GERMINATION OF *Ficus microcarpa* L. ON LIMESTONE FOR RESTORING MINES

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Research Title Germination of *Ficus microcarpa* on limestone for restoring mines.

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Abstract

For limestone mining in Thailand, it is stipulated that the site must be reclaimed to the original vegetation after mining is completed. The study area was the Muang Poon semi-opencast limestone mine, operated by Siam Cement Group in Lampang Province, northern Thailand. Forest restoration is hindered by no top soil and the stony nature of the mine substrate. I hypothesized that *Ficus microcarpa* should be able to grow in the limestone mine and facilitate forest restoration because, its strong root system can penetrate and break up the mine substrate and because it is the keystone species in tropical forests in northern Thailand, and should attract seed dispersing animals into the mine. This project therefore tested germination of *Ficus microcarpa* using hydrogel for successful in reclamation. For this research, germination was tested in a nursery on substrate gathered from the mine. The experiment was a randomized complete replicate for 5 treatments; i.e. control (seeds sown without hydrogel or additives), hydrogel (H), hydrogel with slow release fertilizer (14-14-14) (HFe), hydrogel with fungicide (HFu) and, hydrogel with slow release fertilizer and fungicide (HFeFu). Highest survival rate (34.5%) was achieved with the HFeFu treatment, whilst lowest survival occurred with the control (19%). Germination percent for the H, HFe, and HFu treatments were (25%, 28.5%, and 27%, respectively). Differences among treatments were not significant (ANOVA, $p<0.05$). In conclusion, *Ficus microcarpa* has potential for direct seeding to establish fig trees on mine sites and that hydrogel may increase success.

หัวข้อปัญหาพิเศษ การงอกของ *Ficus microcarpa* L. บนหินปูนเพื่อการฟื้นฟูเหมืองแร่

ชื่อผู้เขียน นางสาวณัตติพร ยะบึง

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บทคัดย่อ

การทำเหมืองแร่ในประเทศไทยมีข้อกำหนดให้มีการฟื้นฟูพื้นที่หลังจากการทำเหมืองให้กลับสู่สภาพเดิมโดยได้ทำการศึกษาในพื้นที่เหมืองปูนซึ่งสัมปทานโดยบริษัทเครือซิเมนต์ไทย จำกัด จ.ลำปาง แต่การฟื้นฟูพื้นที่ดังกล่าวมีข้อจำกัด คือ หน้าดินถูกทำลาย และดินมีการอัดตัวกันอย่างหนาแน่น ซึ่งในการศึกษานี้คาดว่า *F. microcarpa* น่าจะมีความเหมาะสม ในการฟื้นฟูพื้นที่ดังกล่าว เนื่องจากในธรรมชาติ *F. microcarpa* สามารถเจริญได้บนหินปูน ซึ่งพืชชนิดนี้มีระบบรากที่แข็งแรง สามารถแทรกและทำลายชั้นหินได้ ทั้งยังเป็นพืชที่มีความสำคัญ ทางนิเวศวิทยา (keystone species) ในป่าเขตร้อนทางตอนเหนือของไทย ซึ่งจะดึงดูดสัตว์ ที่ช่วยแพร่กระจายเมล็ดเข้ามาในพื้นที่ฟื้นฟู การศึกษาในครั้งนี้จึงมีวัตถุประสงค์ เพื่อทดสอบการงอกของ *F. microcarpa* ร่วมกับการใช้ Hydrogel โดยวิธีหยอดเมล็ด ในเรือนเพาะชำ โดยใช้ดินจากพื้นที่ศึกษา แบ่งออกเป็น 5 ชุดการทดลอง คือ ชุดควบคุม (ดินจากเหมือง), Hydrogel (H), Hydrogel ร่วมกับปุ๋ยเม็ดละลายช้า สูตร 14-14-14 (HFe), Hydrogel ร่วมกับยาฆ่าเชื้อรา (HFu) และ Hydrogel ร่วมกับปุ๋ย และยาฆ่าเชื้อรา (HFeFu) พบว่าเมล็ดที่ปลูกบน HFeFu มีอัตราการงอกสูงสุดที่ 34.5% ขณะที่ชุดควบคุมมีอัตราการงอกต่ำที่สุดคือ 19% ส่วนอัตราการงอกของเมล็ดบน H, HFe, และ HFu เท่ากับ 25%, 28.5% และ 27% ตามลำดับ ซึ่งไม่มีความแตกต่างอย่างมีนัยสำคัญ (ANOVA, $p<0.05$) ดังนั้น *F. microcarpa* สามารถปลูกโดยการหยอดเมล็ด ในการตั้งต้นบนพื้นที่เหมืองปูนและ Hydrogel อาจจะเพิ่มโอกาสที่จะประสบความสำเร็จ ของการฟื้นฟูพื้นที่ได้

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LIST OF ABBREVIATION

°C	degree Celsius
$Al_2(SO_4)_3$	aluminium sulphate
CEC	cation exchange capacity
cm	centimeter
cm^3	cubic centimetre
CMC	carboxymethyl cellulose
cmol(+)/kg	centimol positive charge per kilogram of soil
CMU	Chiang Mai
cP	centipoises
FORRU-CMU	Forest Restoration Research Unit of Chiang Mai University
g	gram
H	hydrogen
HFe	hydrogel with slow release fertilizer (14-14-14)
HFeFu	hydrogel with both slow release fertilizer and the fungicide
HFu	hydrogel with fungicide
K	potassium
m	meter
M	molar
mg	milligram
min.	minute
P	phosphorus
ppm	parts per million

LIST OF ABBREVIATION (CONTINUE)

RCBD	randomized complete block design
SCG	Siam Cement Group
total N	total nitrogen
w/w	mass/mass

CHAPTER 1

INTRODUCTION

Mines present one of the harshest set of conditions for forest restoration, particularly open cast mine, where the top soil has been destroyed and the underlying substrate has become compacted and depleted in nutrients (Almendro-Candel *et al.*, 2007). The study described here was carried out in collaboration with the Siam Cement Group (SCG) and the Forest Restoration Research Unit of Chiang Mai University (FORRU-CMU) to provide reclamation guidelines at the Muang Poon semi-opencast limestone mine, Lampang province. This area is mined for calcareous materials for cement manufacture, so the pH of the substrate is high and it is stony, which reduces infiltration of rain water and organic matter content. Furthermore, soil surface temperatures are high. These conditions present considerable obstacles to plantation establishment.

One species which might be able to overcome these problems is *Ficus microcarpa,* which is able to survive in little or no soil; such as in building crevices, sidewalk cracks, and rocks (Lin *et al.*, 2008). Seeds of this species can adhere well to substrates such as rocks and trees and it has a strong, dense, root system, capable of breaking down rock (FORRU, 2006). Furthermore, the genus *Ficus* is well tested for framework tree species (FORRU, 2006). They produce figs eaten by many seed dispersing animals such as birds, monkeys, bats, gibbons, dears, and wild pigs. In addition, *Ficus* spp. are keystone species in tropical rain forest ecosystem, because they produce fruits all-year-around, which help to maintain populations of seed-dispersing animals. Thus, they are important for reforestation (FORRU, 2006). For these reasons, I hypothesized that *F. microcarpa* would be a suitable species for mine reclamation.

Direct seeding is explored in this research, because of its ease of use and low cost. Hydrogel, carboxymethyl cellulose (CMC) polymer, was included as a treatment in trials, to protect the seeds from desiccation and to mimic the effect of seed deposition, which is naturally achieved via animal faeces. The benefits of hydrogel are i) support seed adherence to the substrate ii) water absorption, and iii) nontoxic to seed and environmentally friendly (Nnadi and Brave, 2011).I hypothesized that hydrogel would increase germination of fig seeds and would be applicable to steep slopes on mine areas, which are difficult for conventional tree planting and susceptible to soil erosion.

Consequently the aim of this study was to achieve acceptable germination rates of fig seeds on limestone substrates. The specific objective was to determine the best combination of treatments to maximize germination of *F. microcarpa* seeds and develop an appropriate protocol for establishing this tree species in the field.

CHAPTER 2

LITERATURE REVIEW

Reclamation on limestone mines

Every country has legal requirements, regarding pose-mining management. Mines in Virginia, USA are required to carry out reclamation of surface-mined land, by re-establishing only unmanaged forest (non-commercial forest land) because such action encourages wild life, environment benefits and self sustainability. However, unmanaged forests provide no long-term commercial values for the landowner, so managed forests (commercial forest land) are provided together. For example, planting of 400 trees per acre of commercial species is mixed with 40 trees per acre of wildlife trees (Burger and Zipper, 2002). In the past, hydroseeding with nurse species (grasses, legumes) was used to re-establish vegetation cover on mines, but it often fails because nurse species are dense, which are a seedling problem for establishment.Moreover grasses compete with crop tree. Hence, the method has not been used to restore mine lands in Virginia (Preve, 1983).

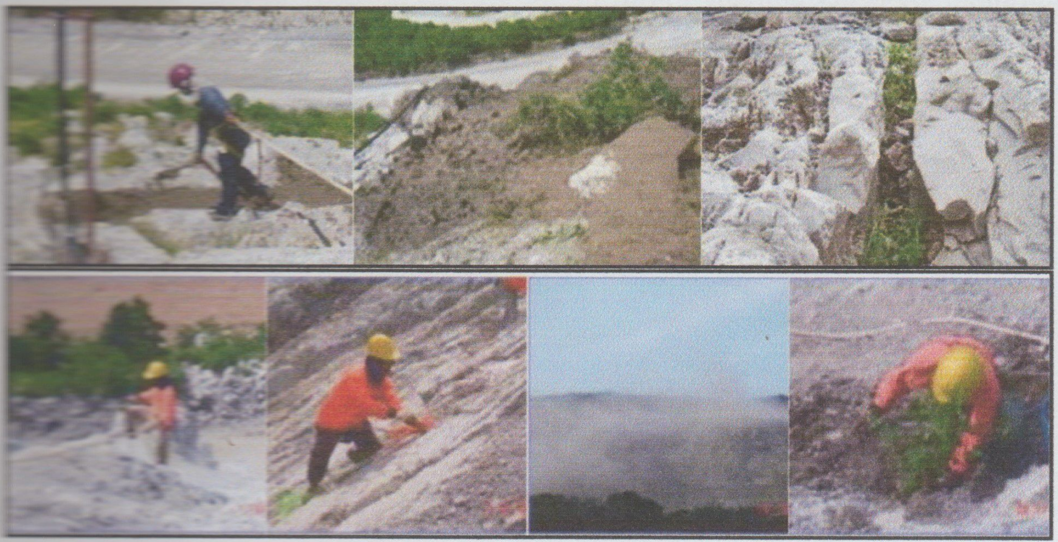
Figure 1 Hydroseeding

Even though primary forest many grew close to a mine, seed dispersers rarely visit restoration sites, since on-going mining operations create barriers, such as desolate open areas and roads with heavy traffic so natural seed-dispersal does not usually facilitate tree species recruitment. Working at an opencast bauxite mine in Central Amazonia, Parrotta and Knowles (1999) screened a range of native tree species for reforestation programs "where natural succession is retarded by physical, chemical and/or biological barriers", in order to "replicate, in an accelerated fashion, natural forest successional processes that lead to complex, self-sustaining forest ecosystems". Restoration sites were levelled and covered with 15 cm of top soil, within a year of forest clearance and bauxite extraction. They were deep ripped to 90 cm depth (1 m between rip lines) and tree propagules (direct seeding, wildlings or nursery-raised seedlings) were planted along alternate rip lines at 2 x 2 m spacing (2,500 plants/ha). At least 70 species were planted, ensuring that trees of the same species were not planted adjacently. The experiments resulted in a two-stage maximum diversity approach, using mostly sun-tolerant pioneers to create the conditions needed for subsequent addition of all other tree species, representative of the target forest ecosystem. But the cost of this technique was high (2,500 US$/ha in 1985) (Parrotta *et al.*, 1997). Therefore the appropriate method is direct seeding which has survival rate ≥ 75% and suitable restore in long time (Parrotta and Knowles, 2001).

Mined lands are rather difficult to restore, because soil compaction and microclimatic factors limit germination and survival rate. Burger and Zipper (2002) showed that soil compaction at the Poweel River Project Mine site in Virgimia reduced rain water infiltration, which in turn reduces moisture availability to plants, increased the risk of soil erosion, and inhibited root growth. In addition, the top soil was removed out and digging increased the slope in the land, which reduced soil nutrients for planted trees. Burger and Zipper (2002) suggested reducing slope steepness (slope less than 20 percent and lengths on mined areas to less than 33 metres long) and adding fresh soil to promote plant growth and increase survival rate, because of the function of fungi and other microorganisms. Nevertheless, success depends on soil quantity. If there is a lot of soil, the chances of reclamation success are increased.

Figure 2 Typical grading and tracking-in operations for non-forest post mining land use

There is a need for further study about native species and environmental factors, so that data are available to develop appropriate methods for restoration. In Muang Poon at Saraburi provice, SCG used Mud Slurry Seeding method for reclamation which consist e.g. water: soil: fertilizer: seed (*Pithecellobium dulce, Ricinus communis, Plerocarpus indicus, Leucaena leucocephala, Brachiaria ruziziensis, Dalbergia cochinchinensis, Stylosanthes hamata* ect.) with proportions 3: 4: 2: 0.5. Seeds germinated over about 50% of the reclaimed area but this method had problems e.g. Ingredients did not mix well with and seeds which were very scattered because of its high pressure of its. Moreover, they blow up soil for plant so this method has high cost and use a lot of labours (SCG).



a

b

Figure 3 Methods of reclamation mines by SCG a) Mud Slurry Seeding Method
b) Blow up Soil for Plant Method

Direct seeding

Direct seeding has been tried as a method of forest restoration on post-mine lands, as well as former agricultural and industrial sites and after deforestation (Bonilla-Moheno and Holl, 2010) The main advantage of the technique is low cost, because a nursery is not needed for seedling production :-

1. reduced labour requirements.
2. easy to transport seeds to the restoration sites compared with seedlings therefore reduced transportation costs
3. the root system can grow and develop better than for nursery-raised saplings because it is not constrained within a container (Woods and Elliott, 2004).

On the other hand, planting nursery-raised tree seedlings is expensive, because of the high labour cost for seed collection, work in nursery and field and taking care of the trees after planting (Hardwick *et al*, 2000). However, direct seeding is limited by soil moisture (i.e. desiccation of the seeds before they can germinate) and weeds (Vanderwoude, 1995). Several factors affect direct seeding success, including species and seed characteristics:

1. rapid growth which is essential for survival and competition against herbaceous weeds
2. seed coat thickness
3. soil moisture content (Tunjai and Elliott, 2012)

Tunjai and Elliott (2012) suggested that medium (0.1 – 4.99 g dry mass) to large seeds (> 5.0 g dry mass) with round shape were appropriate for direct seeding, because large seeds can tolerate environmental stresses (i.e. drought, heat etc.) better than small seeds due to their large energy reserves . Direct seeding cannot be used under all conditions (Coomes, 2003; Muller-Landan, 2010), but several forest tree species are suitable for direct seeding (Tunjai and Elliott, 2012) since natural seed dispersal is like direct seeding. Burying the seeds can also promote direct seeding success.This increases germination and promotes early seedling establishment, because soil maintains moisture around the seed and prevents seed predation (Doust *et al.,* 2006; Woods and Elliott, 2004). Similarly McLaren and McDonald (2003) showed that shading and be used for moisture support seed germination. Finally, reforestation by direct seeding should native species and should be done at the start to the rain season (FORRU, 2006).

Direct seeding involves:

1. collecting seeds from native trees in the target forest ecosystem and if necessary storing them until sowing;
2. sowing them in the restoration site, at the optimal time of year for seed germination and
3. manipulating field conditions to maximize germination.

Direct seeding is relatively inexpensive, since there are no nursery and planting costs (Doust *et al.*, 2006; Engel and Parrotta 2001). Transporting seeds to the restoration site is obviously easier and cheaper than trucking in seedlings, so the method is particularly suitable for less accessible sites. Trees, established by direct seeding, usually have better root development and grow faster (Tunjai, 2011a) than nursery-raised saplings, since their roots are not constrained within a container.

In nature, a very low percentage of dispersed tree seeds germinate and even fewer seedlings survive to become mature trees. The same is true of direct seeding (Bonilla-Moheno and Holl, 2010; Cole *et al.*, 2011). The biggest threats to direct sown seeds and seedlings are: i) desiccation, ii) seed predation, particularly by ants and rodents (Hau, 1997) iii) seedling predation, perticulaly snail and vi) competition from herbaceous weeds. By counteracting these factors, it is possible to achieve higher rates of germination and seedling survival than would occur with naturally dispersed seeds.

The problem of desiccation can be overcome by selecting tree species with seeds that are tolerant or resistant to desiccation (i.e. those with thick seed coats) and by burying seeds or laying mulch over the seeding points (Woods and Elliott, 2004).

Burying can also reduce seed predation, by making the seeds more difficult to find. Pre-sowing seed treatments, to accelerate germination, reduce the time available for seed predators to find the seeds. Once germination commences the nutritional value of seeds and their attractiveness to predators rapidly decline. However sometimes, treatments that break the seed coat and expose the cotyledons may actually increase the risk of desiccation or make seeds more attractive to ants (Woods and Elliott, 2004).

Seedlings germinating from seeds are tiny, compared with planted, nursery-raised saplings, so weeding around the seeding points is especially important and it must be carried out with extra care. Such meticulous weeding can greatly increase the cost of direct seeding (Tunjai, 2011a). Species that tend to be successfully established by direct seeding are generally those with large (> 0.1 g dry mass), spherical seeds with medium moisture content (36-70%) (Tunjai, 2011b). Large seeds have large food reserves, so they can survive longer than smaller seeds and produce more robust seedlings. Seed predators find it difficult to handle large, round or spherical seeds, especially if such seeds also have a tough and smooth seed coat. For *F. Microcarpa* seeds are small seeds but they can germinate and establish on less nutrient substrate e.g. building crevices, sidewalk cracks, and on rock (Wee, 1992)

***Ficus microcarpa* L.**

Ficus microcarpa L. (Moraceae) is native to Asia, Australia and New Caledonia (Wagner *et al.*, 1999). The species produces figs all year round (1-4 crop per year) (Lin *et al.,* 2008). Seeds are dispersed by birds, ants, and other vertebrates (Kawakami *et al.*, 2009)

Figure 4 Characteristics of *F. Microcarpa* a) multiple fruit b) simple leaf c) stem d) aerial roots

Characteristics of *F. microcarpa*

- Evergreen tree to 15-20 m, with a rounded dense crown, smooth gray bark, milky sap, dangling aerial roots
- Leaves alternate, simple, leathery, deep glossy green, oval-elliptic, the leaf blades measure about 3–12 by 1.5–9 cm.
- Flower tiny, unisexual, numerous, hidden within the fig, the symbiotic pollinating fig wasp is *Eupristina verticillata* (Farache *et al.*, 2009).

- Fruit a fleshy, specialized receptacle that develop into a multiple fruit (syconium), this green turning to yellow or dark red when ripe, sessile, in pairs at leaf axils, small, to 1 cm in diameter.

F. microcarpa is suitable for restoration mine lands because it grows fast and is able to grow in nutrient poor soils. Jim (1998) showed that *Ficus* spp. establish well in old stone walls in Hong Kong, especially *F. microcarpa* which accounted for almost 50% of the wall-tree population, because they produce dense strong roots, capable of strong attachment to rock. Lin *et al.*, (2008) demonstrated a high germination rate of *F. microcarpa* on limestone, beside forest soil, with pH 9.29 and pH 6.82, respectively. In addition, fig trees are a keystone food resource, which attracts birds and thus promote seed dispersal into restoration sites. Twenty Ficus species (of 47 native species to northern Thailand) have been tested to restore tropical forests by FORRU (Elliott *et.al.*, 1998 and CMU Herbarium Database, 2000). Furthermore, Legumes and fig tree species can be used as nurse plantation species to reclaim destroyed land, because the strong root system can break down compacted soil or even rock on mines. The function of Legumes is nitrogen fixation in soil, which increases soil nutrient status. In combination these species improve both soil structure and soil chemistry (FORRU, 2006).

Hydrogel

Hydrogels are polymers, which absorb considerable quantities of water. Research is underway to develop biopolymers as substitutes for petroleum-base polymers, because they are biodegradable and non toxic (Stahl *et al.* 2000; Weerawarna, 2011). Carboxymethyl cellulose (CMC) is a biopolymer that is interesting because of low cost.

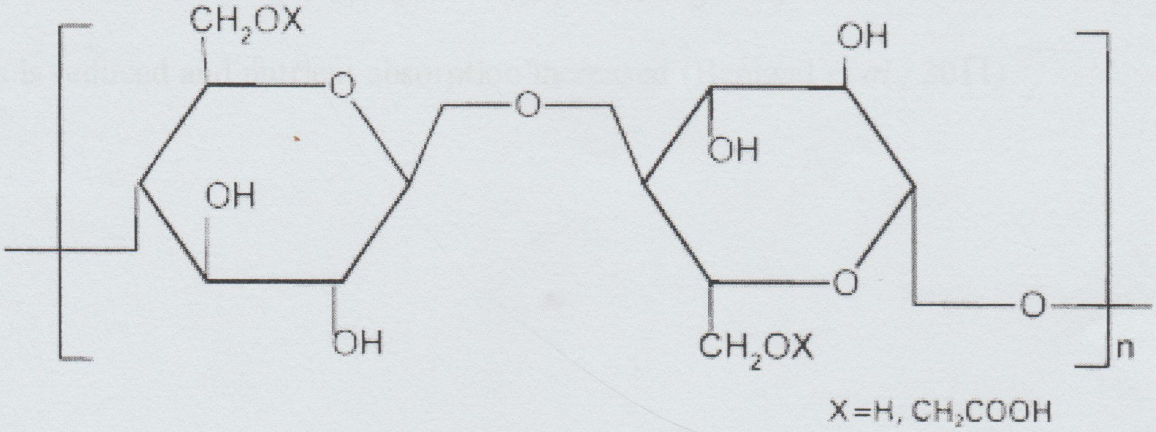


Figure 5 Structure of carboxymethyl cellulose

Nnadi and Brave (2011) reported that hydrogel, which is biopolymer was used to restore a limestone mine. The required characteristics of a hydrogel are high water absorption, sticky texture, and nontoxic, which make the substance suitable for mines with high slopes and high surface temperatures.

CMC polymer complexes from cross-linkages with aluminium ions to form non-permanent bonds, which swell when in contact with water. In addition, water retention is optimum with 2.3% aluminium sulphate $Al_2(SO_4)_3$ by weight. Thus, hydrogels are used for agriculture, horticulture, waste management, electronics and construction, because they are capable of maintaining water in soil (Barbucci *et al.,* 2000; Nnadi and Brave, 2011). Miller (1979) also reported that hydrogels help to absorb water in sand. Furthermore, hydrogels encourage mycorrhiza in soil planted with Beech trees (*Fagus sylvatical* L.), promoting early establishment, because water loss is reduced and nutrient absorption increased (Beniwal *et al.,* 2011).

CHAPTER 3

MATERIALS AND METHODS

1. Hydrogel Preparation

Commercial cassava starch powder was used, without further purification. Carboxymethyl cellulose sodium salt, with an average molecular weight of 90000 and dissolved solids = 0.7 ppm, was mixed with starch to make hydrogel polymers (Zohuriaan-Mehr and Kabir, 2008) in 6/10 proportions. Reagent grade aluminum sulfate ($Al_2(SO_4)_3$) was added 2.3%w/w to crosslink the polymer complex (El Salmawi, 2007; Wang and Wang, 2010).

a. Preparation of hydrogel polymer

Hydrogel preparation followed procedures described by Suo *et al.* (2007), Weerawarna (2009) and Nnadi and Brave (2011). Cassava starch (1.2 g) was gelatinized in 50 mL of distilled water at 80 ˚C for 45 min. Carboxymethyl cellulose sodium salt (CMC) (20 g) was mixed with 2.0 L of distilled water and stirred, using a magnetic stirrer. Next, 2.3 % w/w aluminum sulfate ($Al_2(SO_4)_3$) was mixed with the CMC solution again by magnetic stirrer. Finally, the gelatinized starch was added to the mixed CMC solution for 60 min. The hydrogel was adjusted at pH 7 by add 1 M KOH.

2. Germination trials

a. Study site

Experiments were carried out in a small nursery in the Biology Department, Science Faculty, Chiang Mai University (18° 80'N, 98° 95'E) at 335 m elevation, during the cool season from November to December, 2012. Substrate was collected from the Muang Poon semi-opencast limestone mine, operated by Siam Cement Group in Lampang Province, northern Thailand (18° 29'N, 98° 37'E) at 350 – 500 m elevation. The collection site had been cleared of its original mixed deciduous forest. The bedrock was compacted. The substrate consisted of limestone rocks and clay.

b. Seed collection

Seeds were randomly collected from 4 large *F. microcarpa* trees in October 2012, in Chiang Mai University campus and in the parking zone at Lotus Pang Suan Kaew Hotel.

This species is a "strangling" tree, found along streams in bamboo deciduous, mixed evergreen deciduous and evergreen forests from 350 to 1050 m elevation. Seed germination usually exceeds 90% with a median length of dormancy of 18 days. Performance of the species in planting trials has been excellent. Figs are produced from 3 years after planting out and the species is an excellent suppressor of weeds and is resilient after fire damage (FORRU, 2006)

c. Treatments

Five treatments were tested i.e. i) control (seeds sown without hydrogel or additives), ii) hydrogel (H), iii) hydrogel with slow release fertilizer (14-14-14) (Luxecote) iv) (HFe), hydrogel with fungicide (chlorothalonil; a broad spectrum contact fungicide) (HFu) and v) hydrogel with both slow release fertilizer and the same fungicide (HFeFu). Slow release fertilizer was mixed with hydrogen and fungicide was mixed with water for watered seeds. Seeds, subjected to hydrogel treatments, were injected into the substrate by means of a syringe.

d. Germination trials in the nursery

Experiments in the nursery tested the ability *F. microcarpa seeds to germinate* on limestone mine substrate. The five treatments, described above, were replicated over 4 blocks, within a randomized complete block design (RCBD). One replicate of each treatment consisted of 50 seeds sown into limestone mine substrate in germination baskets (20×30×10 cm^3). Seeds were watered every 3 days by hand, using a fine spray and further hydrogel (with no addition seeds) was injected into the substrate again after 1 week.

Germination was monitored every 2-3 days for 4 weeks, and the numbers of germinating seeds (defined as emergence of the cotyledons) was recorded.

e. Data analysis and interpretation

Data were analyzed using ANOVA: RCB in SPSS version 17.0 program.

CHAPTER 4

RESULTS

Visible seedlings

The graphs showed visible seedlings in each block in each treatment. For control treatment, H treatment, and HFe treatment were variable in each block, particular, during 17 – 29 day after sowing seed. In the other hand, HFu treatment and HFeFu treatment had same tendency that raised seedlings and deceased until stabled in every blocks.

Comparison among the 5 treatment showed that HFeFu treatment had the highest visible seedling, followed by HFe treatment, HFu treatment, H treatment, and control had lowest visible seedling.

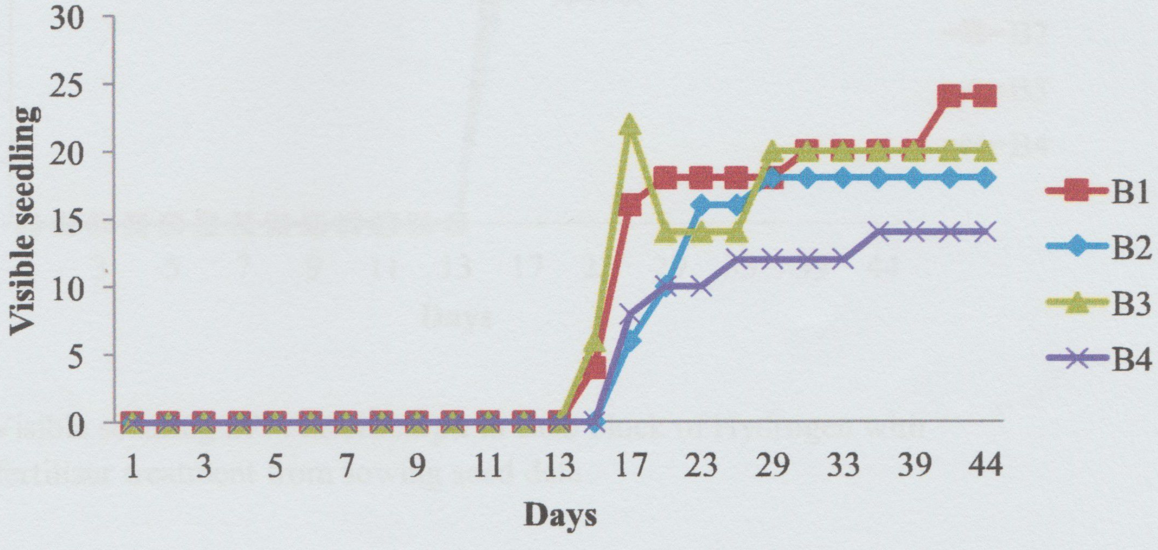


Figure 6 Visible seedling of *F. Microcarpa* in each block of control treatment from sowing seed date

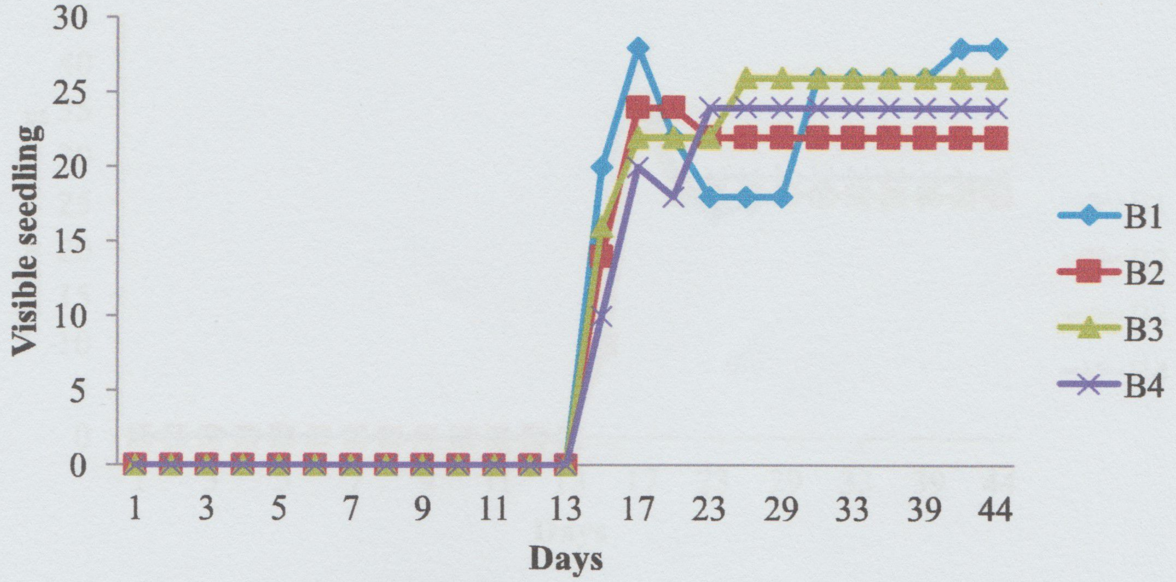


Figure 7 Visible seedling of *F. Microcarpa* in each block of Hydrogel treatment from sowing seed date

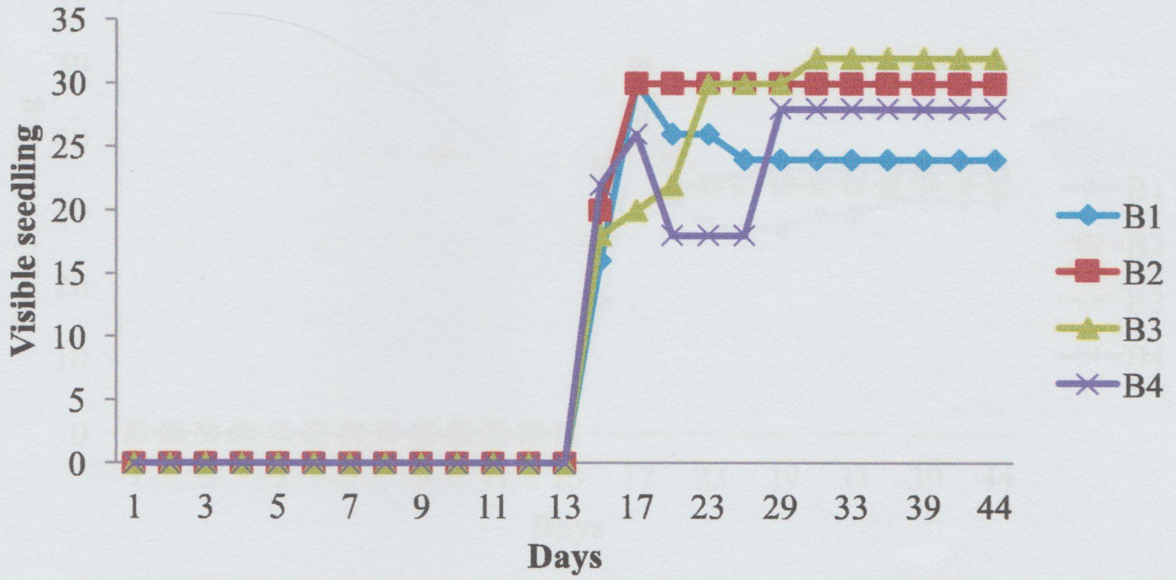


Figure 8 Visible seedling of *F. Microcarpa* in each block of Hydrogen with fertilizer treatment from sowing seed date

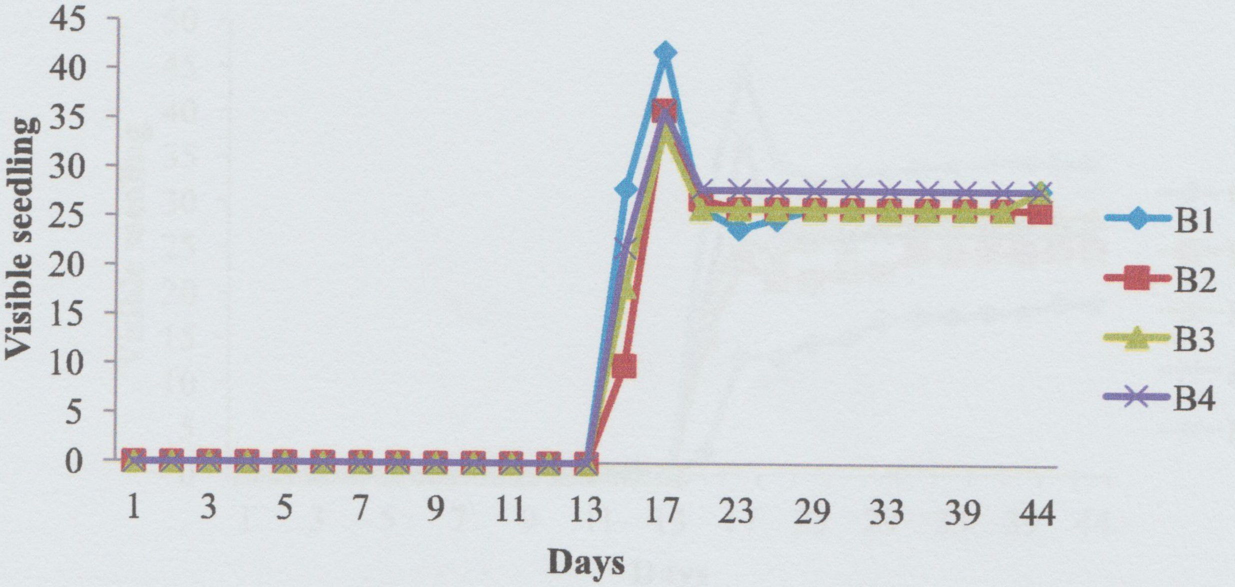


Figure 9 Visible seedling of *F. Microcarpa* in each block of Hydrogen with fungicide treatment from sowing seed date

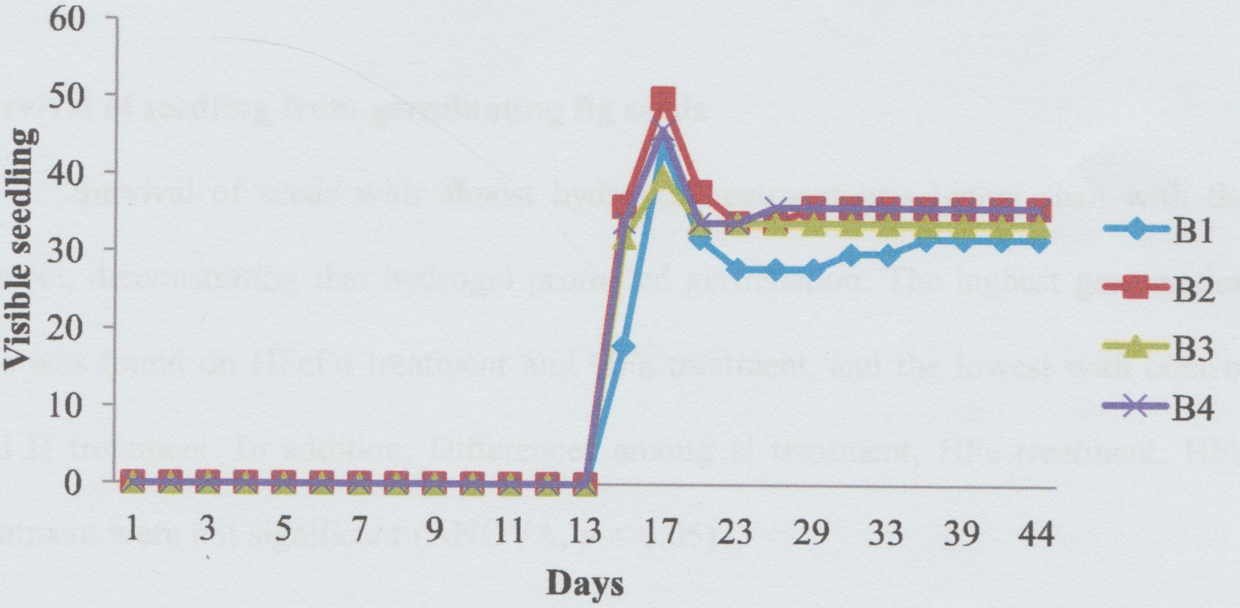


Figure 10 Visible seedling of *F. Microcarpa* in each block of Hydrogen with fertilizer and fungicide treatment from sowing seed date

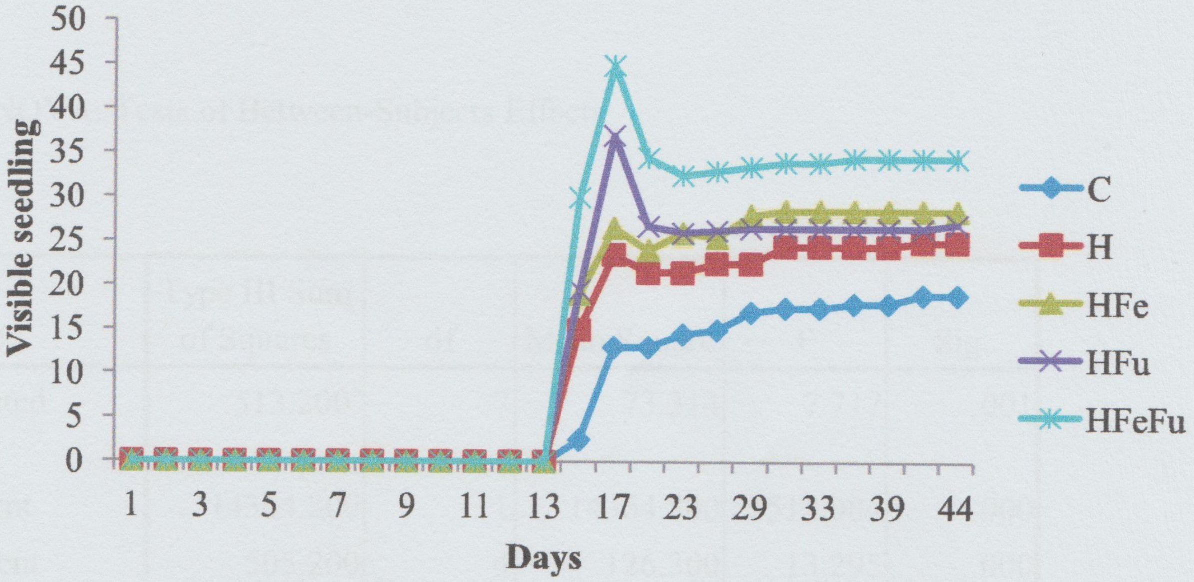


Figure 11 Visible seedling of *F. Microcarpa* in each treatment from sowing seed date

Survival of seedling from germinating fig seeds

Survival of seeds with almost hydrogel treatment was higher than with the control, demonstrating that hydrogel promoted germination. The highest germination rate was found on HFeFu treatment and HFe treatment, and the lowest with control and H treatment. In addition, Differences among H treatment, HFe treatment, HFu treatment were not significant (ANOVA, $p < 0.05$)

Table 1 ANOVA: Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	513.200[a]	7	73.314	7.717	.001
Intercept	14364.800	1	14364.800	1512.084	.000
treatment	505.200	4	126.300	13.295	.000
block	8.000	3	2.667	.281	.838
Error	114.000	12	9.500		
Total	14992.000	20			
Corrected Total	627.200	19			

a. R Squared = .818 (Adjusted R Squared = .712)

Table 2 Tukey HSD[a,,b]

treatment	N	Subset		
		1	2	3
control	4	19.0000a		
H	4	25.0000a	25.0000b	
HFu	4		27.0000b	
HFe	4		28.5000b	28.5000c
HFeFu	4			34.5000c
Sig.		.103	.521	.103

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square (Error) = 9.500.

a. Uses Harmonic Mean Sample Size = 4.000.

b. Alpha = 0.05.

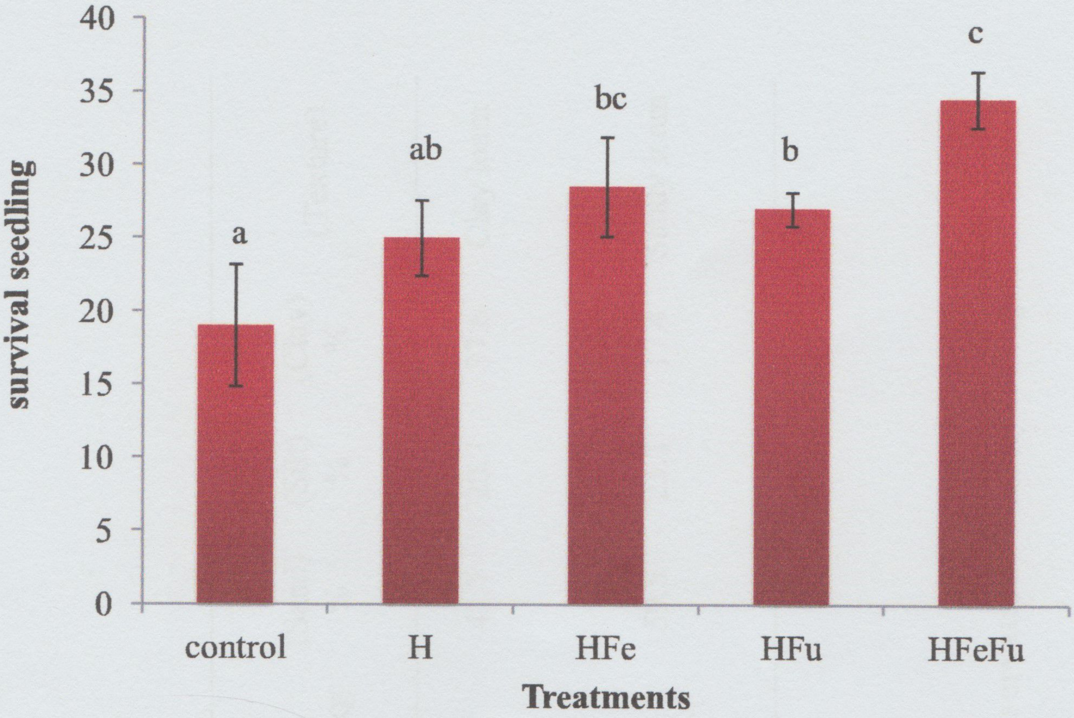


Figure 12 Effect of different treatments on seedling survival from germinating *F. microcarpa* seeds 4 weeks after first seed germinated.

Soil analysis

Soil properties in the mine land were fewer nutrients (table 3) and have pH about 8 that mean medium pH. For plant tree, should add fertilizer.

Table 3 Soil analysis report

Sample Designation	pH	(Organic matter) g/100g	(Total N) g/100g	(P) mg/kg	(K) mg/kg	(CEC) cmol(+)/kg	(Sand) %	(Silt) %	(Clay) %	(Texture)
S.1	7.98	0.588	0.680	9.584	34.80	10.90	41.7	20.7	37.6	Clay loam
S.2	8.09	0.836	0.722	0.599	31.49	5.87	59.5	23.1	17.4	Sandy loam

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

Viscosity of hydrogel

This figure showed viscosity of hydrogel which mean thickness. The hydrogel had a very high viscosity when compare with water which has a viscosity as well as 0 centipoise (cP).

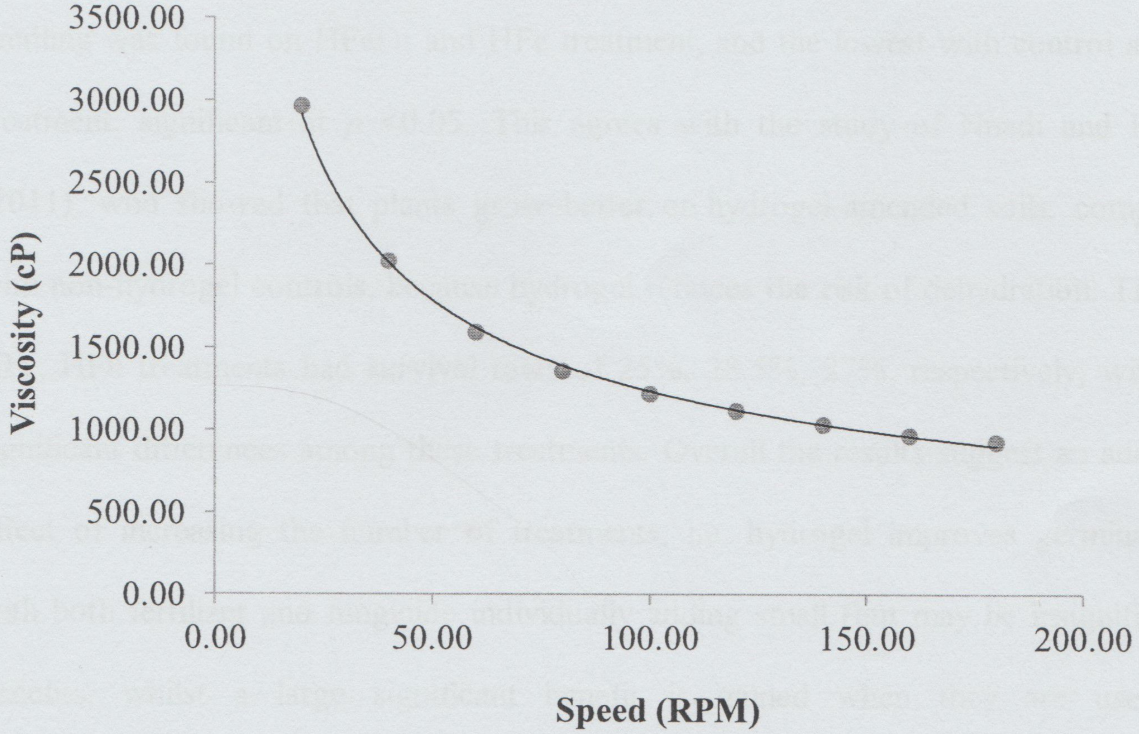


Figure 13 Relationship between the viscosities with speed in the hydrogel at pH 7 (Industrial Chemistry Department, Faculty of science, Chiang Mai University)

CHAPTER 5

DISCUSSION

Germination with almost hydrogel treatments was higher than with the control, demonstrating that hydrogel promoted germination. The highest visible seedling was found on HFeFu and HFe treatment, and the lowest with control and H treatment, significant at $p < 0.05$. This agrees with the study of Nnadi and Brave (2011), who showed that plants grow better on hydrogel-amended soils, compared with non-hydrogel controls, because hydrogel reduces the risk of dehydration. The H, HFe, HFu treatments had survival rates of 25%, 28.5%, 27%, respectively, with no significant differences among these treatments. Overall the results suggest an additive effect of increasing the number of treatments, i.e. hydrogel improves germination, with both fertilizer and fungicide individually adding small (but may be insignificant) benefits, whilst a large significant benefit is gained when they are used in combination.

The insignificance of the fertilizer result suggests that nutrients are not a prerequisite for seed germination, but may affect early seedling establishment. Tunjai and Elliott (2012) noted that small seeds have low food storage capacity, which tends to result in seedlings with low stress-tolerance. However, their seedlings are rather small and easy damaged from fungi (root rot) so that using fungicide can increase germination rate (FORRU, 2006). Although, fertilizer was not necessary for seed germination, it increased growth and fleshy seedlings. Similarly, Hobbas and Atkins (1988) showed that plant growth and establishment were increased greatly by adding fertilizer.

However, the experiment was complicated due to invasion of some seed germination trays by the exotic Giant African Snail, *Achatina fulica* which it caused high variation of germination for the HFe treatment. During the first 3-5 days germination period, the number of visible seedlings decreased and deteriorated, but after molluscicide pellets were used, the seedling number became stable or increased slightly, since further destruction of seedlings by the snails was halted.

A. fulica is an edible African terrestrial mollusk, which has been spread to Southeast Asia, the Pacific Islands, Australia, Japan, and American, as an exotic species. It is usually found in soil, on trees and in decomposing material (Ohlweiler *et al.,* 2010). It goes in search of food at night and hides before daylight. When conditions are unfavorable the snail burrows into the ground (Mead, 1979). It is a threat to agriculture and the environment throughout its expanded range (Anon, 2007). The eggs can be transported with plant material and soil, while the adults can "hitch-hike" on vehicles, machinery, equipment, cargo containers, and packing material or through direct introduction of snails (Mead, 1979). This species is highly adaptable to a wide range of environments, modifying its life cycle to suit local conditions. It prefers environments which are rich in calcium carbonate, such as limestone, marl, and developed areas with abundance of cement or concrete (Anon, 2007). It is therefore possible that *A. fulica* will damage young tree seedlings, established by direct seeding, on limestone mine sites. In the nursery, metaldehyde was used for control the snails. Anon (2007) suggests that plowing the soil twice year reduces populations of achatinids in open fields. An interesting possibility would be to mix metaldehyde into the hydrogel. This should be tested by further experiments.

Treatments that included fungicide (both HFu and HFeFu) produced germination curves markedly different from those for treatments that did not include fungicide. Visible seedlings increased to a peak and then decreased, since mortality exceeded emergence of new seedlings. This pattern was reproduced with low variability among all blocks, in contrast with the other (non-fungicide) treatments which exhibited much higher variability among blocks. The fungicide used was chlorothalonil; a broad spectrum contact fungicide. It has a non-systemic action and is applied as a foliar spray. It is the third most commonly used fungicide in the world, after sulphur and copper, and has been in use since 1967 (Gianessi and Anderson, 1995). Chlorothalonil is a multi-site inhibitor of various enzymes and other metabolic functions, thereby preventing spore germination and causing death of growing cells. Adverse effects of chlorothalonil have been demonstrated. Earwigs exposed to chlorothalonil residues on peanut foliage suffered 10 to 20 percent mortality. Earwigs are controller of insects that are agricultural pests and eat eggs and larvae (Toxicity, 1997). So a more immediate may affect such as the smell of the fungicide attracting the snails.

F .microcarpa was chosen for this trial because it is often found in rain gutters, building crevices, sidewalk cracks, and on rock. Jim, 1998 reported that *F. microcarpa* commonly roots in stone walls. . Other *Ficus* species also capable of rooting in rocky substrates include *F. superba, F. hispida, F. virens, F. variegata, and F. religiosa*. In general, the occurrence of *Ficus* spp. in rocky substrates is due to their dense, tough and vigourous root systems, efficient seed dispersal by birds and subsequent secondary dispersal by ants. The results, presented here for *F. Microcarpa,* agree with those of Lin *et al.*(2008) who reported higher germination rates of *F. microcarpa seeds* on limestone (the crushed concrete for road pavement) and limestone mixed with forest soil compared with forest soil.

The soil properties (table 3) in the mine land is a poor nutrients that arranges for low soil fertility because it has organic matter less than 1.5 %. Furthermore, primary macronutrients (nitrogen, phosphorus, and potassium) are low quantity. Thereby, if plant tree in the soil, suggest that need to add fertilizer (Soils, 1993). In addition, the soil has pH about 8 that mean moderately alkaline (Soils, 1993). However, *F. microcarpa* prefer high pH Lin *et al.,* (2008).

For that matter, one thing was used in the trial is hydrogel which is polymer. From figure 13 showed high viscosity when compare with water. So the viscosity fluid is a measure of its resistance to gradual deformation by shear stress or tensile stress. For liquids, it corresponds to the informal notion of "thickness" (Symon and Keith, 1971). Before then, gelatinize starches were tested but they failed because they had not crosslink that are non-polymer, water-soluble, and decomposed by bacteria or contaminated with Fungi. Moreover, agar was tested too, but it is not sticky enough.

CHAPTER 6

CONCLUSION

Restoration of mine lands is challenging because of poor environmental conditions for plant establishment. So the method and plant species were specialized, and for this study site had slope, high temperature, soil at high pH, no top soil, and the stony nature of the mine substrate. Hence, using saplings for restoration were high cost and require a lot of labour. Direct seeding with hydrogel were tested in this study because the method is cheap and hydrogel supports seed germination. *F. microcarpa* was chosen because it is a native species, and is both a framework species and a keystone species (FORRU, 2006), with a strong root system and is able to grow on limestone or rock. Highest germination was achieved when all treatments (hydrogel, fungicide and fertilizer) were used in combination. Results suggest a field trial using the HFeFu treatment and direct seeding of *F. microcarpa* seeds at the mine site may produce positive results. Moreover, *A. fulica* can be problem for seed germination in this site so molluscicide may be used with hydrogel for control them.

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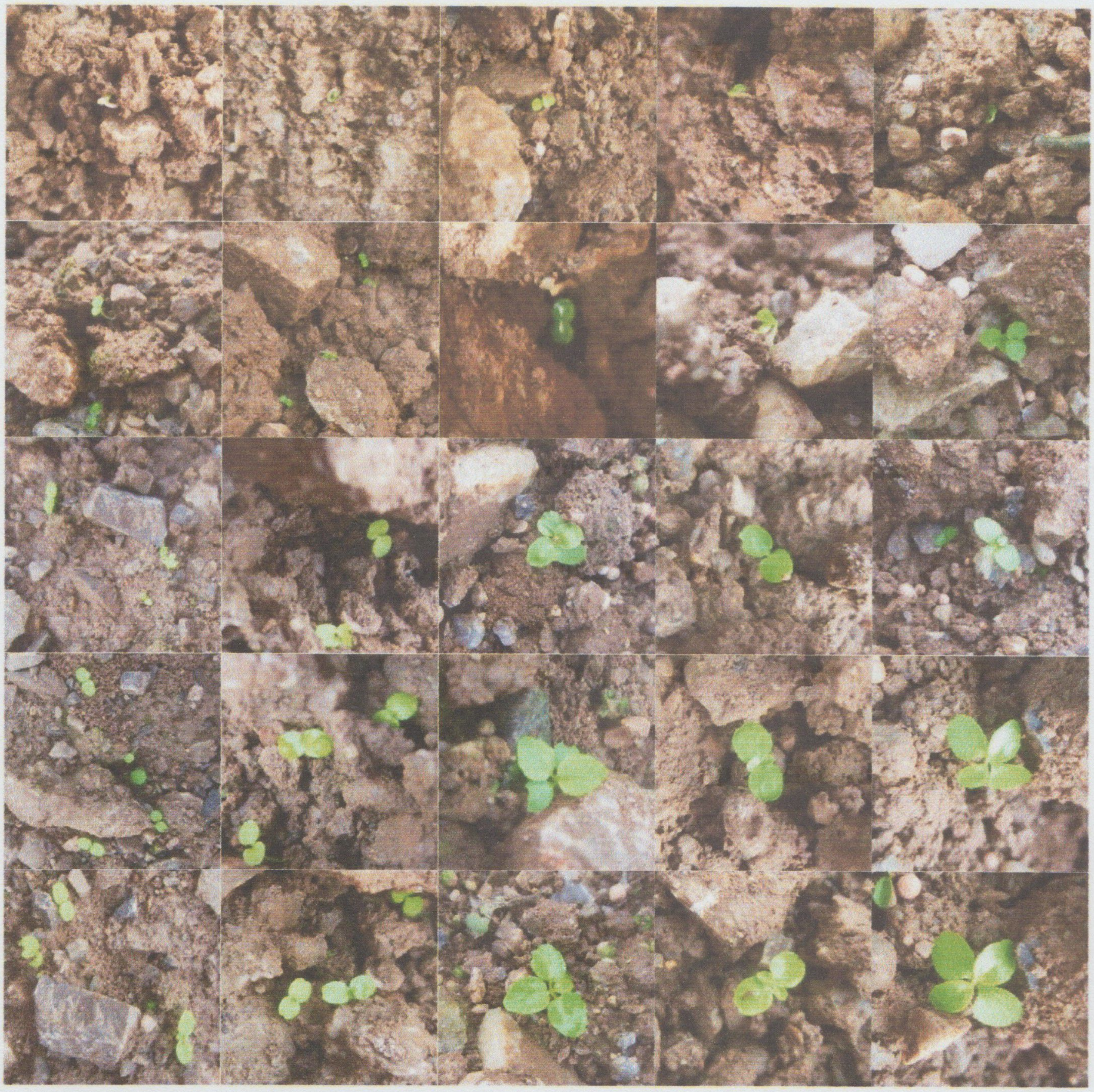

Figure 14 Germination of *F. microcarpa* on each treatment (C = control; H = hydrogel; HFe = hydrogel with fertilizer; HFu = hydrogel with fungicide; HFeFu = hydrogel with fertilizer and fungcide) a) 0 day b) 7 days c) 14 days d) 21 days e) 28 days

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