Allelopathic Effects of *Prunus cerasoides* Buch.-Ham ex. D. Don Leaves on Common Weeds in Forest Restoration Sites

Punnat Changsalak

Bachelor of Science

Biology

Department of Biology, Faculty of Science

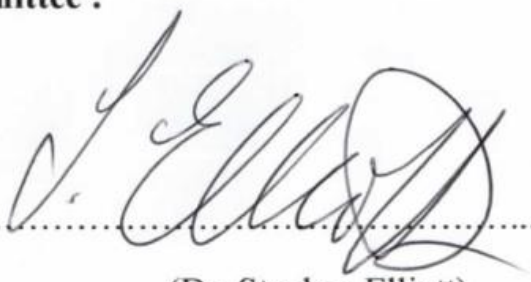
Chiang Mai University

Allelopathic Effects of *Prunus cerasoides* Buch.-Ham ex. D. Don Leaves on Common Weeds in Forest Restoration Site

Punnat Changsalak

This special project has been approved to be
partial fulfillment of the requirements for
the Degree of Bachelor of
Science in Biology.

Examining Committee :



..... Chairperson

(Dr. Stephen Elliott)

..... Member

(Assist. Dr. Sutthathorn Chairuangsri)

..... Member

(Assist. Dr. Pimonrat Tiansawat)

31 March 2020

Acknowledgement

This research could not have been finished without the help of my Advisor, FORRU-CMU staff and friends. I would like to thank you all of my success in this step.

First, I would like to thank my Advisor, Dr. Stephen Elliott, for his invaluable help on this research, especially in writing period. Similarly, I am also grateful to other advisor, including Assistant Professor Dr. Sutthathorn Chairuangsri and Assistant Dr. Pimonrat Tiansawat.

Thanks due to The Development and Promotion of Science and Technology Talented Project (DSTP), for financial support during studying in bachelor degree at Chiang Mai University.

I wish to express my appreciativeness for The Forest Restoration Research Unit (FORRU-CMU) for facilities support during research. I thank to all FORRU-CMU staff who helped with experiments in the nursery as well.

In addition, Thanks are due to Bhanuwat Foojakham, Kotchamon Chamhar, Wittawat Insian and Sarana Chansuebsri, who encouraged and helped me in material collection from the field.

Furthermore, I would like to acknowledge the Department of Biology, Faculty of Science, Chiang Mai University which gave me a lot of new experiences and beneficial knowledge for the further education.

Research Title Allelopathic effects of *Prunus cerasaides* Buch.-Ham ex. D. Don leaves on common weeds in forest restoration site

Author Mister Punnat Chnagsalak

B.S. Biology

Examining Committee

Lecturer Dr. Stephen	Elliott	Chairperson
Assist. prof. Dr. Sutthathorn	Chairuangsri	Member
Assist. Dr. Pimonrat	Tiansawat	Member

Abstract

Weeding by hand tools is the most expensive part of forest restoration projects, so herbicides are an attractive alternative weeding technique. However, synthetic herbicides may have negative impacts on the environment and human health. *Prunus cerasoides* Buch.-Ham. Ex D.Don produces allelochemicals, which may enable the tree to compete with herbaceous weeds such as *Chromoleana odorata* (L.) R.M.King & H.Rob. and *Bidens pilosa* L.. Consequently, it may be possible to develop more environment-friendly "bio"-herbicides from *P. cerasoides* allelochemicals. This project investigated the inhibitory potential of aqueous leaf extract (ALE) from *P. cerasoides* leaves on seed germination and seedling growth of *C. odorata* and *B. pilosa* - two abundant weeds of forest restoration sites. ALE at various concentrations (0.75-5.00 wt%) was applied to weed seeds to test the intensity and duration of its inhibitory effect on germination. ALE at 0.75 and 1.25 wt% significantly inhibited germination of *C. odorata* and *B. pilosa*, respectively ($P \leq 0.05$), with the degree of inhibition increasing with increasing concentration. It also delayed germination for a few days (1-4 days). ALE had no significant substantial inhibitory effect on seedling survival and biomass per plant irrespective of development stage. Consequently, *P. cerasoides* ALE should be further investigated as a pre-emergent herbicide. It is unlikely to be useful as a general weed killer on forest restoration sites.

หัวข้อปัญหาพิเศษ ผลจากอัลลีโลพาธีของใบ *Prunus cerasoides* Buch.-Ham ex. D. Don ต่อวัชพืชที่พบได้บ่อยภายในแปลงฟื้นฟู

ชื่อผู้แต่ง นายปุณณัตถ์ ช่างสลัก

วิทยาศาสตรบัณฑิต สาขาวิชาชีววิทยา

คณะกรรมการสอบปัญหาพิเศษ

อ. ดร. สตีเฟน	**เอลเลียต**	**ประธานกรรมการ**
ผศ. ดร. สุทธาธร	**ไชยเรืองศรี**	**กรรมการ**
ผศ. ดร. พิมลรัตน์	**เทียนสวัสดิ์**	**กรรมการ**

บทคัดย่อ

การกำจัดวัชพืชโดยการตัดด้วยเครื่องมือกำจัดวัชพืชแบบทั่วไปเป็นค่าใช้จ่ายที่สำคัญในโครงการฟื้นฟู สารกำจัดวัชพืชจึงเป็นทางเลือกที่น่าสนใจ แต่งานวิจัยพบว่าสารกำจัดวัชพืชอาจส่งผลกระทบต่อสิ่งแวดล้อมและสุขภาพของมนุษย์ *Prunus cerasoides* Buch.-Ham. Ex D.Don มีกลไกอัลลีโลพาธีในการต่อสู้กับวัชพืชพื้นล่างที่มีอยู่ในตามธรรมชาติ หากนำความสามารถนี้ของ *P. cerasoides* มาใช้พัฒนาเป็นสารกำจัดวัชพืชที่เป็นมิตรต่อสิ่งแวดล้อม อาจเป็นประโยชน์แก่โครงการการฟื้นฟูป่าในลำดับต่อไป ปัญหาพิเศษนี้จึงจัดทำขึ้นเพื่อทดสอบความสามารถของสารสกัดหยาบจากใบ (ALE) ของ *P. cerasaides* ต่อการงอกของเมล็ดและการเจริญเติบโตของต้นกล้าวัชพืชที่พบได้บ่อยในแปลงฟื้นฟู ได้แก่ *C. odorata* และ *B. pilosa* โดยใช้ ALE ระดับความเข้มข้นตั้งแต่0.75-5.00 wt% ในการทดสอบการยับยั้งการงอกเมล็ดวัชพืช ทำให้ทราบถึงระดับความเข้มข้นที่มีความสามารถยับยั้งเมล็ด *C. odorata* และ *B. pilosa* ได้ดีที่สุด คือ 0.75 และ 1.25 wt% ตามลำดับ และผลจาก ALE ยังสามารถชะลอการงอกของเมล็ดทั้ง 2 ชนิดได้ (ประมาณ 1-4 วัน) จากนั้นใช้ระดับความเข้มข้นที่ได้ทดสอบกับต้นกล้าอายุ 2 เดือนของวัชพืช ทำให้ทราบว่า ALE ไม่มีผลต่ออัตราการตาย

และน้ำหนักแห้งของต้นกล้าทั้ง 2 ชนิด จึงมีการเพิ่มระดับความเข้มขึ้นเป็น 4 เท่าจากเดิม (ซึ่งระดับความเข้มข้นที่ใช้กับต้นกล้า *C. odorata* และ *B. piloas* คือ 3.00 และ 5.00 wt% ตามลำดับ) เพื่อทดสอบกับต้นกล้าวัชพืชที่มีระยะการพัฒนาแตกต่างกัน ซึ่งที่ความเข้มข้นดังกล่าว มีผลทำให้เกิดการเปลี่ยนแปลงอัตราการตายและน้ำหนักแห้งในต้นกล้าบางระยะของ *C. odorata* เท่านั้น ในขณะที่ไม่พบการเปลี่ยนแปลงใด ๆ ในต้นกล้า *B. pilosa* ทุกๆระยะ จากการศึกษาครั้งนี้ ทำให้ทราบว่า ALE มีความสามารถในการยับยั้งการงอกของเมล็ดวัชพืชแต่ไม่มีผลต่อต้นกล้าวัชพืชจึงยังไม่เหมาะสมสำหรับการใช้ควบคุมวัชพืชในแปลงฟื้นฟู

Table of Contents

Table of Contents (Continued)

List of Tables

List of Figures

List of Figures (Continue)

Chapter 1

Introduction

1.1 Introduction

Weed growth is one of the most serious factors that prevent the success of forest restoration projects, particularly invasive exotic weed species. Weeds inhibit growth and survival of both naturally regenerating and planted trees, due to competition for nutrients, water and light (Harper 1982). Some of the most ubiquitous weeds that impede forest restoration in northern Thailand are *Chromoleana odorata* (L.) R.M.King & H.Rob. and *Bidens pilosa* L. (both Compositae /Asteraceae). Originally from the New World, these species are well known as invasive exotic species in SE Asia (Pallewatta, Reaser, and Gutierrez 2003) forming dense stands, which prevent the establishment of other plant species (USDA, 2007) including tree seedlings, due to their allelopathic properties (GISD, 2015). Furthermore, both species grow rapidly in the rainy season, which is particularly inhibitory to tree saplings, planted at that time, for forest restoration.

Weeding is the most expensive part of restoration even on a small sites, because the weeds must be dug out by the roots; otherwise they quickly regrow (Forest Restoration Research Unit, 2005). Therefore, herbicides are an attractive alternative to weeding by hand tools. The most widely used herbicide in forest restoration projects is glyphosate or ©Roundup (Xu et al., 2019). It is a broad-spectrum, non-residual and highly efficient herbicide (Qiu et al. 2020). Even though glyphosate breaks down quickly when it in soil, it can be harmful to human health in high doses (Williams, Kroes, and Munro 2000) and environmentally damaging. High concentrations in human cells are cytotoxic (Townsend et al. 2017). Furthermore, run off or leaching of glyphosate into aquatic habitats, can kill some fish species (de Brito Rodrigues et al. 2019).

Allelopathy is "the chemical inhibition of one plant by another, due to the release into the environment of substances acting as germination or growth inhibitors" (Cheng and Cheng 2015a). Chemicals may be released out from various parts of plant tissues by volatilization, exudation from roots and decomposition of senescent leaves (Latif, Chiapusio, and Weston 2017). Allelopathy is one of several mechanisms, by which invasive weeds become super-abundant in open areas (Chengxu et al. 2011). Trees may also be allelopathic and it may also be possible to exploit their allelopathy to develop more user- and environment-friendly herbicides for forest restoration projects. For example, most *Prunus* spp. (Rosaceae) are rich in flavonoids and their glycosides (Jangwan and Kumar 2015) , which are stored in various parts of plant (Joseph, Anjum, and Tripathi 2018) including: stem, bark, fruits, seeds and other tissues (Jang et al., 2018; Jangwan & Bahuguna, 1989). *Prunus cerasoides* Buch.-Ham. Ex D.Don (Rosaceae) is a wild cherry tree species, common on Doi (=mountain) Suthep-Pui in

northern Thailand (Hardwick et al. 1997). It is a fast-growing pioneer tree species, categorized as a framework species, valuable for forest restoration (Elliott et al., 2002; 2003). *P. cerasoides* stores allelochemicals, as flavones and flavanone glycoside in the stem bark (Tripathi 2018), as kaempferol in leaves (Bhatt and Todaria 1990) and naringenin in seeds (Shrivastava 1982). All these allelochemicals are derivative of flavonoids ,which can reduce electron transport chain activity, leading to cell death (Moini et al. 1999). When some parts of *P. cerasoides* like leaves decompose on the ground, the allelochemicals are released enabling the tree to compete with understory weeds in their natural habitat.

Although, several allelopathic effects of *P. cerasoides* on crops have been studied, the allelopathic effects of *P. cerasoides* on weed species that are typical of deforested sites, are unknown. Consequently, for this special project, I carried out experiments to investigate the potential effects of allelopathic chemicals from *P. cerasoides* leaves on seed germination and seedlings growth of two weeds species that are abundant in forest restoration sites. I tested the hypothesis that aqueous leaf extract (ALE) of *P. cerasoides* contains allelopathic chemicals that inhibit seed germination and early seedling growth of *C. odorata* and *B. pilosa.* (both Compositae). The project is part of a long-term effort to develop environmentally friendly "bio"-herbicides, to replace glyphosate during forest restoration projects (Elliott 2016).

1.2 Objective

To determine the effects of allelopathic chemicals in aqueous leaf extract (ALE) of *Prunus cerasoides* leaves on seed germination and seedling growth of *Chromoleana odorata* (L.) R.M.King & H.Rob. and *Bidens pilosa* L (Compositae).

Chapter 2

Literature Review

2.1 Chemical herbicides

Herbicides are synthetic chemicals manufactured to kill weeds or inhibit their growth (Gupta, 2011). According to Au, 2003, herbicide can be classified according to their molecular structure or chemical activity, toxicity, and their mode of action. Two major categories, of herbicides, classified by mode of action, are contact herbicides and translocated herbicides.

Contact herbicides affect only on the parts of the plants that they cover. Absorption of the chemical into the plant is minimal. Contact herbicides must cover most of the foliage of the target plants to be effective e.g. diclofop, dinoseb, diquat, and paraquat. The actions of contact herbicides differ. For example, diquat and paraquat generate phytotoxic free radicals that interfere with lipid metabolism, which ultimately leads to death, whereas diphenyl ether inhibits photosynthesis, causing chlorosis and necrosis. Furthermore, some of biochemical mechanisms of contact herbicides are not yet clearly understood, such as cacodylic acid. Various environmental factors may interfere with the action of some contact herbicides, particularly soil particles (for diquat and paraquat).

In contrast, translocated or systemic herbicides are absorbed and travel along the vascular system to roots and other pant parts where they interfere with the normal plant biochemistry. Systemic herbicides are applied to foliage or soil. They are translocated to to their point of action where they bind to a specific location and disrupt physiological processes, plant growth and development. Examples of translocated herbicides are atrazine, glyphosate 2,4-dichlorophenoxyacetic acid (2,4-D) and simazine. They have modes of action at the molecular level. Chlorinated aliphatic acid herbicides, such as trichloroacetic acid (TCA), modify protein structure, causing chlorosis and necrosis. Amide herbicides, such as alachlor and metachlor, interfere with both protein and nucleic acid synthesis. Thiocarbamates and dithiocarbamates inhibit lipid synthesis, whilst carbamates inhibit protein synthesis, Phenoxy herbicides, such as 2,4-D, stimulate protein and RNA synthesis, which accelerate plant growth and mortality. Only triazine herbicides, directly block photosynthesis.

The most widely used herbicide in forest restoration projects in the tropics is glyphosate. It is a non-residual, highly effective and broad-spectrum herbicide. It breaks down rapidly in the environment and does not accumulate in the soil. It is rated least dangerous, compared with other herbicides and pesticides (Forest Restoration Research Unit, 2005). Its acute toxicity to mammals is low. However, its potential carcinogenic properties are being debated. In March 2015, the World Health Organization's International Agency for Research on Cancer (IARC) classified glyphosate as "probably carcinogenic in humans" (category 2A)

(Cressey, 2015), whereas the European Food Safety Authority concluded in November 2015 that "the substance is unlikely to pose a carcinogenic threat to humans". A joint WHO-FAO committee stated that the use of glyphosate formulations does not constitute a health risk below stated admissible daily maximum intake limits (one milligram/kg of body weight per day) for chronic toxicity (Bellon, 2019). Furthermore, the European Chemicals Agency (ECHA) classified glyphosate found no evidence implicating it as a carcinogen, a mutagen, toxic to reproduction, nor toxic to specific organs (Is et al., 2016).

Nevertheless, public perception of glyphosate as a health hazard, due to recent high profile court cases in the USA, is generating opposition to its continued use (regardless of the scientific evidence). Consequently, more benign, nature-based alternatives for weed control must be found urgently.

Once diluted for spraying, glyphosate has low toxicity to mammals (including humans), but it is toxic to aquatic animals when contaminated in streams or lakes. The herbicide negatively impacts aquatic plants, amphibian larvae and the ratio of predatory benthic invertebrates, which can change the ecological balance of aquatic habitats (Baker et al., 2014). High glyphosate levels may damage animal reproduction and nervous systems (Jingwen et al., 2019). Emerging evidence suggests that glyphosate might affect soil organisms other than plants (Neli et al., 2019).

However, these potentially damaging effects of the chemical on the environment must be balanced against also the damaging environmental consequences of not restoring forest ecosystems where they have been destroyed.

In terms of effects on human health, safety procedures can minimize the risk, Users are advised to wash with large amounts of water and see a doctor if the chemical is sprayed on to the skin or in eyes. As soon as possible after spraying, workers are advised to shower, wash all clothes worn during spraying and clean all of the equipment used (backpacks, boots and gloves etc.).

2.2 Allelopathy and allelochemicals

The term “allelopathy” was originally conceived specifically to denote the inhibitory chemical effects of one plant upon another. It is derived from the Greek allelo- (meaning “from one to the other) and pathy (meaning suffering or disease). It first appeared in press in 1937 in the book, “Der Einfluss einer Pflanze auf die andere – Allelopathie” (The Effect of Plants on Each Other - Allelopathy) by the Austrian professor, Hans Molisch. He used the term to describe biochemical inhibition of plants by neighbouring plants. Although the term has been greatly expanded over the years, to include a broad range of chemical interactions among living

organisms, both positive and negative, the original definition of the term is retained for the purpose of this report.

Allelochemicals are secondary plant metabolites, which, once synthesized take no further part in metabolism. Allelochemicals are released into the environment from plant organs such as roots, rhizomes, leaves, stems, bark, flowers, fruits and seeds. Allelopathic compounds affect on germination and growth of neighboring plants by disrupting various physiological processes including photosynthesis, respiration, water and hormonal balance. The underlying cause of their action is mainly inhibition of enzyme activity. Moreover, allelochemicals are an important factor in the success of many invasive plants (Chen et al., 2017). It benefit invasive plants by enabling them to acquire more resources (such as nutrients, water or light). The production of allelochemicals depends on biotic factors such as nutrient availability, and abiotic factors, such as temperature and pH-level. Plants producing allelochemicals are termed the "donor" plants, whilst those upon which the allelochemicals act are termed the "target" (or acceptor) plant. Most allelochemicals penetrate the soil as already plant-active compounds, e.g. phenolic acids, cyanimides, momilactones and heliannuols, etc. Some are modified into the active form by microorganisms or by specific environmental conditions (pH, moisture, temperature, light, oxygen etc.), e.g. juglone, benzoxazolin-2-one (BOA), 2-amino-3-H-phenoxazin-3-one (APO) (Li et al., 2015).

Allelochemicals can be classified into 10 categories according to their different structures and properties (Soltys et al., 2013):

1. Water-soluble organic acids
   (straight-chain alcohols, aliphatic aldehydes, and ketones)
2. Simple lactones
3. Long-chain fatty acids and polyacetylenes
4. Quinines
   (benzoquinone, anthraquinone and complex quinines)
5. Phenolics
6. Cinnamic acid and its derivatives
7. Coumarins
8. Flavonoids
9. Tannins
10. Steroids and terpenoids
    (sesquiterpene lactones, diterpenes, and triterpenoids)

2.3 Chemicals isolated from the *P. cerasoides*

P. cerasoides has been extensively investigated for its phytochemical constituents and a considerable number of chemical constituents of diverse classes including steroids,

terpenoids, flavonoids, polyphenolics, glycosides, etc. have been reported from different parts of the plant. The example of chemical constituents in *P. cerasoides* have been isolated and characterised as following part (Joseph et al., 2018):

Stem heartwood: dihydrotectochrysin, pinocembrin, dihydrowogonin, chrysin, naringenin, kaempferol, aromadendrin, quercetin, taxifolin, Carasinone, Carasidin and Carasin

Stem sapwood: puddumin-A [7-O-(β-D-glucopyranosyl)-5-O-methylnaringenin], genistein, prunetin, n-pentacosane, triacontane, noctacosanol,β-sitosterol, ursolic acid, oleic, palmitic and stearic acids, afzelin, kaempteritrin, naringenin and β-sitosterol-β-D-lucoside

Stem bark: padmakastein and its derivatives; β-sitosterol behenate, leucocynidin, chrysophenol, emodin, 8-β-D glucosides, orientalone, physcion, β- sitosterol glucoside, amygdalin, prunasetin, sakuranetin, puddumetin, Puddumin-B, sakuranetin, neosakuranin, leucocyanidin, taxifolin, prunetin, genistein, and genkwanin

Leaves: Quercetin-3-rhamnoglucoside, and kaempferol

Fruit: 2, 4, 4'-dihydroxy- 6-methoxy chalcone-4-O-β-D-glycopyranosyl (1→4) + α-L-rhamnopyranoside

Seeds: tectochrysin, genistein, leucocyanadin, genkwanin, prunetin, Sakuranetin, genkwanin-4'-glucoside, naringenin-5-O-α-L- rhamnopyranoside, 4′-O-methyl-liquiritigenin-7-O-α-L- rhamnopyrano-side, naringenin 4′-methylether 7- xyloside, β-sitosterol-3-O-D-galacto-pyranoside and 7-O-β-D-galactopyranosyl-5-O-methyl naringenin

Some chemicals such as flavonoids and their derivatives, act as allelochemicals. The majority of their functions result from their strong anti-oxidative properties (Moini et al., 1999). Specifically, kaempferol (flavonoids' derivatives) inhibits transport at the flavoprotein site of plant mitochondria, due to kaempferol binds to complex 1 and inhibits the transfer of hydrogen electrons from Complex 1 to Coenzyme Q. The oxidation of NADH is therefore decreased, and in turn increases the concentration of NAD. As the result, the phosphorylation process of ADD is decreased, and ATP synthesis decreases respectively, which limiting cellular respiration throughout the plant.

2.4 Forest restoration

Forest restoration is: "directing and accelerating ecological succession towards an indigenous target forest ecosystem of the maximum biomass, structural complexity, biodiversity and ecological functioning that can be self-sustained within prevailing climatic and soil limitations." It may involve protecting natural regeneration, assisting its growth and/or augmenting it by tree planting or direct seeding (Elliott, 2013). However, competition from

herbaceous weeds, particularly aggressive, invasive, exotic species, can slow or completely halt forest succession, by suppressing growth and survival of tree seedlings and saplings, thus rendering efforts “direct and accelerate” succession ineffective.

The restoration technique, for upland evergreen forest in northern Thailand, developed by Chiang Mai University’s Forest Restoration Research Unit (FORRU-CMU) begins with clearing the restoration site of weeds

The weed-pressing technique or mulching around the existing naturally regenerating trees can be employed successfully during assisted or accelerated natural regeneration (ANR), particularly on sites dominated by soft (non-woody) grasses and herbs.

Where weed-pressing is ineffective, slashing is often substituted but slashing encourages weed species to re-sprout and thus absorb more water and nutrients from the soil than if they had never been cut in the first place. This actually intensifies root-competition with any existing and the planted trees (Schenk, 2006). To prevent this weeds must be dug out and their roots exposed. Unfortunately, that disturbs the soil, increasing the risk of soil erosion. The risk of damaging naturally established tree seedlings or saplings is high and the work is labor-intensive and therefore costly.

Using a slow-acting, broad-spectrum, systemic herbicide, such as glyphosate is a more cost-effective alternative (Baylis, 2000). Herbicide usage reduces costs and avoids the need to disturb the soil, but it cannot be used for weeding after tree planting because broad spectrum herbicides kill trees as well as weeds. Only a few tree species are resistant to glyphosate. Plants absorb glyphosate through their leaves and it is then translocated to all other parts of the plant including the roots. The affected plants die slowly, gradually turning brown over 1–2 weeks. Weed regeneration from seed takes 6–8 weeks, so the treatment creates weed-competition free conditions for establishment of planted trees (Elliott, 2013).

Cummings, et al. (2012) suggested that allelopathy may be a key mechanism by which some native trees could reduce the abundance and impact of exotic species and that exploiting allelopathy in native species could improve forest restoration success and the re-establishment of natural successional dynamics. He found that allelochemicals from native pioneer tree species may be particularly effective at controlling the spread invasive exotic herbaceous weed species, since invading exotic weeds may never have been exposed to such chemicals in their evolutionary history (the so-called “homeland security” hypothesis). Consequently, the exploitation of native pioneer tree species (either planting them or extracting allelochemicals from them) may provide opportunities for finding nature-based weed control strategies that do not rely on synthetic herbicides. However, Cummings et al. studied the use of allelopathy to control weeds in agroforestry systems. Very few studies have explored the possibility of exploiting the technique for ecosystem restoration.

Chapter 3

Materials & Method

3.1 Preparation of Aqueous leaf extract (ALE)

P. cerasoides leaves were collected from various trees in October to December, 2019 before deciduous period. Small, young, light green leaves was discarded. The remaining mature, dark green leaves were sun-dried for 3 days at room temperature or left another few days when air humidity was high (Figure 18), then ground into powder using high-speed grinder. The crude powder was sieved creating a fine powder with particle size ~ 0.1 mm. The powder was then kept inside the freezer at -10 ±10˚C of refrigerator before being used in the following experiments.

Figure 1 Dried leaves (Left), grinding the leaves into powder (middle) and sieving to make fine powder (right).

3.2 Application of aqueous leaf extract (ALE)

ALE was prepared at various concentrations by adding various amounts of *P. cerasoides* leaf powder as required to water in the following quantities in a 200-ml beaker (Figure 20).

Table 1 Amount of water and powder in different concentration treatment.

ALE wt %	Amount of water (ml)	Amount of powder (g)
0.75	100	0.755
1.25	100	1.265
2.50	100	2.564
5.00	100	5.263

The amount of water was increased when the ALE was used for seedling experiments and the amount of powder was multiplied as the ratio of increasing water amount. The suspension of fine powder was left over night at the room temperature with plastic wrap before use (around 12 hours). Sediment was removed, as required by the treatments (as shown in 3.6).

The suspension was transferred to seed germination experiments using a dropper and to seedlings growth experiments using a watering can. The pH of all batches of the suspensions was measured before use.

3.3 Application of acidic treatment

All suspensions were acidic, so an acid treatment was added to the experiments to separate the effects of acid from those of the allelochemicals. Thirty-seven percent HCl, diluted with tap water, was prepared to match the pH-level of the ALE. The acid was prepared on the treated date and used immediately.

3.4 Selected weed collection

C. odorata and *B. pilosa* seeds were collected from gravel roadsides and open areas within Chiang Mai University in the fruiting season, just before the rainy season. Visibly immature and damaged seeds were removed, and the good seeds were then put into plastic zip-lock nags and stored at room temperature, before their use in experiments. The time period between collection and sowing was not longer than one week, to prevent fungal growth.

3.5 Preparation of seedlings in nursery.

All seedlings of the 2 selected weeds species were grown in the nursery of The Forest Restoration Research Unit (FORRU), located on Chiang Mai University campus. Seeds were germinated in petri-dishes (Figure 19), before being transplanted into modular germination trays. All seedlings were watered with a watering can every day, until they had grown big enough for experiments. The seedlings were arranged by growth stage (same number of nodes) (as show in figure 3).

Figure 2 Seedlings after transplanted into germination trials.

3.6 Experimental Design

A completely randomized design (CRD) was used for seed germination experiments whilst a randomize complete block design (RCBD) was used for seedlings growth experiments.

Experiment I - To determine the lowest concentration of ALE that inhibits germination. 50 seeds of *C. odorata* and *B. pilosa* were treated with ALE solutions at concentrations of: 5 wt% (as recommended by Suphannika Intanon, Naresuan University pers com.), 2.5 wt%, 1.25 wt% and 0.75 wt% both with and without sediment removed. A control, with 100% tap water, an acid treatment were also set up for comparison. Treatment were applied every other day. The number of seeds germinated was recorded daily. Seed germination was defined as emergence of radical to more than half of the seed length. The experiment ended at 9 days, when the number of seed germinating was constant. Every treatment was replicated 3 times.

Experiment II - To determine the duration of inhibition effect of aqueous leaf extract (ALE). 50 seeds of *C. odorata* and *B. pilosa* were treated with ALE solution at the lowest effective concentration derived from experiment I, which was 0.75 wt% and 1.25 wt% in *C. odorata* and *B. pilosa*, respectively and replicated both with and without sediment. A control, with 100% tap water was also used to compare ALE solutions. All seeds were treated with ALE solution on the first day of the experiment only, and watered with tap water daily thereafter. The number of seed germinated was recorded daily. The experiment ended at 14 days, when no further seeds had germinated for 3 consecutive days. Every treatment was replicated 3 times.

Experiment III - To determine the inhibitory effects of ALE on 2-month-old seedlings of *C. odorata.* and *B. pilosa* at the lowest concentration that inhibited seed germination (from experiment I; 0.75 wt% and 1.25 wt%, respectively). 50 seedlings of each species were treated with ALE, both with and without sediment removed. A control, with 100% tap water, was also used to compare ALE treatments. Every treatment were grouped into a block, which located inside, near the edge and outside of the nursery roof (totally 3 replications). Treatments were applied every 4 days (totally 8 times), with daily watering with tap water. The number of seedlings deaths was recorded every other day, with death defined as unhealthy-brown stem and leaves dropped. The experiment was terminated at 31 days, due to the limitation of semester duration. The number of live seedlings and dry weight were recorded at the end of experiment.

Experiment IV - To determine if the inhibitory effects of ALE were related to seedling development stage (number of nodes), 20 seedlings of. *C. odorata* and *B. pilosa* were sorted into 1-, 3- and 5-node development stages. All of seedlings were treated with ALE at 4 times the minimum concentration that bring about seed germination inhibition from experiment 1 (which was 3.00 wt% and 5.00 wt% in *C. odorata* and *B. pilosa*, respectively). For this experiment sediment was not removed. A control, with 100% tap water and acid treatments

were also set up for comparison. Every treatment were grouped into a block, which located inside, near the edge and outside of the nursery roof (totally 3 replications). Treatments were applied every four days (totally 4 times). All seedlings were watered with tap water daily, even on untreated days. The number of seedlings that had died was recorded every other day, with death defined as unhealthy-brown stem and leaves dropped. The experiment was terminated 15 days, due to the limitation of semester duration. The number of live seedlings, dry weight and seedling height were recorded at the end of experiment.

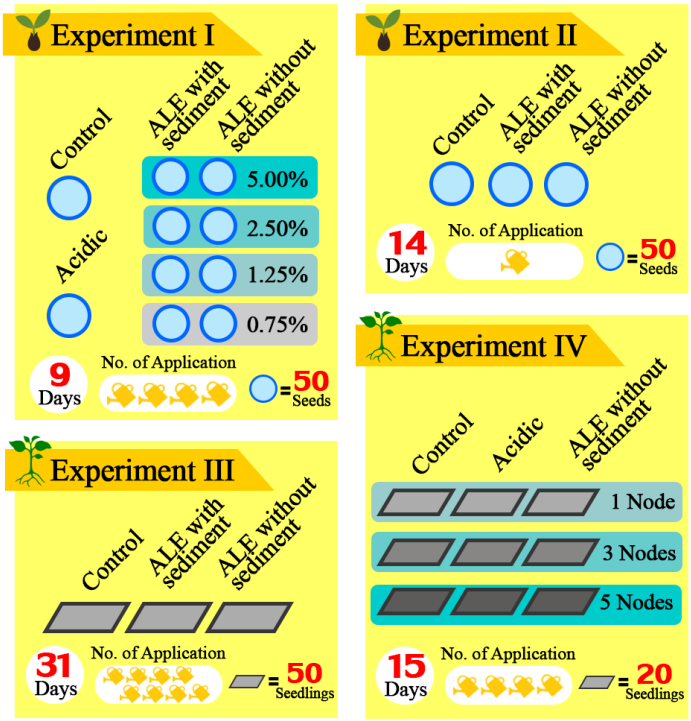


Figure 3 All Experimental design diagrams (replicated 3 times)

3.7 Dry weight measurement

At the end of experiment III and IV, dry weight of seedlings or the biomass per plant were recorded by harvest method. *C. odorata* and *B. pilosa* seedlings were removed out of each modular germination tray, then washed out soil from root and put into oven at 80 °C for 48 hours until a constant mass. All seedlings on each germination tray were weighed together and recorded as total dry weight before calculated into the biomass per plant.

3.8 Seedling height measurement

All live seedlings of experiment IV of both weed species were measured height individually. Seedlings were not straightened whilst measurement, which measured from the ground point at root collar level to the highest point of shoot (not the tip of the leaf).

3.9 Data analysis

Data were subjected to an analysis of variance (ANOVA) using SPSS and the Analysis ToolPak in Microsoft Excel to separate the effects of block position from treatments. The dependent variables were percentage seed germination, mortality rate, dry weight (biomass per plant) and seedling height. The independent factors were treatment and replication. When ANOVA revealed significant difference were present among treatments, Tukey's HSD (Honest Significant Difference) test was applied to compare mean values between treatments at $P \leq 0.05$.

Chapter 4

Results

4.1 Seed germination

Experiment I - To determine the lowest concentration of ALE that inhibits seed germination.

The germination percentage of *C. odorata* after application of *P. cerasoides* ALE of various concentrations (0.75-5.00 wt%). ANOVA revealed significant differences in germination percent among the treatments ($P \leq 0.05$) (Figure 4). ALE significantly inhibited germination of *C. odorata* seeds at all concentrations of 0.75-5.00 wt% (with or without sediment), which the effect was almost complete germination inhibition ($F_{2,9}=58.19$, $P \leq 1.79 \times 10^{-11}$).

Acid treatment had no effect on germination ($P>0.05$). Sediment removal had no significant effect on the inhibitory effects of ALE at all concentrations ($P>0.05$).

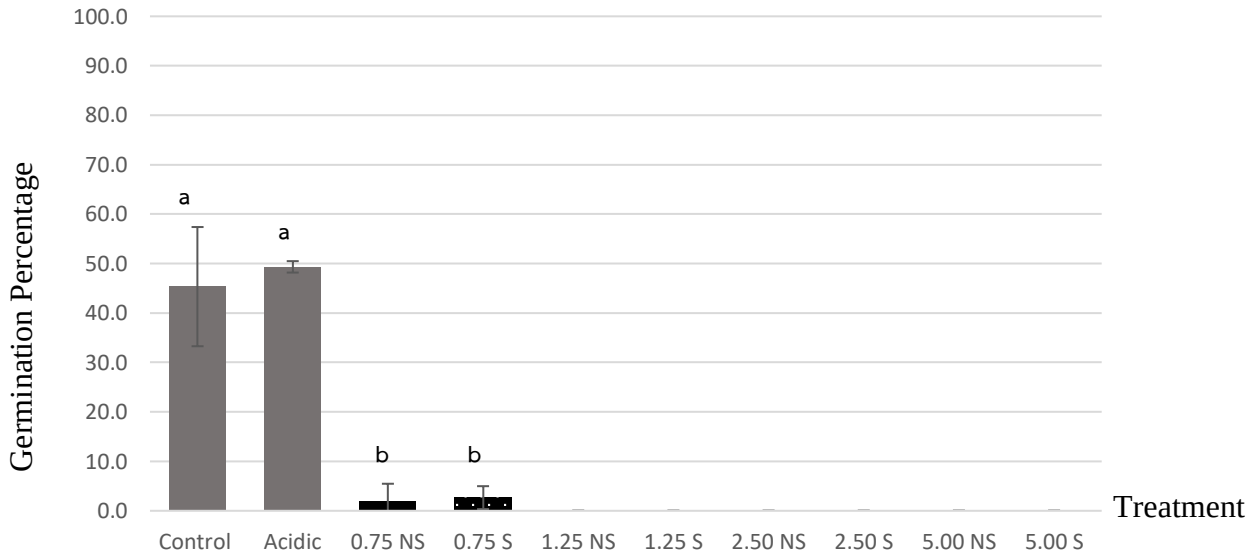


Figure 4 Germination percentage of *C. odorata* seeds with application of ALE after 9 days. Control = water only. Acid treatment (pH c.5.45). Solid columns (NS) = no sediment. Hashed columns (S) = with sediment. Errors bars are ± 1 standard deviation. Columns not sharing the same superscripts are significantly different ($P \leq 0.05$). Absence of visible columns indicate 0.

The germination percentage of *B. pilosa* after the application of *P. cerasoides* ALE of various concentrations (0.75-5.00 wt%). ANOVA revealed significant differences in germination percent among the treatments ($P \leq 0.05$) (Figure 5). ALE significantly inhibited germination of *B. pilosa* seeds (compared with the $P \leq 3.01 \times 10^{-11}$control) at concentrations of

2.50-5.00 wt% (with or without sediment) and at 1.25 wt% (with sediment) ($P \leq 0.05$). The inhibitory effect increased in magnitude markedly with increasing ALE concentration, with the highest concentration bringing about almost complete germination inhibition ($F_{2,9}=54.81$, $P \leq 3.01 \times 10^{-11}$).

Acid treatment had no effect on germination ($P>0.05$). Retaining sediment significantly increased the inhibitory effect at lower concentrations of ALE (0.75-1.25 wt%) ($P \leq 0.05$), but it had no additional effect on the already powerful inhibitory effect of higher concentrations (2.50-5.00 wt%) ($P>0.05$).

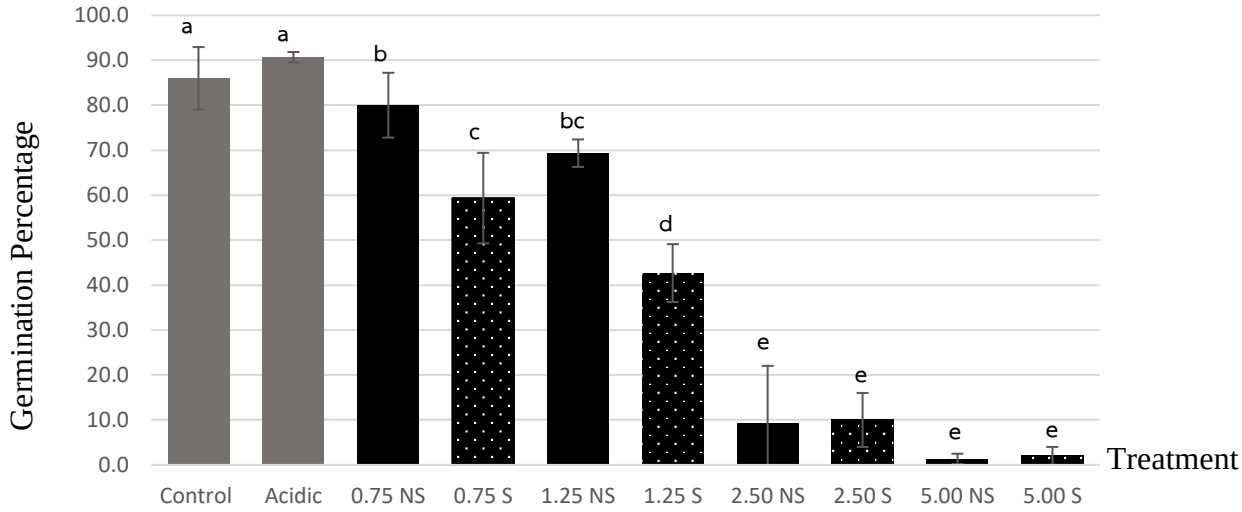


Figure 5 Germination percentage of *B. pilosa* seeds with application of ALE after 9 days. Control = water only. Acid treatment (pH c.5.45). Solid columns (NS) = no sediment. Hashed columns (S) = with sediment. Errors bars are ± 1 standard deviation. Columns not sharing the same superscripts are significantly different ($P \leq 0.05$). Absence of visible columns indicate 0.

Experiment II - To determine the duration of inhibition effect of ALE.

As experiment I showed that ALE concentration of 0.75 wt% was the lowest to bring about significant germination inhibition, that concentration was used to determine the degradation of ALE effectiveness over time. Figure 6 shows germination of *C. odorata* seeds over 14 days with a single application of ALE on day 1. ANOVA revealed significant differences among the treatments cumulative germination percent at 14 days ($P \leq 4.15 \times 10^{-3}$). ALE at 0.75 wt% both with and without sediment significantly inhibited germination of *C. odorata* seeds over the full 14 days and delayed the start of germination by 4 and 1 day(s), respectively. Sediment removal had no effect on the inhibitory action of the ALE ($P>0.05$).

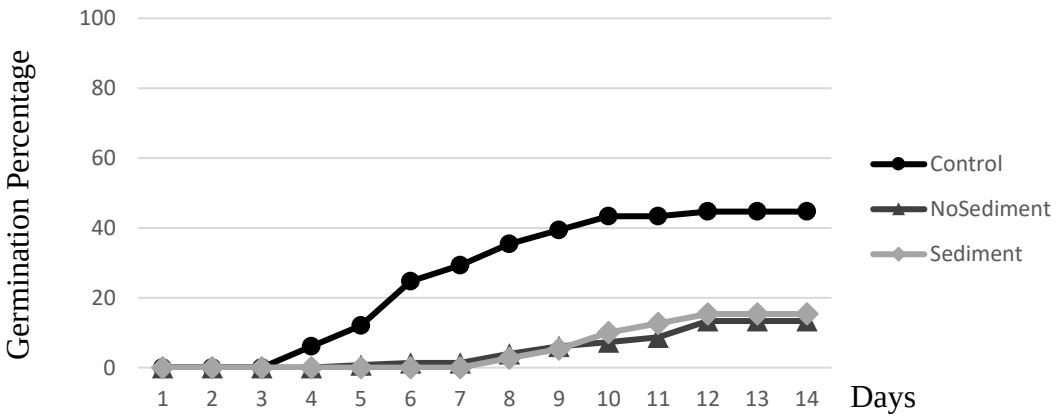


Figure 6 Cumulative germination of *C. odorata* seeds over 14 days, with application of 0.75 wt% concentration *P. cerasoides* ALE on day 1. N=3.

Similarly, 1.25 wt% concentration showed highest potential inhibiting the germination of *B. pilosa*, was used to determine the degradation of ALE effectiveness over time. Figure 7 shows germination rate of *Bidens pilosa* seeds during 14 days. ANOVA revealed significant differences in accumulative germination percent within 14 days among the treatments ($P \leq 8.98 \times 10^{-3}$).The mean percent germination of *B. pilosa* seeds with sediment treatment was 52.00 ± 10.58 and without sediment was 76.00 ± 2.00, while mean percent germination in the control was 88.67 ± 5.03. ALE with sediment inhibited germination of *B. pilosa* seeds, which delayed germination rate for 3 days. The inhibitory effect of ALE without sediment treatment had no significant difference when compared with control ($P > 0.05$), but delayed germination rate for 2
days.

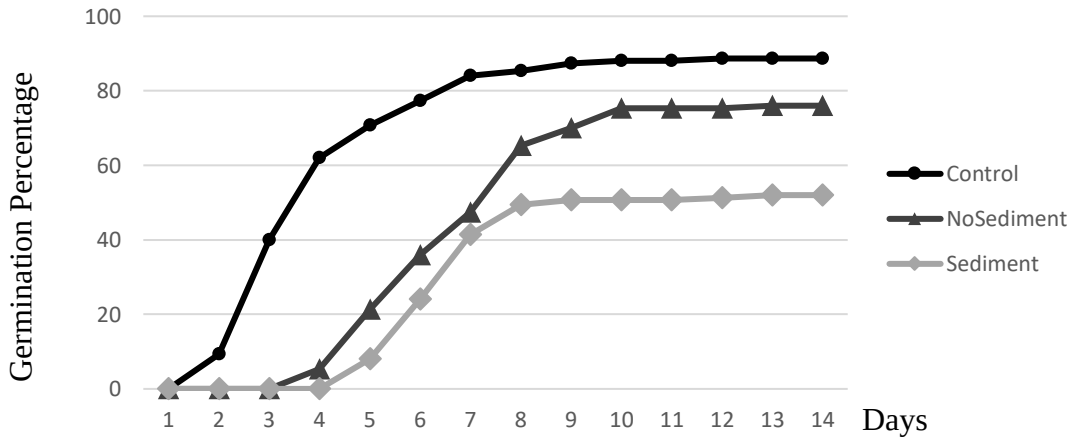


Figure 7 Cumulative germination of *B. pilosa* seeds over 14 days, with application of 0.75 wt% concentration *P. cerasoides* ALE on day 1. N=3.

4.2 Seedling growth

Experiment III - To determine the inhibitory effects of ALE on 2-month-old seedlings.

The optimized ALE concentration (0.75 and 1.25 wt%) for *C. odorata* and *B. pilosa*, respectively, both with and without sediment, did not significantly increase mean mortality of *C. odorata* compared with the control (P>0.05) (Figure 8).

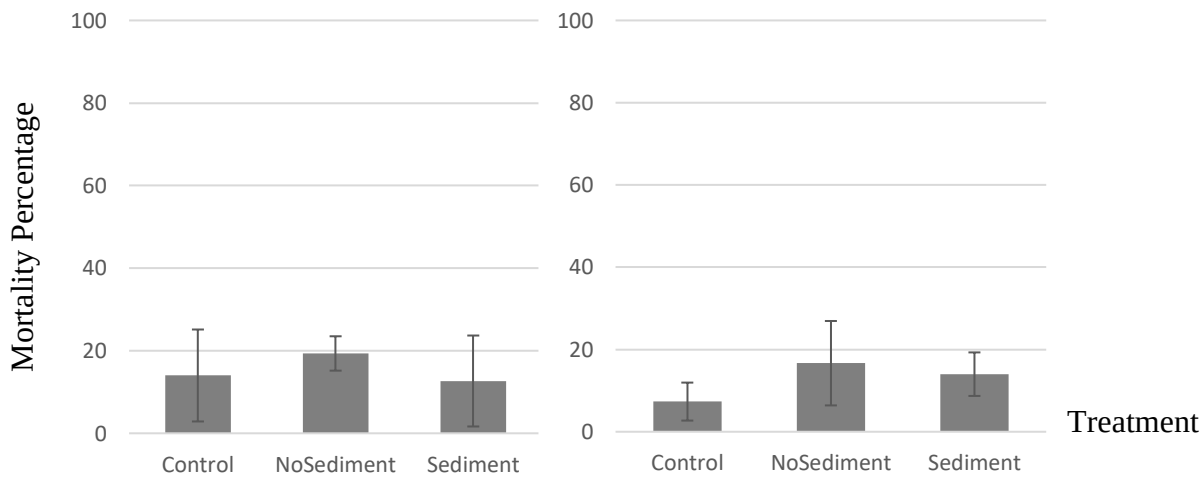


Figure 8 Mortality of *C. odorata* (left) and *B. pilosa* (right) seedlings over 31 days, with the application of *P. cerasoides* ALE at concentrations of 0.75 and 1.25 wt%, respectively, every 4 day. N=3. No significant difference among all treatments.

ALE treatment (both with and without sediment) had no significant effect on biomass per plant of both *C. odorata* and *B. pilosa* seedlings (P>0.05) compared with the control group (Figure 9). Mean biomass per *C. odorata* seedling in control groups was 0.24 ± 0.01 g compared with 0.23 ± 0.05 g for ALE treatment with sediment and 0.17 ± 0.02 g without. The mean biomass per seedling of *B. pilosa* in control groups was 0.57 ± 0.01 g compared with 0.44 ± 0.07 g for ALE-treated seedlings with sediment and 0.64 ± 0.13 g without.

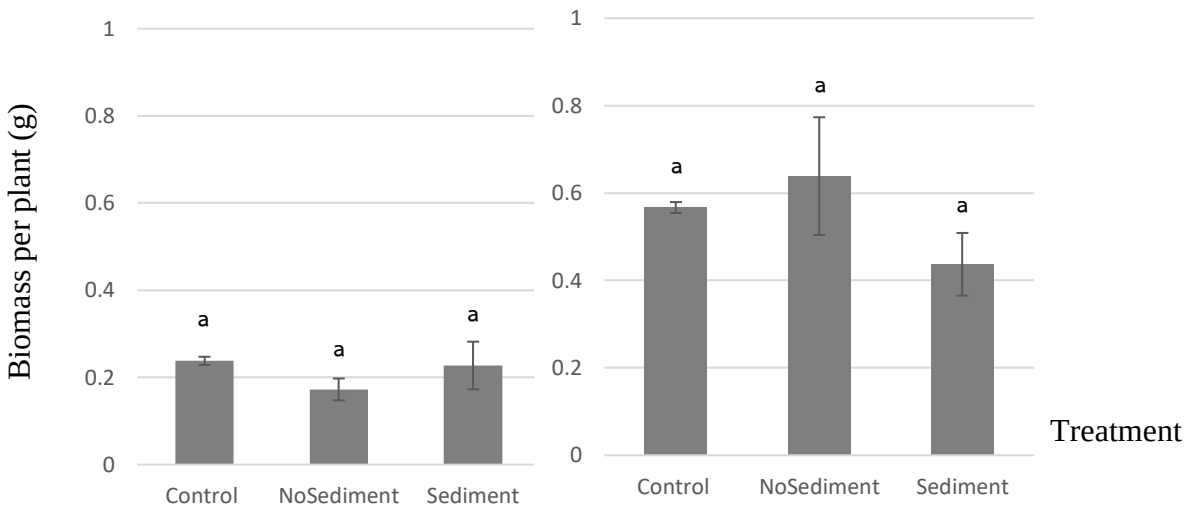


Figure 9 Biomass per plant of *C. odorata* (left) and *B. pilosa* (right) seedlings after 31 days, with the application of *P. cerasoides* ALE at concentrations of 0.75 and 1.25 wt%, respectively, every 4 day. N=3. Differences among treatments were all insignificant (P>0.05). Error Bars indicate standard deviation.

Experiment IV - To determine the inhibitory effects on seedling development stage.

When testing seedlings of 3 sizes with the high-dose treatment (4 times the optimal concentration with sediment) mortality was generally very low (over 15 days) and differences among treatments were insignificant, except for 1-node *C. odorata* seedlings. The control suffered high mortality (80%), which the ALE treatment significantly reduced ($P \leq 9.13 \times 10^{-3}$) by about 60%, contrary to expectations. The acid treatment had no effect (Figure 10).

The main cause of death of 1-node *C. odorata* seedlings in the control group was damping off disease, which raises the possibility that ALE might inhibit growth of the fungi that cause damping off disease.

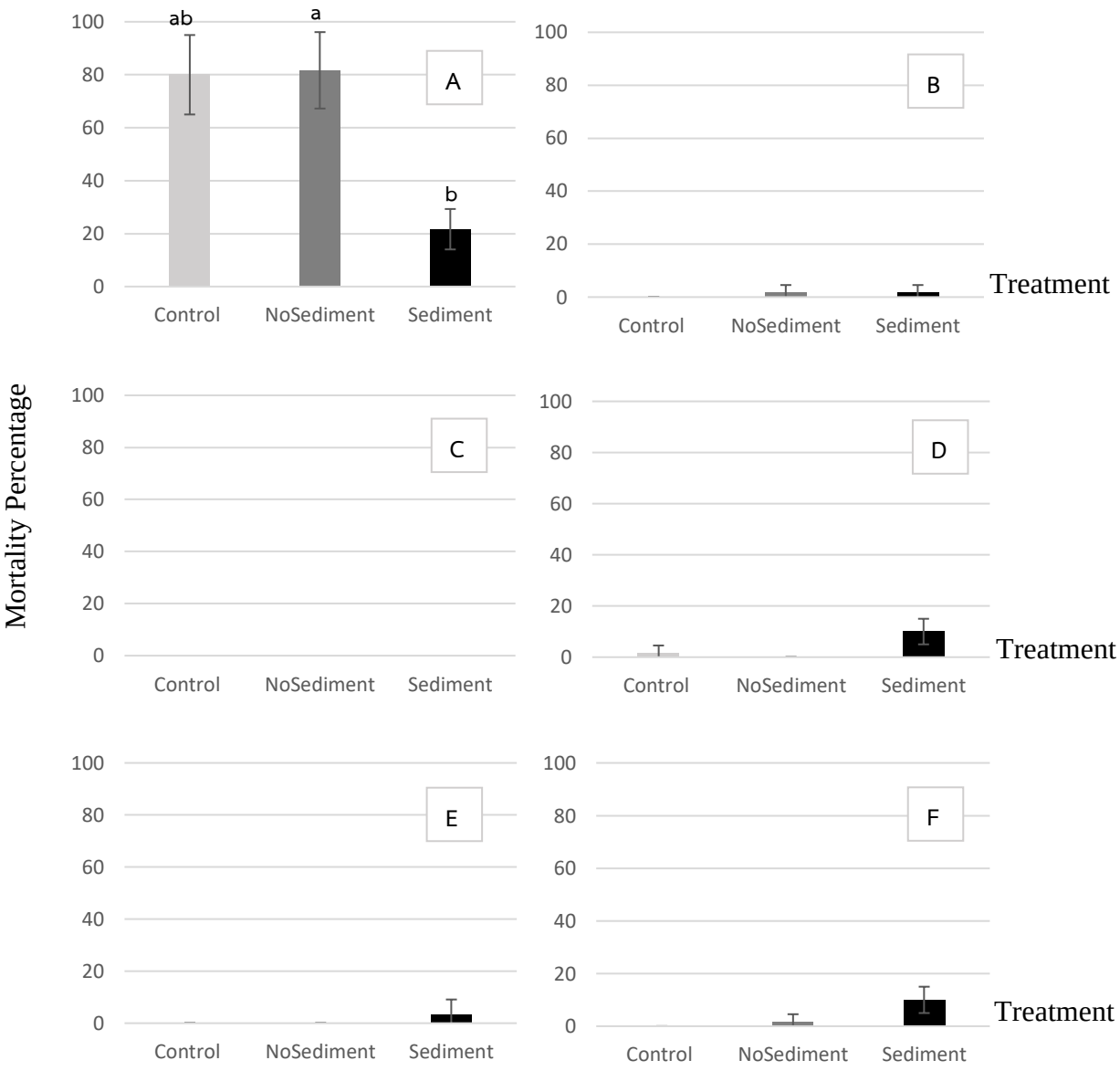


Figure 10 Mortality of *C. odorata* seedlings (left) and *B. pilosa* seedlings (right) over 15 days, with *P. cerasoides* ALE of 3.00 and 5.00 wt%, respectively, applied every 4th day; 1-node seedlings (A and B), 3-node seedlings (C and D) and 5-node seedlings (E and F). Differences among treatment not significant except for (A) ($P>0.05$). N=3.

The high-dose ALE-with-sediment treatment reduced mean biomass of *C. odorata* seedlings, compared with the control group. The reduction was statistically significant for the largest 5-node seedlings over 15 days ($P \leq 1.55 \times 10^{-2}$), whilst acidic treatment had no significant effect on biomass at all stages of development (Figure 11).

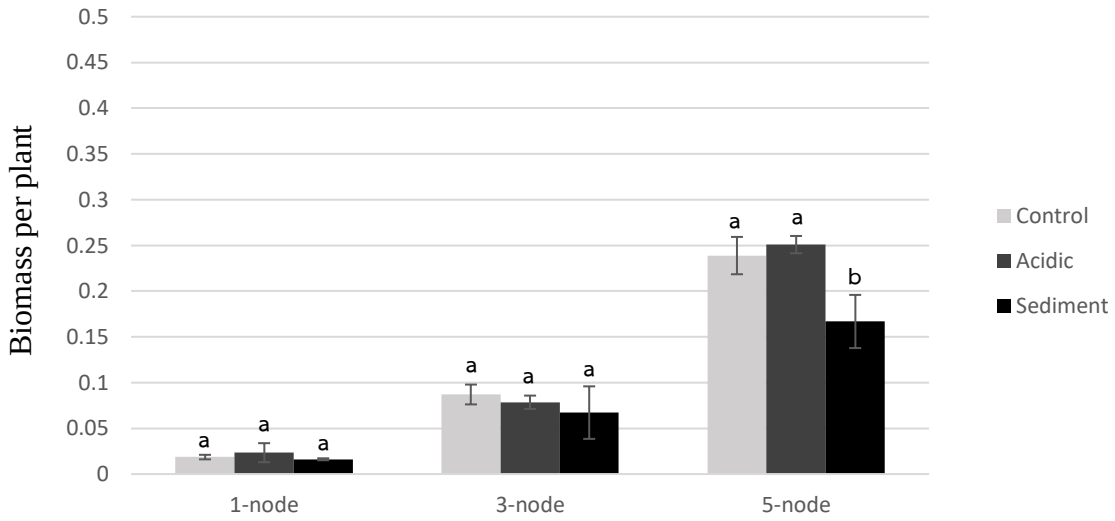


Figure 11 Biomass of *C. odorata* seedlings after 15 days, with the application of 3.00 wt% *P. cerasoides* ALE, every 4th day. Columns within size classes, not sharing the same superscript, are significantly different (P>0.05). N=3.

Neither the ALE treatment nor the acid treatment had any significant effects on biomass of *B pilosa* seedlings, at all stages of development (Figure 12).

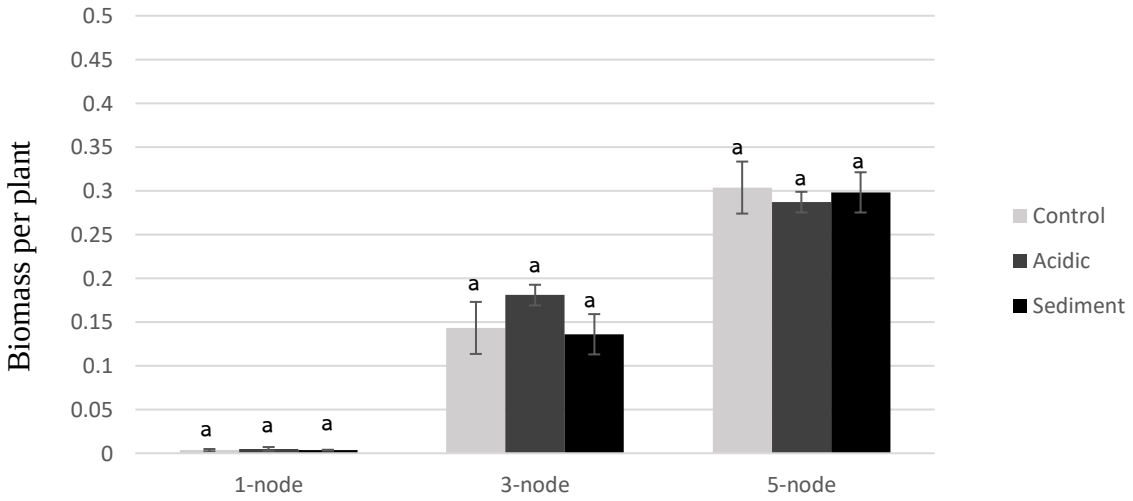


Figure 12 Biomass per plant of *B. pilosa* seedlings after 15 days, with the application of 5.00 wt% concentration *P. cerasoides* ALE, every 4th day. The same letter indicates no significant difference (P>0.05).

The effects of both ALE and acidic treatments on mean height of *C. odorata* and *B. pilosa*, seedlings at all stages of development, after 15 days were not significant (P>0.05), compared with the control (Figures 13-14), although mean heights of *C. odorata* seedlings, treated with ALE, at all stages of development, were consistently slightly lowered (Figure 13) and 23% lower than the control at the 5-node stage.

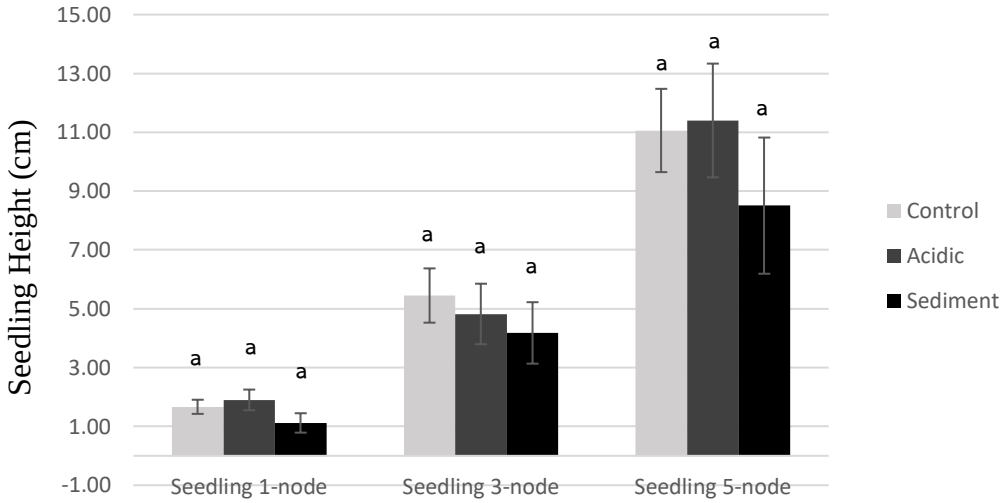


Figure 13 Seedling height of *C. odorata* seedlings after 15 days, with the application of 3.00 wt% concentration *P. cerasoides* ALE, every 4th day. No significant difference among treatments with development cases (P>0.05). N=3.

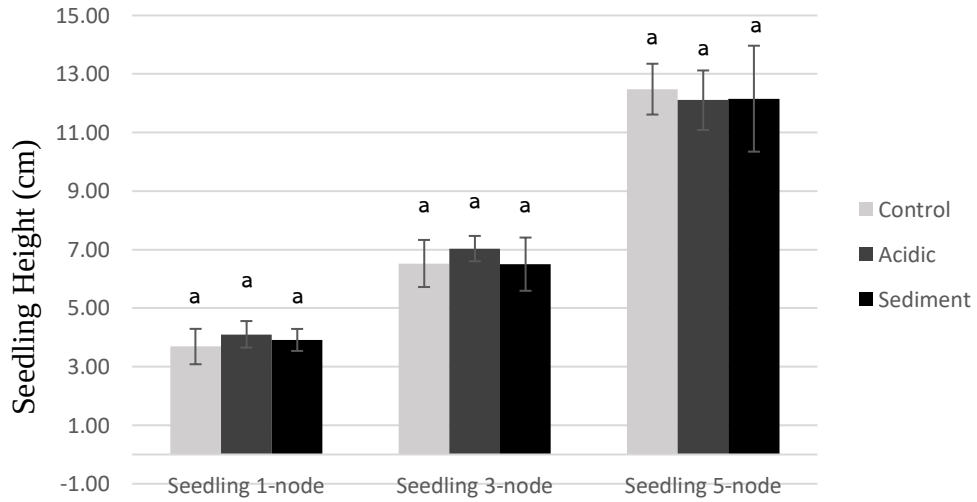


Figure 14 Seedling height of *B. pilosa* seedlings after 15 days, with the application of 5.00 wt% concentrations *P. cerasoides* ALE, every 4th day. The same letter indicates no significant difference (P>0.05). N=3.

Chapter 5

Discussion

P. cerasoides ALE reduced germination of both *B. pilosa* and *C. odorata* substantially and significantly, but had only a minor effect on seedlings mortality and biomass of both weed species.

5.1 Seed germination

The inhibitory effects of *P. cerasoides* on weed seed germination differed markedly between the weed species that were tested and was strongly dose-dependent. The effect was not due to the low p_H of the ALE and was therefore most likely due to allelochemicals in the extract. Retention of sediment increased the inhibitory potency of the ALE.

P cerasoides ALE brought about almost complete inhibition of germination of *C. odorata* seeds at 0.75 wt% concentration (the lowest concentration in the experiment). Substantial inhibition of *B. pilosa* seed required a much higher concentration - 1.25 wt% which was twice that required to inhibit germination of *C. odorata* seeds. The inhibitory effect increased in magnitude markedly, with increasing ALE concentration. Belel & Belel (2015), reported similar results – with the inhibitory effects of nutgrass (*Cyperus tuberosus*) leaves the germination of cowpea (*Vigna unguiculata* (L.) Walp) being proportional to the extract concentration. Various authors have reported that the inhibitory effects of allelopathic plant extracts on seed germination varies among target plant species (Chon et al., 2005; Hong et al., 2003; Cummings et al., 2012), as was shown with *C. odorata* and *B. pilosa* in the present study.

The shortest germination experiment was terminated at 9 days, because the ALE, which was applied every other day, started to ferment inside the petri-dishes. The sediment turned brown and emitted an odor, which may affect to germinated seed apart from allelochemicals. This result agrees with Ma (2019), who reported that ethanol fermentation of rice straw affect tomato productivity act as plant growth promoter. On the other hand, the second germination experiment was terminated at 14 days, because the germination per cent had become stable, following application of ALE on the first day only.

The second experiment showed that for some of the seeds the inhibitory effects of the ALE was temporary, since germination was delayed but not completely prevented. ALE also decreased seed viability germination percent was significantly lowered for both weed species. This result agrees with Khan (2011), who found that the allelopathic effect of *Rhazya stricta* leaves significantly decreased seed viability by inhibiting radical growth of *Zea mays*. Roots are more sensitive to allelopathic extracts than other parts of the plant (Jalata et al., 2005).

The inhibitory effect of ALE on seed germination was almost certainly due to allelochemicals rather than other factors. The inhibitory effect of ALE did not depend on its acidity, since acid controls had no effect on seed germination of both weed species. Önen (2018) suggested that acidity affects *Sicyos angulatus* seed germination, but interactions were not significant among populations. Furthermore, the effect was not due to the physical effects of particulate matter coating the seeds because ALE without sediment also had an inhibitory effect. However, the effect was stronger with sediment, probably because allelochemicals continued to leach out from the particles throughout the experiment. Consequently, to control weed seed germination, ALE with sediment is recommended. Furthermore, filtering out the sediment takes time and increases preparation costs.

Radhakrishnan (2018) reported that allelochemicals, when absorbed by weed seeds, damage cell membranes, DNA, mitosis, amylase activity and other biochemical processes which delay or inhibit seed germination.

For convenience, seed germination trials were carried out in petri dishes. However, this techniques has been criticized, e.g. by Csiszár (2014) who reported that seed germination experiments in petri dishes can greatly overestimate allelopathic effects, compared to more realistic field conditions, as was shown with *C. odorata* seeds in the present study.

5.2 Seedling experiments

Although optimized ALE concentration did not significantly increase mortality and biomass of both *C. odorata* and *B. pilosa* 2-month-old seedlings within 31 days, the increased mortality of *C. odorata* seedlings was getting larger by the end of the experiment and the effect may have become significant had the experiment been run for longer. No similar effect was observed with *B. pilosa*.

The lack of significant effect may have been due to various factors that broke down the allelochemicals, such as soil (Anaya, 1999; Mishra et al., 2012; Li et al., 2015) or season (Ahmad, 2019). Barto & Cipollini (2009) also reported that the allelopathic potential of *Alliaria petiolate* leaf extract had short half-lives (the longest half-life was only 45.5 h), due to degradation of the compounds in the soil. In addition, because of uncontrolled factors within the soil, both abiotic factors and biotic factors during the experiment (Belz, 2007), may also degraded the allelochemicals them.

In the final experiment, ALE at concentration of 3.00 and 5.00 wt% on *C. odorata* and *B. pilosa*, respectively, had no significant effect on mortality, biomass per plant and seedling height at all seedling development stages. This result agrees with Cheng & Cheng (2015), who found that the inhibitory effect of ALE depended on the plant variety and species, not only development stage and environment factors.

C. odorata seedlings treated with ALE did not grow as much as the control/acid seedlings did. However, 15 days was not long enough for mortality to become significant, although the trend was towards increasing mortality of ALE treated seedlings and the effect may have become significant if the experiments had been run longer. Once again, retention of sediment had a greater inhibitory effect in seedlings, comapred with celar ALE, similarly to seed germination.

The possible inhibitory effects of ALE on 5-node seedlings, may have been due to sediment or/and allelochemicals blocking photosynthesis. This explanation is supported by Yu (2006), who found out allelochemicals were barriers to chlorophyll synthesis in eggplant seedlings. Furthermore, ALE sediment or/and allelochemicals may have changed the soil nutrient status, as proposed by Mohammadkhani and Servati (2017).

ALE may have inhibited growth of fungi that cause damping off disease in 1-node *C. odorata* due to retention of sediment from ALE treatment on soil surface. Many fungal diseases are soil-borne, as reported by Ampt (2019). A layer of ALE sediment covering the soil surface may have prevented infection of fungal spores into the seedlings. In addition, 1-node seedlings of *C. odorata* were at their most vulnerable stage, resulting in higher chances of death by disease.

In this study, the equipment used to apply ALE with sediment, could not do so evenly, since a sediment caused blockages. This may have had an effect on our seedling experiment. Apart from that, different locations of replication blocks (inside, near the edge and outside of the nursery roof), may have caused unnecessary variation among blocks, which resulted in insignificant mortality, biomass and height of both weed seedlings. Another source of error may have been the long time that ALE powder was kept inside the freezer, causing it to lose some of its allelopathic effects (N. Hong et al., 2003).

Since *P. cerasoides* ALE significantly inhibited seed germination but had little or no effect on seedling growth and mortality, it use on forest restoration projects will be limited to preventing regrowth of the weeds from the soil seed bank, following manual cutting of the weeds. At this stage, the treatment cannot be recommended for controlling the vegetative spread of weeds, because it is not worth enough in cost and it has limitation of inhibitory effect (efficient only on weed seed).

Chapter 6

Conclusions

Conclusion & Advices

1. *P. cerasoides* ALE strongly inhibited seed germination, but only slightly inhibited seedling survival and growth of *C. odorata* and *B. pilosa.*
2. Retaining sediment increases the potency of the ALE. Consequently equipment must have capacity to apply *P. cerasoides* ALE without becoming clogged.
3. Sediment particles might encourage weed growth by acting as fertilizer. Further experimentation is required.
4. Use of *P. cerasoides* ALE in the field may affect non-target species in the soil seed bank. So further testing under field conditions will be necessary.
5. The effects of environmental factors on the action of *P. cerasoides* ALE requires further experiments.

Boundaries

Some limitation of the project design are outlined below.

- All experiments were done in a nursery for rapid and low cost confirmation of presence or absence of allelopathic effects. Subsequent, field trials would be needed to determine the practical use of any such allelopathic effects.
- *Prunus cerasoides* is a deciduous tree, so we needed to collect mature leaves (towards the end of the rainy season) and store them in a freezer as a dried powder before being used in experiments. Such treatments may have affected concentrations of allelochemicals.
- Both of the weeds species, used for this project, grew rapidly and variably due to changes in the surrounding environment. The difference of air humidity, light period length of each month affected to weed quality. Thus, the surrounding environment's change become one of the factors in this experiment.

References

Ahmad, Rameez, Anzar A. Khuroo, Maroof Hamid, Akhtar H. Malik, and Irfan Rashid. 2019. Scale and Season Determine the Magnitude of Invasion Impacts on Plant Communities." *Flora* 260:151481.

Ampt, E. A., van Ruijven, J., Raaijmakers, J. M., Termorshuizen, A. J., & Mommer, L. (2019). Linking ecology and plant pathology to unravel the importance of soil-borne fungal pathogens in species-rich grasslands. *European Journal of Plant Pathology*, *154*(1), 141–156.

Anaya, Ana Luisa. 1999. Allelopathy as a Tool in the Management of Biotic Resources in Agroecosystems. *Critical Reviews in Plant Sciences - CRIT REV PLANT SCI* 18:697–739.

Au, A. M. 2003. PESTICIDES AND HERBICIDES | Types, Uses, and Determination of Herbicides. *Encyclopedia of Food Sciences and Nutrition* 4483–87.

Baker, Leanne, Joseph Mudge, Dean Thompson, Jeff Houlahan, and Karen Kidd. 2014. The Direct and Indirect Effects of a Glyphosate-Based Herbicide and Nutrients on Chironomidae (Diptera) Emerging from Small Wetlands. *Environmental Toxicology and Chemistry*.

Barto, E., and Don Cipollini. 2009. Half-Lives and Field Soil Concentrations of Alliaria Petiolata Secondary Metabolites. *Chemosphere* 76:71–75.

Baylis, A. D. 2000. Why Glyphosate Is a Global Herbicide: Strengths, Weaknesses and Prospects. *Pest Management Science* 56:299–308.

Belel, Mustapha Dauda, and Rahimatu Dauda Belel. 2015. Allelopathic Effect of Leaf and Seed Extract of Nutgrass (Cyperus TubeBelel, M. D., & Belel, R. D. (2015). Allelopathic Effect of Leaf and Seed Extract of Nutgrass (Cyperus Tuberosus) on the Germination of Beans (Vigna Unguiculata (L.) Walp). Cogent Food . *Cogent Food & Agriculture* 1(1):1–7.

Bellon, T. (2019) In Roundup case, U.S. judge cuts $2 billion verdict against Bayer to $86 million. Neuters. Available at: https://www.reuters.com/article/us-bayer-glyphosate-lawsuit/in-roundup-case-u-s-judge-cuts-2-billion-verdict-against-bayer-to-86-million-idUSKCN1UL03G Accessed March 15, 2020

Belz, Regina. 2007. Allelopathy in Crop/Weed Interactions - An Update. *Pest Management Science* 63:308–26.

Bhatt, B. P., and N. P. Todaria. 1990. Studies on the Allelopathic Effects of Some Agroforestry Tree Crops of Garhwal Himalaya. *Agroforestry Systems* 12(3):251–55.

de Brito Rodrigues, Laís, Gessyca Gonçalves Costa, Emanoela Lundgren Thá, Lucas Rafael da Silva, Rhaul de Oliveira, Daniela Morais Leme, Marta Margarete Cestari, Cesar Koppe Grisolia, Marize Campos Valadares, and Gisele Augusto Rodrigues de Oliveira. 2019. Impact of the Glyphosate-Based Commercial Herbicide, Its Components and Its Metabolite AMPA on Non-Target Aquatic Organisms. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 842:94–101.

Cheng, Fang, and Zhihui Cheng. 2015. Research Progress on the Use of Plant Allelopathy in Agriculture and the Physiological and Ecological Mechanisms of Allelopathy. *Frontiers in Plant Science* 6 (NOVEMBER):1–16.

Chengxu, Wang, Zhu Mingxing, Chen Xuhui, and Qu Bo. 2011. Review on Allelopathy of Exotic Invasive Plants. *Procedia Engineering* 18:240–46.

Chon, Sang-Uk, Hong-Gi Jang, Doochul Kim, Yonjung Kim, and Hee-Ock Boo. 2005. Allelopathic Potential in Lettuce (Lactuca Sativa L.) Plants. *Scientia Horticulturae - SCI HORT-AMSTERDAM* 106:309–17.

Cressey, D. (2015). Widely used herbicide linked to cancer. Nature, International weekly journal of science. Available at: https://www.nature.com/news/widely-used-herbicide-linked-to-cancer-1.17181 Accessed March 15, 2020

Csiszár, Ágnes. 2014. Study on Allelopathic Potential of Some Invasive and Potentially Invasive Neophytes.

Cummings, Justin A., Ingrid M. Parker, and Gregory S. Gilbert. 2012. Allelopathy: A Tool for Weed Management in Forest Restoration. *Plant Ecology* 213(12):1975–89.

Elliott, S., D. Blakesley, and K. Hardwick. (2013). Restoring tropical forests: a practical guide. Royal Botanic Gardens, Kew, UK.

Elliott, Stephen. 2016. The Potential for Automating Assisted Natural Regeneration of Tropical Forest Ecosystems. *Biotropica* 48(6):825–33.

Elliott, Stephen, Cherdsak Kuarak, Puttipong Navakitbumrung, Sudarat Zangkum, Vilaiwan Anusarnsunthorn, and David Blakesley. 2002. Propagating Framework Trees to Restore Seasonally Dry Tropical Forest in Northern Thailand. *New Forests* 23(1):63–70.

Elliott, Stephen, Puttipong Navakitbumrung, Cherdsak Kuarak, Sudarat Zangkum, Vilaiwan Anusarnsunthorn, and David Blakesley. 2003. Selecting Framework Tree Species for Restoring Seasonally Dry Tropical Forests in Northern Thailand Based on Field Performance. *Forest Ecology and Management* 184(1–3):177–91.

Forest Restoration Research Unit, 2005. How to Plant a Forest: The Principles and Practice of Restoring Tropical Forests. Biology Department, Science Faculty, Chiang Mai University Thailand.

Global Invasive Species Database (GISD) (2015) Species profile Bidens pilosa and Chromoleana odorata. Available at: http://www.iucngisd.org/gisd/. Accessed November 20, 2019

Gupta, P. K. 2011. Herbicides and Fungicides. *Reproductive and Developmental Toxicology* 503–21.

Hardwick, Kate, John Healey, Stephen Elliott, Nancy Garwood, and Vilaiwan Anusarnsunthorn. 1997. Understanding and Assisting Natural Regeneration Processes in Degraded Seasonal Evergreen Forests in Northern Thailand. *Forest Ecology and Management* 99(1–2):203–14.

Harper, F. 1982. Weed–Crop Competition – A Review. By Robert L. Zimdahl. Oregon: International Plant Protection Center (1980), Pp. 195, US$9.00. *Experimental Agriculture* 18(1):101.

Hong, Nguyen Huu, Tran Dang Xuan, Tsuzuki Eiji, Terao Hiroyuki, Matsuo Mitsuhiro, and Tran Dang Khanh. 2003. Screening for Allelopathic Potential of Higher Plants from Southeast Asia. *Crop Protection* 22(6):829–36.

Is, W. H. Y., There Concern, and Where D. O. Epa. 2016. May 2015 FACT SHEET GLYPHOSATE AND CANCER RISK : FREQUENTLY ASKED QUESTIONS. (May 2015).

Jalata, Mulatu, Gezahegn Yadessa, and Befekadu Bulti. 2005. Allelopathic Effects of Parthenium Hysterophorus Extracts on Seed Germination and Seedling Growth of Lettuce. *Tropical Science* 45:159–62.

Jang, Ga Hee, Heon Woong Kim, Min Ki Lee, So Young Jeong, A. Ram Bak, Dong Jin Lee, and Jung Bong Kim. 2018. Characterization and Quantification of Flavonoid Glycosides in the Prunus Genus by UPLC-DAD-QTOF/MS. *Saudi Journal of Biological Sciences* 25(8):1622–31.

Jangwan, J. S., and R. P. Bahuguna. 1989. Puddumin-B, a New Flavanone Glycoside from Prunus Cerasoides. *International Journal of Crude Drug Research* 27(4):223–26.

Jangwan, J. S., and Naveen Kumar. 2015. Isolation and Characterization of New Flavonoid Glycoside from the Seeds of Prunus Cerasoides. 3(March 2009):20–22.

Joseph, N., N. Anjum, and Y. C. Tripathi. 2018. Prunus Cerasoides D. Don: A Review on Its Ethnomedicinal Uses, Phytochemistry and Pharmacology. *International Journal of Pharmaceutical Sciences Review and Research* 48(1):62–69.

Khan, Musharaf. 2011. Allelopathic Effects of Rhazya Stricta Decne on Seed Germination and Seedling Growth of Maize. *AFRICAN JOURNAL OF AGRICULTURAL RESEEARCH* 6.

Latif, Sajid, G. Chiapusio, and L. A. Weston. 2017. Allelopathy and the Role of Allelochemicals in Plant Defence. in *Advances in Botanical Research*.

Li, Yang-Ping, Yu-Long Feng, Chen Yajun, and Yao-Hua Tian. 2015. Soil Microbes Alleviate Allelopathy of Invasive Plants. *Chinese Science Bulletin* 60:1083–91.

Ma, X., Hu, J., Wang, X., Choi, S., Zhang, T.-A., Tsang, Y. F., & Gao, M.-T. (2019). An integrated strategy for the utilization of rice straw: Production of plant growth promoter followed by ethanol fermentation. *Process Safety and Environmental Protection*, *129*, 1–7. https://doi.org/10.1016/J.PSEP.2019.06.004

Mishra, Sandhya, Aradhana Mishra, Puneet Chauhan, Madhuree Kumari, Abhishek Niranjan, and C. Nautiyal. 2012. Pseudomonas Putida NBRIC19 Dihydrolipoamide Succinyltransferase (SucB) Gene Controls Degradation of Toxic Allelochemicals Produced by Parthenium Hysterophorus. *Journal of Applied Microbiology* 112:793–808.

Mohammadkhani, Nayer, and Moslem Servati. 2017. Nutrient Concentration in Wheat and Soil under Allelopathy Treatments. *Journal of Plant Research* 131.

Moini, H., A. Arroyo, J. Vaya, and L. Packer. 1999. Bioflavonoid Effects on the Mitochondrial Respiratory Electron Transport Chain and Cytochrome c Redox State. *Redox Report : Communications in Free Radical Research* 4(1–2):35–41.

Natural Resources Conservation Service Caribbean Area (USDA) (2007) *Chromolaena odorata* habitat description. Available at: https://www.nrcs.usda.gov/wps/portal /nrcs/detail/pr/newsroom/factsheets/?cid=nrcs141p2_037321.
Accessed November 20, 2019.

Önen, Huseyin, Shahid Farooq, Sonnur Tad, Cumali Özaslan, Hikmet Gunal, and Bhagirath S. Chauhan. 2018. The Influence of Environmental Factors on Germination of Burcucumber (Sicyos Angulatus) Seeds : Implications for Range Expansion and Management.

Pallewatta, Nirmalie, Jamie Reaser, and Alexis Gutierrez. 2003. *Invasive Alien Species in South-South East Asia, National Reports and Directory of Resources*.

Qiu, Shengnan, Huiyang Fu, Ruiying Zhou, Zheng Yang, Guangdong Bai, and Baoming Shi. 2020. Toxic Effects of Glyphosate on Intestinal Morphology, Antioxidant Capacity and Barrier Function in Weaned Piglets. *Ecotoxicology and Environmental Safety* 187:109846.

Radhakrishnan, Ramalingam, Abdulaziz A. Alqarawi, and Elsayed Fathi Abd_Allah. 2018. Bioherbicides: Current Knowledge on Weed Control Mechanism. *Ecotoxicology and Environmental Safety* 158:131–38.

Romano-Armada, Neli, María Amoroso, and Veronica Rajal. 2019. Construction of a Combined Soil Quality Indicator to Assess the Effect of Glyphosate Application. *Science of The Total Environment* 682:639–49.

Schenk, H. 2006. Root Competition: Beyond Resource Depletion. *Journal of Ecology* 94:725–39.

Shrivastava, S. P. 1982. A Flavanone Glycoside from Prunus Cerasoides. *Phytochemistry* 21(6):1464–65.

Soltys, Dorota, Urszula Krasuska, Renata Bogatek, and Agnieszka Gniazdowska. 2013. Allelochemicals as Bioherbicides — Present and Perspectives.

Townsend, Michelle, Connor Peck, Wei Meng, Matthew Heaton, Richard Robison, and Kim O'Neill. 2017. Evaluation of Various Glyphosate Concentrations on DNA Damage in Human Raji Cells and Its Impact on Cytotoxicity. *Regulatory Toxicology and Pharmacology* 85:79–85.

Tripathi, Yogesh Chandra. 2018. Prunus Cerasoides D. Don: A Review on Its Ethnomedicinal Uses, Phytochemistry and Pharmacology. (January).

Williams, Gary M., Robert Kroes, and Ian C. Munro. 2000. Safety Evaluation and Risk Assessment of the Herbicide Roundup and Its Active Ingredient, Glyphosate, for Humans. *Regulatory Toxicology and Pharmacology* 31(2):117–65.

xu, Jingwen, Shayna Smith, Gordon Smith, Weiqun Wang, and Yonghui Li. 2019. Glyphosate Contamination in Grains and Foods: An Overview. *Food Control* 106:106710.

Yu, Jihua, Yun Zhang, Caixia Niu, and Jianjian Li. 2006. Effects of Two Kinds of Allelochemicals on Photosynthesis and Chlorophyll Fluorescence Parameters of Solanum Melongena L. Seedlings. *Ying Yong Sheng Tai Xue Bao = The Journal of Applied Ecology / Zhongguo Sheng Tai Xue Xue Hui, Zhongguo Ke Xue Yuan Shenyang Ying Yong Sheng Tai Yan Jiu Suo Zhu Ban* 17:1629–32.

Appendices

Appendix A

Plant species in this research

***Bidens pilosa* L.**

Common Names: Black-Jack, Beggar-Ticks, Cobbler's Pegs, Sticky Beaks, Farmer's Friends and Spanish Needle.

Taxonomic classification Division : Magnoliophyta (Flowering plants)

Class : Magnoliopsida (Dicotyledons)

Order : Asterales

Family : Asteraceae

General Information:

Habit - erect, annual herb which stands from 0.3-2 m high

Stems - reddish tinged; 4-angled, simple, or branched.

Leaves - opposite, pinnately compound, broadly ovate leaves with 3-20 cm. long and 2.5-12 cm. wide. Each Leaf is ovate to lanceolate lobed or bi-lobed at the base with margins crenate-serrate and apices acute. Petioles 10-30 mm.

Flower - Head solitary or in paniculate cymes at the ends of the main stem and lateral branches. Its flower usually radiate, 5-12 mm broad with 2 rows of involucral bracts, outer ones 7-10, spathulate, reflexed at anthesis, 3-4 mm long, inner ones ovate lanceolate; ray flowers absent or 4-8, sterile, corolla 7-15 mm long, white to yellow or pinkish, disk flowers with 3.5-5 mm long, yellow corolla.

Fruit/Seed - achenes which are black, 4-8 ribbed, linear, 6-16 mm long, with 2-3 retrorsely barbed bristles of 2-4 mm. long.

Habitats:

B. pilosa has capable of invading a vast range of habitats. It thrives in disturbed areas, high sunlight, and moderately dry soils including grassland, streamlines, roadsides, plantations areas, pasture and agriculture areas. It tolerates to droughts with a required annual rainfall range is 500-3500 mm , tolerant to a pH range of 4-9 and also high salinities of up to 100 mM NaCl. It prefers temperatures above 15°C and below 45°C, low to high altitudes of up to 3,600 m.

Distribution:

Tropical and subtropical regions.

***Chromoleana odorata* (L.) R.M.King & H.Rob.**

Common Names: Siam Weed, Christmas Bush, Devil Weed, Triffid, and Common Floss Flower,

Taxonomic classification Division : Magnoliophyta (Flowering plants)

Class : Magnoliopsida (Dicotyledons)

Order : Asterales

Family : Asteraceae

General Information:

Habit - a perennial herbaceous that forms dense tangled bushes 1.5-2.0 m. in tall. It occasionally reaches its maximum height of 6m (as a climber on other plants).

Stems - branch freely, with lateral branches in pairs from the axillary buds. The older stems are brown and woody near the base; tips and young shoots are green and succulent.

Leaves - opposite, which are flaccid-membranous, velvety-pubescent, deltoid-ovate, acute, 3-nerved, very coarsely toothed; blade mostly 5-12 cm. long and 3-6 cm. wide, capitula in sub-corymbose axillary and terminal clusters; peduncles 1-3cm long,; involucre of about 4-5 series of bracts, pale with green nerves, acute, the lowest ones about 2 mm. long; hairy, glandular and give off a pungent, aromatic odor when crushed. Petiole slender, 1-1.5cm long.

Flower - heads which are borne in terminal corymbs of 20 to 60 heads on all stems and branches; form masses covering the whole surface of the bush; florets all alike (disc-florets), pale purple to dull off-white, the styles extending about 4 mm. beyond the apex of the involucre, spreading radiate; florets about 20-30 or a few more, 10-12 mm. long; corolla slender trumpet form; pappus of dull white hairs 5mm long.

Fruit/seed – small, achenes with 3-5mm long and 1 mm. wide.

Habitats:

C. odorata grows on a wide range of soils. Most abundantly appears on the edge of forested areas than under story shade. In shady areas it becomes etiolated and behaves as a creeper, growing on other plants; altitudes up to 1,000 m.

Distribution:

Native to tropical South America north to Mexico and to the Caribbean Islands. Then, widespread to Tropical and subtropical regions.

***Prunus cerasoides* Buch.-Ham. ex D.Don**

Common Names: Padam, Wild Himalayan Cherry and Dwarf Cherry

Taxonomic classification Division : Magnoliophyta (Flowering plants)

Class : Magnoliopsida (Dicotyledons)

Order : Rosales

Family : Rosaceae (Rose family)

General Information:

Habit - medium sized deciduous plant, up to 10 m high.

Stem - reddish brown to grey or dark brown with circular strips (pustular-lenticels), outer layer thin and crack horizontally with age.

Leaves - elliptic or ovate-lanceolate, apex acuminate, both surfaces glabrous, dark glossy, serrate with toothed margin; stipules long; conduplicate in bud. Petioles 1.2-2 cm. long.

Flowers - pinkish white or crimson, appearing before the leaves in umbellate fascicles and are the rich sources of nectar and pollen for bees; pedicels 0.5-2cm long. Calyx is bell shaped, 5-lobed, ovate-acute. It flowers in autumn and winter. The flowers generally appearing on bare branches, or with young leaves.

Fruit/Seed - yellow, maturing to red, ovoid shape, supported by base of calyx tube and contain one large seed.

Habitats:

It grows at evergreen-deciduous forest, evergreen-pine forest and deciduous forest, altitudes of 1200-2400 m. (3, 900-7, 900 ft.) above sea level.

Distribution:

Himalayas, from Himachal Pradesh in North-central India to Sikkim, Burma, Nepal, Bhutan, Myanmar, West China and Thailand.

Figure 15 *C. odorata* and *B. pilosa* seedlings.

Figure 16 *P. cerasoides* flower (left), bark (middle) and leaves (right).

Appendix B

Picture about the Experiments

Figure 18 Mature, dark green leaves *P. cerasoides* were dried for 3 days at room temperature

Figure 19 Germinated *C. odorata* and *B. pilosa* seeds.

Figure 20 Various concentrations *P. cerasoides* ALE powder, before mixing with water (left) and after mixing of water (right).

Figure 21 *B. pilosa* seeds treated with 0.75, 1.25, 2.50 and 5.00 wt% *P. cerasoides* ALE, with and without sediment (left), compared with control and acidic treatment, respectively (right).

Figure 22 Experimental Design of *B. pilosa* (top row) and *C. odorata* (bottom row) seedlings, located inside (left), near the edge (middle) and outside of the nursery roof (right).

Figure 23 Comparison of *C. odorata* seedlings from control, acidic and ALE treatment, respectively, at the end of Experiment III (after 31 days).

Figure 24 Comparison of *B. pilosa* seedlings from control, acidic and ALE treatment, respectively, at the end of Experiment III (after 31 days).

Appendix C

Seed germination and seedling growth data

Table 2 Percent of seed germination of *C. odorata* after 9 days

Replication	Seed germination (%)									
	Control	Acidic	0.75 NS	0.75 S	1.25 NS	1.25 S	2.50 NS	2.50 S	5.00 NS	5.00 S
R1	44	50	6	4	0	0	0	0	0	0
R2	34	50	0	4	0	0	0	0	0	0
R3	58	48	0	0	0	0	0	0	0	0
Mean	45.3	49.3	2.0	2.7	0.0	0.0	0.0	0.0	0.0	0.0
SD	12.05543	1.154701	3.464102	2.309401	0	0	0	0	0	0

Table 3 Percent of seed germination of *B. pilosa* after 9 days

Replication	Seed germination (%)									
	Control	Acidic	0.75 NS	0.75 S	1.25 NS	1.25 S	2.50 NS	2.50 S	5.00 NS	5.00 S
R1	78	90	82	50	70	40	24	4	2	0
R2	90	92	72	70	66	50	2	16	2	4
R3	90	90	86	58	72	38	2	10	0	2
Mean	86.0	90.7	80.0	59.3	69.3	42.7	9.3	10.0	1.3	2.0
SD	6.9	1.2	7.2	10.1	3.1	6.4	12.7	6.0	1.2	2.0

Table 4 Cumulative germination of *C. odorata* seeds over 14 days

Day	No. of seed germination								
	Control			Particle			Non-Particle		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
1	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0
4	2	3	4	0	0	0	0	0	0
5	3	7	8	0	0	0	0	1	0
6	8	10	19	0	0	0	0	1	1
7	11	12	21	0	0	0	0	1	1
8	14	15	24	1	2	1	1	3	2
9	15	20	24	4	2	2	3	4	2
10	17	22	26	7	3	5	4	5	2
11	17	22	26	7	5	7	5	5	3
12	17	24	26	8	7	8	6	8	6
13	17	24	26	8	7	8	6	8	6
14	17	24	26	8	7	8	6	8	6

Table 5 Percent of seed germination of *C. odorata* after 14 days

Replication	Seed germination (%)		
	Control	0.75 NS	0.75 S
R1	34	12	16
R2	48	16	14
R3	52	12	16
Mean	44.66667	13.33333	15.33333
SD	9.451631	2.309401	1.154701

Table 6 Cumulative germination of *B. pilosa* seeds over 14 days

Day	No. of seed germination								
	Control			Particle			Non-Particle		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
1	0	0	0	0	0	0	0	0	0
2	3	6	5	0	0	0	0	0	0
3	26	22	12	0	0	0	0	0	0
4	35	31	27	0	0	0	1	2	5
5	40	32	34	5	6	1	3	14	15
6	42	35	39	11	13	12	17	19	18
7	44	38	44	25	18	19	21	25	25
8	44	40	44	29	23	22	33	34	31
9	44	42	45	30	24	22	35	36	34
10	44	42	46	30	24	22	38	38	37
11	44	42	46	30	24	22	38	38	37
12	44	42	47	31	24	22	38	38	37
13	44	42	47	32	24	22	38	39	37
14	44	42	47	32	24	22	38	39	37

Table 7 Percent of seed germination of *B. pilosa* after 14 days

Replication	Seed germination (%)		
	Control	0.75 NS	0.75 S
R1	34	12	16
R2	48	16	14
R3	52	12	16
Mean	44.66667	13.33333	15.33333
SD	9.451631	2.309401	1.154701

Table 8 Percent mortality of *C. odorata* seedlings after 31 days

Replication	Seedling mortality (%)		
	Control	No Sediment	Sediment
R1	10	30	18
R2	2	10	16
R3	10	16	8
Mean	7.333333	18.66667	14
SD	4.618802	10.2632	5.291503

Table 9 Percent mortality of *B. pilosa* seedlings after 31 days

Replication	Seedling mortality (%)		
	Control	No Sediment	Sediment
R1	12	18	12
R2	26	24	24
R3	4	16	2
Mean	14	19.33333	12.66667
SD	11.13553	4.163332	11.01514

Table 10 Biomass per plant of *C. odorata* seedlings after 31 days

Replication	Biomass per plant (g)		
	Control	NoSediment	Sediment
R1	0.248889	0.201053	0.196341
R2	0.232245	0.154	0.290476
R3	0.233111	0.162619	0.195435
Mean	0.238082	0.172557	0.227417
SD	0.009369	0.025051	0.054612

Table 11 Biomass per plant of *B. pilosa* seedlings after 31 days

Replication	Biomass per plant (g)		
	Control	No Sediment	Sediment
R1	0.577727	0.579512	0.435227
R2	0.553243	0.543947	0.509474
R3	0.569583	0.792857	0.366122
Mean	0.566851	0.638772	0.436941
SD	0.012469	0.134621	0.071691

Table 12 Percent mortality of *C. odorata* in each development stage seedlings after 15 days

Replication	Seedling mortality (%)								
	1-node seedling			3-node seedling			5-node seedling		
	Control	No Sediment	Sediment	Control	No Sediment	Sediment	Control	No Sediment	Sediment
R1	0	0	5	5	0	10	5	0	10
R2	0	5	0	0	0	5	0	0	5
R3	0	0	0	0	0	15	0	0	15
Mean	0	1.666667	1.666667	1.666667	0	10	1.666667	0	10
SD	0	2.886751	2.886751	2.886751	0	5	2.886751	0	5

Table 13 Percent mortality of *B. pilosa* in each development stage seedlings after 15 days

Replication	Seedling mortality (%)								
	1-node seedling			3-node seedling			5-node seedling		
	Control	No Sediment	Sediment	Control	No Sediment	Sediment	Control	No Sediment	Sediment
R1	95	90	30	0	0	0	0	0	0
R2	65	65	15	0	0	0	0	0	0
R3	80	90	20	0	0	0	0	0	10
Mean	80	81.66667	21.66667	0	0	0	0	0	3.333333
SD	15	14.43376	7.637626	0	0	0	0	0	5.773503

Table 14 Biomass per plant of *C. odorata* in each development stage seedlings after 15 days

Replication	Biomass per plant (g)								
	1-node seedling			3-node seedling			5-node seedling		
	Control	Acidic	Sediment	Control	Acidic	Sediment	Control	Acidic	Sediment
R1	0.02	0.015	0.016429	0.094	0.0855	0.034	0.231	0.2435	0.199
R2	0.015714	0.02	0.017059	0.0925	0.071	0.0835	0.2235	0.2475	0.1425
R3	0.02	0.035	0.015	0.0745	0.079	0.084	0.262	0.2615	0.158889
Mean	0.018571	0.023333	0.016162	0.087	0.0785	0.067167	0.238833	0.250833	0.166796
SD	0.002474	0.010408	0.001055	0.010851	0.007263	0.028724	0.02041	0.009452	0.029068

Table 15 Biomass per plant of *B. pilosa* in each development stage seedlings after 15 days

Replication	Biomass per plant (g)								
	1-node seedling			3-node seedling			5-node seedling		
	Control	Acidic	Sediment	Control	Acidic	Sediment	Control	Acidic	Sediment
R1	0.003695	0.00406	0.003605	0.175042	0.18139	0.153311	0.23412	0.249945	0.208139
R2	0.002115	0.007368	0.00288	0.13855	0.192255	0.109905	0.35579	0.255942	0.286876
R3	0.004755	0.0035	0.003915	0.116145	0.168665	0.144806	0.321	0.355115	0.399416
Mean	0.003522	0.004976	0.003467	0.143246	0.18077	0.136007	0.303637	0.287001	0.298144
SD	0.001329	0.002091	0.000531	0.029728	0.011807	0.023002	0.062666	0.059065	0.096135

Table 16 Seedling height of *C. odorata* in each development stage after 15 days

Replication	Seedling height (cm)								
	1-node seedling			3-node seedling			5-node seedling		
	Control	Acidic	Sediment	Control	Acidic	Sediment	Control	Acidic	Sediment
R1	2.1	2.2	2.042857	5.835	4.89	4.355	10.96	11	9.945
R2	2.271429	2.483333	2.042857	5.094737	5.38	4.81	10.16	12.09	8.46
R3	2.575	2.9	2.1	5.4	4.088235	3.37	12.06	11.11	7.11
Mean	2.32	2.53	2.06	5.45	4.82	4.18	11.06	11.40	8.51
SD	0.240544	0.35211	0.032991	0.922585	1.028351	1.044776	1.415555	1.93347	2.3141

Table 17 Seedling height of *B. pilosa* in each development stage after 15 days

Replication	Seedling height (cm)								
	1-node seedling			1-node seedling			1-node seedling		
	Control	Acidic	Sediment	Control	Acidic	Sediment	Control	Acidic	Sediment
R1	3.63	3.615	4.136842	6.142105	7.48	6.144444	13.325	11.375	11.57778
R2	3.115	4.189474	3.48	5.985	7.01	5.826316	11.59	11.67368	10.70588
R3	4.32	4.51	4.125	7.45	6.615	7.535294	12.53	13.265	14.18421
Mean	3.69	4.10	3.91	6.53	7.04	6.50	12.48	12.10	12.16
SD	0.604614	0.453465	0.375856	0.804311	0.433042	0.908871	0.868509	1.016005	1.809809

Curriculum vitae

Author's name	Mister Punnat Changsalak
Date/Year of Birth	October 10, 1997
Education	2015 High school, Benchamarachuthit Chanthaburi School, Chanthaburi, Thailand
Scholarship	Development and Promotion of Science and Technology scholarship (DPST scholarship)
Address	51 M.6 Bang Kacha Sub-District, Muang District, Chanthaburi, 22000
E-mail	nchangsalak@gmail.com
Phone number	065-615-4944