이학석사 학위논문

Ecological study of *Vitex rotudifolia* community on Masian beach and its allelopathic influence

마시안해변 순비기나무 군락의 생태학적 연구와 알렐로패시적 영향

2021 년 2 월

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February 2021

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Abstract

Vitex rotundifolia is a sprawling shrub in the family of Verbenaceae and one of the representative coastal sand dune plants. It appears on the front and back of coastal sand dunes. It has been used as medicine and landscape plants worldwide due to various chemicals from it and attractive foliage and flower. Furthermore, as its deep root system contributes to stabilizing sand dunes, it has been suggested to be a rehabilitation plant for the degraded areas. Despite its usefulness, there are not enough ecological studies about *V. rotundifolia.* In this study, species within *V. rotundifolia* community were investigated in August on Masian beach where a coastal sand dune was with well-preserved vegetation. Species investigation was done by quadrat and soil samplings at depth of 0-5 cm and 5-10 cm were done randomly in each quadrat. Also, soil properties were measured to figure out the habitat condition of *V. rotundifolia* community and further, correlation with environmental factors and the structure of *V. rotundifolia* community was confirmed by NMDS. There were 12 species within the community, including *Lathyrus japonicus* and *Carex kobomugi.* The habitat of *V. rotundifolia* community is a typical coastal sand dune, with less soil moisture and high salinity. According to the result by NMDS, soil hydrophobicity and organic matter were significantly correlated to *V. rotundifolia* community structure. With these results, it was found that various coastal sand dune plants that appear on foredune and stabilized sand dune at the same time were within *V. rotundifolia* community, showing the community on Masian beach has complex vegetation and Masian beach is partially stabilized. Thus, *V.rotundifolia* community might be an indicator to show the degree of stability of dunes. Also, it was shown that its soil characteristics corresponded to the coastal sand dune environment, and among them, soil hydrophobicity and organic matter, which are known to be related to the dominant strategy of *V. rotundifolia,* have a significant correlation with the community structure. *V. rotudifolia* has various chemicals and some of them like phenolic and flavonoid compounds are known as allelochemicals. In this study, allelopathic influence of *V. rotundifolia* on other plants, especially coastal sand dune plants, was investigated with laboratory and in soil condition. *Lactuca sativa* var. *capitata,* the commonly used species for allelopathic experiment, *Oenothera odorata,* one of naturalized coastal sand dune plants, and *Calystegia soldanella,* one of the native coastal sand dune plants were studied to figure out allelopathic influence of *V. rotundifolia* through leachate with different concentrations, 0%, 10%, 50%, and 100%. In laboratory condition, the results showed there was no meaningful effect in terms of germination percentage but radicle elongation was stimulated more with increasing concentration. Also, in soil condition, *L. sativata* var. *capitata* with 50% leachate treatment showed the highest germination percentage. Their radicles got longer as the concentration of leachate increased. However, *O. odorata* and *C. soldanella* did not have significant differences among their results. Even though stimulation seems to affect other species positively, it would be deleterious to them in the long term, increasing seedling mortalities. With the present studies, it was found that *V. rotundifolia* has a relationship with its surroundings. The species composition of *V. rotudnifolia* community shows the state of coastal dune and its environmental factors like soil organic matters and soil hydrophobicity have a correlation with the community. Also, *V. rotundifolia* fruits might allelopathic influence on neighboring species even far from the community as they could spread easily by wind or current. These studies would be helpful to provide basic information for the management of *V. rotudnifolia* community and restoration of degraded coastal dune.

Keyword: *Vitex rotundifolia,* coastal sand dune, coastal sand dune plants, soil environment, allelopathy, leachate, Manhyungja, vegetation

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Chapter 1. General Introduction

1.1 Characteristics of *Vitex rotundifolia*

Vitex rotundifolia L. fil., also called beach vitex, is a deciduous sprawling shrub in the family of Verbenaceae (Lee, 1980; Hu et al., 2007b; Cousins et al., 2010a,b; 2017; Park&Park, 2001). Also, it is perennial and salt-tolerant so that it usually occurs in coastal sand dunes, both the front and back (Cousins et al., 2010b; Murren et al., 2014). It is an obligate beach-dune species and one of the representative coastal sand dune plants (Kim, 2005). With its deep root system, it has played a role as dune stabilizer. Also, its beautiful flower and foliage and fruits containing various chemical components have been used in various practical ways. It is native to tropical and temperate regions (latitudes ranging from 0° to 38°) including Korea, China, Japan, India, Taiwan, Thailand, Indonesia, Malaysia, Papua New Guinea, Philippines, Australia, Fiji, New Caledonia and Hawaii (Hu et al., 2007; True, 2009; Whitwell et al., 2016; Murren et al., 2014; Cousins et al., 2010a; Lee et al., 2007). Besides, it has been introduced in a lot of countries such as the United States, England and Germany (Cousins et al., 2017; Whitwell et al., 2016).

As it is distributed worldwide, there are various common names; round-leaved chaste tree (English) (Cousins et al., 2010a), dan ye man jing (Chinese), sunbiginamu (Korean), pohinahina (Hawaiian) (Bornhost&Rauch, 2003). It was reported that *V. rotundifolia* communities were located on dry, unstable, or semi-stable coastal sand dunes as well as near windbreak forest (Lee et al., 2007). *V. rotundifolia* appears along the coastlines of Korea; Goheung-gun, Yeosu-si, Wando-gun, Haenam-gun Jeollanam-do and Jeju at the southern coast, Gangneung-si, Goseong-gun, Donghae-si, Samcheok-si, Yangyang-gun Gangwon-do, Busan, Namhae-gun, Changwon-si Gyeongsangnam-do and Yeongdeok-gun, Ulleung-gun, Uljin-gun, Pohang-si

Gyeongsangbuk-do at the eastern coast and Ansan-si Gyeonggi-do, Ongjin-gun, Ganghwa-gun, Jung-gu Incheon, Muan-gun, Sinan-gun, Jindo-gun Jeollanam-do, Gunsan-si, Buan-gun Jeollabuk-do, Dangjin-gun, Boryeong-si, Seosan-si, Seocheon-gun, Taean-gun and Hongseong-gun Chungchangnam-do at the western coast (Kim et al., 2008; Lee et al., 2007; Kim&Park, 1998; Park et al., 2009; Shim et al., 2009; Jung&Kim, 2000; KOEM, 2018; 2019) (Figure 1-1). Even though there are numerous reports about habitats of *Vitex rotundifolia* in South Korea, a lot of habitats have been destroyed by development of coastal area and sand dune (Kim et al., 2008)

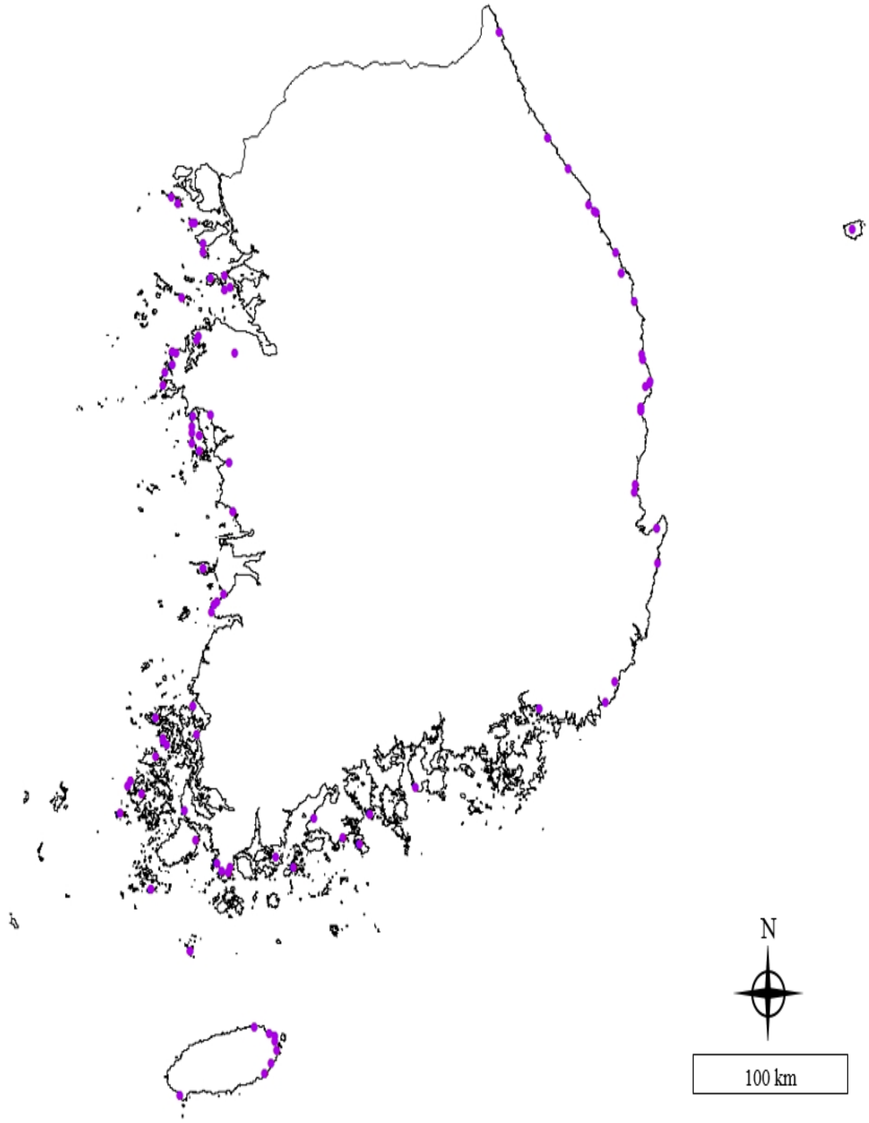

Figure 1-1. Distribution of *Vitex rotundifolia* in South Korea. Purple dots indicate the site where *V. rotudndifolia* was found. 98 sites, including Yeongjong-do Incheon, were known as the habitat of *V. rotundifolia*.

Young stems are square and green or purple, fleshy at the tips, and as the stems mature develop into round, brown, and woody. Branches from running stems are erect that grows 10 to 92 cm tall. Bark cracks and fissure with age (Cousins et al., 2017). It grows crawling on sandy land (Kim&Park, 1998) and spreads to about 10 m diameter, rooting at the nodes from long vegetative stems (Munir, 1987; Whitwell et al., 2016). Leaves are typically simple but can be palmately trifoliate or 2-lobed (Munir, 1987). Leaf blades are 2-5 cm long by 1.5-3.0 cm wide; its margin is entire and shape is round or obovate. It is coriaceous and there are white hair densely on the back of leaves (Lee, 1980; Park&Park, 2001). Leaves synchronously abscise during the winter in temperate region; but, *V. rotundifolia* is evergreen in tropical areas (Murren et al., 2014; Cousins et al., 2010a; Figure1-2).

Figure 1-2. Seasonal landscapes of *Vitex rotundifolia* community. (A) *V. rotundifolia* community in April, early spring. (B) Community in May, spring. (C) Community in August, summer. (D) Community in September, fall. (A), (B), (C) were photographed in 2020 and (D) was in 2019.

Flowers bloom from July to September, panicles are terminal and inflorescences measure 4 to 7 cm long (Lee, 1980). Calyx is cup-shaped and 4-4.5 cm long. Corollas are zygomorphic, purple to blue-purple, and short pedunculated. They are two lipped and funnel form (8mm in length) (Cousins et al., 2017; Munir, 1987). These flower and foliage are attractive so it is widely used as a landscaping plant and also for lei, a Hawaiian wreath, making (Whitwell et al., 2016; True, 2009; Bornhorst and Rauch, 2003; Cousins et al., 2010a; Raulston, 1993; Figure 1-3). Flies, butterflies, ants, honey bee and native bee are flower visitors and are likely to transfer pollen. Since anthers and stigmas of *V. rotundifolia* are separate from each other, restricting self-pollination, they are potential pollinators (Abe, 2006a, b; Cousins et al., 2017; Yeeh et al., 1996; Murren et al., 2014).

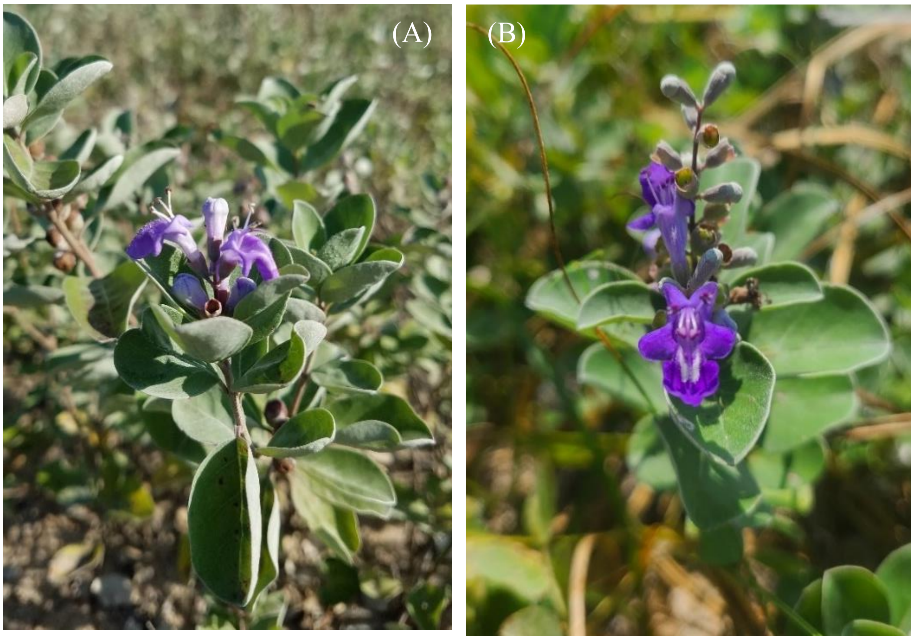

Figure 1-3. Flowers of *Vitex rotundifolia* (A) Lateral appearance of *V. rotundifolia* flower. Terminal panicles and cup-shaped calyx are shown. (B) Frontal appearance of the flower. It is two-lipped and zygomorphic with separate anthers and stigmas.

Fruits are green during expansion and then turn yellow and red before maturing to bluish black (Cousins et al., 2017). They are spherical and non-fleshy drupes which ripen on September and October (Whitwell et al., 2016; Cousins et al., 2010a). Their diameter is about 5 mm and they have waxy coating which allow them to resist water penetration (Cousins et al., 2009). They are really light, about 0.02 g per fruit (author's observation). There are 4 locules within fruits and each holds one seed; however, seeds are not always present in each compartment (Cousins et al., 2010b; True, 2009). BV produced 2,730 fruits/m^2 on average (Gresham&Neal, 2004). Fruits readily float on the water for several weeks. It was observed that fruits floated in the ocean and in beach drift (Munir, 1987; Cousins et al. 2010a). Many studies reported that there are a lot of types of chemical compounds like flavonoids, phenolic compounds and essential oils in extracts from fruits. These chemical compounds relate to various pharmacological and physiological activities of *V. rotundifolia* such as anti-mutant, anti-cancer, anti-allergic, anti-fungal, anti-oxidant, anti-malaria, antiprotozoal, vasorelaxant activity (Park et al., 2012; Ono et al., 1999; Miyazawa et al., 1995; Shin et al., 2000; Alam et al., 2002; Kawazoe et al., 2001; Kobayakawa et al., 2004; Lim et al., 2007; Gu et al., 2004; Hu et al., 2007b; Kim et al., 2011; Kupeli et al.,, 2006; Guerrero et al., 2002). In China and Japan, its fruit is herbal medicine to prevent and treat colds, headache, migraine, eye-pain, asthma, chronic bronchitis and gastrointestinal infections, including bacterial dysentery and diarrhea (Sun et al., 2019; Hu et al., 2007b; Ko et al., 2000). Also, in Korea, this fruit, called 'Man hyung ja' is used to relieve headache caused by upper respiratory infection and to treat various allergic diseases (Shin et al., 2000; Jang et al., 2002; Park et al., 2004). Lee et al. (2008) reported fruit and stem of *V. rotundifolia* contain high level of sugars, various amino acids and amino acid derivatives, essential amino acids, a lot of minerals like K, Ca and Mg. They also found that fruits contain taurine which is effective to fatigue recovery. As *V. rotundifolia* has high contents of various nutrients, it is expected to be functional food. Figure 1-4 shows the characteristics of fruits of *V. rotundifolia* described above.

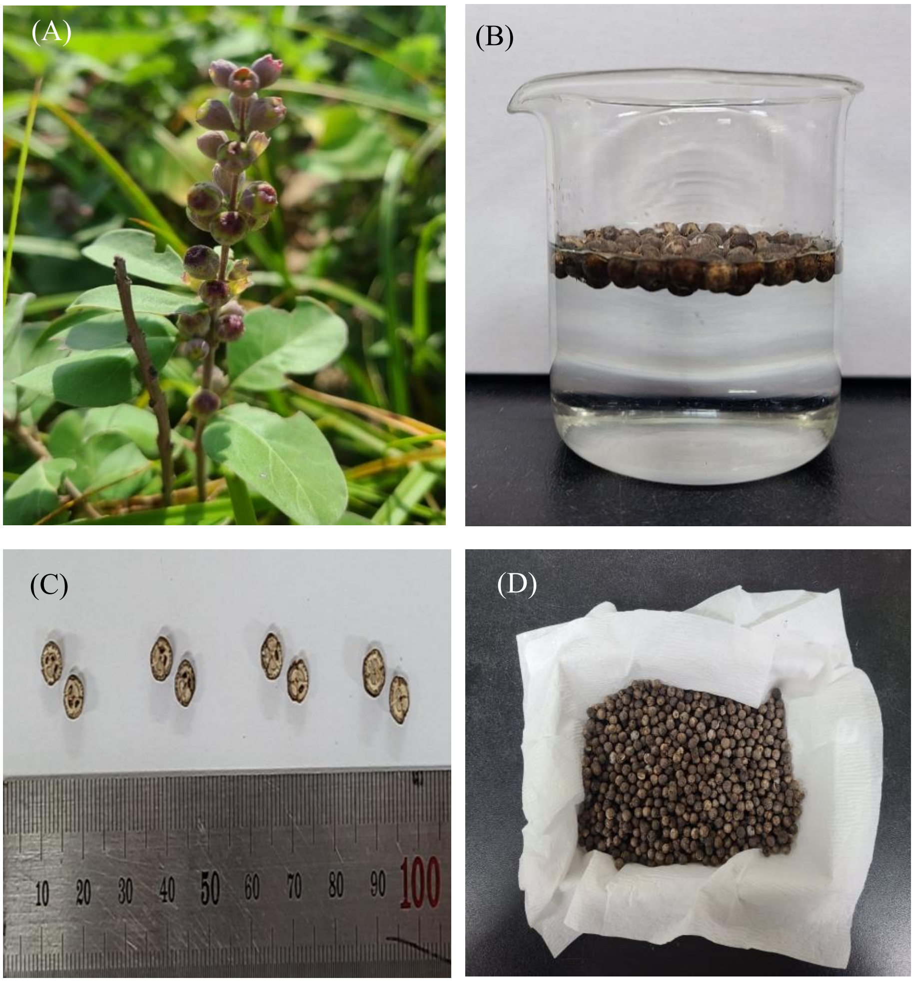

Figure 1-4. Fruits of *Vitex rotundifolia.* (A) Fresh fruits of *V. rotundifolia.* When they expand, their color is green. (B) Fruits could float on the water. (C) Cross sections of fruits. There are 4 locules in fruits which are non-fleshy and hard. Fruit can have 0 to 4 seeds within it. (D) Mature fruits of *V. rotundifolia.* Their color is brown or bluish black.

Fruits and leaves have unique and strong scent which is so aromatic that they have been used as bathing preparations for a long time (Park et al., 2012; Park et al., 2009; Cousins et al., 2017). Also, there are a waxy substance on the surface of leaves and fruits, making soil hydrophobic. Increasing soil hydrophobicity might be a competitive strategy of *V. rotundifolia* (Cousins et al., 2009; Whitwell et al., 2016). Many similar phenolic acid and flavonoid compounds from leaves and fruits have been shown to possess allelopathic qualities and to work as plant growth regulators (Correa et al., 2000; Wu et al., 2000; 2001; Cousins et al., 2017; Yoshioka et al., 2004; Shiwari et al., 2017).

V. rotundifolia has a deep root system at least 60 cm, preventing beach erosion so it has a considerable value as a beach-stabilizer (Munir, 1987; Kim, 2005; Gresham&Neal, 2004). Also, it is responsible for providing large amounts of water and preventing desiccation on the dune, allowing them survive well in harsh environment like coastal sand dunes (Munir, 1987; Cousins et al., 2009; 2017). Park and Kim (2004) revealed that *V. rotundifolia* could survive at soil with 5% moisture, showing that it is tolerant to drought, one of characteristics of coastal sand dune. They also found that the survival rate was high in the weathered granitic soil, red silt loam and sea sand. It showed that it can be well-grown and adapted in various conditions of nature, suggesting it is useful as an environmental afforestation plants (Park et al., 2009).

V. rotundifolia reproduces by seed (sexual reproduction) and by rooting at the nodes from long vegetative stems (asexual reproduction) (Whitwell et al., 2016). It usually propagates through long runner branches, growing at a rate of more than 3 m per year (Cousins et al., 2017). As it was reported that fruits float on river and ocean, it might spread out further by water dispersal (Munir, 1987; Cousins et al., 2017).

Seeds require stratification for germination, indicating they have physiological dormancy mechanism (Cousins et al., 2010b). Also, soaking period seems to be necessary for water to penetrate the hydrophobic exocarp (Cousins et al., 2009; 2010b). Even though germination rate is low, its fecundity and seed viability are high (Murren et al., 2014; Cousins et al., 2010a; 2010b). Park and Park (2001) reported that its germination rate is 30.3% in sea sand, showing high survivability of seedling in sea sand. After germination, small seedlings grow long roots and small shoots in the first season but they do not frequently survive the first year in the harsh dune environment (Cousins et al., 2010a; Cousins et al., 2017). Seed bank could be able to produce seedlings even 4 years after vegetation removal (Cousins et al., 2010b)

Also, its attractive foliage and flower and deep root system make it compelling landscape plants. It would serve to maintain dune stability by root and showcase its leaves and floral characteristics in the landscape (Kim&Park, 1998; Cousins et al., 2010a; 2017; Raulston, 1993). It was first introduced into the United States as early as 1955 and its hypothesized origin is Korea (Cousins et al., 2010a; Murren et al., 2014; Raulston, 1993). It was widely planted to stabilize frontal dunes along the east coast of the United States in the aftermath of Hurricane Hugo in 1989 (Cousins et al., 2010b; Murren et al., 2014; Gresham&Neal,2004). *V. rotundifolia* was also evaluated as a suitable species to form a vegetative belt on the outer fringe of a coastal forest since it was reported that ground coverage was achieved completely one year after planting (Gresham&Neal, 2004).

Vitex rotundifolia has been used in various ways. Its fruits have been used as oriental medicine to treat various illness in Korea, China and Japan for a long time. Also, it has been recommended to protect coastal sand dunes which have been in danger of being destructed by construction and various activities.

Even though *V. rotundifolia* has been considered valuable, there is no specific management or conservation plan for it. Furthermore, the habitats of *V. rotundifolia* have been continuously reduced in South Korea (Park et al., 2009). There are a lot of factors to threaten *V. rotundifolia* populations; the increased use of the plant as a medical resource, coastal overexploitation, environmental destruction like constructional and recreational developments. Considering the danger that *V. rotundifolia* has faced, China and Japan have recorded it in the List of the Important Wild Plants for Conservation (Sun et al., 2019).

1.2 Previous research on *Vitex rotundifolia*

V.rotundifolia has been used as medicine for hundreds of years in Asian countries, especially Korea, China and Japan (Cousins et al., 2017; Park et al., 2004; Ko et al., 2000; Lee et al., 2009; Jang et al., 2002; Hu et al., 2008). Many studies have been focused on the isolation of chemical compounds from its leaves, stem and fruits and the identification of its physiological and pharmacological activity such as antitumor, anti-allergic, anti-fungal, vasorelaxant effects. Various types of chemicals, such as flavonoid, phenolic compound and essential oil, have been isolated and their physiological effect have been studied. Hesperidin, *n*-hydrocarbons, polyoxygenated flavonoids like vitexicarpin, artemetin and luteolin, and vanillic acid were isolated and identified from fruits of *V. rotundifolia* (Kang et al., 1994).

Watanabe et al., (1995) isolated rotundial from the leaves of *V. rotundifolia* which has the mosquito repelling activity. This study compared the mosquito repelling activity of rotundifolial to that of DEET and the result showed that it is superior to DEET. According to Miyazawa et al. (1995), a compound in a methanol extract from *V. rotundifolia*, identified as (+)-polyalthic acid, has suppressive effect on the mutagenicity of Trp-P-1. Okuyama et al. (1998) reported that there are components having analgesic effects, relieving pain. Labdane-type diterpeneds and abietane-type diterpenoids were isolated from the fruits of *V. rotundifolia* and among them, ferruginol has antioxidant activity stronger than BHA (Ono et al., 1999; 2001; 2002). Shin et al., (2000) studied the effect of aqueous extract of *V. rotundifolia* fruits and their research showed that it has immediate-type allergic reactions. Ko et al. (2000) confirmed three polymethoxyflavonoids from the fruit of *V. rotundifolia* and its inhibitory activity against proliferation HL-60 cells, inducing apoptosis in human myeloid leukemia cells. Rotundifuran, one of labdane type diterpenes, was isolated from the fruit of *V. rotundifolia* and was considered as a potential chemo-preventative and chemotherapeutic agent them the next year.

Kawazoe et al., (2001) isolated nine phenylnaphthalene compounds such as vitrofolal A from the subterranean part of *V. rotundifolia* and confirmed that some of these compounds has antibacterial activity against methicillin-resistant *Staphylococcus aureus*. Also, 76 types of essential oils including monoterpene and sesquitepene were isolated and identified by Jang et al. (2002). Among these components, vitexicarpin, also called as casticin, is known to be able to block effects of histamine released from sensitized mast cells by stabilizing the mast cells membrane function (Alam et al., 2002). It also has an inhibitory effect on human lung cancer cells (PC-12) and human colon cancer cells (HCT116) (Ono et al., 2002). Kobayakawa et al., (2004) studied its antitumor activity, discovering the correlation of casticin with G2-M arrest. Haïdara et al., (2006) confirmed this correlation as well. Furthermore, Hu et al. (2007a) discovered that it reduced the abnormally high level of prolactin in the blood.

Yoshioka et al. (2004) revealed that phenolic and flavonoid compounds from fruits and leaves of *V. rotundifolia* are plant growth regulator, altering root growth. According to the study by Gresham and Neal (2004), its fresh leaf extract and litter extract and beach soil extract had a slight beneficial effect to radish sprout growth. Since there are many similar phenolic acid and flavonoid compounds possessing allelopathic qualities (Correa et al., 2000; Wu et al., 2001; 2002), it might be caused by allelopathy. Hu et al., (2007c; 2007d) found that essential oil from the fruits of *V. rotundifolia* shows estrogen-like biological activity, which may be helpful to regulate the hormone levels to treat premenstrual syndrome. Lee et al. (2008) analyzed the nutritional and functional materials from its fruit and stem. Not only nutrients but also biologically active substance were identified so that it has been evaluated as a source of functional food. In 2009, they also reported that extracts from the fruits has tyrosinase inhibition activities, indicating that the extract has an effectivity antioxidant activity.

Kim et al. (2011; 2014) isolated luteolin, vitexcarpin, artemetin, xanthotoxin, vitetrifolin F, vitetrifolin E and vitetrifolin D from extracts of *V. rotundifolia*. Xanthotoxin has vasorelaxant and antioxidant effect and luteolin has been well-known for its antioxidant activity. Lim et al., (2006) did a research on the luteolin-mediated regulation of cell cycle progression and apoptosis in the HT-29 human colon cancer cell line. It was reported that luteolin promotes both cell cycle arrest and apoptosis in this cell line, suggesting its antitumor activities. Park et al. (2012) isolated agnuside, chrysosplenol B, artemetin and studied the antifungal activities of these three compounds. They figured out these chemicals from fruits of *V. rotundifolia* shows strong antifungal activity to rice blast disease, gray mold of tomato, late blight of tomato, leaf rust of wheat. Lee et al., (2012) did a research on anti-inflamatory, antitumor, and analgesic properties of Manhyungja and demonstrated that it inhibits ROS-NF-κB pathway in vascular endothelial cells, thus reducing vascular inflammation.

In Korea, Japan and China where *V.rotundifolia* is native, there are a lot of studies conducted besides chemical and pharmacological studies. Kim and Park (1998) investigated ecological habitat and morphological characteristic of the species. Jung (2000) conducted a comparative study of the Viticetea rotundifoliae in South Korea and Japan. Park and Park (2001) studied about the growing characteristics, propagation and cultivation of *V. rotundifolia*.

Kim and Park (2004) focused on its usability as a rehabilitation plant, investigating the difference of seed germination depending on stage of seed collection and the influence by method of cutting propagation. Park et al. confirmed that micropropagation is an effective and useful for mass production, suggesting this technique might be helpful to restore eroded areas in the dunes by increasing *V. rotundifolia* populations the same year.

Cho investigated the soil environmental characteristic of the habitat and the method of reproduction and found that soil moisture content was higher in *V. rotundifolia* habitat than non-habitat. Also, he suggested that it could be used readily as a resource plant in 2006. Hu et al., (2007b) studied association between chemical and genetic variation of *V. rotundifolia* populations and confirmed these two variations are closely related to each other. Also, they (Hu et al., 2008) analyzed population genetic structure of *V. rotundifolia* and detected a relatively high genetic differentiation among populations which is likely to be caused by its sexual/asexual reproduction and limited gene flow, supporting the study conducted by Yeeh et al. in 1996. Park et al. (2009) did research on the ecological characteristics of the *V. rotundifolia* communities in Korea. They reported the community habitats were destroyed seriously because of the absence of specific management and conservation plans. Soils of the *V. rotundifolia* communities had high salinity but low contents of organic matter, indicating that it could survive at barren environment. Also, there were various species in the community habitat, showing biodiversity.

In 2010, Setoguchi et al. compared leaf blade thickness of coastal *V. rotundifolia* to that of inland individuals. Leaves of inland individuals tended to be thinner than coastal individuals, showing differentiation as a result of adaptation to environments. Park et al. (2010) investigated the influence to the amount of the absorbed inorganic component and growth of *V. rotundifolia* by

NaCl treatment. Sun et al. (2019) analyzed genetic diversity and population structure and found that *V. rotundifolia* has a low level of genetic diversity, clear genetic differentiation among populations and an insignificantly different phylogeographical structure.

Since *V. rotundifolia* has been known for dune-stabilizer because of its deep root system, it was introduced to the horticulture landscape industry in North and South Carolina in the 1980s (Whitwell et al., 2016). After Hurricane Hugo in 1989, it was planted widely in the United States to restore the damaged frontal dune (Cousins et al., 2010b; Murren et al., 2014; Gresham&Neal, 2004). However, its rapid growth by running stems and vegetative reproduction allowed it to dominate the dune ecosystems and to exclude native species, proving to be highly invasive in the United States (Cousins et al., 2009; 2017).

Since it became invasive species, there have some research to study its invasiveness and how to control it. Gresham and Neal (2004) studied the invasive potential of the species. In 2009, True conducted experiment to study the efficacy of herbicides, including aminopyralid, on *V. rotundifolia.* Cousins et al. (2009) studied about mechanisms of invasiveness of *V. rotundifolia,* and they argued that soil hydrophobicity which was present under the cover of the species might be one of mechanisms. Also, their study (2010b) showed that it has both physical and physiological dormancy mechanisms, implying its potential to reestablish from seed after vegetation removal. Murren et al. (2014) observed vegetative reproduction, sexual reproduction, and viable seed set rates are high, even though germination rate is low. Also, they found pollinator activity was consistent with increased fruit and seed set, suggesting a possible mechanism to make sexual reproduction success. With these observation, they concluded that *V. rotundifolia* might have a lag phase, a potential to expand further, supporting its invasiveness. Whitwell et al. (2016) carried out experiment to figure out which foliage and cut stem herbicide application is effective to control it. However, in 2019, Hawkins and Robacker discovered the positive relation between *Vitex* plants and pollinators, especially bee populations, suggesting that these plants could be a valuable resource to support pollinators which have decreased across the world in an urban area.

1.3 Objectives of the study

Many researchers have studied the chemical components from *V. rotundifolia* and their physiological activity which could be applied to pharmacology and medical science. Also, it was found that it has allelopathic influence to regulate the growth of other species (Yoshioka et al., 2004; Gresham&Neal, 2004; Shinwari et al., 2017). Its deep root system, attractive foliage and flowers make it a good dune-stabilizer and rehabilitation plant. Even though it has considerable value, it is considered as invasive species in the United States because of its rapid growth, so some studies evaluate it negatively. Unlike physiological and medical studies, only a few of ecological and morphological studies for the habitat conservation of the plant species have been found (Park et al., 2009). Most of ecological studies conducted in Korea are about the condition of habitat and the growth characteristics to evaluate its ability to restore the destroyed area. Thus, research is required to investigate the correlation between surroundings and *V. rotundifolia*.

The purpose of this research is to verify a hypothesis that *V. rotundifolia* has an influence to surroundings. Even if it is not verified, this research might discover the strategy of *V. rotundifolia* to dominate. Unlike the previous study, we did quadrat survey and environmental investigation to understand the species composition within *V. rotundifolia* community and the relation between vegetation in the community and soil environment. Especially, soil hydrophobicity was added as an environmental factor which has not been considered much in Korea before. Also, with germination experiment targeting species that coexist with *V. rotundifolia* in lab and greenhouse at the same time, we would confirm its allelopathic influence. With this research, basic information could be provided for clarifying its interaction with surroundings, which could be applied to the conservation of coastal sand dune.

Chapter 2. Ecological study of *Vitex rotundifolia* community on Masian beach

2.1 Introduction

Coastal sand dunes have a dynamic topographical structure having coastal and terrestrial components at the same time, as they are built by the accumulation of sand which are moved by wind and wave at the coastal margin (Barbier et al., 2011; Short&Hesp, 1982). Coastal sand dunes, with these two different characteristics, provide various ecosystem services, including production of raw material, attenuation of wave, water catchment and purification, habitat for wildlife, carbon sequestration and recreational uses (Barbier et al., 2011; Carter, 1990; Pye&Tsoar, 2008). Exposure to salt spray, strong winds, sand blast and low soil moisture contents which are characteristics of these habitats, affect vegetation on coastal sand dune (Hwang et al., 2016; Ishikawa et al., 1995; Min&Je, 2000). Coastal sand dune plants have adaptability to allow them establish and survive in this harsh environment (Olafson, 1997; Shin et al., 2013). Vegetation increases the stability of the surface soil and landscape by trapping sand, resulting in the formation of coastal sand dunes (Olafson, 1997; Min&Je, 2002).

Ihm et al. (2001) reported that perennial grass communites like *Carex kobomugi*, *Carex pumila* and shrub community of *V. rotundifolia* dominated coastal sand dune. Variation in the species composition from seaward to landward direction is evident (Hwang et al., 2016). In unstable dune like primary dune, *Elymus mollis*, *C. kobomugi*, *Calystegia soldanella*, *Argusia sibirica*, *Ixeris repens*, *Glehnia littoralis* are found, which are herbaceous perennial plants and pioneer species. As a distance increases from the shore line, frequency of their appearance decreases (Cho&Song, 2007; Hwang et al., 2016; Jung et al., 2017)

In stabilized dune which usually is close to land, *Ischaemum anthephoroides*, *Lathyrus japonicus*, *Rosa rugosa*, *V. rotundifolia*, *Miscanthus sinensis* exist. In general, the appearance of *I. anthephoroides*, *R. rugosa* and *V. rotundifolia* means that the surface of soil reaches the stage of stability (Cho&Song, 2007). Especially, *V. rotundifolia* community is a typical shrub on coastal sand dune in Northeast Asia (Han et al., 2013; Lee&Ahn, 2012). It is the indicator species that separate into foredune and fixed dune (Hwang et al., 2016). Also, it stabilizes coastal sand dune efficiently, with its deep root systems (Ihm et al., 2001; Hwang et al., 2016) and tolerate salt spray and sea wind. Thus, it is considered as a climax species of vegetation on coastal sand dune in Korea. Behind dense *V. rotundifolia* community there are a mixture of halophytes including *L. japonicus* and inland plants like *O. odorata* which stabilizes areas (Hwang et al., 2016).

Plant succession tends to follow a landward-decreasing trends (Hesp, 1991). Associations between vegetation and habitats were separated along soil gradient far from the seashore (Hwang et al., 2016). Soil moisture content, pH decrease with increasing distance from coast, but organic matter increases as the distance increases (Hwang et al., 2016). The level of stresses is highest in the foredune environment which is close to the coast and generally decreases with distance from the coast in fully vegetated coastal dune, resulting in plant succession and decrease of species richness toward land (Hesp, 1991). Not only does environment influence vegetation, but also reverse happens (Bakker et al., 1979 in van Meulen et al., 2008; Figure 2-1). For example, the difference in vegetation composition might have changed the soil properties by providing nitrogen to soils (Hwang et al., 2016).

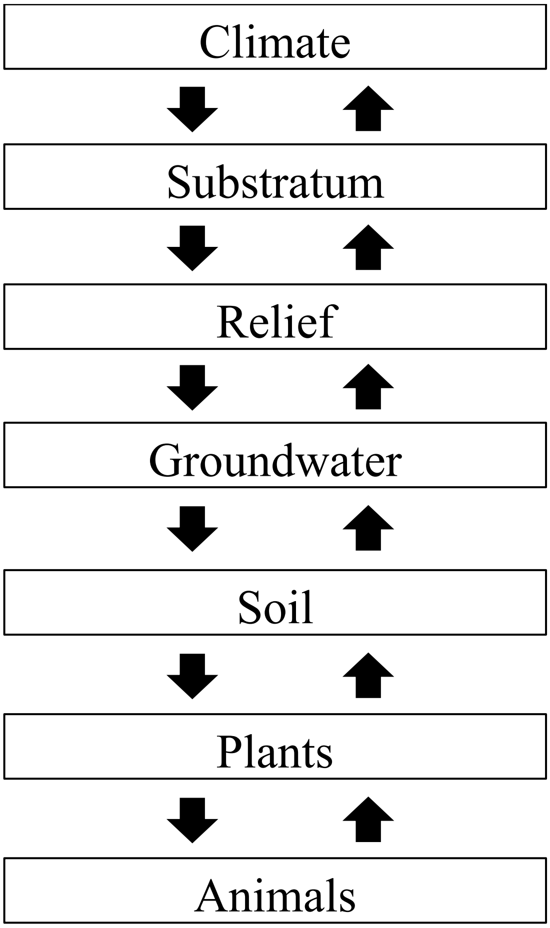

Figure 2-1. Hierarchical model of landscape forming factors (Bakker et al., 1979 in van Meulen et al., 2008).

Soil hydrophobicity, also called as soil water repellency, is one of soil characteristics indicating the decrease of wettability and retention of water in the soil caused by the presence of hydrophobic coatings on soil particles (Hallet&Gaskin, 2007). When soil is hydrophobic, it means that soil tends to resist surface water infiltration, letting more water runoff. It is one of the most important physical properties of soils, having significant effects on the ecohydrological processes of agriculture as well as coastal sand dune (Olonunfemi et al., 2014; Hallet&Gaskin, 2007). Soil hydrophobicity is caused by organic compounds like aliphatic hydrocarbons with elongated carbon chain and polar substances of amphiphilic structure. These compounds are derived from living or decomposing plants or microorganisms (Doerr et al., 2000). Particular vegetation types like trees or shrubs with resins, waxes or essential oils associate with soil water repellency (Doerr et al., 2000).

V. rotundifolia has been usually investigated as one of coastal sand dune plants, although it is one of representative shrub community in coastal sand dune (Ihm et al., 2001). There is little research on relationship among species that coexist with it. Also, its environmental condition has been surveyed but correlation between the community and environmental factors has not.

Thus, the objectives of this chapter are below:

1) To understand which species coexist within *V. rotudifolia* community
2) To understand which environmental factors including soil hydrophobicity might have correlation with *V. rotundifolia* community.

2.2 Material & Method

2.2.1 Site

The site of the study was Masian beach located at 37°25'57.6"N 126°24'57.6"E (662 Yongyu-dong Jung-gu Incheon) (Figure 2-2). It is within a range of the northernmost area of habitat for *V. rotundifolia* (Lee et al., 2007; Cousins et al., 2010a). This site has well-developed coastal sand dune with well-preserved vegetation and large *V.rotundifolia* community might play a vital role to stabilize dune. The area of the community was about 40 x7 m^2. There was a stream behind the dune. Figure 2-3 shows the average temperature (°C) and precipitation (mm) of Incheon from 2010 to 2019, provided by Office of Meteorology.

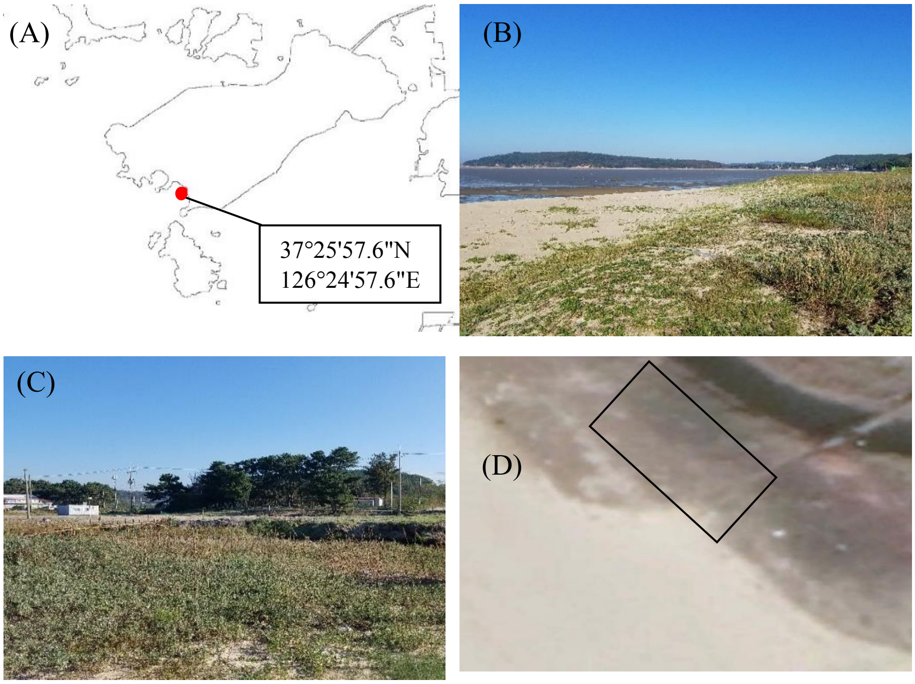

Figure 2-2. Masian beach at Yeongjong-do (A) The location of Masian beach at Yeongjong do. The red dot shows the site of the study. (B), (C) The landscape of *Vitex rotundifolia* community on the coastal sand dune. (B) shows the front of *V. rotundifolia* community and (C) shows the back of the community. There is a stream behind the dune. (D) The box represents the area where the quadrat survey was done (Google Earth).

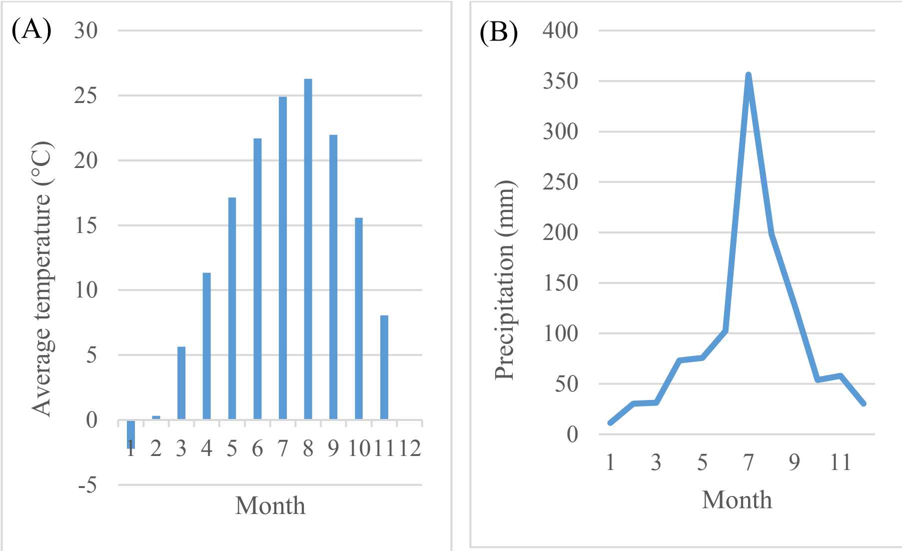

Figure 2-3. 2020 Meteorological data of Incheon. (A) Average temperature (°C) from 2010 to 2019 (B) Average Precipitation (mm) from 2010 to 2019. The data were provided by Office of Meteorology.

2.2.2 Quadrat survey and Soil sampling

Quadrat survey was conducted in August. Two lines were installed where the slope of foredune ends; one was parallel to coastline and the other was vertical to the former line. 1x1m^2 quadrats were placed randomly at intervals of 1 m within the area. Not only cover (%) of every species in each quadrat but also species at the outside of the area were recorded. To analyze the environmental factors, soil were collected at three random points in each quadrat by using stainless soil core with a diameter of 5 cm and a length of 5 cm. Soil at the depth of 0-5 cm and 5-10 cm were collected and put in plastic bags corresponding to its depth.

a. Measuring the environmental factors

After moving soil samples to laboratory, soil moisture content and its organic matter were measured. After drying fresh soil at 105°C for 48 hours, soil moisture content was measured (Kim, 2010).

$$\text{Soil Moisture Content (\%)} = \frac{(weight\ of\ fresh\ soil)\ (g) - (weight\ of\ dried\ soil)(g)}{(weight\ of\ fresh\ soil)(g)} \times 100$$

With Loss on ignition (LIO) method, we measured soil organic matter content. It was done after burning the dried soil at 550°C, using an electric furnace (Thermolyne 67100) (Kim, 2010).

$$\text{Soil Organic Matter Content (\%)} = \frac{(weight\ of\ dried\ soil)(g) - (weight\ of\ burnt\ soil)(g)}{(weight\ of\ dried\ soil)(g)} \times 100$$

Soil were air-dried under the shade at room temperature and then, were 2-mm sieved to eliminate impurities like litter. Air-dried soil and distilled water were mixed in the proportion of one to five prior to 1-hour shaking. Then, pH, EC and salinity of the soil were measured by PC2700 meter. The rest of soil were sealed and stored at room temperature. Also, to determine the soil hydrophobicity, water drop penetration time (WDPT) test was done. 50g of air-dried soil was placed on petri dish and 200ul of distilled water was released from a pipet held 1 cm above the sand surface. Timing started when the drop fell from the pipet and was stopped when the drop was absorbed into the sand. WDPT of three drops were measured for each petri dish and the average of each sample was calculated (Cousins et al., 2009). Using the average WDPT, class of soil hydrophobicity of each sample was determined (Doerr, 1998; Table 2-1).

Table 2-1. Level of soil hydrophobicity depending on water droplet penetration time (Doerr, 1998). WDPT; water droplet penetration time (s; seconds)

Class	Descriptive label	WDPT (s)
1	very hydrophilic	<5
2	hydrophilic	5-10
3	slightly hydrophobic	10-30
4	moderately hydrophobic	30-60
5	strongly hydrophobic	60-180
6	extremely hydrophobic	>180

b. Data analysis

Since the data did not follow the normal distribution, Mann-Whitney-Wilcoxon test was used to analyze significant difference among data at $P < 0.05$. The relation of species within *V. rotundifolia* community was figured out by using NMDS (Non-metric Multidimensional Scaling) which was run on the vegetation data with Bray-Curtis distance measure. NMDS analysis was conducted using metaMDS function of vegan package in R. Three different ellipses were drawn to connect quadrats based on location of quadrats to see the relation of coastal sand dune plants in *Vitex rotundifolia* community on Masian beach. The correlation between *V. rotundifolia* community and environmental data was shown by using envfit function of the package. It calculated the goodness-of-fit values (R^2) and their significances ($P < 0.05$) using 1000 random permutations and fitted environmental vectors onto the NMDS ordination. To see whether significant environmental factors are related to distance from seashore, Kruskal-Wallis test was conducted at $P < 0.05$. When the factor was significant, Dunn's test was done as post-hoc test. All statistical analysis were done by using R program (version 3. 6. 3).

2.3 Result

2.3.1 Composition of species within *V. rotudifolia* community in Masian beach

In August, there were 12 species which was slightly different from the composition of species (Figure 2-4). Among them, *V. rotundifolia* (36.8 ± 5.6%), *Carex kobomugi* (24.2 ± 5.7%), *Diodia teres* (21.5 ± 6.6%), *L. japonicus* (19.1 ± 2.0%) and *Rosa rugosa* (10.3 ± 5.3%) had high cover. Table 2-2 showed the percentage cover of species within *V. rotundifolia* community in each month. *Phragmites australis*, *I. anthephoroides, O. odorata, R. rugosa, Ixeris repens*, *Glehnia littoralis*, *Cuscuta chinensis* were outside the area of investigation.

NMDS results shows which species exist closely within *V. rotundifolia* community in Masian beach (Figure 2-5). *L. japonicus* was the closest to *V. rotundifolia* in August, showing close relation between two species. *D. teres* and *C. kobomugi* high cover within the community, but only *D. teres* was close to *V. rotundifolia* in the plot. *C. kobomugi* was not close to *V. rotundifolia* but close to *C. soldanella. R. cordifolia* and *O. odorata* were located the farthest from it, with low coverage. According to the plot, it was shown that *C. kobomugi* and *C. soldanella* usually existed close to seashore. Also, *L. japonicus, D. teres* and *V. rotundifolia* were shown to be placed close to land.

Table 2-2. Cover (%) of species within *Vitex rotundifolia* community in August. Average (± SE) cover of species in August (n = 21).

Family	Scientific name	Cover (%)
Fabaceae	*Lathyrus japonicus*	19.14 ± 1.97
Rosaceae	*Rosa rugosa*	10.33 ± 4.29
Onagraceae	*Oenothera odorata*	0.57 ± 0.29
Rubiaceae	*Diodia teres*	21.48 ± 6.29
	Rubia cordifolia	1.10 ± 0.88
Convolvulaceae	*Calystegia soldanella*	2.52 ± 0.79
Asteraceae	*Xanthium strumarium*	6.76 ± 2.74
Verbenaceae	*Vitex rotundifolia*	36.76 ± 6.12
Chenopodiaceae	*Corispermum stauntonii*	2.33 ± 0.57
Liliaceae	*Asparagus oligoclonos*	2.81 ± 0.86
Cyperaceae	*Carex pumila*	6.00 ± 1.50
	Carex kobomugi	24.19 ± 5.85

Figure 2-4. Species within *Vitex rotundifolia* community in Masian beach

(A) *Calystegia soldanella* flower in May. Bee visited its flower in May. (B) *Lathyrus japonicus* flower in May (C) *Diodia teres* in August (D) *Xanthium strumarium* in August

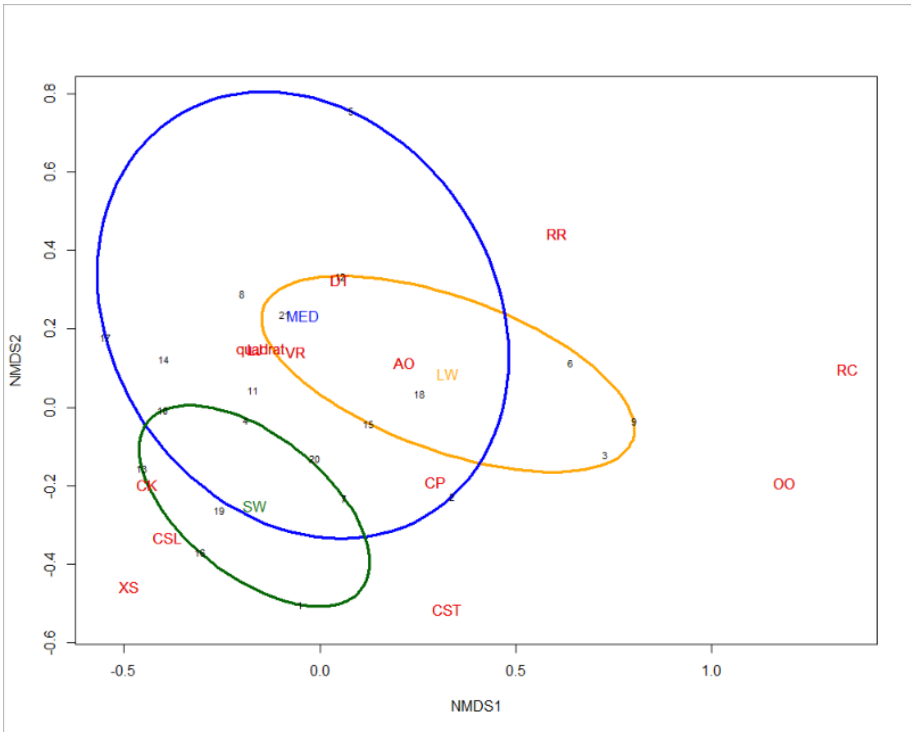

Figure 2-5. Result obtained by NMDS running with 2-dimensions for the vegetative data of *Vitex rotundifolia* community at Masian beach in August. NMDS ordination diagram for the data in August. It was ran with Bray distance and its STRESS value is 0.1444203. Small black numbers correspond quadrat and red-colored abbreviations do individual species. Each ellipse groups quadrats whose location is seaward, landward and median. AO; *Asparagus oligoclonos,* CSL; *Calystegia soldanella,* CK; *Carex kobomugi,* CP; *Carex pumila;* CST; *Corispermum stautonii,* DT; *Diodia teres,* LJ; *Lathyrus japonicus,* OO; *Oenothera odorata,* RR; *Rosa rugosa,* RC; *Rubia cordifolia,* VR; *Vitex rotundifolia,* XS: *Xanthium strumarium,* SW; seaward location, MED; median location, LW; landward location

2.3.2 Soil properties of *V. rotundifolia* community in Masian beach

In *V. rotundifolia* community, average soil moisture and soil organic matter were quite low at the both depth of soil (Table 2-3), but only soil moisture had a significant difference depending on the depth (soil moisture; p-value = 1.056e-05, soil organic matter; p-value = 0.4697). pH at the depth of 0-5 cm was 7.11 and one at the depth of 5-10 cm was 7.09, indicating soil was neutral (p-value = 0.9799). At the depth of 0-5 cm and 5-10 cm, electrical conductivity were 19.67 dS/m and 15.54 dS/m, respectively, showing that soil was highly saline. Upper level had higher EC than lower one but there was no significant difference between them (p-value = 0.1188). Soil hydrophobicity of both depth described that soil of *V. rotundifolia* was slightly hydrophobic, but within area, strong hydrophobicity happened. There was no significance between them (p-value = 0.2654). Even though the significance depending on the depth was only shown in terms of soil moisture, each component shows significant difference within the community ($P < 0.05$).

Table 2-3. Soil characteristics of *Vitex rotudnfiolia* community at Masian beach in August. Data show minimum, maximum and average (±SE) of each soil characteristic (n = 21). SM; soil moisture, OM; soil organic matter, EC; electrical conductivity, WDPT; water droplet penetration time, SHCL; class of hydrophobicity, 1; 0-5 cm of depth, 2; 5-10 cm of depth. '*'; $P < 0.05$

	Min	Max	Average (± SE)
SM1 (%)	0.18	0.45	$0.29 \pm 0.02^{*}$
SM2 (%)	0.18	1.6	$0.68 \pm 0.08^{*}$
OM1 (%)	0.42	0.79	$0.54 \pm 0.02^{*}$
OM2 (%)	0.28	0.95	$0.56 \pm 0.03^{*}$
pH1	6.35	8.04	$7.11 \pm 0.13^{*}$
pH2	6.3	7.92	$7.09 \pm 0.13^{*}$
EC1 (dS/m)	12.92	67.13	$19.67 \pm 2.54^{*}$
EC2 (dS/m)	12.86	20.65	$15.54 \pm 0.50^{*}$
SHCL1	1	5	$2.95 \pm 0.31^{*}$
SHCL2	1	5	$2.52 \pm 0.28^{*}$

2.3.3 Correlation between environmental variables of *V. rotundifolia* community in Masian beach

In August, the cover of *V. rotundifolia* was not correlated significantly with the community, unlike in May (envfit; R^2 = 0.0266, p-value = 0.798202). Only soil moisture at the depth of 0-5 cm had a weak correlation with community but soil moistures at both depth did not influence significantly the community (envfit; soil moisture (0-5 cm): R^2 = 0.00226, p-value = 0.829171; soil moisture (5-10 cm): R^2 = 0.2100, p-value = 0.121878). The correlations between soil organic matter and the community structure at both depth were significant, being strong predictors (envfit; soil organic matter (0-5 cm): R^2 = 0.3936, p-value = 0.008991; soil organic matter (5-10 cm): R^2 = 0.4049, p-value = 0.010989). pH, EC and salinity were not correlated with the community, even though they had quite long arrows (envfit; pH (0-5 cm): R^2 = 0.1495, p-value = 0.226773; pH (5-10 cm): R^2 = 0.1859, p-value = 0.156843; EC (0-5 cm): R^2 = 0.0672, p-value = 0.539461; EC (5-10 cm): R^2 = 0.2116, p-value = 0.122877; salinity (0-5 cm): R^2 = 0.0582, p-value = 0.587413; salinity (5-10 cm): R^2 = 0.2110, p-value = 0.120879). However, soil hydrophobicity at the both depth was correlated with vegetative structure, also having strong correlations (envfit; soil hydrophobicity class (0-5 cm): R^2 = 0.3855, p-value = 0.010890; soil hydrophobicity class (5-10 cm): R^2 = 0.3949, p-value = 0.012987). Thus, the data showed that both soil hydrophobicity and soil organic matter were strongly correlated with the community structure (Figure 2-6).

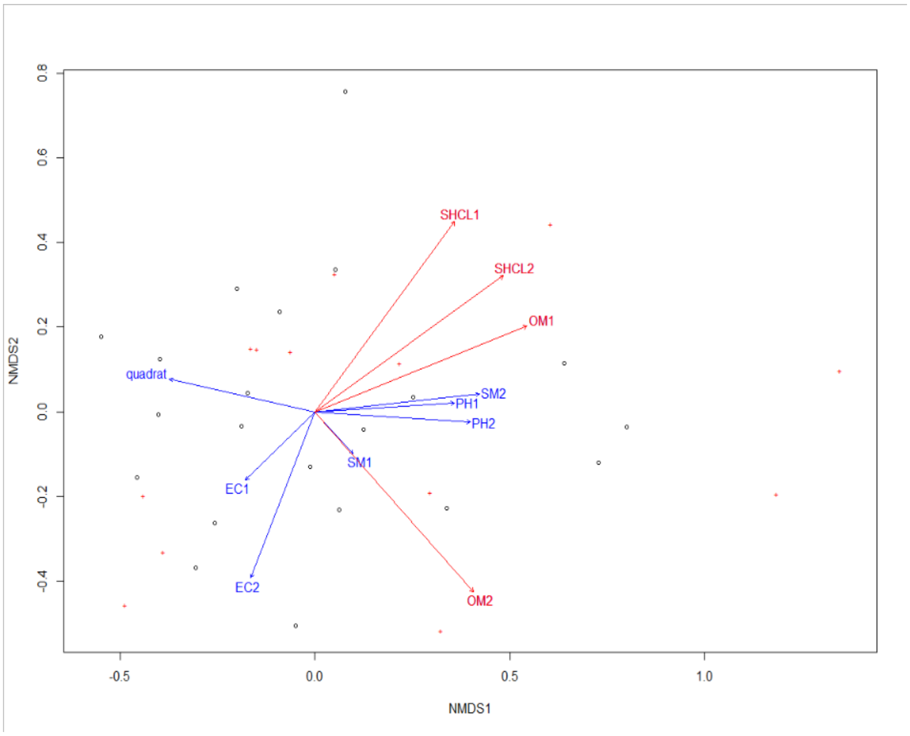

Figure 2-6. NMDS ordination diagram of environmental factors in August. NMDS was run on 2-dimensions and with Bray Curtis distance measure. SM; soil moisture, OM; soil organic matter, EC; electrical conductivity, SHCL; class of hydrophobicity, POSS; seaward; POSL; landward, 1; 0-5 cm of depth, 2; 5-10 cm of depth. Red-colored arrow identifies the significant correlation with ordination ($P < 0.05$).

2.4 Discussion

2.4.1 Species relationship within *V. rotundifolia* on Masian beach

In this study, species relationship within *V. rotundifolia* was shown. This finding is important to know the relation of *V. rotundifolia* with surrounding species, and it seemed to depend on distance from land. NMDS ordination showed the relation among species within *V. rotundifolia* community, grouping them based on their positions. *L. japonicus* had a close relationship with *V. rotundifolia*, indicating that they share similar habitat, the rear of sand dune with stabilization (Hwang et al., 2016). Usually, herbaceous plants are suppressed under the dominance of woody perennial plants (Kutiel et al., 2000). However, in Masian, *V. rotundifolia,* coastal shrub, and *L. japonicus,* herbal type, coexisted. This coexistence was observed by Kim and Lee (2012), either. Also, even though *C. kobomugi* had a higher cover than *L. japonicus,* it was quite far from *V. rotundifolia,* rather close to *C. soldanella. C. kobomugi* and *C. soldanella* known as foredune species, usually exist dune ridge and leeward slope of foredune (Hwang et al., 2016; Kim&Lee, 2012; Cho&Song, 2007). Also, on stable dunes, they can grow on the seaside of *V. rotundifolia* population, distributed in harsher environmental conditions (Min&JE, 2002; Ishikawa et al., 1995). Both of them were observed at the seaside margin of the community on the field. Since pioneer stage consisting of herbaceous plants indicates that dune is unstabilized (Kumler, 1969), the part where *C. soldanella* or *C. kobomugi* existed was still rather unstable within *V. rotundifolia* community in Masian. *V. rotundifolia* on Masian beach did not dominate completely, thus allowing co-existence with several different species. This community contained foredune and land species at the same time, indicating *V. rotundifolia* community on Masian beach has complex structure of vegetation. Also, its composition depending on the distance from seashore shows the degree of dune stability. Thus, when *V. rotundifola* community gets larger with increasing shrub size, this co-existence would gradually vanished, reaching the climax stage of coastal sand dune vegetation with dune stabilization (Bai et al., 2019; Min&Je, 2002).

2.4.2 Correlation between environmental factors and *V. rotundifolia* community in Masian beach

In *V. rotundifolia* community, soil organic matter and soil hydrophobicity were significantly correlated with the community structure. The leading and most documented contributor to soil hydrophobicity is organic matters which are derived from living or decomposing plants or microorganisms (Doerr et al., 2000; Olonunfemi et al., 2014). Especially, plants are predominant source of organic compounds in the soil, strongly influencing the abundance and distribution of soil organic matter (Mao et al., 2019). Also, the occurrence of soil hydrophobicity has been associated with particular vegetation types, especially shrubs with resins, waxes or essential oils (Doerr et al., 2000). Since *V. rotundifolia* is known to contain a lot of essential oils (Jang et al., 2002), the presence of soil hydrophobicity within the community might be caused by decomposition and leaching of *V. rotundifolia,* making this result reasonable. Cousins et al. (2009) argued that *V. rotundifolia* could cause soil hydrophobicity with its fruits and leaves which containing numerous *n*-alkanes. They claimed that increasing soil hydrophobicity might be one of strategy for *V. rotudifolia* to dominate. Also, Adams et al. (1970) insisted that hydrophobic soils under some California desert shrubs contribute to the exclusion of annuals surrounding the soils.

Despite of the lack of significance, the electrical conductivity, pH at upper and lower level were strong predictors, having long arrows on the NMDS ordination. Soil moisture-salinity interaction has been widely recognized as the most important factor in the distribution of the salt-tolerant plants (Cantero et al., 1998). The chemical and hydrophysical characteristics of soils also affect the diversity and structure of the vegetation (Cantero et al., 1998).

Insignificance might be related to cover of *V. rotundifolia* which blocks salt spray from the coast.

2.5 Conclusion

In this study, species within *V. rotundifolia* community and its environmental factor were investigated. *L. japonicus,* one of stabilized dune plants, was highly related to *V. rotundifolia* with high cover. Although *C. kobomugi* had the second high cover in the field, it has close relationship with *C. soldanella* which is foredune species just like *C. kobomugi*. *V. rotundifolia* community on Masian beach contained foredune species at the seaside and land or stabilized dune species at the landside simultaneously, showing the community has complex vegetative structure and partial dune stability of Masian beach. Thus, the appearance of *V. rotundifolia* might imply the dune would be stabilized gradually with various species within the community. Also, within the community, soil hydrophobicity and soil organic matter at the depth of 0-5 cm and 5-10 cm were significantly correlated with community structure. It might be caused by the organic compounds derived from *V. rotundifolia* by leaching and decomposition.

Chapter 3. Allelopathic influence of *Vitex rotundifolia*

3.1 Introduction

Allelopathy is both stimulatory and inhibitory effects of secondary metabolites from plant upon another including microorganisms, resulting in regulation of plant growth and development (Rice, 1984; Chou, 1999). As research found that allelopathic interactions happen not only by plants and microbes but also soil (Blum et al., 1993; 1994; Blum&Shafer, 1988), the definition of allelopathy is clarified by the International Allelopathy Society in 1996 as "any process involving secondary metabolites produced by plants, algae, and fungi that influences the growth and development of agriculture and biological systems" (Chou, 2006; Far et al., 2018). It plays a significant role in plant dominance, succession, formation of plant communities and climax vegetation, and crop productivity (Muller, 1969; Rice, 1984; Chou, 1999; 2006; Wardle et al., 1998). Many studies reported the presence of allelopathy and its stimulatory and inhibitory effects to germination and seedling growth (Correa et al., 2000; Nilsson&Zackrisson, 1992; Anaya, 2010; Heisey, 1990; Imatomi et al., 2013; Inderjit&Dakshini, 1994; Carballeira&Reigosa, 1999; Ravlić et al., 2013; Oleszek&Jurzysta, 1987; Schumacher et al., 1983; Park et al., 2006). It is almost universally observed that stimulation happens at low allelochemical concentration and inhibition does with increasing concentration (An et al., 1993; Liu et al., 2003; Far et al., 2018; Figure 3-1).

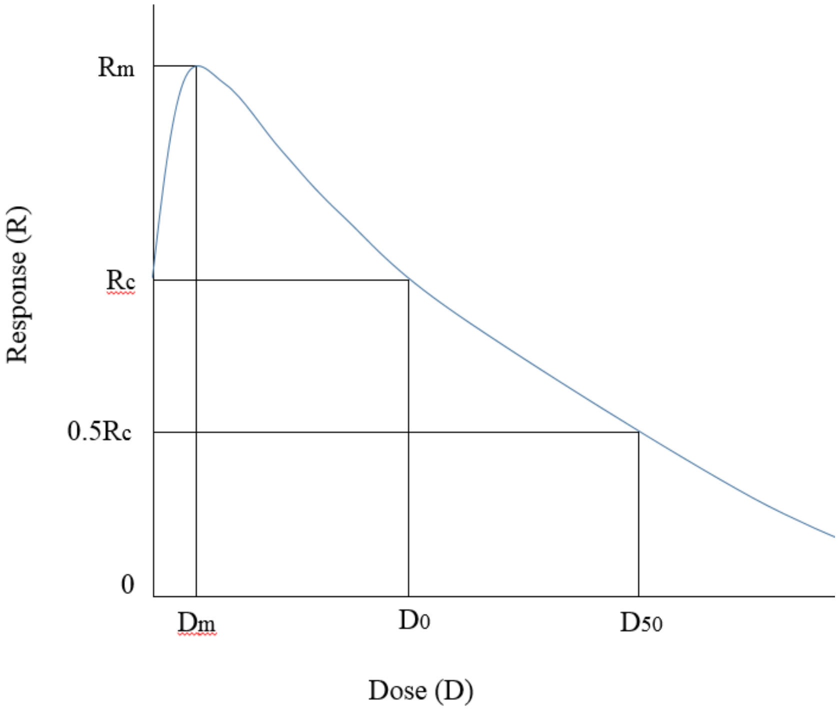

Figure 3-1. A hypothetical allelochemical dose-response curve (Liu et al., 2003). R_m; the maximum stimulating peak. R_c; response of control. D_m; the dose that induces peak of stimulation. D_0: the dose with no effect. D_{50}: the inducing 50% inhibition of control.

Allelopathic compounds, called as allelochemicals (Whittaker&Feeny, 1971), are secondary metabolites released into environment through volatilization, leaching, root exudation and decomposition (Rice, 1984; Inderjit&Dakshini, 1994; Inderjit, 1996; Chou, 1999; 2006; Blum et al., 1993; 1994; Ferguson&Rathinasabapathi, 2003). Most of allelopathic compounds like phenolic acids, alkaloids, flavonoid compounds are hydrophilic, meaning that they leachate from plants by fog drip, rain or any other water source (Chou, 1999; Cutler&Cutler, 1999; Del Moral&Muller, 1969; Rice, 1984).

Most allelopathic studies used laboratory bioassays to figure out allelopathy which are perfectly controlled to eliminate all possible interfering factors, so lab bioassays could not reflect the condition of field (Inderjit&Dakshini, 1995; Inderjit&Weston, 2000). Also, some studies used ground root or leaves as treatment, but preparing leachate or extracts with water is preferred which is likely form of allelopathy (Inderjig&Dakshini, 1995; Schmidt, 1990). As germination experiment is used to investigate the allelopathy activity of species, lettuce is the most common target species because of its sensitivity and rapid germination and seedling growth. However, to clarify allelopathic influence, Inderjit and Weston (2000) argued that researchers should choose species as a receiver whose habitat condition is similar to donor and prepare at least three levels of allelopathic material.

V. rotundifolia has known to contain various chemical compounds like alkaloids and flavonoids in their leaves and fruits (Kobayakawa et al., 2004; Lim et al., 2007; Gu et al., 2004; Hu et al., 2007b). Many phenolic acid and flavonoid compounds from it have been shown to contribute to allelopathic qualities of *V. rotundifolia* (True, 2009; Cousin et al., 2017). Especially, according to the study by Yoshioka et al. (2004), it was found that *V. rotundifolia* leaves and fruits contain both inhibitory and stimulatory plant growth regulators. Especially, within *V. rotundifolia* fruits, there are 4-hydroxybenzoic acid methyl ester, vanillic acid methyl ester, 4-hydroxy benzaldehyde, 4-hydroxybenzoic acid, ferulic acid, luteolin, vitexicarpin and artermetin. Shinwrari et al. (2017) reported allelopathic potential of *V. rotundifolia* with petri dish assay.

Previous research on the allelopathic influence of *V. rotundifolia* did laboratory experiment with lettuce. Lettuce is an inland species, meaning that it is not a representative of the natural condition that *V. rotundifolia* encounters, coastal sand dunes. Also, laboratory experiment could not reflect the natural condition because of lack of soil (Inderjit&Weston, 2000).

Thus, the objectives of this chapter are below:

1) To investigate allelopathic influence of *V. rotundifolia* to coastal sand dune plants in laboratory condition
2) To investigate allelopathic influence of *V. rotundifolia* to coastal sand dune plants in soil

3.2 Material and Method

3.2.1 Collection of seeds of coastal sand dune plants and fruits of *V. rotundifolia*

Target species were *Lactuca sativata* var. *capitata*, *Oenothera odorata* and *Calystegia soldanella*. *O. odorata* represents the naturalized species in coastal sand dune and *C. soldanella* is a representative of native coastal sand dune plants. *V. rotundifolia* was excluded since its germination percentage was too low. When pre-test was conducted, only one of 15 seeds was germinated. *L. sativata* var. *capitata,* one of the most used species for allelopathy experiment, was added to check the allelopathic influence of *V. rotundifolia*, even though it is not a coastal sand dune plant. In September, October, 2019 and May, 2020, the seeds of *C. soldanella* and fruits of *V. rotundifolia* were collected. Seeds that collected in 2019 were stored at 4°C for vernalization. Seeds of *O. odorata* and *L. sativata* var. *capitata* were bought online at Danong (http://www.danong.co.kr).

3.2.2 Pre-treatment of *C. soldanella* seeds

Prior to the allelopathy experiment, seeds of all target species were sterilized by soaking them in 1% NaClO solution for 10 minutes and rinsing them with distilled water five times. *C. soldanella* seeds have hard coat, indicating that it has physical dormancy mechanism (Baskin & Baskin, 1998). To break this kind of dormancy, chemical scarification is required and it is done by using H_2SO_4 solution (Baskin&Baskin, 1998; Walmsley&Davy, 1997; Ko et al., 2004; Mariko, 1992). *C. soldanella* seeds were soaked into 95% H_2SO_4 solution for 4 hours and then, rinsed by distilled water five times.

3.2.3 Making leachate of fruits of *V. rotundifolia*

40 fruits of *V. rotundifolia* (about 10 g) and 100mL of distilled water were put into falcon tube and then shaken at 110rpm for 24 hours. Then, extracts were filtered through Whatman No.2 filter paper (Suman et al., 2002). This extract was set as 100% and then, it was diluted by distilled water to make 10% and 50% of leachate when we started the experiment (Kil, 2003). Until starting the experiment, the extract had been stored at 4°C.

3.2.4 Laboratory experiment

Whatman No.2 filter paper was placed on each petri dish (100x20 mm) and then, 15 seeds of each target species were put on it. 3mL of 0% (distilled water as control) 10%, 50% and 100% leachate were added to each dish and each treatment was done by 5 replicates (Figure 3-2). To reduce contamination, 1mL of 0.05g/100mL benomyl solution was added at the first day of the experiment. Then, petri dish were located randomly in the growth chamber whose temperature was set as 25/15°C. Whenever filter paper got dried, a little amount of distilled water was added. When a radicle broke through seed coat, it was regarded as germinated (Park et al., 2006). Germinated seedlings were counted every day until the germination was over. On the last day of the experiment, 15 individuals of seedlings were randomly selected and then, the length of radicle of each individual was measured by mm ruler.

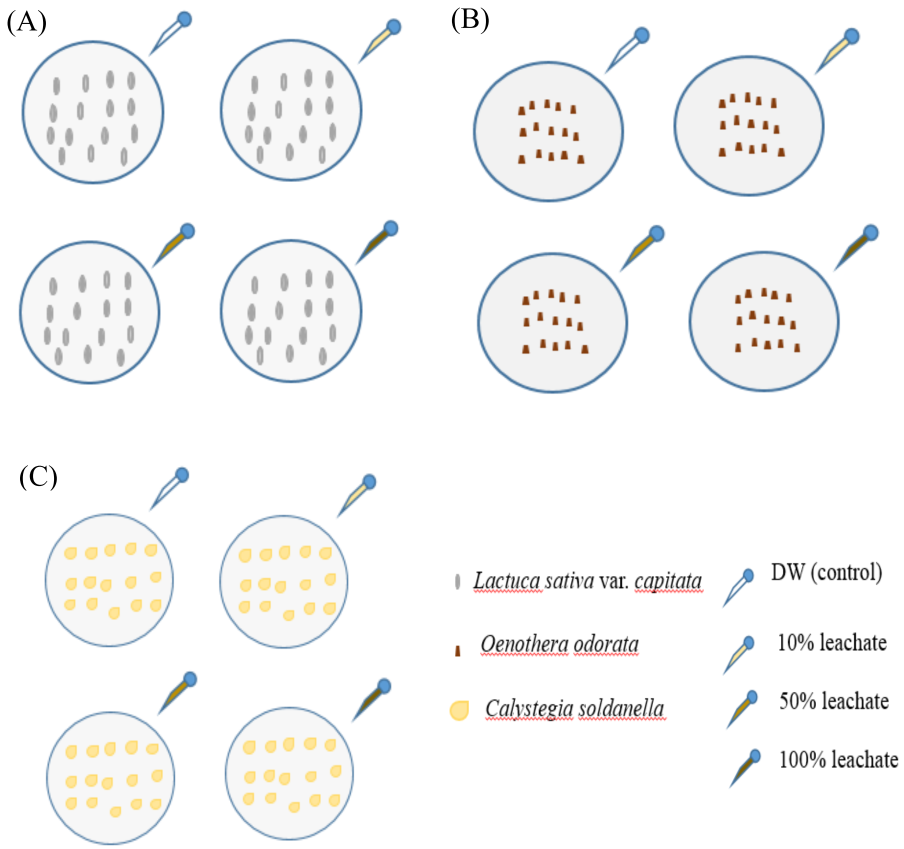

Figure 3-2. Design of allelopathy experiment with petri dish. (A) *Lactuca sativata* var. *capitata.* (B) *Oenothera odorata.* (C) *Calystegia soldanella.* Each species was treated by different concentrations of leachate and each treatment was replicated 5 times.

3.2.5 Greenhouse experiment

In soil assay was conducted at greenhouse. Soil was from Masian beach and was collected at the area where *V. rotundifolia* did not exist. After drying, it was put into pots (10x10x10 cm^3). 25 seeds of each target species were sown at the depth of 0-3 cm of each pot (Figure 3-3). The water was added as much as the surface of soil got well-moistened and then, 5mL of 0%, 10%, 50% and 100% leachate was added to each pot. It was done by 5 replicates. Water was added whenever the surface was dried and 5mL of each leachate and control was added every 10 day. Germinated individuals were counted every. When a radicle broke through seed coat or it came up above the surface, it was determined to be germinated. The experiment was terminated when germination did not happen anymore. After the end of the experiment, length of radicle of 15 individual seedlings which were randomly selected were measured by using mm ruler.

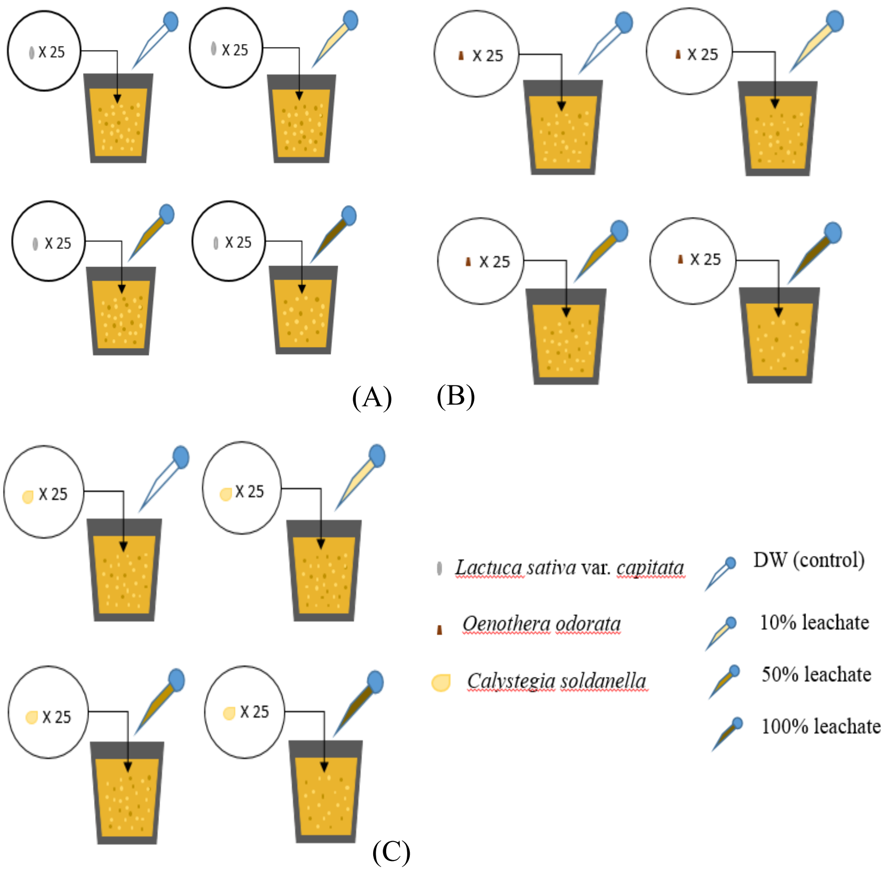

Figure 3-3. Design of allelopathy experiment in greenhouse. (A) *Lactuca sativata* var. *capitata.* (B) *Oenothera odorata.* (C) *Calystegia soldanella.* Different concentrations of leachate was added to each species by 5 replicates.

3.2.6 Data analysis

Germination percentage was calculated by GermPerctent function from germinationmetrics package. As the distribution of the result did not appear to be normal, the significance of data was verified through Kruskal-Wallis test ($P < 0.05$). Then, if the Kruskal-Wallis test is significant, Dunn's test was conducted as post-hoc test to determine differences between each other group ($P < 0.05$). All statistical analysis were run by using R (version 3. 6. 3).

3.3 Result

3.3.1 Laboratory experiment

Figure 3-4 and 3-5 show germination percentage and radicle length of each species treated with leachate or control at the last day of the experiments. In the case of *L. sativata* var. *capitata*, germination percentage for 10% leachate treatment (85.33 ± 2.49%) was higher than one with control (81.33 ± 7.12%). Germination percentage for 50% leachate treatment (81.33 ± 5.33%) was same as control and one for 100% leachate (78.67 ± 4.90%) was lower than control. Radicle length of *L. sativata* var. *capitata* increased with the concentration of *V. rotundifolia* fruit leachate (control; 10.13 ± 1.37 mm; 10%; 27.6 ± 2.89 mm; 50%; 58.67 ± 5.75 mm; 100%; 65.93 ± 5.52 mm). Germination percentages of *O. odorata* and *C. soldanella* of 10% and 50% leachate treatment were higher than control of each species (*O. odorata* (control); 69.33 ± 5.42%; *O.odorata* (10%); 70.67 ± 3.40%, *O. odorata* (50%); 82.67 ± 4%; *C. soldanella* (control); 68 ± 7.42%, *C. soldanella* (10%); 73.33 ± 7.60%; *C. soldanella* (50%); 72 ± 4.90%). However, with 100% leachate treatment, both species had lower germination percentage than control (*O. odorata* (100%); 64 ± 7.48%, *C. sodanella* (100%); 65.33 ± 2.49%). Radicle of *O. odorata* seedlings were getting longer as the concentration of leachate increased (control; 13.66 ± 1.68 mm, 10%; 14.33 ± 1.26 mm; 50%; 35.13 ± 3.19 mm, 100%; 35.73 ± 3.93 mm). Radicles of *C. soldanella* were longer when 10% or 100 % leachates were added than when distilled water was added, but *C. soldanella* seedlings had shorter radicle with 50% leachate than control (control; 44.67 ± 5.24 mm; 10%; 48.73 ± 6.22 mm, 50%; 38.13 ± 5.76 mm; 100%; 60 ± 5.53 mm). No species had significance difference by the concentration of leachate in terms of germination percentage (*L. sativata* var. *capitata*: p-value = 0.8516; *O. odorata*: p-value = 0.07697, *C. soldanella*: p-value = 0.8068), but all of their radicle lengths were affected by the leachate (*L. sativata* var. *capitata*: p-value = 1.78e-09; *O. odorata*: p-value = 2.53e-07; *C. soldanella*: p-value = 8.74e-04; $P < 0.05$).

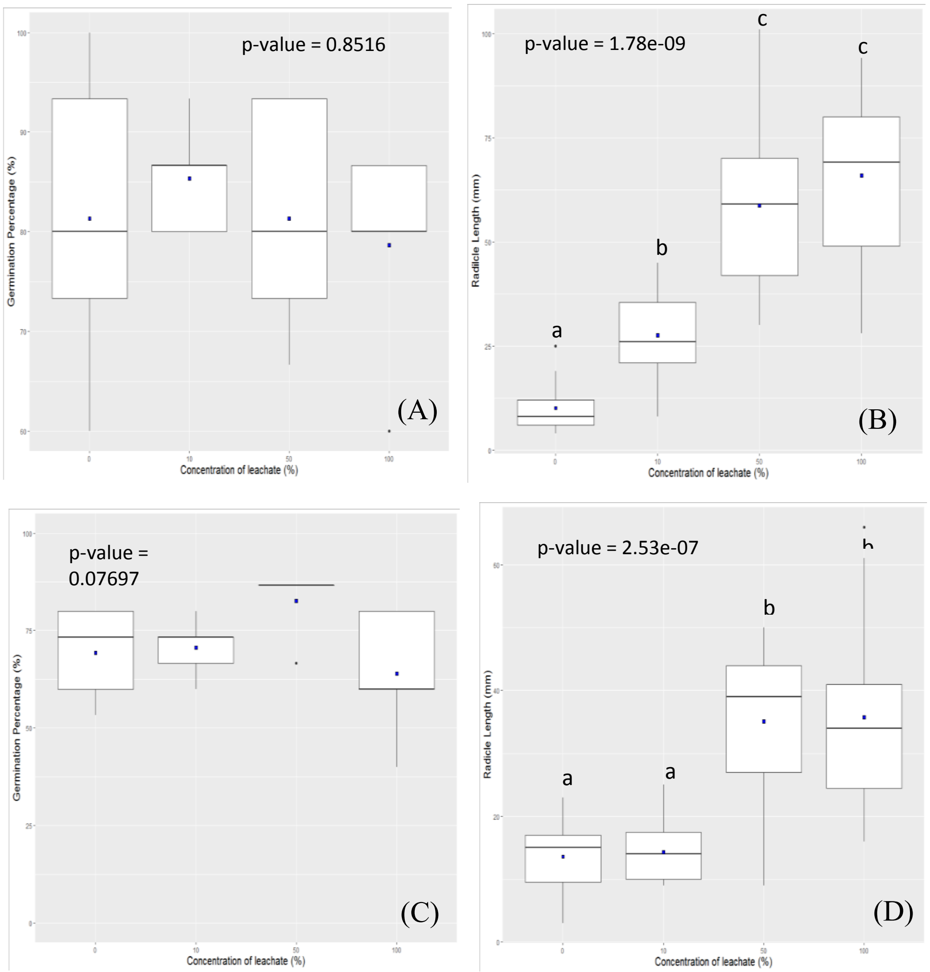

Figure 3-4. Germination percentage (%, *n* = 5) and radicle length (mm, *n* = 15) of *Lactuca sativata* var. *capitata* and *Oenothera odorata* in laboratory condition (A) Germination percentage of *L. sativata* var. *capitata.* (B) Radicle length of *L. sativata* var. *capitata* (C) Germination percentage of *O. odorata* (D) Radicle length of *O. odorata.* Boxes depict groups of germination percentage data or radicle length data through their quartiles. Line on each box shows median value and small blue square-shaped dot does average. Lines extending from boxes mean the upper and lower quartiles. Individual points out of boxes are outliers. Different letters indicate significant differences at $p < 0.05$.

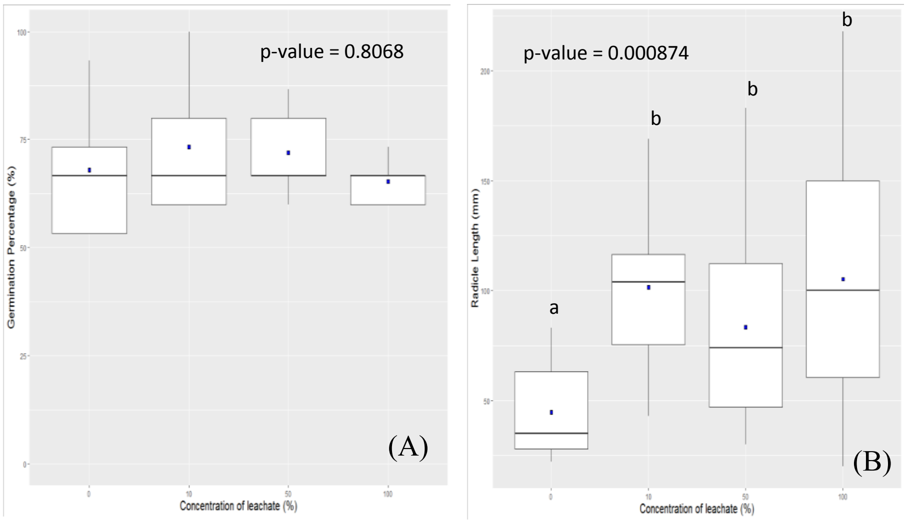

Figure 3-5. Germination percentage (%, *n* = 5) and radicle length (mm, *n* = 15) of *Calystegia soldanella* in laboratory condition. (A) Germination percentage of *C. soldanella.* (B) Radicle length of *C. soldanella.* Boxes depict groups of germination percentage data or radicle length data through their quartiles. Line on each box shows median value and small blue square-shaped dot does average. Lines extending from boxes mean the upper and lower quartiles. Individual points out of boxes are outliers. Different letters indicate significant differences at p < 0.05.

3.3.2 Greenhouse experiment

Germination percentages of *L. sativata* var. *capitata* treated by 10% leachate (57.6 ± 5.6%) or 100% leachate (56.8 ± 3.88%) were slightly lower than control (58.4 ± 4.66%). However, when it was treated by 50% leachate, germination percentage was higher than one with control (50%; 75.2 ± 2.94%). The tendency of germination percentage of *O. odorata* and *C. soldanella* responding to the leachate was similar. When they were treated with the leachate, they had higher germination percentage than control and with 50% leachate, the percentages of both species were the highest (*O. odorata* (control); 56.8 ± 4.96%, *O. odorata* (10%); 58.4 ± 5.15%, *O. odorata* (50%); 62.4 ± 6.01%, *O. odorata* (100%); 58.4 ± 3.71%, *C. soldanella* (control); 17.6 ± 5.74%; *C. soldanella* (10%); 17.6 ± 5.00%, *C. soldanella* (50%); 23.2 ± 4.27%, *C. soldanella* (100%); 15.2 ± 4.8%). Radicle length of *L. sativata* var. *capitata* increased with the concentration of leachate (control; 79.13 ± 3.89 mm, 10%; 80.73 ± 4.01 mm; 50%; 93 ± 3.29 mm, 100%; 97.8 ± 7.93 mm). In the case of *O. odorata,* seedlings with 10% and 50% leachate had longer radicles than control, and seedling with 100% leachate had same length of radicle as control (control; 60 ± 5.87 mm, 10%; 64 ± 5.90 mm, 50%; 61.1 ± 2.54 mm, 100%; 60 ± 7.04 mm). Compared to radicles of *C. soldanella* seedlings treated by control (63.47 ± 5.32 mm), 10% leachate and 50% leachate were getting longer as the concentration of leachate increased (10%; 65.13 ± 5.26 mm, 50%; 71.2 ± 1.79 mm). However, *C. soldanella* seedlings had the shortest radicle when they were treated by 100% leachate (57.8 ± 5.28 mm). Only *L. sativata* var. *capitata* had significant correlation with the leachate in terms of both germination percentage and radicle length (germination percentage; p-value = 0.02209, radicle length; p-value = 2.99e-06; $P < 0.05$). Figure 3-6 and 3-7 show the germination percentage and radicle length of each species at the last day of experiments.

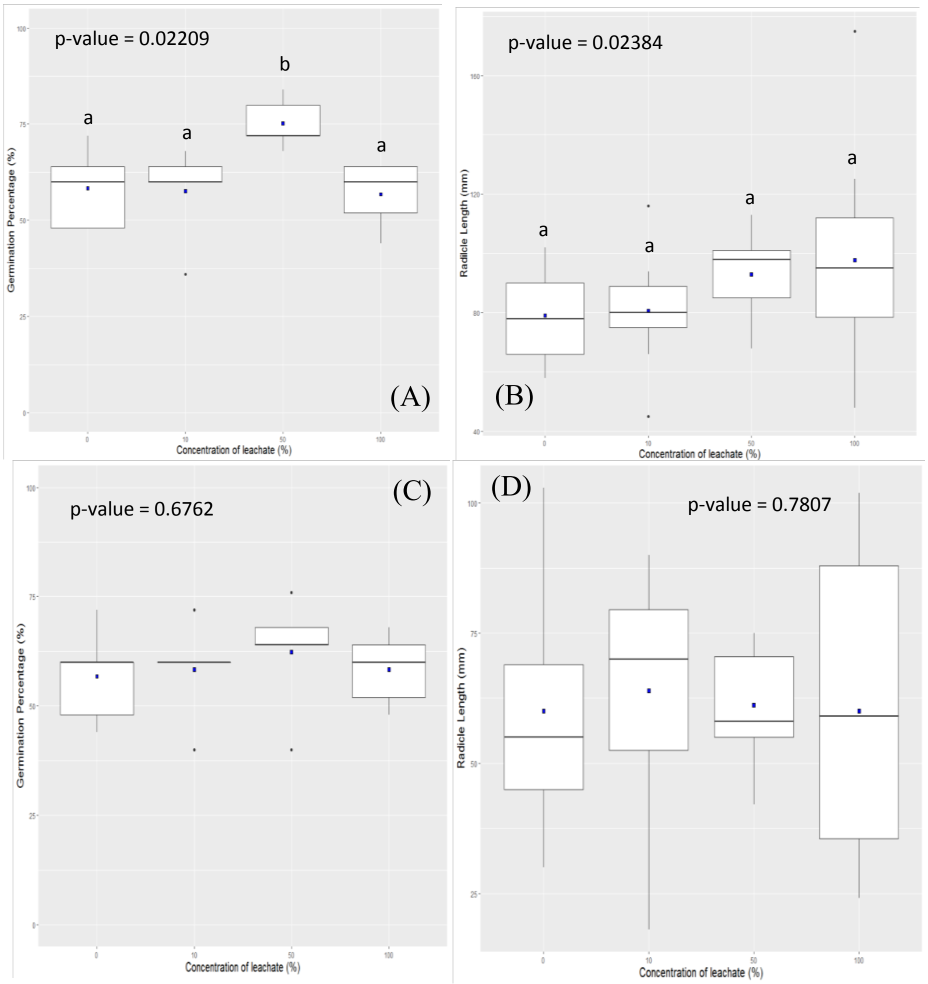

Figure 3-6. Germination percentage (*n* = 5) and radicle length (*n* = 15) of *Lactuca sativata* var. *capitata* and *Oenothera odorata* in soil condition (A) Germination percentage of *L. sativata* var. *capitata.* (B) Radicle length of *L. sativata* var. *capitata* (C) Germination percentage of *O. odorata* (D) Radicle length of *O. odorata.* Boxes depict groups of germination percentage data or radicle length data through their quartiles. Line on each box shows median value and small blue square-shaped dot does average. Lines extending from boxes mean the upper and lower quartiles. Individual points out of boxes are outliers. Different letters indicate significant differences at $p < 0.05$.

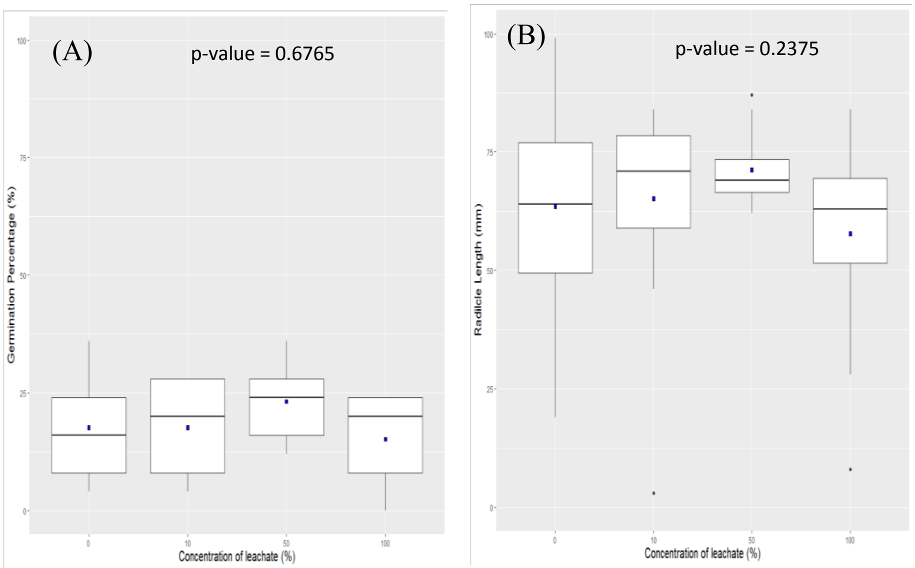

Figure 3-7. Germination percentage (*n* = 5) and radicle length (*n* = 14) of *Calystegia soldanella* in soil condition. (A) Germination percentage of *C. soldanella.* (B) Radicle length of *C. soldanella.* Boxes depict groups of germination percentage data or radicle length data through their quartiles. Line on each box shows median value and small blue square-shaped dot does average. Lines extending from boxes mean the upper and lower quartiles. Individual points out of boxes are outliers. Different letters indicate significant differences at $p < 0.05$.

3.4 Discussion

In laboratory condition to see allelopathic influence of *V. rotundifolia*, *L. sativata* var. *capitata, O. odorata* and *C. soldanella* showed similar response to the leachate treatments in terms of germination percentage. All of three had higher germination percentage with 10% leachate and 50% leachate treatments, compared to the control. With 100% leachate which is similar to possible concentration at the boundary of *V. rotundifolia* community where it interact with other species (author's observation), their germination percentage were lower than control. However, p-values of all three cases in terms of germination percentage were over 0.05, meaning insignificant differences. Thus, it could not be seemed to have allelopathic influence on germination in petri dish condition.

In greenhouse, in soil assay was used to investigate allelopathic influence of *V. rotundifolia.* Since soil was involved in allelopathy as well, this assay is meaningful to see allelopathic influence in natural condition. *L. sativata* var. *capitata, O. odorata* and *C. soldanella* had the highest germination percentage with 50% leachate treatment. *L. sativata* var. *capitata* showed slightly inhibition with 10% leachate and 100% leachate treatments. With 10% leachate treatment, *L. sativata* var. *capitata, O. odorata* and *C. soldanella* showed slight inhibition, stimulation and no effect, respectively. Slight inhibition of *L. sativata* var. *capitata* with 10% leachate was similar to no effect, as the average number of seedlings with 10% leachate and control was 14.4 and 14.6, respectively. With 100% leachate, the germination of *L. sativata* var. *capitata* and *C. soldanella* were inhibited but one of *O. odorata* was stimulated. However, compared to 50% leachate treatment, *O. odorata* had lower germination percentage, showing allelopathic influence.

Radicle length or seedling growth are secondary effects responding to allelechemicals as they reflect primary metabolic disruption (Lovett et al., 1989). Young seedlings may be most susceptible to biologically active chemicals (Lovett et al., 1989). Especially, radicle length is a sensitive indicator showing influence (Romeo&Weidenhamer, 1998). In laboratory condition, all of target species showed more radicle elongation with increasing concentration of leachate, except *C. soldanella* with 50% leachate treatment. This error might be caused by cotyledon of *C.soldanella.* Its cotyledons are quite large, making them lopsided to bottom and radicles to top. This made radicles difficult to absorb water, affecting radicle growth. If radicles had been on right position, length of radicle would have been about 55 mm, showing stimulation.

In the case of greenhouse experiment, all with only one exception of *C. soldanella* with 100% leachate treatment showed stimulation showed stimulatory effect on radicle growth by *V. rotundifolia* leachate. *L. sativata* var. *capitata* radicles were elongated more as the concentration of leachate increased. *O. odorata* radicle growth showed stimulation with leachate treatment, decreasing with the increase of concentration. *C. soldanella* radicle growth followed allelopathic tendency, stimulation at low concentration and inhibition at high. Responses of each test species were different unlike in laboratory condition and these were observed before (Lorenzo et al., 2010). It might be attributed to the presence of soil. Blum et al. (1993) found the allelopathic interactions in soil environments could involve the interaction with neutral substance, promoters and/or inhibitors of plant growth. Also, after the release of allelochemicals from leaves, fruits and/or litters into the environment, a cascade of biochemical events may follow, affecting each step of the allelopathic process (Anaya, 2010).

In these two conditions of experiments, only radicle length of three species in laboratory condition and *L. sativata* var. *capitata* results in soil condition showed significance, having p-value less than 0.05. First, in the case of petri dish assay, it might be explained by sensitivity of radicle elongation. Romeo and Weidenhamer (1998) said that radicle elongation was a more sensitive indicator than germination to show influence. Moreover, radicle came in contact with leachate directly in petri dish, so it might be more reliable indicator to see the influence by *V. rotundifolia* leachate. Also, in soil assay, only *L. sativata capitata* showed significant differences in terms of germination percentage and radicle growth. It might be explained by type of species. *L. sativata capitata* is an annual plant, *O. odorata* is a biennial and *C. soldanella* is a perennial species. Unlike *O. odorata* and *C. soldanella, L. sativata capitata* germinates soon and grows rapidly. Furthermore, it is usually chosen to be allelopathic indicator because of its high sensitivity. *O. odorata* and *C. soldanella* might need longer time to see allelopathic influence confidently with in soil assay. Unlike laboratory condition, germination percentage of *L. sativata* var. *capitata* showed significant difference with different treatments. It might be caused by the presence of soil which is involved in allelopathic interaction in nature.

Stimulatory effect could be considered nonsense but it may have deleterious effects at population level in long term since too early stimulation of seeding growth during periods of poor resources could lead to increasing seedling mortality (Lorenzo et al., 2010). Especially, coastal sand dune has harsh environment with low moisture, low organic matter and high salinity. Stimulatory effect through allelopathy helps more plants to germinate but after germination, seedling would encounter another hurdle, competing siblings for limited resource. Also, radicle elongation of other coastal sand dune plants by

V. rotundifolia leachate might be beneficial for it, since deep root would stabilize coastal sand dune, a dynamic ecosystem with wind and wave.

Fruits of *V. rotundifolia* are light so they roll easily by wind, spreading outside community. Allelochemical might be leached from these fruits by rain, fog drip or dew. At the initial stage, there might be few of fruits, making low concentration of leachate. This low concentration would stimulate germination and radicle growth of other coastal sand dune plants, resulting in the increase of stability of coastal sand dune. *V. rotundifolia* seeds have physical and physiological dormancy (Cousins et al., 2010b), meaning their germination do not readily happen. Even if germination would happen, their seedlings are too weak to survive harsh environment (Cousins et al., 2010a). This means that after coastal sand dune gets stabilized, they would survive. Considering the results, allelopathy through fruit leachate might be a helpful for *V. rotundifolia* to settle and dominate.

3.5 Conclusion

Allelopathy is a critical factor to operate ecological processes, including community dynamics (Muller, 1966). Previous studies found that *V. rotundifolia* possesses allelopathic influence by using only lettuce as a receiver. In this chapter, germination experiments with petri dish assay and in soil assay were conducted to confirm allelopathic influence of *V. rotundifolia* fruit leachate to three species, *L. sativata* var. *capitata, O. odorata* and *C. soldanella*. According to results, it was shown that *V. rotundifolia* fruits have allelopathic influence on radicle growth of all three species in laboratory condition. Also, they affected germination and radicle length of *L. sativata* var. *capitata* in soil condition. In short term, stimulation by *V. rotundifolia* seems to be beneficial to receiver species, but in long term, it would lead to premature plant growth in the condition of lack of supply and the increase of seedling mortality, leaving the stabilized dune with their long roots behind. Through this process, coastal sand dune might become suitable environment for *V. rotundifolia* seeds to germinate and seedlings to survive.

Chapter 4. General conclusion

4. Conclusion

Vitex rotundifolia is a sprawling shrub in the family of Verbenaceae (Lee, 1980). It is an obligate beach-dune species which usually occurs on coastal sand dunes, both the front and back (Murren et al., 2014). According to previous studies introduced in chapter 1, *V. rotundifolia* contains various chemical compounds including phenolic compounds. Among them, some of chemicals from *V. rotundifolia* relate to strategy to dominate. It is known that *V. rotundifolia* induces the increase of soil hydrophobicity through *n*-alkane from its fruit and leaves. Also, some studies found that it has plant growth regulator, resulting in allelopathic influence. Compared to chemical and pharmacological studies, there are not enough ecological studies about *V. rotundifolia.* Most of them were about the condition of habitat and the growth characteristics. It means that there is few paper about the correlation between surroundings and *V. rotundifolia.* As it is both dune stabilizer to preserve coastal dune and medicine to treat various illness, more specific studies should be conducted.

The purpose of the present study is to confirm the relation between *V. rotundifolia* and surrounding. First, the species and environmental factors within *V. rotundifolia* were investigated to know the ecological meaning of the presence of *V. rotundifolia.* Usually, it appears on the stabilized dune or landside of foredune so its existence might be reflect vegetative structure and the degree of stability of dune. Then, allelopathic influence of *V. rotundifolia* was confirmed in two conditions, laboratory and in-soil conditions. Especially, since it has been suggested to be used as rehabilitation plant, its influence to other coastal plants should be identified. With these, the basic information about *V. rotundifolia* could be provided to manage coastal sand dune where it appears.

In chapter 2, species and soil characteristics of *V. rotundifolia* community were investigated. The investigation was done at Masian beach where coastal sand dune with well-preserved vegetation exists in August. There were 12 species within *V. rotundifolia* community and among them, *D. teres, C. kobomugi* and *L. japonicus* had high cover. According to NMDS diagram, *L. japonicus* appears close to *V. roundifolia,* sharing habitat. On the other side, even though *C. kobomugi* had high coverage, it is close to *C. soldanella* which is a pioneer species like it. The results show the dune on Masian beach has a complex vegetation struction within *V. rotundifolia* community which is dominant species. Also, soil characteristics of the area show that the *V. rotundifolia* community exist on a typical coastal sand dune with low moisture and high salinity. According to the ordination, it was found that soil organic matter and soil hydrophobicity have significantly correlation with *V. rotundifolia* community structure. According to Cousins et al. (2009), soil hydrophobicity increases with the presence of *V. rotundifolia* which contains *n*-alkane, the organic compound.

In chapter 3, allelopathic influence of *V. rotundifolia* was confirmed. Germination experiments in both laboratory and in soil conditions were conducted with *L. sativata* var *capitata, O. odorata* and *C. soldanella.* The results showed that *V. rotundifolia* has allelopathic influence on its target species by its leachate differently depending on experimental condition. In petri assay, radicle growth of all species showed significant responses but in soil assay, only *L. sativata* var. *capitata* was affected by leachate significantly in both aspects, germination percentage and radicle length. Usually, stimulatory effect by leachate was observed in the present study. According to the allelpathic quality model, with low concentration, stimulatory effect occurs but with high concentration, inhibitory effect does (Liu et al., 2003; An et al., 1993). Especially, stimulation could be regarded as loss but in long term, it might not be. In long term, stimulation of germination might lead to the increase of seeding mortality because of premature growth in the condition of lack of supply (Lorenzo et al., 2010). Also, elongated root might be helpful to stabilize dune, making a better environment for *V. rotundifolia* seedlings to survive.

With the present research, it was found that within *V. rotundifolia* communities, there are various plant species appear and their composition might reflect the state of coastal sand dune. Also, *V. rotundifolia* has significant correlations with soil organic matter and soil hydrophobicity, which are related to its strategy to dominate. These indicates that *V. rotundifolia* might be a good indicator to show stabilizing process and plant succession of coastal sand dune. Also, as fruits are really light to easily spread and contain allelochemicals, they have allelopathic influence on neighboring species, usually stimulating germination percentage and radicle elongation. Thus, *V. rotundifolia* might have influence on surroundings and this study would be helpful to manage a dune that encounters threat to be destructed.

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국문 초록

순비기나무는 마편초과에 해당하는 기어서 자라는 관목으로 해안 사구 대표 식물 중 하나이다. 이는 해안사구의 전면부와 후면부에서 나타난다. 순비기나무에서 나오는 다양한 화학물질로 인해 약제로 사용되어왔고 아름다운 잎과 꽃 덕에 세계적으로 경관식물로 사용되고 있다. 그에 더해, 깊게 자라는 뿌리를 통하여 사구를 안정화시키는데 기여하여 손상된 지역에 복원 식물로 제안되어 오고 있다. 이러한 유용성에도 불구하고 순비기나무에 대한 생태학적 연구는 부족한 실정이다. 본 연구에서는 8 월에 식생이 잘 보존되어있는 사구가 있는 마시안 해변의 순비기나무 군락의 종 조성에 대해 조사하였다. 방형구 조사가 시행되었으며 각 방형구에서 임의적으로 0-5 cm 와 5-10 cm 깊이의 흙을 채집하였다. 또한, 순비기나무 군락 서식지 조건을 알기 위해 토양 특성이 측정되었고 NMDS 를 통하여 순비기나무 군락 구조와 환경 변수간의 상관 관계가 확인되었다. 마시안 해변 순비기나무 군락에는 갯완두와 통보리사초를 비롯하여 12 종의 식물이 존재하였다. 해당 군락의 서식지 환경은 적은 수분과 높은 염도를 나타내며 전형적인 해안사구의 특성을 보였다. NMDS 결과에 따르면 환경 변수 중 토양 소수성과 유기물이 군락 구조가 유의미한 상관관계를 가짐을 알 수

있었다. 이러한 결과를 바탕으로 전사구와 안정화된 사구에서 나타나는 다양한 해안 사구 식물이 군락 내에 공존함을 알 수 있었고 이는 마시안 해변의 해당 군락이 복잡한 식생을 가지고 있으며 어느 정도 안정화된 사구임을 알 수 있었다. 또한, 군락의 토양 특성이 해안 사구 환경과 일치했음을 보였으며 그 중에서도 순비기나무의 우점 전략과 관련되었다고 알려진 토양 소수성과 유기물이 유의미하게 군락 구조와 상관관계를 이룸을 알 수 있었다. 순비기나무는 다양한 화학 성분들을 가지고 있으며 그 중 일부는 페놀릭 화합물이나 플라보노이드 화합물과 같은 알렐로케미컬이라고 알려진 물질들이 해당된다. 본 연구에서는 순비기나무가 다른 해안 식물들에 알렐로패시적 영향을 주는지 실험적 조건과 토양 조건에서 실험하였다. 알렐로패시 실험에 가장 흔히 사용되는 상추, 해안 사구 대표 귀화 식물인 달맞이꽃 그리고 해안 사구 자생 식물 중 하나인 갯메꽃이 순비기나무의 알렐로패시적 영향을 확인하는데 사용되었으며 순비기나무 침출액은 0%, 10%, 50%, 100%를 사용하였다. 실험실 조건에서는 발아율 관점에서는 의미있는 효과는 보지 못하였지만 유근 생장은 농도가 진해질수록 촉진되었다. 또한, 토양 조건에서 50% 침출액 처리 시, 상추의 발아율은 가장 높았으며 유근은 농도가 증가함에 따라 더 길어졌다. 하지만, 달맞이꽃과 갯메꽃의 경우 유의미한 차이를 볼 수는 없었다. 비록 촉진 효과가 다른 종들에게

긍정적으로 작용하는 것처럼 보이나 이는 장기적으로 봤을 때 유묘들의 사망률을 높이기에 유해할 수 있다. 본 연구를 통하여 순비기나무 군락의 그 주변과 관계를 갖고 있음을 알 수 있었다. 순비기나무 군락의 종 조성은 해안 사구의 상태를 보여주며 토양 유기물과 토양 소수성과 같은 환경 요인은 군락과 상관관계를 가진다. 또한, 순비기나무 열매는 바람과 해류에 의해 쉽게 퍼지면서 군락에서 멀리 떨어진 주변 식물들까지 알렐로패시적 영향을 미친다. 본 연구는 순비기나무 군락 관리와 손상된 해안 사구 복원에 필요한 기본 정보를 제공하는데 그 의미가 있다.

주요어: 순비기나무, 해안 사구, 해안 사구 식물, 토양 환경, 알렐로패시, 침출액, 만형자, 식생

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