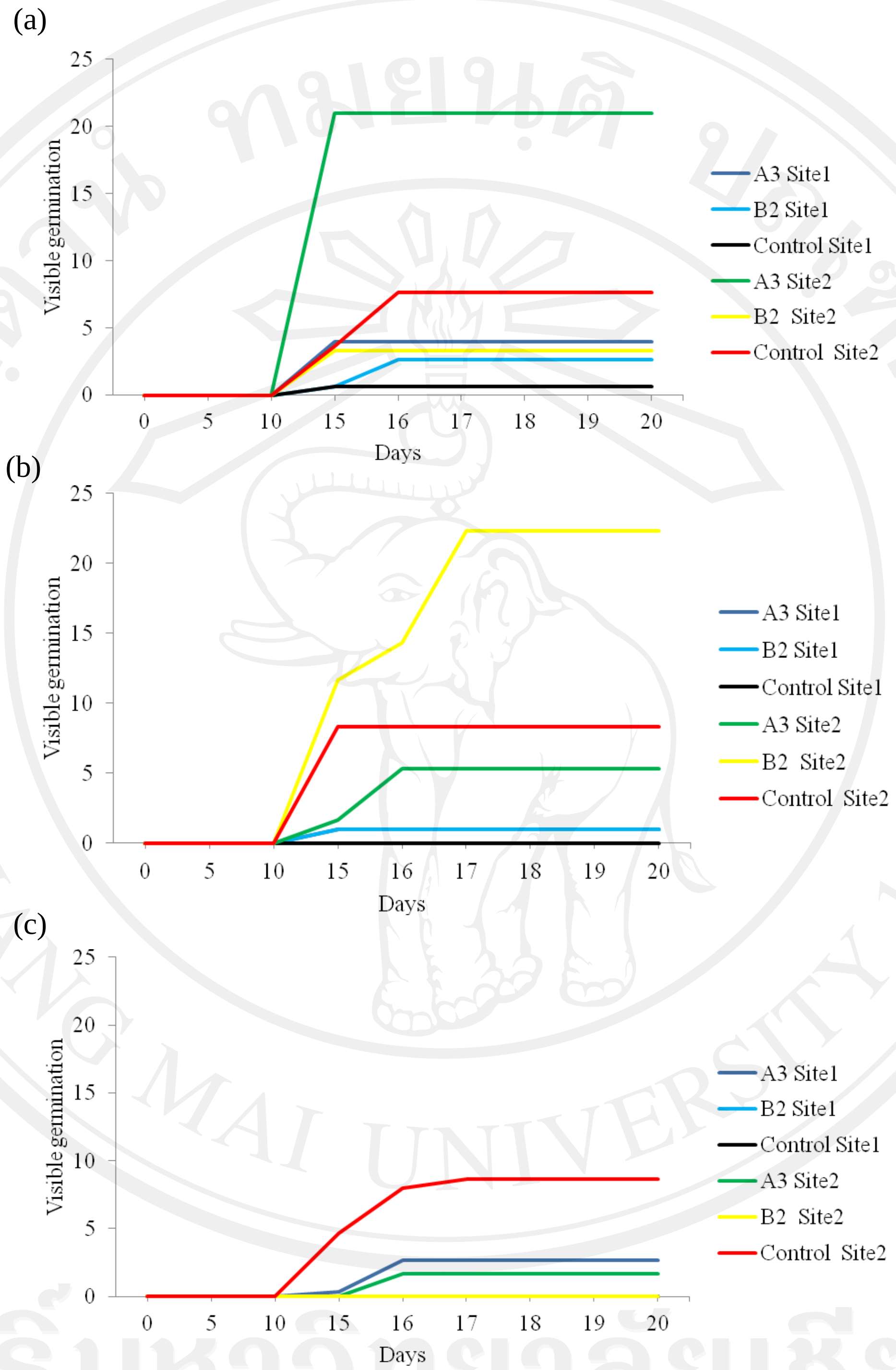


Figure 4.18 Visible germination in limestone mine from approximately 10,000 seeds of *F. benjamina* (a), *F. hispida* (b) and *F. semicordata* (c)

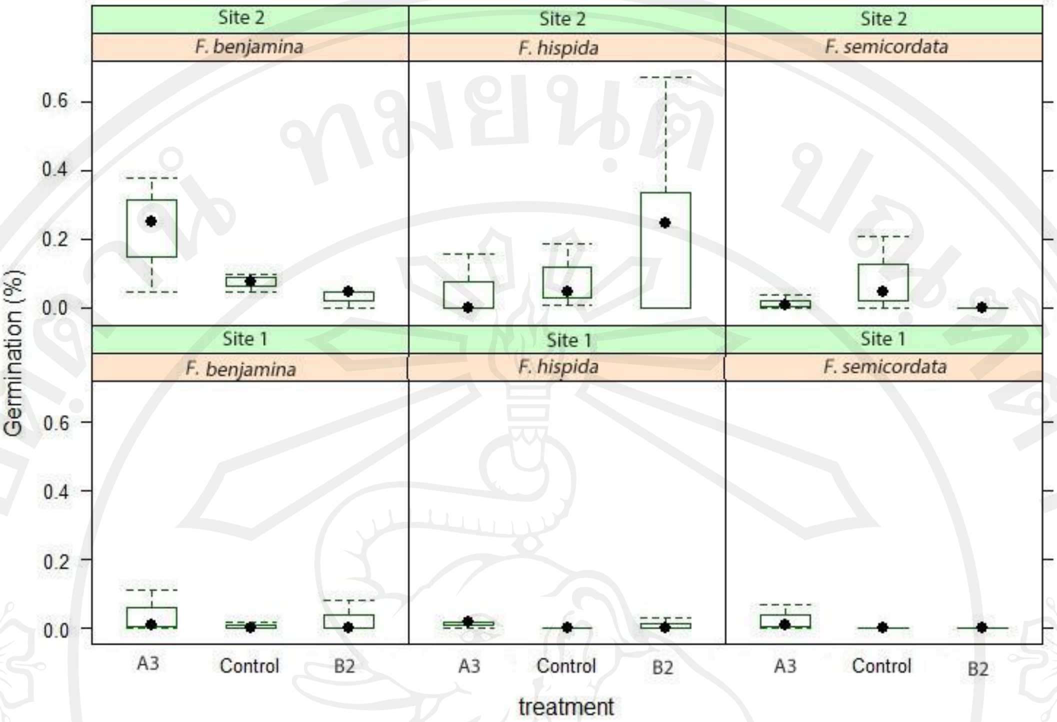


Figure 4.19 Per cent germination in limestone mine site

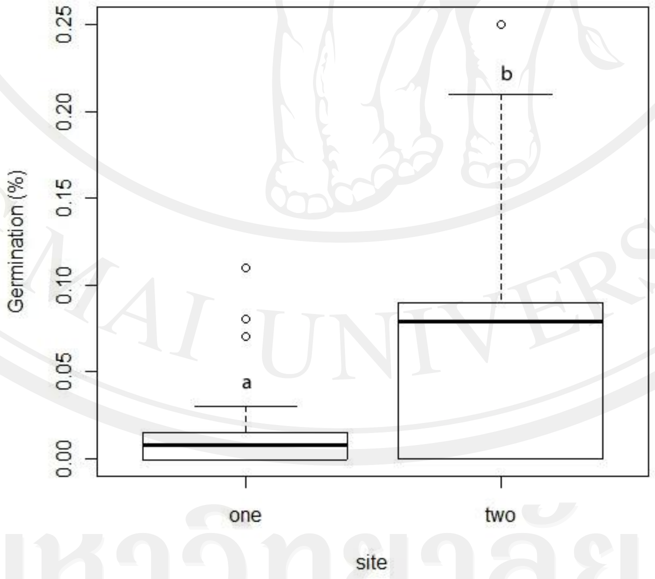


Figure 4.20 Per cent germination of *Ficus* spp. at 2 sites in limestone mine

Differences in MLD among hydrogel treatments and sites were not significant (*p* < 0.05, Figure 4.18) and there was no interaction between site and hydrogel treatment ($p < 0.05$). In contrast differences in MLD were significant among species ($p < 0.05$). *F. hispida* had the shortest dormancy (13.37a days ± 1.48), followed by *F. benjamina* (13.41a days ± 1.92), and *F. semicordata* (16.16b days ± 1.75) (Figure 4.21).

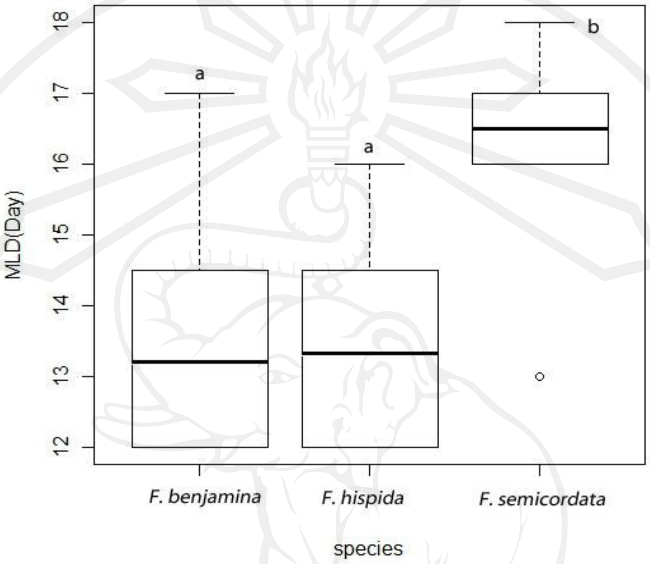


Figure 4.21 MLD of *Ficus* spp. in limestone mine site

Seedling survival did not differ significantly between the study sites and there was no interaction between study site and hydrogel treatment (ANOVA, $p < 0.05$), within each species, *F. benjamina*, *F. hispida* and *F. semicordata,* each hydrogel treatment had the same effect on per cent survival ($p < 0.05$, Figure 4.22).

At site 1, comparison among A3, B2 and control showed that control treatment in *F. benjamina* had the highest survival percentage, followed by B2 and A3, respectively ($p < 0.05$), On the other hand, seedling survival with every treatment declined dramatically deceasing tend until no seedlings survived after no new seedling occur for 60 days (Figure 4.23).

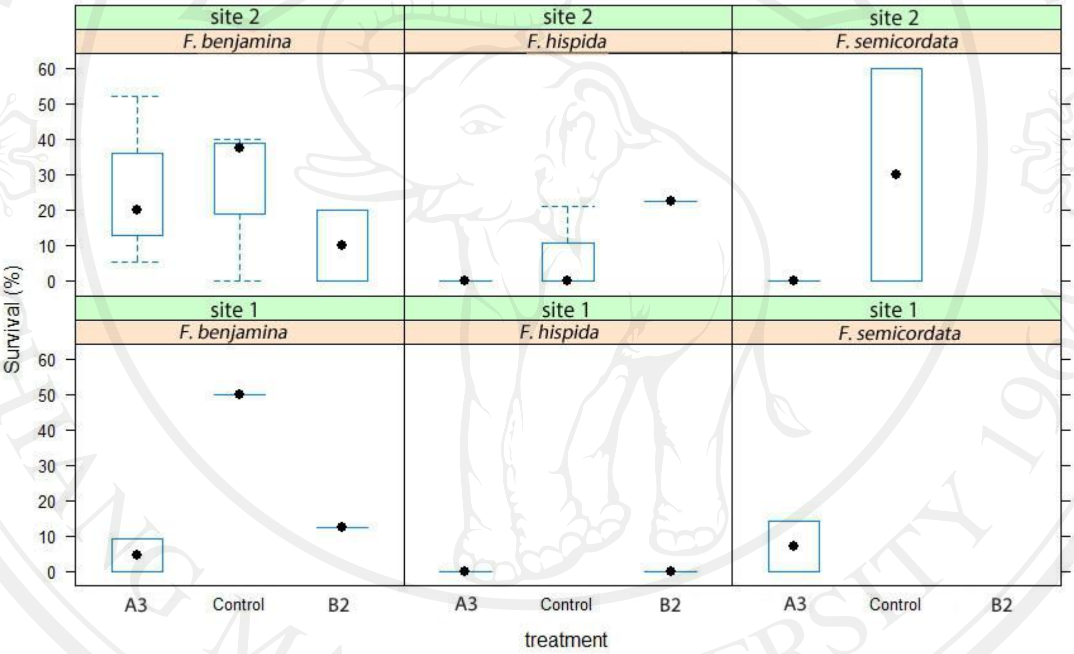


Figure 4.22 Per cent survival of *Ficus* spp. in limestone mine site after first seed germinated for 15 days

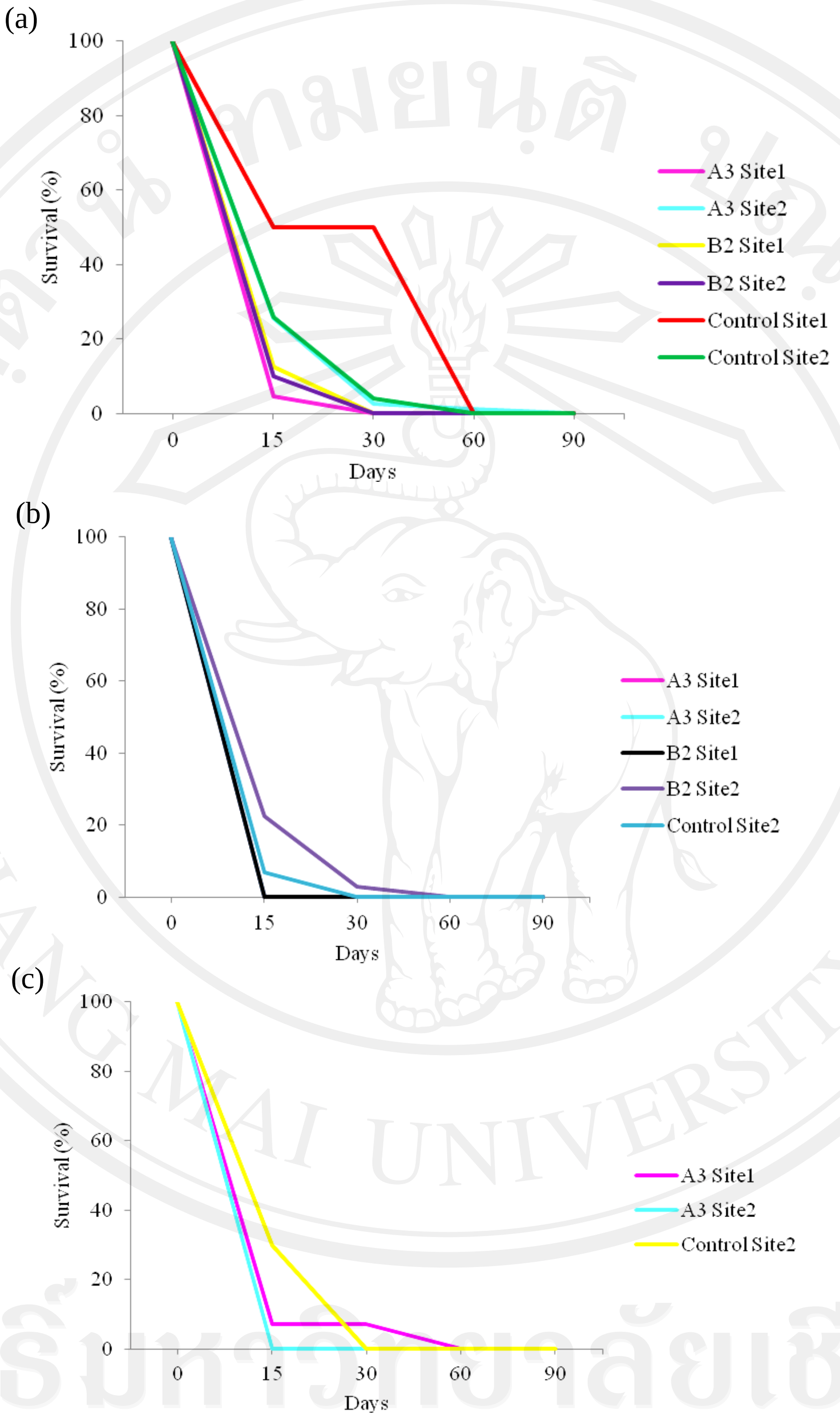


Figure 4.23 Survival percentage for 90 days of *F. benjamina* (a), *F. hispida* (b) and *F. semicordata* (c) in limestone mine condition

CHAPTER 5

Discussion

5.1 Suitability of the hydrogel

The substrate and environmental conditions in the limestone quarry were very harsh for *Ficus* spp. to germinate by hydroseeding. The study site was steep and lacked top soil.

Tests showed that increasing NaCMC in the hydrogels increased water holding capacity. High NaCMC content increased equilibrium water content and swelling ratio. It terms of swelling ratio, the highest water uptake was found with 100% NaCMC. Although both agar and corn have water holding properties, the lowest values were found with high proportions of corn starch and agar in the hydrogel. Guobin *et al*. (2011) reported that in NaCMC molecular chains, there are numerous carboxyl groups with negative charge, carboxyl anions (-COO-) and hydroxyl groups (-OH). Interactions among negative charges result in more space within the polymer chains and consequently the creation of a porous layer and more swelling, since water molecules can easily diffuse into the small pores.

Hydrogel must be sticky enough to hold all components, remain attached to the mine substrate on slopes and can attach at the mine slope to enable the establishment of *Ficus* seedlings. The slopes of restored mining site were normally higher than 30 degrees (SCG Cement and WWF Thailand, 2009). Tests on the hydrogels showed that the thickness of hydrogel was tested then the result showed that corn starch mixed with high composition of NaCMC was stickier than agar with and corn starch were sticky than agar. Because NaCMC has large amounts of carboxyl groups, created those linked to the NaCMC chains. Higher contents of NaCMC side chains which bonded into a network and increased viscosity (Sannino *et al*., 2009). Furthermore, corn starch is long polymer, comprised of amylose and amylopectin. The basic repeating unit for both types of starch is connected by glycosidic bonds. The polymer chains in corn starch and their formations of the intermolecular network result in a thick gel (Corn Refiners Association, 2006). On the other hand, agar has only hydroxly groups which are weakly linked (Lahaye and Rochas, 1991).

Temperatures at the mine were above than 30 ^{o}C, so drying of the hydrogels was studied. Drying occurred more rapidly at higher temperatures. When the hydrogel dried out, it became a thin film which covered the substrate surface. This may inhibit germination due to lack of oxygen and moisture. Therefore, thin film of the hydrogel must have the ability to transfer moisture to the seed. The treatments (A1-A5 and B1-B4) were evaluated for transfer of air and moisture through the films. The measurement of water vapor permeability (WVP) under various levels of relative humidity (RH) increased with increasing corn starch proportion in the hydrogel but agar content had no effect on WVPs. More mixing CMC to rice starch films reduced WVP at RH 90% and 25°C. Accordingly, WVP decreased because the carboxylate acid groups of CMC reacted with the hydroxyl groups of the starch to form an ester bond through dry heating. This formation would lead to high solid structure, more compact molecule and diminish the number of available hydroxyl groups, thereby impeding diffusion of water vapor.

Dry films were also determined moisture sorption by various humidities. Result of water sorption isotherms in value of equilibrium moisture contents (Me) showed that NaCMC blended with agar increased significantly different than blended with corn starch at higher RH ($p < 0.05$).

GAB monolayer moisture contents (Mo) were indicated the maximum amount of water that layer could be absorbed (Balakrishnan *et al.*, 2005). Mo was higher in NaCMC with agar than corn starch formulae (Table 4.2). SEM images can be indicated that combination of agar blended with NaCMC exhibited rough surface which were not homogeneous formation but corn starch blended with NaCMC were more compatible in homogeneous layer. Probably difference polymer, agar and corn starch, made different structure form.

5.2 Effect of hydrogel on *Ficus* spp. germination

Hydrogels with a high proportion of NaCMC did not support germination and seeds of *Ficus* spp. germinated best under control conditions. Germination percentage decreased as the NaCMC concentration increased. At 100% NaCMC, *Ficus* spp. germination was very low. In contrast high content of agar or corn starch promoted seed germination.

NaCMC may inhibit germination due to its low osmotic pressure due to sodium salts (Na) in the NaCMC molecule. Moreover, seeds of *F. aurea* also decreased germination at lower water potentials by effect of salts taken up to seed made lower osmotic potential in seed tissue (Swagel *et al.*, 1997).

pH of hydrogel was tested as a chemical property which had pH about 6 that means moderately acidic. While, composition in hydrogel has an important role to their pH because fertilizers (ammonia-N), NaCMC (carboxylate acid groups), agar (sulfate group) or corn starch was listed in acidic substrate (Li *et al*., 2008; Praiboon *et al.*, 2006; Corn Refiners Association, 2006). Although, the *Ficus* spp. could grow well in high pH substrate (Elliott *et al.*, 2013a) but pH in hydrogel might not be effect on germination because pH were insignificantly different. Whereas, Petri dish test found difference among hydrogel and lower NaCMC content had better germination.

Hydrogel treatments had no effect on germination both in nursery and field trials. Hydrogels did regain water after they dried out but even so they might not have provided enough moisture for seeds to germinate, especially under field conditions. If there was no rainfall, the hydrogel in this research could not hold sufficient water for early fig seed germination, because germination in the field trials began after 2 weeks, 13 days of MLD in *F. hispida*, 13 days in *F. benjamina* and 16 days in *F. semicordata*. Therefore, hydrogel was influenced by heat it became more drying fast at the initial period and can be indicated with the result of drying of hydrogel. During the drying process of a hydrogel is less able to contain amount of water than liquid form. Thus, the absorbed water by the hydrogel can release water and nutrients to the soil and plant root as needed.

The lack of *Ficus* spp. establishment might have been due to the hydrogel treatments not regaining water enough. Therefore, absorbent hydrogel in field experiment gained only moisture from natural precipitation that can be only maintained water supply. So that low precipitation made lack of seed germination probably due to shortage of water surround seed environment. Though care during and after planting is one of successive consideration. In conclusion, irrigation may be needed to support hydroseeding.

5.3 Effects of species

F. semicordata had high germination and the highest seedling survival. *F. hispida* had the highest germination but seedling survival was lower than that of *F. semicordata*. *F. benjamina* had both the lowest germination and survival, possibly because this species is a hemi-epiphyte requiring humus and physical support (Harrison *et al*., 2003). The nursery results for *F. hispida* and *F. semicordata* in this experiment were lower than that achieved by direct seeding by Kuaraksa and Elliott (2012), possibly because the latter used a more suitable substrate, 1:1 mixture of sand and charcoalized rice husk. Therefore, germination in this experiment was lower than that recommended for seedling production (35%) (Elliott *et al*., 2002). However, *F. hispida* and *F. semicordata* have been used in direct seeding for forest restoration work in other studies (Burns, 2005; Pandey *et al*., 2005; Kuaraksa and Elliott, 2012).

In the field, *Ficus* spp. had very low germination (less than 0.5%) and low survival by no viable seedlings remaining by 2nd month after the last germination, This was a similar result to that reported by Kuaraksa and Elliott (2012) who tested *Ficus auriculata*, *F. fulva*, *F. hispida*, *F. oligodon*, *F. semicordata*, and *F. variegata*. Nearly all seedlings died by 1 month after germination, during the first rainy season, except *F. hispida*, of which only 1.7% remained alive at the end of second rainy season. Therefore, fig seeds were therefore not suitable for conventional direct seeding in degraded sites, because of high temperatures and light levels and low humidity (Yanes *et al*., 1995).

Furthermore, *Ficus* spp. had none of the recommended seed traits for suitable direct seeding by the study of Tunjai and Elliott (2012). In order to be a suitable tree species for direct seeding, seeds should be fairly large or of intermediate size (0.1-5 g) with low to medium moisture content in seeds and be round or oval shaped, with moderate to thick seed coat (0.1–0.5 mm). Mathew *et al*. (2011) stated that fig seeds are orthodox and remain viable for 6 to 18 months so that seeds could germinate later in limestone mine.

Pre-treating the fig seeds might result in better germination during hydroseeding. *Ficus* spp. seeds germinate well under acidic conditions, after passing through the digestive tracts of seed-dispersers which breaks dormancy (Shanahan, 2000). Previous studies show that pre-treating *Ficus* spp. seeds increased germination and decreased dormancy. Garcia *et al*., (2005) reported that potassium nitrate (0.1%) and gibberellic acid (0.2%) accelerated germination of *F. lundellii* at 30 °C at 8 hours of photoperiod. Seed germination was promoted by increase mobilization of nutrients in a suitable chemical concentration (Mathew *et al*., 2011).

Low survival of *Ficus* spp. seedlings might be caused by fungal infection. Kuaraksa and Elliott (2012) state that damping-off diseases in fig seedlings are a serious impediment to their propagation in nurseries. Infections probably arise from natural soil pathogens such as *Penicillium* spp., *Fusarium* spp., *Aspergillus* spp. and *Rhizopus* spp.

5.4 Effects of substrate and condition in study site

MLD and survival mostly did not differ among the substrates tested in this study, although the site 2 substrate did result in higher germination than the site 1 substrate in the field experiment ($p < 0.05$). This might be the site 2 substrate had more rock crevices. However, experiments should investigate more physical parameters, such as runoff and soil erosion. The soil analysis indicated a pH around 8, which is well with the pH range for *Ficus* spp. growth. Lin *et al*. (2008) demonstrated high germination of *F. microcarpa* in limestone soil, and suggested that Ca^{2+} can facilitate seed germination but there were no significant differences among nutrient treatments on seed germination rate. Laman (1995) also suggested that nutrients are not a prerequisite for *Ficus* spp. germination, but water was a more important factor for seedling establishment.

Germination and survival were less in the field than in the nursery. The high light intensity in the limestone mine intensity may have affected the results (Table 5.1). Laman (1995) reported that high light intensity resulted in high *Ficus* spp. survival, where water supply was not a limiting factor. In contrast, high light intensity promotes water stress, which in turn reduces seedling establishment.

Nursery experiments were watered every day and this promoted hydrogel water retention (Table 5.1) that the irrigation may affect on better result in germination and survival rate that might good for retain water to hydrogel. Titus *et al.* (1990) investigated germination under various moisture conditions in *Ficus pertusa* and *F. tuerckheimii*. The highest germination percentages were in Petri plates with frequent watering, which suggests that high humidity is necessary for *Ficus* hydroseeding. Moreover, *Ficus carica* did not germinate if water was lacking or the substrate frequently dried out (Lisci and Pacini, 1994).

Table 5.1 Summary effects and conditions in hydroseeding experiment

Effects	Nursery trial	Field trial
Soil	Equal	Equal
Water supply	Frequent	Lack
Temperature	25.8 -29.5 °C	32.6-33.2 °C
Light intensity (Lux)	Low (2,600-3,000)	High (54,000)
Others (soil erosion, runoff, seed predation and etc.)	None	Maybe

5.5 Efficacy and improvement *Ficus* hydroseeding in limestone mine.

Hydrogel did not promote *Ficus* spp. seedling establishment both in nursery and limestone mine. This experiment planted *Ficus* seeds into a slope-compacted field plot, where most of the topsoil had been removed. Hydroseeding may prove more effective if the hydrogel is mixed with soil. Further research may add top soil with high nutrient levels to improve soil structure, aeration, infiltration and drainage that may enable to seeding success at study site. A soil-hydrogel mix might encourage rooting and supply nutrients. Moreover, mulching after hydroseeding may also improve results. Since, it can maintain soil moisture while improving soil and hydrogel humidity, which in turn maintains good conditions for germination and reduces exposure.

The microbial component is also important to create more effective hydrogel too. Mycorrhiza may help *Ficus* seedling to be more successful establishment at limestone mine. Nowadays, mycorrhiza is typically used in agriculture to help plants survive drought, extreme temperatures and soil infertility (U.S. Army Corps of Engineers, 2009).

Mixing NaCMC hydrogels with divinylsulphone or aluminuim sulfate can create cross links and increase hydrogel effective period (Omidian, 1997; Nnadi and Brave, 2000). More cross links would help keep seeds in place or protect the seed form run off or washed away. In this direction, hydrogel in limestone mine will be effective where soil has poor infiltration and drainage system.

Using benomyl as chemical treatment can prevent damping off disease at early growth of seedlings (Elliott *et al*., 2006). So that high germination and survival may increased after the seeds chemical treatments because of harmless from infections.

CHAPTER 6

Conclusion

1. This thesis tested the potential of hydrogels, based on NaCMC mixed with agar or corn starch for hydroseeding *Ficus* spp. into mine sites because of their properties as hydrophilic biopolymers. However, the hydrogel treatments did not significantly increase germination over the control and showed no promise for use in hydroseeding.

2. The hydrogel treatment did not facilitate *Ficus* spp. seed germination in Petri dishes. Furthermore, NaCMC inhibited germination, probably due to its effects on osmotic potential and ion exchange.

3. Hydrogel treatments were also not effective in nursery experiments.

4. Tests of the physical properties of the hydrogels showed they were sticky enough and could hold themselves in the field experiment. Seeds were probably lost due to washing away or by being buried by soil erosion, but such losses could not be quantified. I suggest using a net, to hold the seed and hydrogel on the slopes and to reduce soil erosion.

5. Soil mixing with the hydrogel should be developed, as this could possibly increase the functioning of hydrogels. Further research can be done on adding soil to help young seedlings establish root structure. Moreover, micro-organism may influence the success of hydroseeding. Mycorrhizal fungi could be added to hydrogels to increase germination and early seedling survival. On the other hand, added fungicide, especially benomyl could be used as a chemical pre-treatment, to reduce the risk of damping off disease killing *Ficus* spp. seedlings.

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APPENDIX A

Species Studied Descriptions

Ficus benjamina Linn. (Moraceae)

Habit Evergreen “strangling” tree to 20 m, rarely killing its host, with slender, drooping branches & few aerial roots. **Leaf** Size 5-12x1.6-5 cm, narrowly elliptic with long-tapering tip & blunt or slightly pointed base, no teeth. Mature leaves completely smooth with many (>17) pairs of very thin, ±parallel side veins, joined in a thin marginal vein Stalks 0.6-1.8 cm, slender, with pointed stipules, 0.8-1.1 cm. **Flower** solitary or paired at leaf axils, orange or dark red, globose or ovoid, smooth, no stalks, 3 tiny blunt or rounded bracts at base. **Distribution** fairly common in the wild, frequently planted in towns. var. benjamina has globose fruits <1 cm, whereas var. nudus has obovoid fruits 1.2-1.8 cm. **Fruit** drupelet, green color in unripe fruit and yellow in ripen, 17.7(±0. 5) mm in length, width 15.96(±1.377) and thickness 15.82(±1.35). **Seed** achene, smooth testa with brown color, 2 mm in length, 1mm of thickness and 1 mm in width, 0.00015 g of wet weight and 0.000075 in dry with nearly zero in per cent of moisture.

Leafs and ripen fruits of *Ficus benjamina* Linn.

***Ficus hispida* Linn. (Moraceae)**

Habit small independent evergreen or partly deciduous tree to 12 m. **Leaf** 11-28x5-13 cm usually opposite, narrowly ovate, obovate or oblong with shortly pointed tip & blunt or rounded base, sometimes slightly heart-shaped, usually finely toothed especially in upper half. Mature leaves roughly hairy especially veins below. 5-9 pairs of prominent arched side veins, basal pair <1/5 length of leaf. Stalks 1.5-4(10) cm, with persistent stipules, 1-2.5 cm. Twigs stout, hollow when younger. **Flower** 2.5-4 cm, clustered on long stems hanging from trunk & main branches, sometimes in leaf axils on young trees, greenish-yellow with pale dots, pear-shaped or obconical, narrowed at base, flattened & slightly sunken at top with 7-9 inconspicuous darker ribs radiating from mouth, finely hairy, often with scattered scales. Stalks 0.6-2.5 cm with 3 small, triangular bracts, flower times all year and **Distribution** very common in open areas, 6-1525 m in elevation. **Fruit** drupelet, green color in unripe fruit and light greenish-yellow in ripen, 35.52 (±1.98) mm in length, width 27.24 (±0.64) and thickness 26.48 (±0.96), fruiting all year. **Seed** achene, smooth testa with light brown color, 1 mm in length, 1mm of thickness and 1 mm in width, .000275g of wet weight and .000175 in dry with nearly zero per cent of moisture.

Unripe and ripe fruits of *Ficus hispida* Linn.

Ficus semicordata **Buch. (Moraceae)**

Habit Trees, 3-10 m tall, d.b.h. 15-25 cm, crown flat, spreading and umbrellalike. **Bark** gray, smooth. Branchlets white or brown pubescent. Stipules red, lanceolate, 2-3.5 cm, membranous, subglabrous. **Leaves** distichous; petiole thick, 5-10 mm, densely covered with stiff hairs; leaf blade oblong-lanceolate, strongly asymmetric, 18-28 × 9-11 cm, papery, abaxially densely covered with stiff short hairs and small yellowish brown convex spots, adaxially coarse with stiff hairs on veins, base obliquely cordate on one side and auriculate on other side, margin with small teeth or entire, apex acuminate; basal lateral veins 3 or 4 on auriculate side of leaf blade, and extending into auriculate base, secondary veins 10-14 on each side of midvein, flowering time in May to October. **Fruit** drupelet, green color in unripe fruit and dark red-brown in ripen, 26.78 (±2.3) mm in length, width 26.10 (±2.3) and thickness 22.03 (±1.83). **Seed** muricate testa with light brown color, 0.5 mm in length, 0.5 mm of thickness and 0.5 mm in width, .00045 g of wet weight and .0004 g in dry with 11.11 % of moisture.

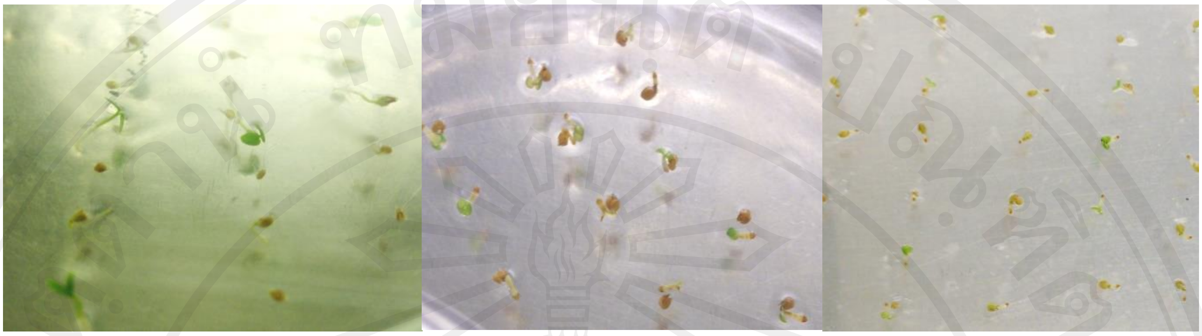
Ripe fruits of *Ficus semicordata* Buch.

APPENDIX B

Pictures in Experiments

Hydrogel treatments, A1-A5, left-right hand.

Hydrogel treatments, B1-B4 and A1, left-right hand



Emergences of root and shoot of *Ficus benjamina*, *Ficus hispida* and *Ficus semicordata* in Petri dishes with various types of hydrogel, left-right hand.

Substrate in trays for hydroseeding experiment in nursery.

Sowing *Ficus* spp. seeds by hydroseeding technique in nursery.

Ficus spp. establishment on mine substrate in nursery.

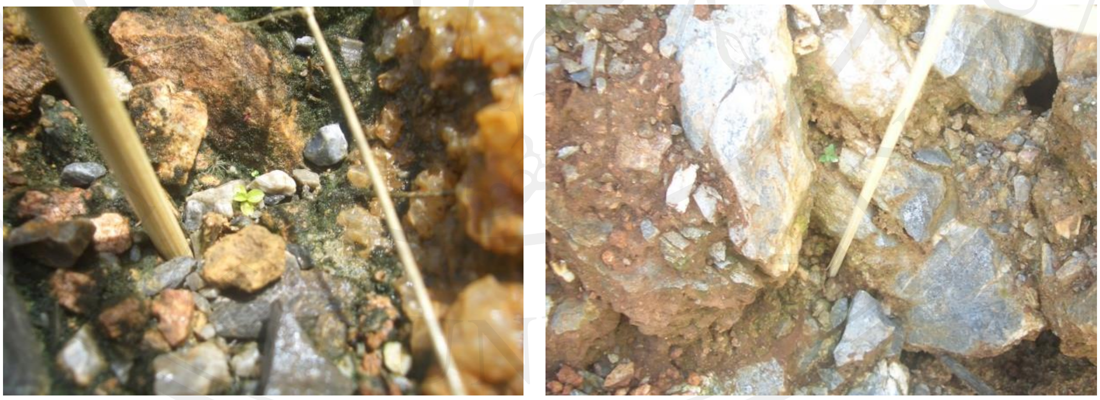
Emergences of shoot of *Ficus benjamina*, *Ficus hispida* and *Ficus semicordata* after hydroseeing in nursery for one month, left-right hand.

Wilt then becoming death of *Ficus sp.*in nursery.

Plots on a slope at limestone mine.

Hydroseeding application at limestone mine by PVC water pistol.

Ficus sp. establishment after hydroseeding at limestone mine.



Survivals after germination for 60 days, *F. semicordata* with A3 treatment and *F. benjamina* with A3 treatment, left-right hand.

CURRICULUM VITAE

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