T.R.
NIGDE OMER HALISDEMIR UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF PLANT PRODUCTION AND TECHNOLOGIES

MASTER THESIS

STUDIES ON THE SEED BIOLOGY, SOIL SEED BANKS AND NATURAL RECRUITMENT OF RUSSIAN OLIVE (*ELAEAGNUS ANGUSTIFOLIA*) AND SEA BUCKTHORN (*HIPPOPHAE RHAMNOIDES*)

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Supervisor
Prof. Dr. IAN TIMOTHY RILEY

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The study titled "**Studies on the Seed Biology, Soil Seed Banks and Natural Recruitment of Russian Olive (*Elaeagnus angustifolia*) and Sea Buckthorn (*Hippophae rhamnoides*)"** and presented by **Baqer Hussein Abbas Al Aabbas** under the supervision of **Prof. Dr. Ian Timothy Riley** has been accepted as Master thesis by the jury at the **Department of Plant Production and Technologies** of Nigde Omer Halisdemir University, Graduate School of Natural and Applied Sciences.

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CONFIRMATION

This thesis has been found appropriate at the date of ..... /..... /2022 by the jury mentioned above who have been designated by the board of Directors of Graduate School of Natural and Applied Sciences and has been confirmed with the resolution of Board of Directors dated ..... /..... /2022 and numbered .....

..... /..... /2022

Prof. Dr. Murat GÖKÇEK

DIRECTOR

DECLARATION OF THESIS

I certify that this thesis has been written by me. I proved that all information exist in this thesis is scientific and is in accordance with the academic acceptance. Any kind of help and providing of stuffs while conducting thesis research have been acknowledged in the thesis.

Baqer Hussein Al Abbas

ÖZET

KUŞ IĞDESI (*ELEAGNUS ANGUSTIFOLIA*) VE YALANCI IĞDE'NIN (*HIPPOPHAE.RHAMNOIDES*) TOHUM BIYOLOJISI, TOPRAK TOHUM BANKASI VE DOĞAL OLARAK YETIŞTIRILMESI ÜZERINE ÇALIŞMALAR

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Niğde Ömer Halisdemir Üniversitesi

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Bu çalışma, *Elaeagnus angustifolia* ve *Hippophae rhamnoides* tohum bankalarını değerlendirmektedir. Ayrıca, *E. angustifolia* ve *H. rhamnoides* doğal alımını etkileyen koşulların ve her iki türün çimlenmesi için minimum büyüme sıcaklığının değerlendirilmesi yapılmıştır. *E. angustifolia* tohumları Niğde Ömer Halisdemir Üniversitesi, Niğde, Türkiye'den, *H. rhamnoides* tohumları Niğde, Çamardı'dan toplanmıştır. *E. angustifolia* meyve karakterizasyonu, meyve ve endokarp uzunluk, çap ve ağırlığının ağaca ve bölgeye göre değiştiğini göstermiştir. *E. angustifolia* verileri, bitki atık örneklerinin toprak örnekleriyle karşılaştırıldığında daha fazla tohum içerdiğini göstermiştir. Doğal tohum bankalarının sulanmasından elde edilen sonuçlar, sulanan ağaçların tüm lokasyonlardaki tüm ağaçlarda en yüksek çimlenme oranına sahip olduğunu göstermiştir. Her iki tür için de minimum çimlenme sıcaklığı 15°C'de başlamış ve bu sıcaklık aralığının altında tohum çimlenmemiştir. Ekim derinlikleri kullanılarak sulu ve susuz koşullarda simüle edilen tohum bankası verileri, en yüksek çimlenme oranını 20 mm ekim derinliğinde, en düşük çimlenme oranını ise 30 mm ekim derinliğinde gözlemlemiştir.

Anahtar Kelimeler: Tohum Bankaları, Biyoloji, Agroecosystems, *Elaeagnus angustifolia, Hippophae rhamnoide.*

SUMMARY

STUDIES ON THE SEED BIOLOGY, SOIL SEED BANKS AND NATURAL RECRUITMENT OF RUSSIAN OLIVE (*ELAEAGNUS ANGUSTIFOLIA*) AND SEA BUCKTHORN (*HIPPOPHAE RHAMNOIDES*)

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This study aimed to assessed the size and viability of *Elaeagnus angustifolia* and *Hippophae rhamnoides* seed banks. Also, the evaluation of conditions that affect natural recruitment of *E. angustifolia* and *H. rhamnoides* was done and minimum growth temperature for germination of both these species. Seeds for *E. angustifolia* were collected from the Nigde Omer Halisdemir University, Nigde, Türkiye, while seed for *H. rhamnoides*, from Çamardı, Nigde. The fruit characterization of *E. angustifolia* showed that the length, diameter and weight of the fruit and endocarp varied according to tree and site. Data for *E. angustifolia* showed that litter samples contain more seeds as compared to soil samples. Results obtained from watering of natural seed banks showed that watered trees had the highest rate of germination in all trees at all locations. The minimum germination temperature for both species was started at 15°C and below this range of temperature no seed germinated. Data for simulated seed bank in underwatered and unwatered conditions by using sowing depths showed highest rate of germination at 20 mm sowing depth and the lowest germination rate was observed at 30 mm sowing depth.

Keywords: Seed Banks, Biology, Agroecosystems, *Elaeagnus angustifolia, Hippophae rhamnoides.*

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

SYMBOLS AND ABBREVIATION

Symbols	Description
%	Percentage
℃	Centigrade
m^2	Square meters
M	Meter
Mm	Millimeter
Kg	Kilogram
g	Gram
No.	Number

CHAPTER I

INTRODUCTION

A seed bank is the resting place or reservoir of seeds that play important role in plants' persistence. It contains both old seeds that persist for several years and newly scattered seeds. Soil seed banks have become part of theories in plant ecology with reference to evolution, traits and succession since Darwin (1859) noted the presence of seeds that could facilitate plant recruitment in soil of pond ecosystems (Abella et al., 2013). Presently, studies of seed banks have shown key understanding of plant ecology and biological conservation (Abella et al., 2013). In conservation biology, soil seed banks provide means of managing rare species or detecting introduced plant species (Lu et al., 2010). They have become crucial component of research topics in plant and vegetation ecology, composition of new plant recruitment (Wang et al., 2013), and co-determines the direction of secondary succession after disturbances at community level (Pakeman and Small, 2005). In addition, belowground seed banks can be critical indicators of aboveground vegetation response to climate change (Moles and Westoby, 2004). Soil seed banks are also key sources of propagule for vegetation restoration because they produce memory of past vegetation and represents the composition of future populations (Gioria et al., 2012). Also, seed bank enables the coexistence of potential competing species and alleviate the effects of inter or intraspecific competitions for the reason that species have different responses to environmental changes and resources (Gioria et al., 2012).

Recently, estimation of soil seed banks are important components in plant ecology and biological conservation. This is because, reliable estimates of soil seed banks when used to evaluate plant regeneration potential, can provide available resources for seed eating organisms and site history which includes evidence of past disturbances (Abella et al., 2013). Generally, there are two methods to estimate soil seed banks (Gonzalez and Ghermandi, 2012). These are the seedling emergence and seed extraction methods. In seed extraction method, seeds and soil particles are separated based on their different sizes and densities (Abella et al., 2013). The first method, seedling emergence method consists of placing seeds under suitable growth conditions for germination (pots, greenhouse or growth chamber). The germinated seeds are later identified and quantified (Thompson and Grime, 1979). In comparison, seedling emergence method is

commonly used (Ter Heerdt et al., 1996), because it is less demanding and useful for large volume of soil samples (Gonzalez and Ghermandi, 2012). However, seedling emergence can underestimate the density of seed bank due to associated errors with seed dormancy and suitability of the environment for germination (Price et al., 2010). The second method, the extraction method provides a better estimation of the total soil seed bank but this may include non-viable seeds.

Generally, perennial species depend on seeds to set up new colonies away from mother plants. So, seed bank studies are important for perennials plants such as *Elaeagnus angustifolia* L. and *Hippophae rhamnoides* L., which are important small trees or shrubs and have various agroecosystem benefits (Khamzina et al., 2009). These plants are produced for a variety of reasons, including fuel, windbreaks to prevent soil erosion, and shading. Many animals and birds can use these plants as nesting sites. Both species may fix nitrogen in the soil by having bacteria in their root nodules that fix nitrogen. The most important bacterial species that involved in nitrogen fixation is the *Frankia* species. *Frankia* are actinomycetes and fix nitrogen from the atmosphere by forming root nodules in many weedy plants (Adeleke et al., 2019). These plants have a wide range of applications in the recycling and improvement of degraded croplands due to their nitrogen fixation ability (Khamzina et al., 2009).

E. angustifolia is a multi-stemmed tree or shrub native to Eastern Asia and Southern Europe that is used to conserve soil and water resources (Collette and Pither, 2015; Katz and Shafroth, 2003). The seeds of *E. angustifolia* may have an advantage in the establishment, both in disturbed and undisturbed areas due to their significantly larger and heavier size (Katz et al., 2001). Previous research has found that the environmental factors had a substantial impact on the spread of *E. angustifolia* in the western USA and Canada (Nagler et al., 2011), and the low temperature is the most suitable temperature for its occurrence and distribution (Friedman et al., 2005; Guilbault et al., 2012).

The germination of *E. angustifolia* plants needed a specified seed depth. Within 30 days of sowing, 89.6% of seeds germinated and emerged in research where seeds were planted at a depth of 25 mm (USDA, 2014). *E. angustifolia* also does not appear to be capable of emerging from or surviving burial at or beyond a 75 mm depth, according to research. *E. angustifolia* is a generalist tree that can withstand a wide range of abiotic circumstances, such as varying levels of shade, water, and soil composition.

E. angustifolia seed bank tests showed that this plant can store a greater quantity of seeds inside the soil as a soil seed bank (Brock, 2003). In most ecosystems, the dormant seeds are naturally stored in the soil in the form of soil seed bank (Shiferaw et al., 2018). The soil seed bank/reservoir also displays the potential seeds that are present on the surface of an area or that are under dormant conditions in the soil (Li et al., 2017). The upper 15 cm surface mostly contains viable seeds and the litter or humus also exhibited some seeds (Mekonnen, 2016). The soil seed bank serves as a reservoir of several vegetative propagules and viable seeds that are capable of naturally regeneration. In many plant assemblages, a seed bank is a vital part of ecosystem resilience and has a great potential of germination when favorable conditions arise.

H. rhamnoides is a perennial shrub or plant inherent to Asian countries, including Türkiye, India, Pakistan, Tibet, Russia, and European countries, including Spain, the United Kingdom, and Italy, as well as Canadian provinces like British Columbia and Saskatchewan (Beveridge et al., 1999; Guliyev et al., 2004). *H. rhamnoides* is an important plant, especially for land procurement, and it is also economically valuable. It is also used in a variety of ways around the world. This plant has been employed in human, animal, and avian nutrition, as well as pharmaceuticals, agricultural, and decorative purposes (Chen et al., 2010). This plant is utilized in agroecosystems to create protective belts and living fences around cropped areas, to plant greens, and to save soil from cutting and erosion (Thomas and Thomas, 2003; Musayev, 2013). It has the potential to replace and conserve soil in the temperate Himalaya's delicate ecosystem. Its large root system protects the soil from high-velocity wind erosion, which is prevalent in cold deserts. As a result, in the erosion-prone soils of cold and desolate mountains, the plant is thought to be an efficient soil binder (Rajiv et al., 2013).

H. rhamnoides spreads swiftly in the presence of abundant water and quickly cover the ground in some areas. A study showed that large-scale communal water usage resulted in a long-term decrease in soil moisture content (Guo et al., 2003). For example, a study in China stated that since 1999, the Huangfuchuan Watershed has experienced severe droughts with a less than 300 mm annual precipitation, resulting in the shortage of water that ultimately led to the death of *H. rhamnoides* in wide regions and also there are chances of extinction occurring at such areas. As a result, when growing *H. rhamnoides* in arid locations, water relations must be taken into considered (Guo et al., 2003).

In agroecosystems, soil seed banks are considered to have to two different roles in agriculture (Mahé et al., 2021). They are a potential threat to agricultural production because it is the main source of future weed infestation in fields (Mahé et al., 2021). In addition, larger and more varied weed seedbanks facilitate other biota with positive agroecological function in agroecosystems such as nutrient cycling, biological control and regulation of microclimate (Franke et al., 2009). Soil seed banks in agroecosystems also serve as a source of food for many invertebrate and small mammals (Mahé et al., 2021). Despite recent research focus on soil seed bank, particularly, in plant ecology, there is limited literature on seed biology and seed banks of *E. angustifolia* and *H. rhamnoides*, especially in semi-arid regions like Niğde. Therefore, the main objective of the study was to understand seed biology, soil seed bank, and the possibilities of natural recruitment for *E. angustifolia* and *H. rhamnoides* under the semiarid conditions of Niğde. Also, the specific objectives of the study was to (1) assessed the size and viability of *E. angustifolia* and *H. rhamnoides* seed banks, (2) determination of growth-limiting factor for *E. angustifolia* and *H. rhamnoides* germination, (3) evaluation of conditions that affect natural recruitment of *E. angustifolia* and *H. rhamnoides* and (4) determination of minimum growth temperature for germination of *E. angustifolia* and *H. rhamnoides*.

This research addresses five questions through a range of methods and research activities. These research questions include:

1. Does *E. angustifolia* and *H. rhamnoides* have large and viable seed banks?
2. Is water a limiting factor for the germination of *E. angustifolia* and *H. rhamnoides*?
3. Is the recruitment of *E. angustifolia* and *H. rhamnoides* affected by water availability and sowing depth?
4. Is there any temperature limitation (minimum temperature) for germination of *E. angustifolia* and *H. rhamnoides*?

CHAPTER II

LITERATURE REVIEW

2.1 Soil Seed Bank

The seed bank is the viable seeds that are present on the ground or inside the soil as dormant or active forms (Li et al., 2017). These seeds are mostly linked to litter or humus in different densities according to climatic regions and land use (Wakshum et al., 2018). Seed bank serves as a reserve of potential for regeneration in many plant assemblages and is a crucial part of ecosystem resilience. The study of soil seed banks may be used to forecast the composition of new plant recruitment. The properties of seed bank also provide some information about the future plant communities because it represents the partial record of existing plants at a specific site under varied ecologies and agroecosystems. It also provides some records about the responses of plant communities under varied climatic conditions over time (Auld and Denham, 2006).

Soil seed banks play a significant role in the study of plant ecology as a prospective plant population as it is one of the important research issues in studying the plant population and vegetation ecology (Zhou et al., 2005). For example, different plant species overcome the unfavorable environmental conditions by contributing in rich seed banks that help in their wide ecology and long existence. Also, with this strategy, plants are able to preserve their species diversity and retained the information about their structure and dynamics (Aponte et al., 2009).

In agroecosystems (arable/pasture land), the ecological studies on soil seed banks have been deliberated as devising two main roles in agriculture (Begum et al., 2006; Kamoshita et al., 2010). The seed bank constitutes a potential threat to agricultural production, because it is the primary source of future weed infestation in fields (Cavers and Benoit, 1989) and limited weed control practices for managing dormant seeds in the soil. To remove these limitations seed bank study plays an important role in determining the exact weed flora present inside the agricultural areas, natural grasslands and pastures.

Plants and shrubs such as *Elaeagnus angustifolia* and *Hippophae rhamnoides* have several advantages in the plant ecology and also, they play a significant role in

agroecosystems. These plants mostly colonize the other areas by generative expansion especially by zoochories (Bolibok et al., 2018). As these plants produce a huge number of seeds and have fleshy fruits so their seed bank mostly rich with seeds and also has the ability to disperse wider areas. Also, their seed bank plays an important role in the survival and invasion of species in a specific area under varied temperature and environmental conditions. The viability of *E. angustifolia* seeds are an important aspect that helps to survive under a wide range of conditions and assists them in their natural recruitment (Brock, 2003).

The studies about seed banks are mostly done by in situ and ex situ ways (Mesquita et al., 2015). In situ study includes the identification and numbering of emerged seedlings in the field to determine the flora inside the field. But this method is less precise and has some flaws because some species may possess seed dormancy or there may be some species whose seeds germinate but are unable to emerge due to unfavorable environmental conditions. However, the ex situ method or soil seed bank sampling includes the physical separation of seeds, conducting germination tests and determining favorable growth conditions by removing the barriers that cause an eruption in seed germination.

The current study aimed to study the soil seed bank of *E. angustifolia* and *H. rhamnoides*. This study covers the determination number of seeds present in the soil seed bank and their germination percentage according to the samples collected from different locations. This study also determines the status of water as a limiting factor for the germination of *E. angustifolia* and by regularly watering the natural seed bank and recording the data about germination as compared with the samples that are without irrigation. The recruitment of *E. angustifolia* and *H. rhamnoides* affected by water availability and sowing depth was also determined under the current study. And is there any temperature limitation exists (minimum temperature) for germination of *E. angustifolia* and *H. rhamnoides* was also studied.

2.2 Plants in the Elaeagnaceae family

Elaeagnaceae, often known as oleaster, is a plant family that primarily consists of perennial shrubs and small trees (Bartish and Swenson, 2004). The family is largely deciduous indigenous in whole cold as well as temperate zones between 7° W and 122° E longitude and 27° and 69° N latitude (Khan et al., 2011). The plants of this family are native to Australia, Europe, and Asia's northern to southern areas (Nazir et al., 2020). There are three genera (*Elaeagnus*, *Hippophae*, and *Shepherdia*) and about 250 plant species in this plant family (WFO, 2022). The *Elaeagnus* genus contains 98 species of shrubs and small trees (WFO, 2022). The *Hippophae* genus is divided into 6 species with 12 subspecies (Bal et al., 2011) while the *Shepherdia* genus contains only 3 species. The shape, color, and arrangement of fruits and seeds, as well as the color and distribution of leaves on shoots, are all examples of morphological distinctions between genera including the plants of Elaeagnaceae family. Also, there is a difference in the *Hippophae*, *Shepherdia*, and *Elaeagnus* genera in that *Hippophae* is completely wind pollinated while the other two are pollinated by insects (Bartish and Swenson, 2004). The majority of plants in this family have nitrogen-fixing bacteria in their root nodules, which allows them to fix soil nitrogen and make it usable, thereby playing an important function in the agroecosystem. Soil-borne Actinobacteria such as bacteria of the *Frankaria* genus involved in making root nodules and involved in nitrogen fixation (Miller and Baker, 1985) (Table 2.1).

Table 2.1. List of important plants of Elaeagnaceae family along with their key features related ecology and biology of these plants

Species	Common name	Growing areas	Natural habitat	Soil type	Water requirement	Light requirement
Hippophae rhamnoides	Sea buckthorn	Northern hemispher, boreal shield ecozone	River banks, seacoast, open areas	Sand, loam	Normal, moist dry	Sunny areas
Elaeagnus angustifolia	Russian olive	Mixed wood plains, atlantic maritime, montane cordillera, prairies	Meadow/ prairie, forest border, riparian, lakeshores, dessert	Clay, calcic, sand	Normal, dry, moist	Partial shade, sunny
Elaeagnus umbellate	Autumn olive	Mixed wood plains, atlantic maritime, boreal shield	Open areas and fields	Sand, clay	Normal, dry	Sunny areas
Elaeagnus commutate	Wolf-willow and Silverberry	Lakeshore, forest edge, riparian edge, riparian /meadow/ field	Boreal shield, prairies, montane cordillera, hudson plains	Clay, sand	Dry	Sunny, partial shade
Sheperdia argentea	Buffaloberry, Thorny	Prairies	Woodland, saltwater, shoreliens	Sand, loam, clay	Moist, dry, normal	Sunny areas
Sheperdia Canadensis	Soopolallie, silver, buffaloberry	Taiga shield, prairies, mixed wood plains, boreal shield, pacific mari	Lakeshores, swamp, marsh, forest edge, woodland, racky bluff	Sand, clay, calcic, loam	Moist, dry, normal	Partial shade, sun

Source: Adopted from Li and Schroeder (1996), Muma (2013) and Collette and Pither (2015).

The plants of the genus *Elaeagnus* are mostly native to subtropical to temperate zones of the Asia continent while some species extend their distribution to northern Australia (e.g., *E. trifloral* Roxb.) and North America (e.g., *E. commutata* Bernh. Ex Rydb.) (CABI, 2022). Species in the genus *Hippophae* are incredibly diverse due to withstanding exceptionally lower temperatures (Xu et al., 2009) as this plant has a large and aggressive root system. While, the *Shepherdia* genus contains dioecious small shrubs and is native to the Americas (North and South). These plants are well known due to their specific non-leguminous nitrogen-fixing feature (Becking, 1970; Muhammad et al., 2019).

Fruit of species in the Elaeagnaceae resembles a drupe (seed surrounded by a single shell of hardened endocarp), which is a berry with a stony seed that is red-orange or grey in appearance. The seeds of this family are mostly solitary with the hard seed coat or testa (Bartish and Swenson, 2004). The embryo is straight without chlorophyll pigments. The endosperm is mostly absent or scanty while the cotyledons have edible flesh with a plano-convex shape. The seeds come in a variety of colors and shapes, ranging from elliptical such as plants of *H. rhamnoides*, to egg-shaped species *fluviatilis* (Krejcarová et al., 2015). Elaeagnaceae plants produce edible fruit that is eaten by birds and humans, allowing for long-distance spreading. Furthermore, dried fruits float easily in water and can be transported far from their source. Elaeagnaceae plants are thought to have both wind and biotic pollination (i.e., ambophily) (Culley et al., 2002).

2.3 Soil seed banks and natural recruitments of Elaeagnaceae plants

Seed banks are critical for species survival and invasion in a given area under varying temperatures and climatic circumstances (Fisher et al., 2009). Elaeagnaceae fruits contain a lot of pulp, which means they would not stay for a long time inside the soil and will be devoured by rodents and insects. Seeds in the soil have an average life of about two years. Silverberry (*E. commutata*) seeds, for example, can be preserved for over two years if kept cool and dry (at 6% moisture content) (Sheet and Page, 2018). A study on the seed viability showed that the dried stored seeds at room temperature for up to two years were still viable (Schopmeyer, 1974).

The plants of Elaeagnaceae family are able to establish on a wide range of sites (Shafroth et al., 1995). For example, the seeds have a dormancy states and are capable of germination when optimum conditions arise. Also, the seed coat hardness saves the seeds from rodents' attacks due to which they can survive for a long duration (Young and young, 1992). The plants of the Elaeagnaceae family are mostly shade-tolerant, especially at the emergence stage (Collette and Pither, 2015). These plants have the ability to compete with other trees and shrubs, and have the potential to become a dominating climax species in the United States, replacing native cottonwoods and willows as well as water courses. *E. umbellata*, for example, got the most ecological benefits since it is well established between the borders and nearby interiors of forest sites in southern Illinois and considering a big reservoir of natural recruitments (Yates et al., 2004).

2.4 Elaeagnaceae plant in Türkiye

Türkiye is home to some *Elaeagnaceae* species, including *E. angustifolia* (Gokturk et al., 2006). These plants can be found all over Türkiye, primarily in the north and east, from sea level to high heights of roughly 3000 meters. *H. rhamnoides* L. is also found in different regions stretching from Persia's Alborz Mountains to Caucasia and Eastern Türkiye (Aras et al., 2007).

2.5 Soil seed bank and natural recruitment of *Elaeagnus angustifolia*

Because of its ability to grow in a variety of environments, *E. angustifolia* is cultivated from northern Asia to the Himalayas and Europe (Klich, 2000). *E. angustifolia* is a deciduous little tree or shrub that can grow for 80 to 100 years and starts to fruit within 5 to 6 years (Kiseleva and Chindyaeva, 2011). The plant's spines are a gleaming brownish-red color. Silver-white scales cover the stems, leaves, flowers, and fruits (Iriondo et al., 1995). The fragrant flowers are erect or almost erect, with bell-shaped calyx tubes and a prominent, glabrous, conical floral disc that surrounds the base of the style (Mistríková, 2007).

The fruit of *E. angustifolia* is a 12 mm light grayish-green olive-shaped drupe with one firm seed within. The seeds are mostly dispersed by some birds and mammals in the form of droppings. These dropped seeds can survive for up to three years and germinate

in several kinds of soil (Knopf et al., 1988). The germination process is normally aided by seed stratification in damp sand for duration of 90 days at 5°C (Edward, 2014). It produces a large quantity of fruits and seeds under normal conditions but if the annual precipitation is increased to 400 mm, it bears little fruits.

2.6 Germination and growth conditions of *E. angustifolia*

E. angustifolia is mostly grown from seed, but cuttings are employed in locations where there is enough moisture. Seeds of *Elaeagnus* species are dormant and must be pretreated before planting. To overcome embryo dormancy, pre-germination therapies such as cold and warm scarification, floating of seeds in hot water, hot air treatment and chemical or mechanical scarification are the most used methods (Gokturk et al., 2006). Also, pre-chilling seeds for 10 to 90 days at 1 to 5°C (34 to 41°C) aids in dormancy breakdown (Olson, 2006). Pre-chilling is required, implying that embryo dormancy is a barrier to germination. For *E. angustifolia*, the minimum effective stratification duration is 9 to 12 weeks (Jinks and Ciccarese, 1997). According to Göktürk et al. (2006), soaking *E. angustifolia* seeds in flowing water at 15°C for 10 days followed by a 30-day cold scarification treatment enhances germination.

Fruit and seed coats are also engaged in dormancy management, as the endocarp and seed coat of *E. angustifolia* is removed, resulting in rapid germination (Jinks and Ciccarese, 1997). The ability of sulfuric acid to scarify these structures suggests that they may physically limit the embryo's ability to emerge (Jinks and Ciccarese, 1997). However, Hamilton and Carpenter (1976) discovered coumarin-like inhibitors in the endocarp, testa, and embryo of *E. angustifolia*, suggesting that these chemicals are responsible for germination suppression. Morgenson (1990) found that soaking silver-olive seeds at room temperature was not as successful as moist pre-chilling at 4°C (39°C) for 30 to 90 days in overcoming dormancy.

E. angustifolia is a tree that thrives in temperate areas with hot summers and cold to bitterly cold winters. Trees can survive frosts of -30°C, according to Bartha and Csiszár (2008), but terminal shoot damage may occur. Zhang et al. (2018) found that the yearly mean temperature, temperature fluctuations, air humidity level and chilling conditions are the primary environmental parameters restricting its potential distribution range in China, based on simulation studies. It may thrive in a variety of conditions due to its

drought tolerance and ability to grow in salt affected, acidic and less fertile lands (Knopf et al., 1988). It also has an aggressive root system that takes nutrients more efficiently. It doesn't care about the type of soil, the temperature, or the amount of moisture in the air (Zheng, 2004). It thrives along flooded areas and where there is natural soil water flow occurred, for instance, on riverbanks, although it can also be distributed in dry areas such as desserts, although in such areas it grows slowly, especially if the water table is below than four meters

2.7 Seed bank studies and natural recruitment of *E. Angustifolia*

Every year, *E. angustifolia* plants generate a large number of seeds that fall to the ground and make a rich soil seed bank. Birds devour their fruits and move them to other regions because of the edible flesh on the seed surface. Brock (2003) conducted a study to observe the seed bank of *E. angustifolia* and found that in the seed bank for the five different locations, the average seeds were 336 to 494 seeds per meter square with 63% viable and fresh seeds while the remaining 37% seeds had less or no germination potential and often were old.

The viability of *E. angustifolia* seeds are an important feature that allows them to survive in a variety of circumstances and germinate quickly when conditions are appropriate. During the years 1999 and 2000, seed bank research of *E. angustifolia* was conducted at five distinct locations in North-Eastern Arizona (Brock, 2003). For the previous 50 years, these locations have been occupied. The seed banks of *E. angustifolia* were determined using sieved soil samples and direct seed counting. After then, the seed population from the 2000 study samples was divided into three categories: viable, fresh, and pulp seeds. In 1999 and 2000, the average number of seeds in the seed bank was 494 and 336 seeds per meter square, respectively, for the five locations (Brock, 2003).

Lesica and Miles (2001) assessed the process of natural recruitment and normal growth rate of *E. angustifolia* in 46 different stands on the Yellowstone and Marias rivers in Montana State USA. The rate of *E. angustifolia* recruitment from each mature tree among 46 stands has differed from 0 to 4.07 recruits per year with an average of 0.69 recruits per year. Also, it has been observed that there was no significant difference in the annual recruitment rate among the Yellowstone and Marias rivers *E. angustifolia*

plants. The association between the rate of recruitment and the canopy of tall trees was also non-significant ($r^2 = 0.006$, $P = 0.61$). There was also no relationship between *E. angustifolia* recruitment rate per year and related understory vegetation; nor between annual recruitment rate and elevation above the river levels, either for Yellowstone or Mariasrivers (Lesica and Miles, 2001).

Fresh and healthy seeds made up 63% of the seed bank, while the remaining 37% were old and had little or no reproductive power. Of the seeds judged viable, physical inspection showed that 60% possessed fresh endosperm, which would further lower the reproduction capacity of the total seed count for *E. angustifolia* seeds. The remaining number of seeds that can germinate would provide a seed source to assure recruitment of new seedlings to the stands, and provide a seed source that could be transported and moved to another place from the site by water or animals for the incursion of new sites by this highly invasive tree (Brock, 2003).

2.8 Soil seed bank, and natural recruitment of *Hippophae rhamnoides*

Sea buckthorn *H. rhamnoides* is an important plant species of Elaeagnaceae family. These are valuable plants that are freshly cultivated and tamed in the orchards, mainly in Canada, the USA, and Europe. These plants can live under low soil fertility and a wide range of temperatures from -40 to +40 °C (Janceva et al., 2022). That plant is invasive since it has minimum demand for growing settings. It grows in mild humid, river gravel, desert, wet landslips, riverbanks, and snow regions. *H. rhamnoides* is a dioecious and anemophilous plant wind pollination of female blooms is achievable (Beveridge et al., 1999).

H. rhamnoides is considered a dioecious and prickly shrub that attained a height of approximately 3 m and is mostly observed in coastal locations in the British Isles (Beveridge et al., 1999; Binggeli, 2001). This species has been widely observed in the coastal areas of both Asia and Europe among the mountain range of the Himalayas (India, Pakistan, China, Nepal, and Bhutan) and Pyrenees (France and Spain) (Pearson and Rogers, 1962). Although it is considered native in the UK, the natural distribution is specifically on the east coast, between Sussex and Humberside. Planting of this plant, mainly for dune stabilization, has resulted in the formation of colonies on many dunes outside its natural area (Pearson and Rogers 1962). *H. rhamnoides* has a wide horizontal

root structure from which aerial shoots often and rapidly emerge; shoots have been demonstrated to achieve 70 cm growth per annum (Pearson and Rogers 1962). The plant spreads vegetatively by roots that can reach for many meters. Ripe berries are oval in form and commonly yellow, orange, or red in color, depending on the type. Berries typically weigh 4 to 60 g per 100 berries, with certain *Hippophae* kinds weighing significantly more (Piłat et al., 2015). This growth is supported by a high seed viability rate (95 to 100% embryo-containing seeds germinated under experimental conditions (Pearson and Rogers, 1962), which means that *H. rhamnoides* is able to rapidly invade suitable habitats (Binggeli, 2001).

H. rhamnoides fruit have differences in both color and shape according to varieties but are commonly egg (oval) to globose-shaped berries that are bright orange to yellow in color. *H. rhamnoides* seed which is in fact the genuine fruit are found as one seed, extremely rarely as two inside the fruit. It is tiny, oblong, firm, ovoid, and brown fawn to blackish, covered by a thick spermoderm, formed of a lignified skin and two big, semicircular cotyledons. The seed is covered by an edible pericarp called hypanthium. The germination capability is 2 years and the semen rest occur in the fruit, since seeds might germinate immediately after extraction (Beldeanu, 1972). Their seeds require timely cleaning and proper storage space and environment because if seeds remain too long inside the pulp, the rate of germination decreases (Busing and Slabaugh, 2008).

2.9 Germination and growth conditions of *H. rhamnoides*

Plants of *H. rhamnoides* demonstrate epigeal germination. Seed germination in *H. rhamnoides* is restricted by various biotic factors such as stiff seed coats and embryo dormancy (Frochot et al., 2009). This internal particular dormancy needs to be disrupted before sowing. For the disruption of internal dormancy of *H. rhamnoides* seeds of stratification in damp sand for a duration of 90 adays at 2 to 5°C is much more advantageous (Busing and Slabaugh, 2008; Pearson and Rogers, 1962). In autumn sown seeds, 15 days stratification is sufficient (Zafer, 2011). For determining seed viability, germination studies may be performed on properly stratified seeds for 40 days in sand filled containers at a temperature range of 20 to 30°C (Li and Schroeder, 1996).The Study showed that the germination rate was increased marginally when seeds were exposed to higher light levels of 2150 lumens per meter square (Pearson and Rogers 1962). The use of other germination regulators such as soaking the *H. rhamnoides* seeds

in hormones solutions of gibberellic acid, H_2SO_4, or with other chemicals, such as zinc sulphate ($ZnSO_4$), potassium iodide (KI), manganese sulphate ($MnSO_4$), or cobalt sulphate (CoSO4), may also promote the rate of germination (Busing and Slabaugh, 2008). Another study showed that the germination rate of untreated seeds was much lower (6 to 60%) after 60 days of germination tests (Li and Schroeder, 1996). While the results of tests conducted in England and Romania showed that the stratified seeds showed a germination of 75 to 85% and 95 to 100% under optimum growth conditions (Pearson and Rogers, 1962).

H. rhamnoides plants are capable to establish and colonize on nutrient poor, unstable and even in sandy soils (Isermann et al., 2007). *H. rhamnoides* are an epicenter of solutions to desertification, high levels of salinity and drought. This shrub doesn't just tolerate nutrient-poor soils but also gifts the soil by fixing nitrogen, one of the most important nutrients for plant growth.

H. rhamnoides normally grows directly by seed or by transplanted from nurseries in the spring season. The highest growth occurred in well-drained, sandy loam soil that contains the highest proportion of organic matter (Li and Schroeder, 1996). *H. rhamnoides* is considered as a tree of temperate climates that is also able to survive with hot summers and harsh cold winters. A study showed that the trees were able to survive in frost conditions ranges from -20 to -30°C but in some cases the terminal shoot buds showed damage as well (Bartha and Csiszár, 2008). Zhang et al. (2018) found that the mean annual temperature, yearly range of temperature, percentage of humidity and cold index are the primary environmental parameters restricting its potential distribution range in China, based on simulation studies. It may thrive in a variety of conditions due to its drought tolerance and ability to grow in bare, alkaline, saline, and less fertile soils (Knopf et al., 1988). This plant also has a well-established and vigorous root system. It does not care about the type of soil, the temperature, or the amount of moisture in the air (Zheng, 2004). It thrives along the flood affected areas and on riverbanks although it can also be found in sandy areas such as deserts or dry places, though the rate of growth decreases or is slower if the water table is below 4 meters. Once established, it is shade tolerant and has a higher competitive ability as compared with other small trees and shrubs. These plants usually need a high temperature to germinate ranges from10 to 12°C. Li and Schroeder (1996) reported 13.2% germination after 47 days of sown at 10 to 12°C temperature as compared with 95% in 6 days after sowing of seeds at 24 to

26°C. A higher germination rate was also obtained when seeds were soaked in hot water at 70°C for a duration of 24 to 48 hours, and stirring frequently, and then letting them cool at room temperature (Li and Schroeder, 1996). But it is important to be considered that after soaking the seeds, seeds should be air-dried before germination starts.

An old study revealed that untreated seeds may be used for fall planting (Zafer, 2011), but for spring planting, seeds must be stratified (Busing and Slabaugh, 2008). Both broadcasts by hand or by drill sowing is satisfactory and seeds must be covered with about 6 mm of soil. Shading at the early germination stages is proved to be beneficial (Busing and Slabaugh, 2008). This species can also be reproduced by suckers, layers and root cuttings (Shah et al., 2015).

2.10 Seed bank studies and natural recruitment of *H. rhamnoides*

H. rhamnoides seed bank is mostly landslides with soilless areas due to its ability to grow in such areas (Bolibok et al., 2018). A study on *H. rhamnoides* seeds germination after long storage (approximately 10 years) at the normal storage conditions in the cold region of arid trans-Himalayan Ladakh showed that the seeds that were stored for 6 years have no germination difference as compared with the freshly produced seeds but the seeds that were 9 to 10 year old had declined the germination rate by 65 and 66% respectively as compared with one and two year stored seeds that provide 100 and 99% respectively (Korekar et al., 2013).

H. rhamnoides seeds can disperse over long distances and this is the essential phase that occurs in vegetation types and is especially critical for pioneer species whose existence is heavily reliant on populating newly established post-disturbance habitats. Methodological issues make studying the long-distance spread of *H. rhamnoides* on cliff collapses in natural environments challenging.

As per Levey et al. (2008), the frequency of seeds hitting a unit of area declines rapidly with distance especially for long dispersion (over 150 m), with just 1% of overall seed production dispersed across long distances (Cain et al. 2000). Because of the limited zone of landslide as well as the significant dispersion range, only a small fraction of seeds may have reached such a spot. It may be necessary for the populations of this specie to operate, although it is insufficient to explain its increase quantitatively.

CHAPTER III

MATERIALS AND METHODS

A study was conducted to determine the soil seed bank of *Elaeagnus angustifolia and Hippophae rhamnoides* and their natural recruitment. Five experiments were conducted to determine the seed viability, germination, field establishment, and minimum growth temperature for *E. angustifolia and H. rhamnoides.* These experiments are listed as:

- **Experiment 1: Fruit and seed characterization of *E. angustifolia***
- **Experiment 2A: Soil seed bank assessment and germination of *E. angustifolia*.**
- **Experiment 2B: Soil seed bank assessment and germination of *H. rhamnoides*.**
- **Experiment 3: Watering of natural seed banks of *E. angustifolia*.**
- **Experiment 4: Determination of minimum germination temperature for local *E. angustifolia* and *H. rhamnoides*.**
- **Experiment 5: Simulated seed bank for *E. angustifolia* and *H. rhamnoides*.**

The materials and methods used for each experiment are explained below:

3.1 Experiment 1: Fruit and seed characterization of *E. angustifolia*

For fruit and seed characterization, fruits were collected from the selected ten trees on 27 October 2021. Fruits were collected manually from each tree separately and tagged them according to tree number and site. After that samples were brought into the lab and left for drying until two weeks. After drying 50 fruits from each tree were chosen randomly and started the process of characterization. Fruit length, fruit diameter and fruit weight were taken. For fruit length and diameter, a Vernier caliper was used while fruit weight was measured by using an electric balance. After that pulp was removed from the fruits and seeds were obtained. Seed length, seed diameter, and seed weight were also measured.

3.2 Experiment 1: Soil seed bank assessment and germination test of *E. angustifolia*

Ten trees for *E. angustifolia* were selected for the seed bank experiment. The selection of trees was done inside the campus area Nigde Omer Halisdemir University, Nigde, Türkiye.

Trees were chosen based on specified requirements, such as an undisturbed region with no grazing. Also, the area was flat and undisturbed and make sure that remained undisturbed during the whole research time. Selected trees must be healthy and can be able to produce enough seeds so that seed bank assessment could easily be done.

Figure 3.1. Map of the trees selected for studying the seedbank assessment and germination test of *Elaeagnus angustifolia* inside Nigde Omer Halisdemir University, Nigde/ Türkiye. The site 1 has three trees tagged them as trees 1, 2, and 3. The site 2 has two trees tagged as trees 4 and 5, the site 3 has three trees as trees 6, 7 and, 8 while the site 4 has 2 trees tagged as trees 9 and 10.

Table 3.1. Location information, trees height and surrounding vegetation of selected trees for studying the seed bank of *Elaeagnus angustifolia*

Site tree	Latitude	Longitude	Tree height	Location information
1	37.94471	34.62548	6.87 m	Trees 1, 2 and 3 were artificially grown in straight rows near the campus fence
2	37.94489	34.62555	7.56 m	
3	37.94482	34.62561	7.11 m	
4	37.93996	34.63008	5.92 m	Grown in one line and artificially planted inside orchard
5	37.94001	34.63007	6.18 m	
6	37.93999	34.63065	5.62 m	Grown naturally inside the orchard
7	37.93995	34.63071	4.96 m	
8	37.94000	34.63062	7.25 m	
9	37.93927	34.63055	6.84 m	These were naturally grown near open area
10	37.93927	34.63045	5.25 m	

3.2.1 Sampling

Both litter and soil samples of *E. angustifolia* were collected within four 750 × 350 m^2 sampling quadrants around each tree (Figure 3.2). Samples were collected on 15 October 2021.

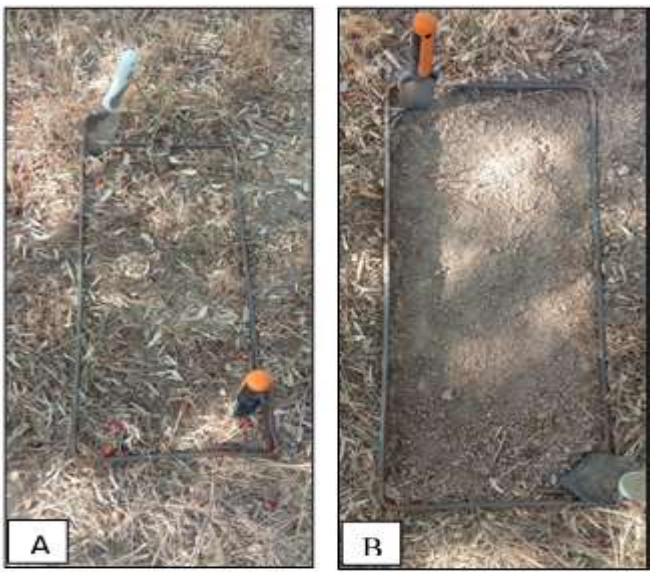

Figure 3.2. Sampling site before (A) and after (B) taking litter samples under the *E. angustifolia* trees.

Samples were taken from 50 mm depth beneath the selected trees by using a small shovel for soil samples and a brush for litter samples. After taking samples each sample was put in a separate polythene bag and tagged properly according to the tree and quadrant number (Figure 3.3). A distance of 1 m was kept between each quadrant. Samples were brought into the lab for further processing.

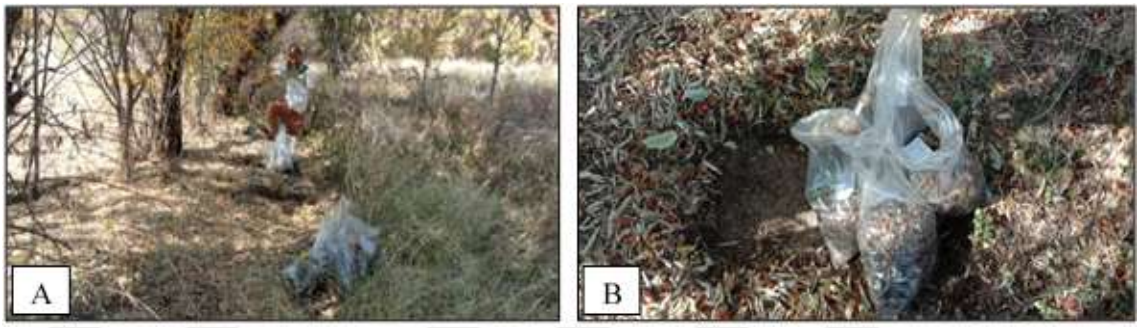

Figure 3.3. Samples collected separately in plastic bags (A, B) and bring them to lab for processing.

3.2.2 Sample processing

After bringing the samples to the lab, samples were keep for 2 weeks under shade drying. After drying litter samples were sieved by using a 2 mm fine sieve to separate dust, sand, soil, stone, and straw particles from endocarp and fruits. Endocarps and fruits were taken, counted, and separated according to fruit new, fruit old, whole endocarp, and broken endocarp. For soil samples, each sample was washed by using tap water to get endocarps and fruits and also categorized according to fruit old, whole endocarp and broken endocarp.

3.2.3 Seed germination test

Before the viability test, the fruits of *E. angustifolia* (both old and new) present inside the sample had pulp that was removed manually to obtain the endocarp for determining their viability. Also, whole endocarp was separated from the damaged/broken endocarps and discarded. Stratification was also done by mixing all the endocarps separately with the sand in plastic bags and putting it inside the cold storage room at 1^{o}C for 40 days.

After 40 of stratification, endocarps were separated from the sand and determine their germination.

If the sample has more than 20 endocarps, 20 endocarps were chosen while for the samples with less than 20 endocarps, all the endocarps were tested for viability. The seed germination test was carried out by using a plastic container from each of the 10 trees. The size of the container was 180 × 110 mm and a peat mixture of 200 g was used in each container for determining the seed viability. Seeds collected from each sample were tested separately. The containers were put inside the lab at a temperature of 15 to 25^{o}C. Each container was moistened according to requirement. The experiment was done until three weeks and after that final germination count was done from each sample separately (Figure 3.4).

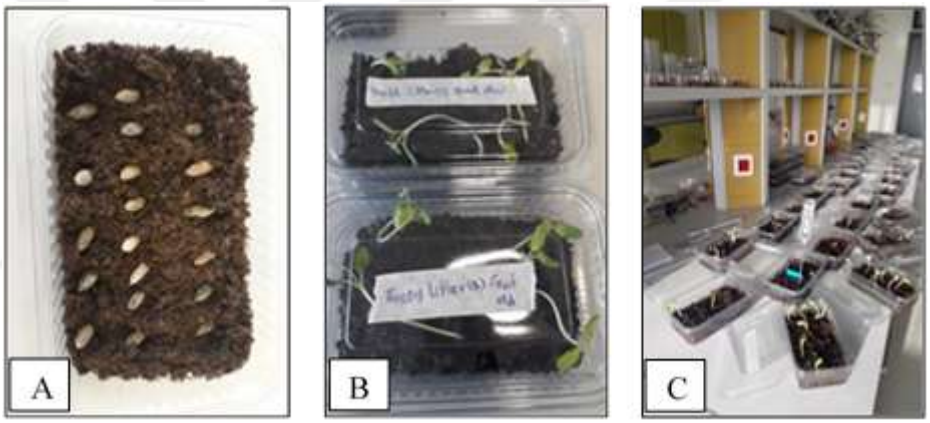

Figure 3.4. The seed germination of *E. Angustifolia*; A, 20 endocarps was kept in container filled with peat while B and C show the germinated after 3 weeks

3.3 Experiment 2: Soil seed bank assessment and germination test of *H. rhamnoides*

Ten sites were selected for the seed bank experiment. The selection of sites was done in Çamardı town, Nigde. Türkiye. (Figure 3.5-3.6, Table 3.2).

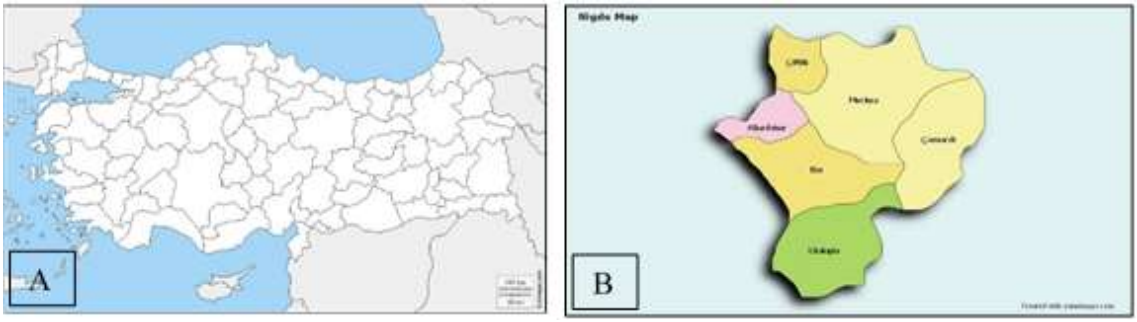

Figure 3.5. A, map of Türkiye; B, map of Nigde Province.

Figure 3.6. Map of the trees site selected for studying the seed bank assessment and germination test of *H. Rhamnoides* in Çamardi. Nigde, Türkiye.

Table 3.2. Latitude and longitude of selected sites for studying the seed bank of *Hippophae rhamnoides* in Çamardi

Site	Google Maps Latitude	Google Maps Longitude	Google Maps Cords
1	37.955045	35.086934	37Â°57'18.2"N 35Â°05'13.0"E
2	37.955299	35.08805	37Â°57'19.1"N 35Â°05'17.0"E
3	37.955625	35.088023	37Â°57'20.3"N 35Â°05'16.9"E
4	37.861604	35.059199	37Â°51'41.8"N 35Â°03'33.1"E
5	37.852145	35.055234	37Â°51'07.7"N 35Â°03'18.8"E
6	37.827273	35.039228	37Â°49'38.2"N 35Â°02'21.2"E
7	37.800522	35.018031	37Â°48'01.9"N 35Â°01'04.9"E
8	37.800386	35.015606	37Â°48'01.4"N 35Â°00'56.2"E
9	37.788466	35.006959	37Â°47'18.5"N 35Â°00'25.1"E
10	37.787245	35.005693	37Â°47'14.1"N 35Â°00'20.5"E

3.3.1 Sampling

Soil samples were collected on 1 March 2022. A random sampling was done using a small shovel. Each sample was about 1 kg in quantity. Reason for choosing random sampling was that the surface behind the trees was sloppy and has rocks, so it was difficult to use meter square for collecting samples. After taking samples, each sample was put in a separate polythene bag and tagged them properly according to site number and sample number. Samples were brought into the lab for further processing.

3.3.2 Sample processing

After bringing samples to lab, they were dried for three weeks under sunlight and after fully dried, weights for each sample were taken. As the samples were taken randomly so

sub-sampling was done from each main sample. The sub-samples were sieved by using two types of sieves. The first sieve was 2 mm and second sieve were 1 mm pore spaces to get seeds of *H. rhamnoides*. The purpose of using 2 mm and 1 mm sieves was to ensure that all the seeds present inside the samples were collected.

3.3.3 Seed germination test

Before germination test, the pulp from the fruits of *H. rhamnoides* (both old and new) present inside the sample was removed manually to obtain the seeds for determining their viability. The whole seeds were separated from the damaged/broken seeds and discarded. Stratification was done by keeping the seeds inside the refrigerator at 1^{o}C temperature for 2 weeks.

All the seeds collected from each sample were observed from their viability due to its comparatively small size and less numbers. The seed viability test was carried out by using a plastic container from each of the 10 trees. The size of the container was 180 × 110 mm and a peat mixture of 200 g was used in each container. A layer of tissue paper was used on peat mixture for placing seed. Seeds collected from each sample were tested separately. The containers were put inside the lab at a temperature of 15 to 25^{o}C. Each container was moistened according to requirement. The experiment was done until three weeks and after that final germination count was done from each sample separately (Figure 3.7).

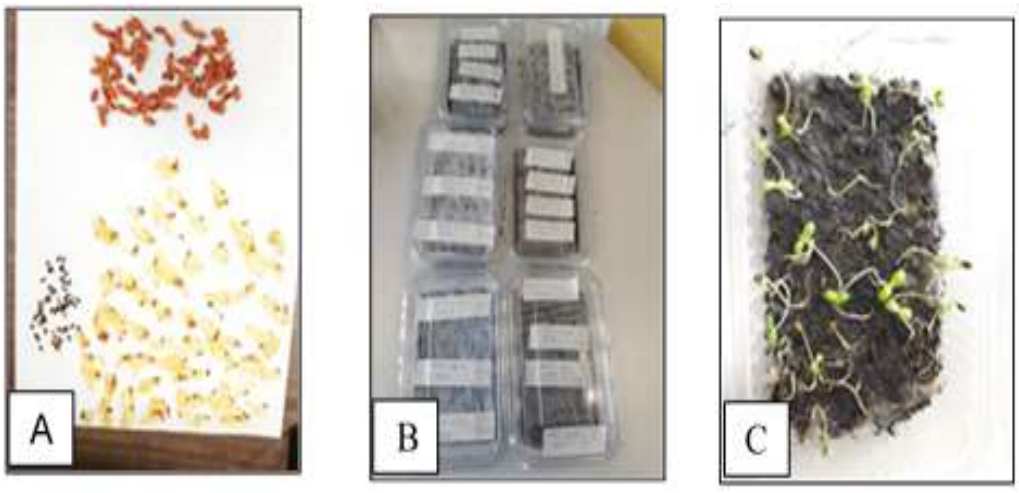

Figure 3.7. Shows the seed viability of *H. rhamnoides*. Seeds with and without pulp (A). The germinated seed after 3 weeks (B and C).

3.4 Experiment 3: Watering of natural seedbanks of *E. angustifolia*

To determine if water is a limiting factor for *E. angustifolia* germination and establishment in natural habitat, four randomly chosen sites of *E. angustifolia* on the Niğde Ömer Halisdemir University campus area was used for the natural seed bank experiment. The first site has three trees tagged them as trees 1, 2, and 3.The second site has two trees tagged as trees 4 and 5, the 3rd site has three trees as trees 6, 7 and , 8 while the fourth site has 2 trees tagged as trees 9 and 10. After sites and trees selection, four quadrants were made around each tree referred to as two with water and two without water. The size for each quadrant was 750 × 350 mm. The experiment was started on 7 April 2022. Quadrant that was mentioned as watered started watering manually by using the plastic bottle on each three days while the quadrant that was mentioned as without water was left as it was and the surface was dry up to 40-50 mm depth at all four selected sites (Figure 3.8). Quadrant selected as with water, water with 2.5 L of water on each 3 days. The experiment continued until 31 May 2022.

Figure 3.8. The sites selected for watering of natural seedbanks of *E. angustifolia*. A is the site 1 while B and C are the site 3 and 4 respectively

3.4.1 Assessment

The assessments done in this experiment were included the temperature and the number of seed germination within the quadrants. Data for germination were recorded on each three days until the end of the experiment. The temperature was measured using a soil thermometer by inserting a thermometer up to a depth of 35 mm (Figures 3.9 and 3.10).

Figure 3.9. Germination of seeds and number of seedlings in watering sites of *E. angustifolia* (A, B and C)

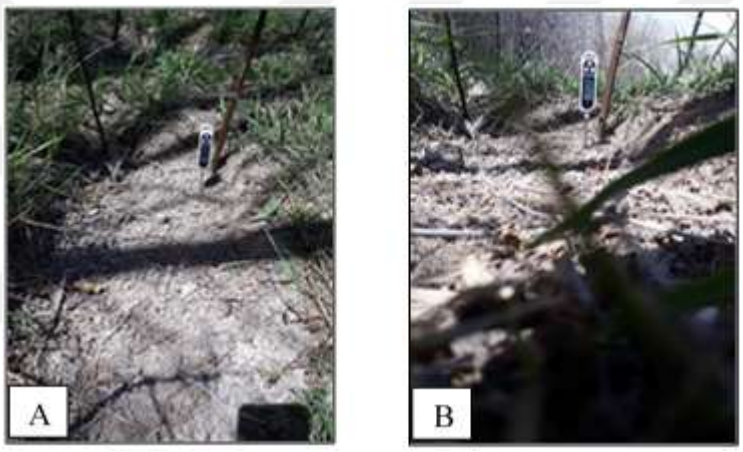

Figure 3.10. Temperature measurement for the sites (A, B) for natural seedbanks of *E. angustifolia*

3.5 Experiment 4. Determination of minimum germination temperature for local *E. angustifolia* and *H. rhamnoides*

Ten Trees of *E. angustifolia* and *H. rhamnoides* were examined for minimum germination temperatures. Fruits for *E. angustifolia* were collected from the campus area Nigde Omer Halisdemir University, Nigde, Türkiye on 15 October 2021. From each tree, fruits were collected separately and tagged properly according to tree number. Fruits were brought to the lab and let them dry for ten days. After drying, the pulp was removed from the fruits to obtain the seeds. As the seeds of *E. angustifolia* have dormancy issues so there is a need to break them. For breaking dormancy, seed stratification was done. For stratification, seeds from each tree were mixed separately

with sand inside a small plastic bag and put inside the cold room at a temperature of 1˚C for 40 days.

After stratification seeds were cleaned from the sand and tested for determining the minimum temperature for germination (Figure 3.11). Seeds from each tree were separately put inside plastic containers (180 × 110 mm) that have a peat of 250 g. Twenty seeds were grown in each container according to Table 1. Each tree has two replicates. These plastic containers were kept at 10, 15, 20, 25 and 30 ˚C inside an incubator. Plastic containers were kept for one week at each temperature level. At the end of each week, germination data were recorded and the temperature of the incubator was changed to the next temperature level. The experiment was started on 7 May 2022 and ended on 9 June 2022.

Figure 3.11. The experiment for the determination of minimum germination temperature (A). The number of germinated seeds for *E. angustifolia* (B and C).

For *H. rhamnoides*, fruits were collected from ten trees, from Çamardı town, Nigde, Türkiye on 9 November 2021. Fruits were put in a plastic bag and tagged according to tree number. After collecting, fruits were brought to the lab and left for 10 days for shade drying. After drying, the pulp was removed from the seeds and kept in lab until start of the experiment. A 100 g peat mixture was used to fill the container. A layer of tissue paper was used on the upper side of the peat mixture. Fifty seeds were grown in each container. Each tree has two replications. These plastic containers were kept at 5, 10, 15, 20, 25 and 30˚C inside an incubator. Plastic containers were kept for one week at each temperature level. At the end of each week, germination data were recorded and

the temperature of the incubator was changed to the next temperature level. The experiment started on 27 March 2022 and ended on 6 May 2022 (Figure 3.12).

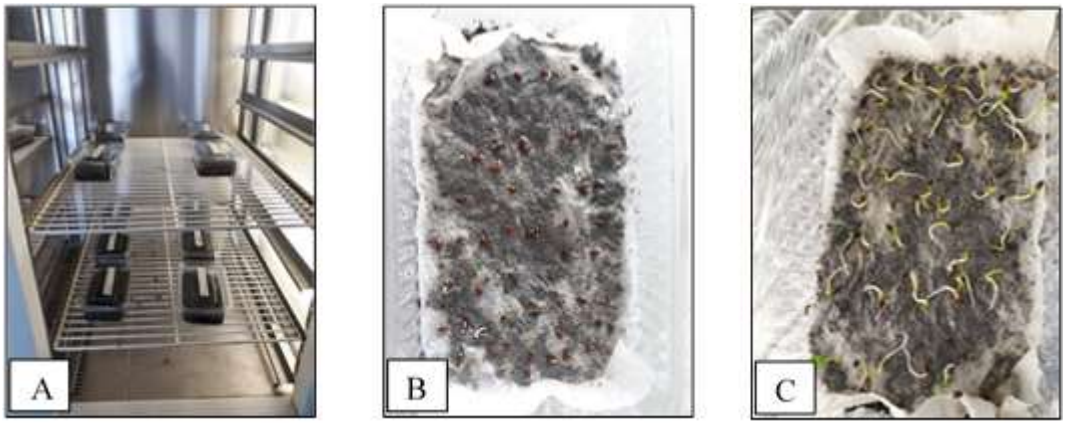

Figure 3.12. The experiment for the determination of minimum germination temperature (A) while B and C show the number of germinated seeds for *H. rhamnoides*

3.5.1 Assessments

The assessments that were done included recording the number of germinated seeds at each temperature level separately.

3.6 Experiment 5: Simulated seed bank for *E. angustifolia* and *H. rhamnoides*

3.6.1 Experiment site and field preparation

The experiment was conducted beside the Faculty of Agriculture, Nigde Omer Halisdemir University, Nigde, Türkiye (37°56’37.3” N, 34°37’40.9” E). The site was cleaned by weeding the area, and cleaning up wastes, debris and big stones. Site was plowed manually by using the shovel and fine plots were prepared for seed sowing according to layout (Figure 3.13).

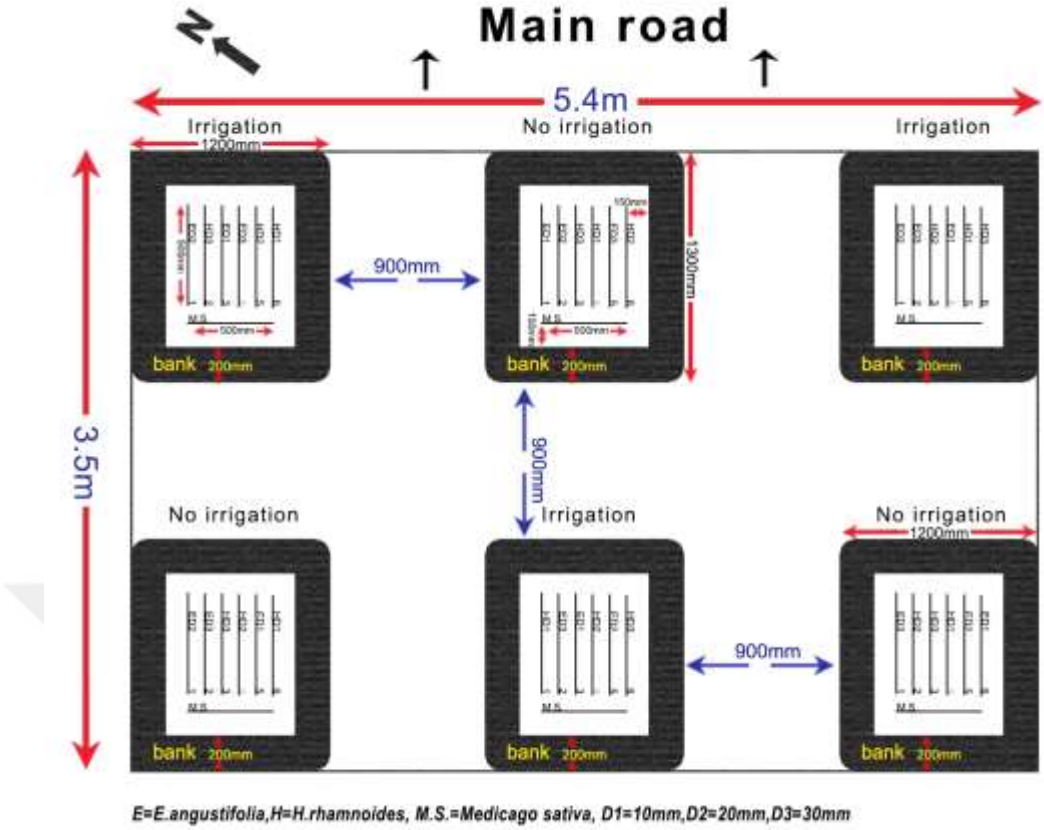

Figure 3.13. Map for field experiment showing the layout of *E. angustifolia* and *H. rhamnoides* grown under irrigated and no-irrigated conditions at three sowing depths.

3.6.2 Seed sowing

Seed sowing of *E. angustifolia* and *H. rhamnoides* was done on 28 December 2021. The experiment was done in a split block design having main plots (irrigated and non-irrigated) separated into subplots (*E. angustifolia* and *H. rhamnoides* grown in rows at three different depths). The total area of the experiment was 5.4 × 3.5 m^2. Each plot has a size of 1200 mm × 1300 mm separated by a bank of 200 mm. The area between two plots was 900 mm. There were six rows in each plot and the length of each row was 500 mm. *E. angustifolia* and *H. rhamnoides* seeds were grown at three different depths as 10 mm, 20 mm and 30 mm and grown in separated rows. The three sowing depths were 10, 20, and 30 mm which were randomized. In each row, 50 seeds were sown (Figure 3.14).

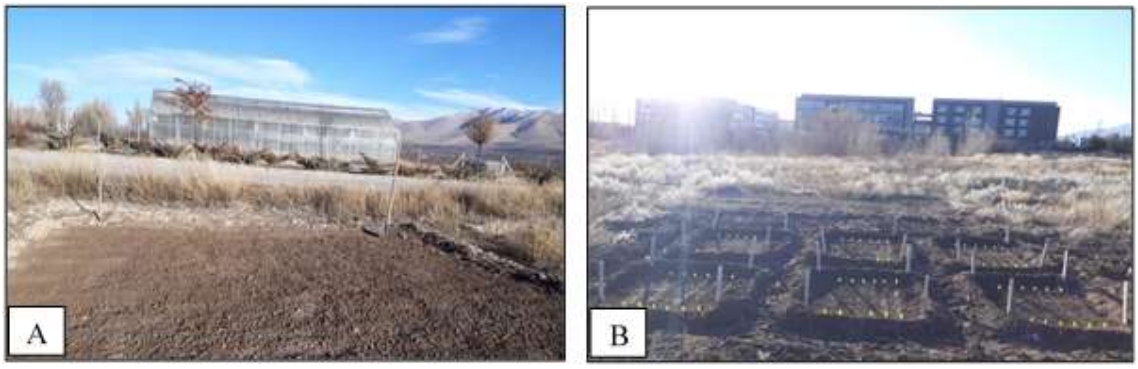

Figure 3.14. Experimental area (A) and seed sowing for studying the simulated seed bank of *E. angustifolia* and *H. rhamnoides* (B)

After sowing, there was a snowy season for almost three months and the temperature was very low (almost +10 to -5˚C) which helped the seeds to get naturally stratification inside the soil (Figure 3.15).

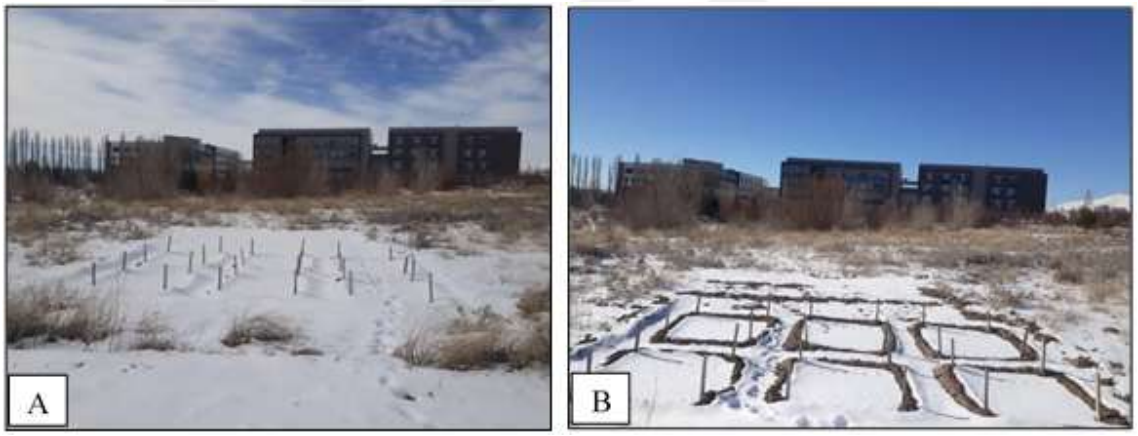

Figure 3.15. Field during snow season getting naturally stratification for *E. angustifolia* and *H. rhamnoides* (A and B)

Irrigation was started on 13 April 2022 and the experiment was irrigated every two days for the irrigated experimental units while the non-irrigated was not watered for the period of the experiment (Figure 3.16). Small plastic bottles were used for watering. In each plot 10 L of water was applied. Weeding of the experiment was also done according to need. A separate row of alfalfa (*Medicago sativa*) was also grown as a control treatment to determine the soil suitability for plants.

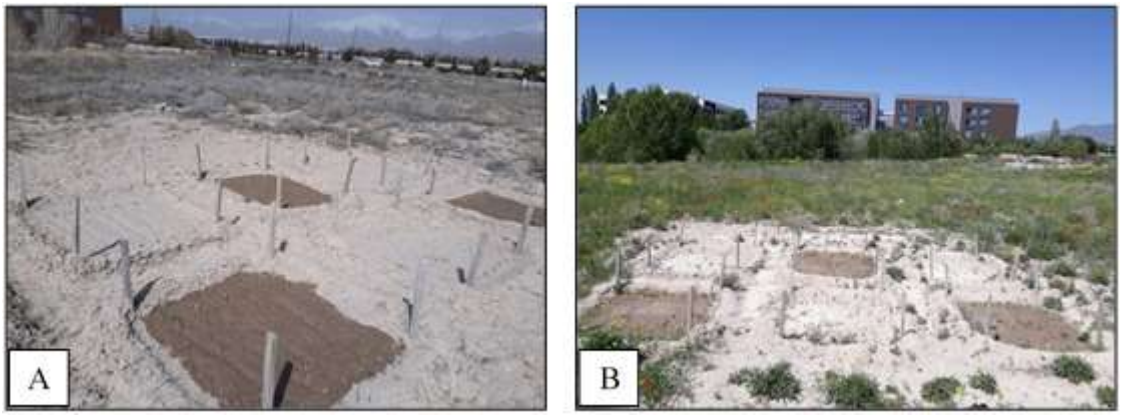

Figure 3.16. Field with irrigated and non-irrigated plots just after starting the experiment (A) and after seedling emergence (B)

3.6.3 Assessment

The assessments that were done include recording of germination, and soil temperature in every two days (Figure 3.17). Data recording was started on 15 April 2022 and continued until 29 May 2022, at the end of the experiment.

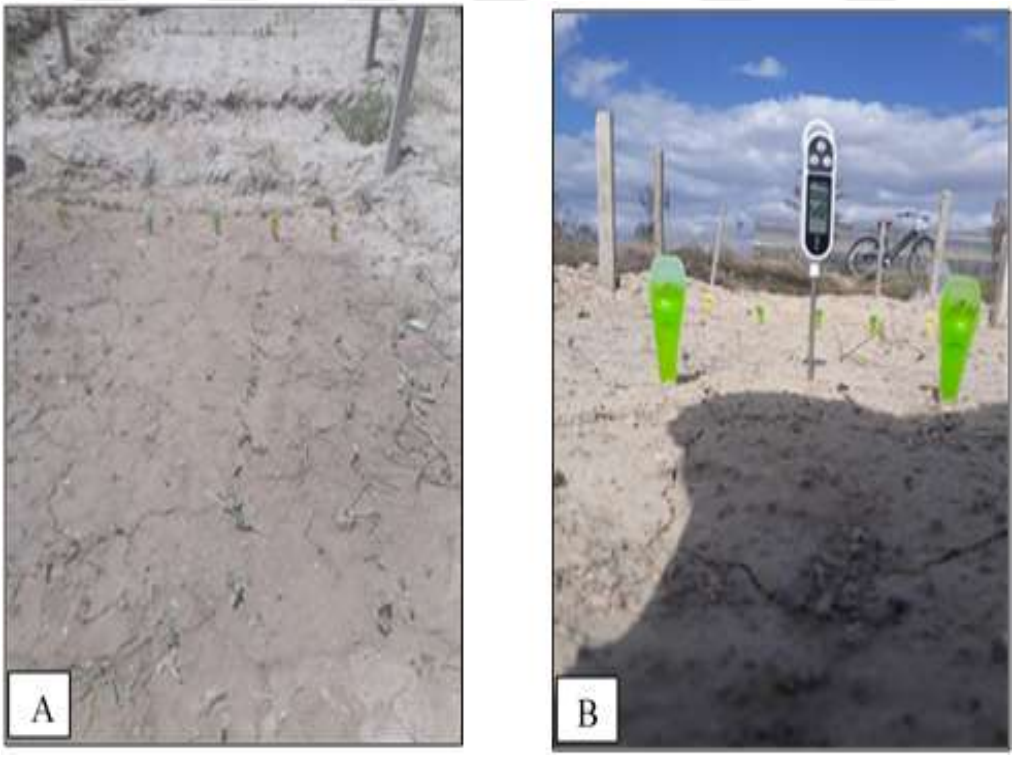

Figure 3.17. Assessment of germination (A) and temperature (B) of *E. angustifolia* and *H. rhamnoides*

3.7 Statistical analysis

Data collected in all experiments were subjected to analysis of variance. The R software platform (R Core Team, 2022) version 4.2.0 was used for analyzing the data and determines the statistical significance of the results each set of experiment.

CHAPTER IV

RESULTS

4.1 Experiment 1: Fruit and endocarp characterization of *Eleagnus angustifolia*

Fruit and endocarp characterization of *E. angustifolia* was done from the selected ten trees. A total of 50 fruits from each tree were chosen randomly for seed characterization. Fruit length (mm) and fruit diameter (mm) was determined by using Vernier caliper while fruit weight (g) was recorded by using weighing balance. Fruit aspect ratio was also calculated by dividing fruit length (mm) on fruit diameter. From the data it has been observed that the highest fruit length was obtained from tree 7 while the lowest fruit length was observed from tree 8 (Figure 4.1). Fruit diameter was significantly the highest in the tree 7 and the lowest the tree 8 (Figure 4.1). Significantly the highest fruit weight (g) was obtained from the tree 7 while the lowest was obtained from the tree 8 (Figure 4.1). The aspect ratio showed that the tree 5 had the highest aspect ratio as compared with other trees while the tree 2 had the lowest aspect ratio (Figure 4.1).

Endocarp length (mm) and diameter (mm) was determined by using Vernier caliper while endocarp weight (g) was recorded by using weighing balance. Endocarp aspect ratio was also calculated by dividing endocarp length (mm) on endocarp diameter. From the data it had observed that the highest endocarp length (mm) was obtained from trees 5 while the lowest endocarp length was observed from tree 8 (Figure 4.2). Endocarp diameter (mm) was significantly the highest in the tree 7 and the lowest the tree 8 (Figure 4.2). Significantly the highest endocarp weight (g) was obtained from the tree 7 while the lowest was obtained from the tree 8 (Figure 4.2). The aspect ratio showed that the tree 4 had the highest aspect ratio as compared with other trees while the tree 2 had the lowest aspect ratio (Figure 4.2).

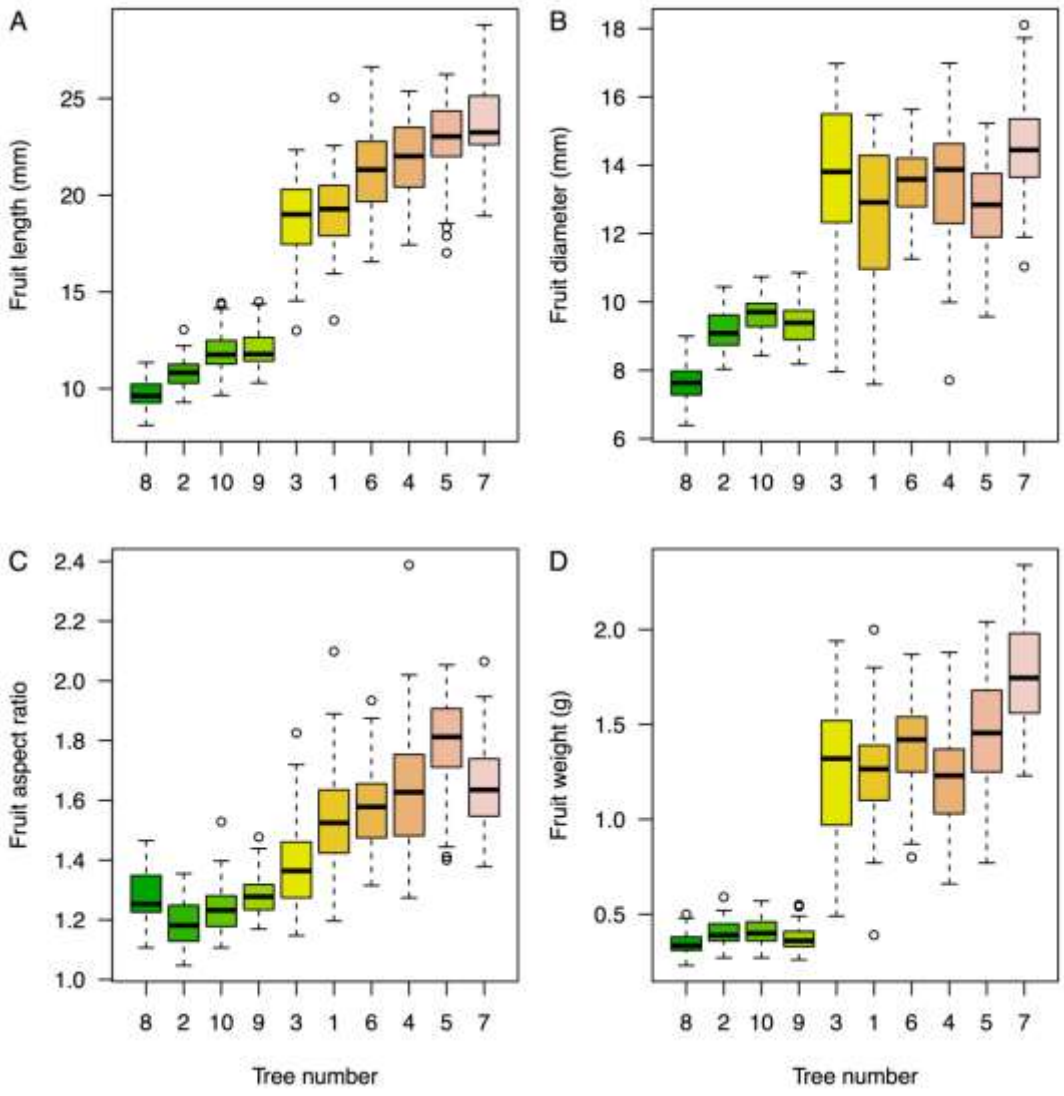

Figure 4.1. Fruit characterization of *E. angustifolia*. A, fruit length (mm); B, fruit diameter (mm); C, fruit weight (g); and D, fruit aspect ratio.

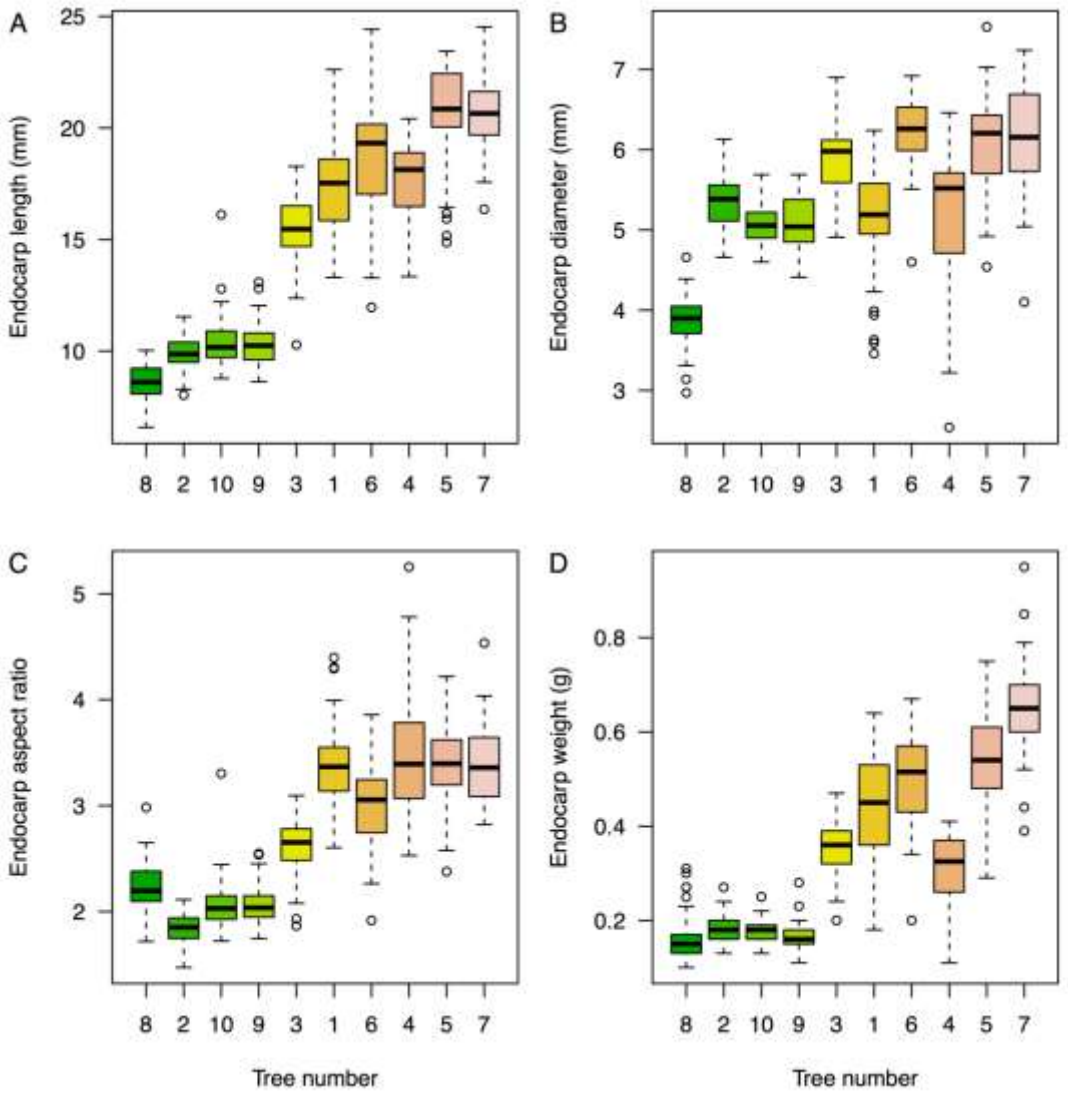

Figure 4.2. Endocarp characterization of *E. angustifolia*. A, the endocarp length (mm); B, endocarp diameter (mm); C, endocarp weight (g): and D, endocarp aspect ratio.

4.2 Experiment 2 A: Soil seed bank assessment and germination test of *E. angustifolia*

Soil seed bank assessment from selected ten trees was done by taking soil and litter sample from an area of 1 m^2. From both samples (i.e., litter and soil) number of new fruit (no./m^2), number of old fruit (no./m^2), whole endocarp (no./m^2), broken endocarp (no./m^2), potential current seed bank and (no./m^2) and potential future seed bank (no./m^2) were determined.

Data in the graph showed that from the litter samples, the highest number new fruits of *E. angustifolia* was obtained from the location of tree 3 and tree 4 while the lowest number of new fruits was obtained from tree 9 and tree 10 (Figure 4.3). From soil samples no new fruits were found that is zero new fruits were found from that location (Figure 4.3).

From litter samples, the highest number of old fruits were observed from tree 3 while the lowest was obtained from tree 9 and 10 (Figure 4.4). From soil samples the highest numbers of old fruits were observed from tree 1 while the lowest from tree 10 (Figure 4.4). When the soil and litter samples were compared collectively, the highest number of old fruits were observed from tree 7 while the least number of old fruits were found at tree 10 samples (Figure 4.4).

From litter samples, the highest numbers of whole endocarps of *E. angustifolia* were observed from tree 6 while the lowest was obtained from tree 9 (Figure 4.5). From soil samples the highest numbers of whole endocarps were observed from tree 3 while the lowest from tree 10 (Figure 4.5). When the soil and litter samples were compared collectively, the highest number of whole endocarps was observed from tree 3 while the least number of whole endocarps was found from trees 9 and 10 samples (Figure 4.5).
From litter samples, the highest numbers of broken endocarps were observed from tree 5 while the lowest was obtained from tree 9 (Figure 4.6). From soil samples, the highest numbers of broken endocarps were observed from tree 5 while the lowest from tree 10 (Figure 4.6). When the soil and litter samples were compared collectively, the highest number of broken endocarps was observed from tree 5 while the least number of broken endocarps was found from trees 9 and 10 samples (Figure 4.6).

Data for potential current seed showed that the litter of tree 6 had the highest potential current seed bank while the tree 9 samples showed significantly lowest potential current seed (Figure 4.7). From soil samples the highest potential current seed bank was observed from tree 3 samples while the lowest was observed from tree 10 samples (Figure 4.7). When the both soil and litter samples were compared collectively, the highest potential current seed bank was observed from tree 3 while the lowest potential current seed bank was found from trees 9 and 10 samples (Figure 4.7).

Data for potential future seed showed that the litter of tree 6 had the highest potential future seed bank while the tree 10 samples showed significantly lowest potential future seed (Figure 4.8). From soil samples the highest potential future seed bank was observed from tree 3 samples while the lowest was observed from tree 10 samples (Figure 4.8). When the soil and litter samples were compared collectively, the highest potential future seed bank was observed from tree 3 while the lowest potential future seed bank was found from trees 10 samples (Figure 4.8).

In general, when all the trees samples were compared collectively, it was observed that each sample had the highest number of endocarp and a smaller number of new fruits, old fruit and broken endocarp (Figure 4.9). This data showed that samples collected under all the locations had the highest potential current seed bank and potential future seed bank (Figure 4.9).

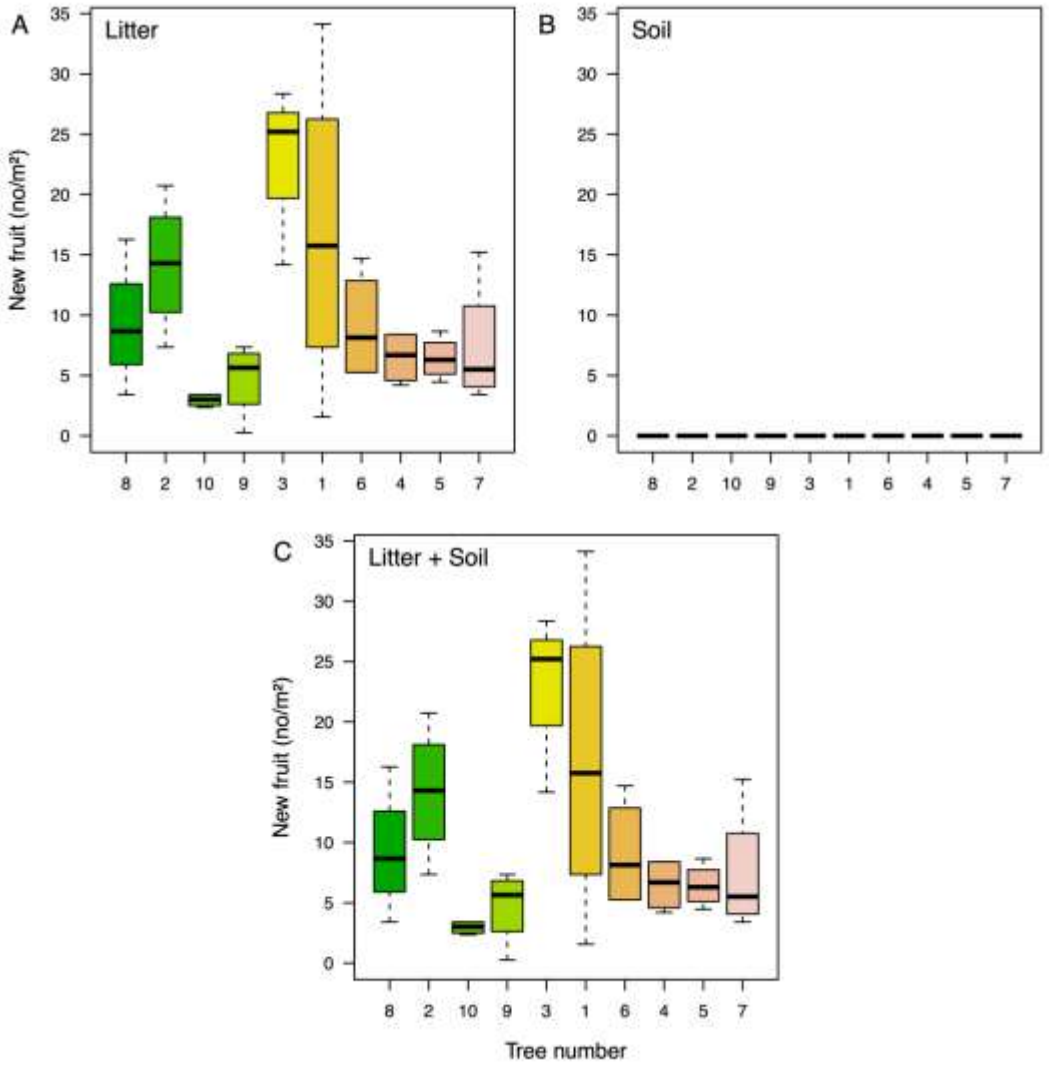

Figure 4.3. Soil seed bank assessment of *E. angustifolia*. A, the number of new fruits/m^2 of litter; B, the number of new fruits/m^2 of soil; and C, the number of new fruits/m^2 of litter + soil.

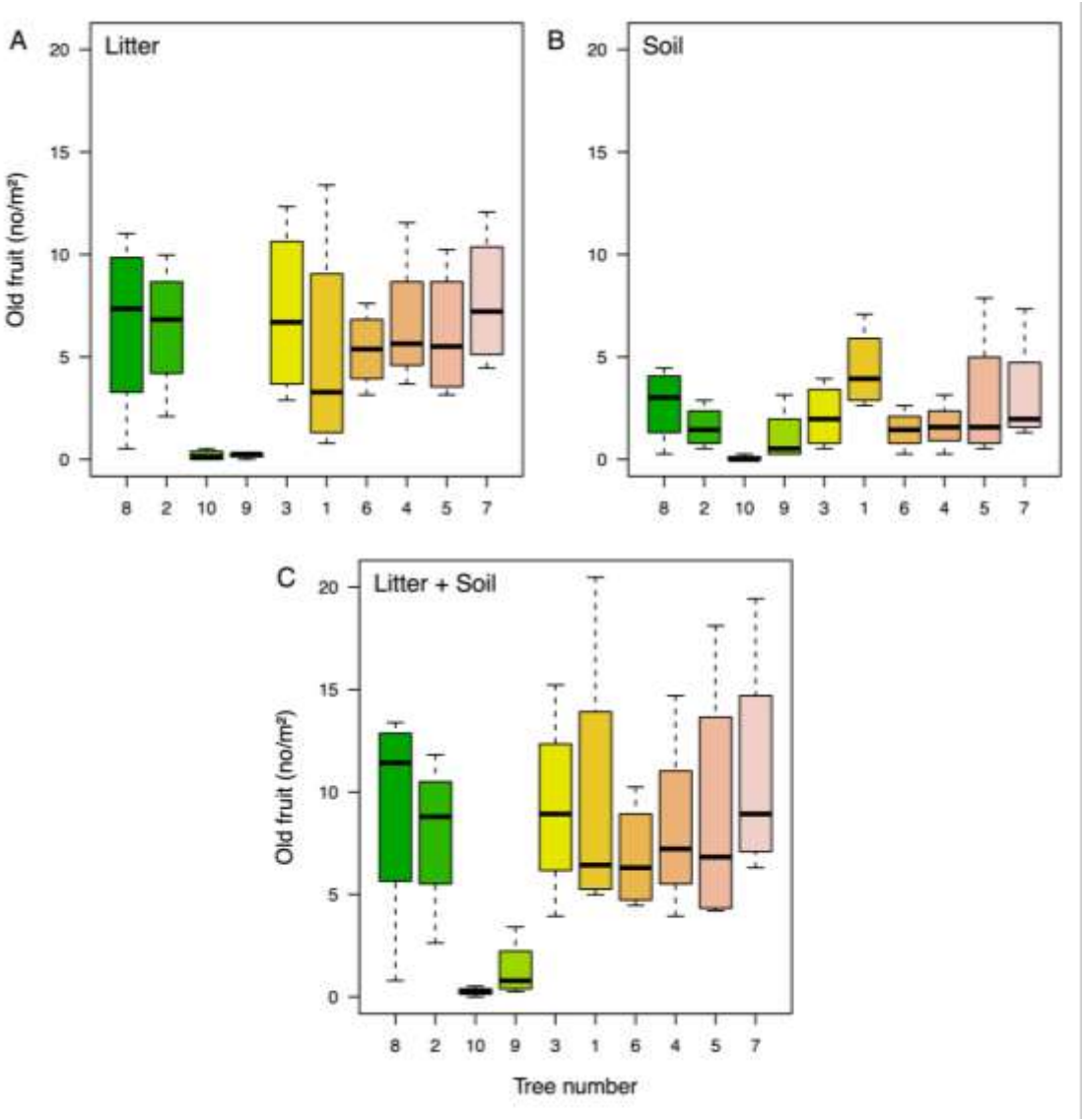

Figure 4.4. Soil seed bank assessment of *E. angustifolia*. A, the number of old fruits/m^2 of litter; B, the number of old fruits/m^2 of soil; and C, the number of old fruits/m^2 of litter + soil.

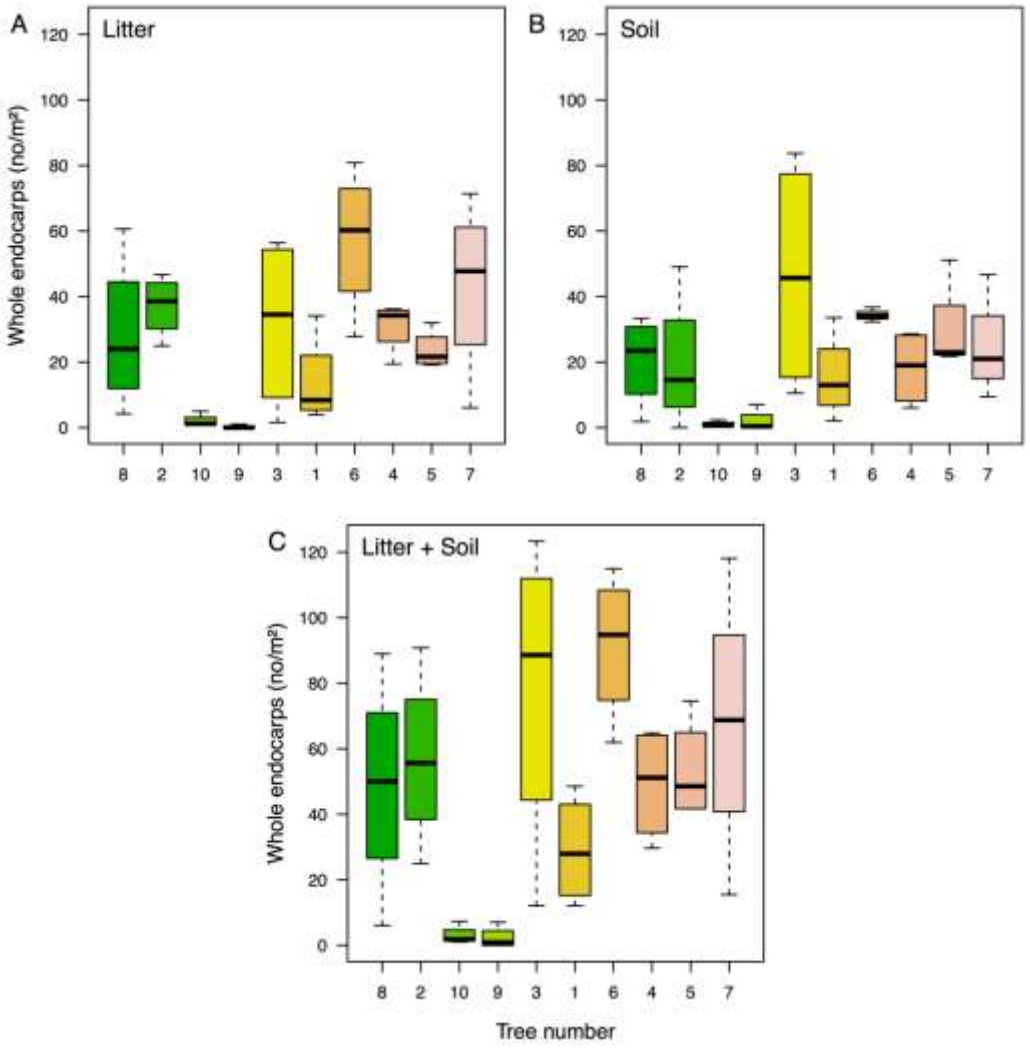

Figure 4.5. Soil seed bank assessment of *E. angustifolia*. A, the number of whole endocarps /m^2 of litter; B, the number of whole endocarps /m^2 of soil; and C, the number of whole endocarps /m^2 of litter + soil**.**

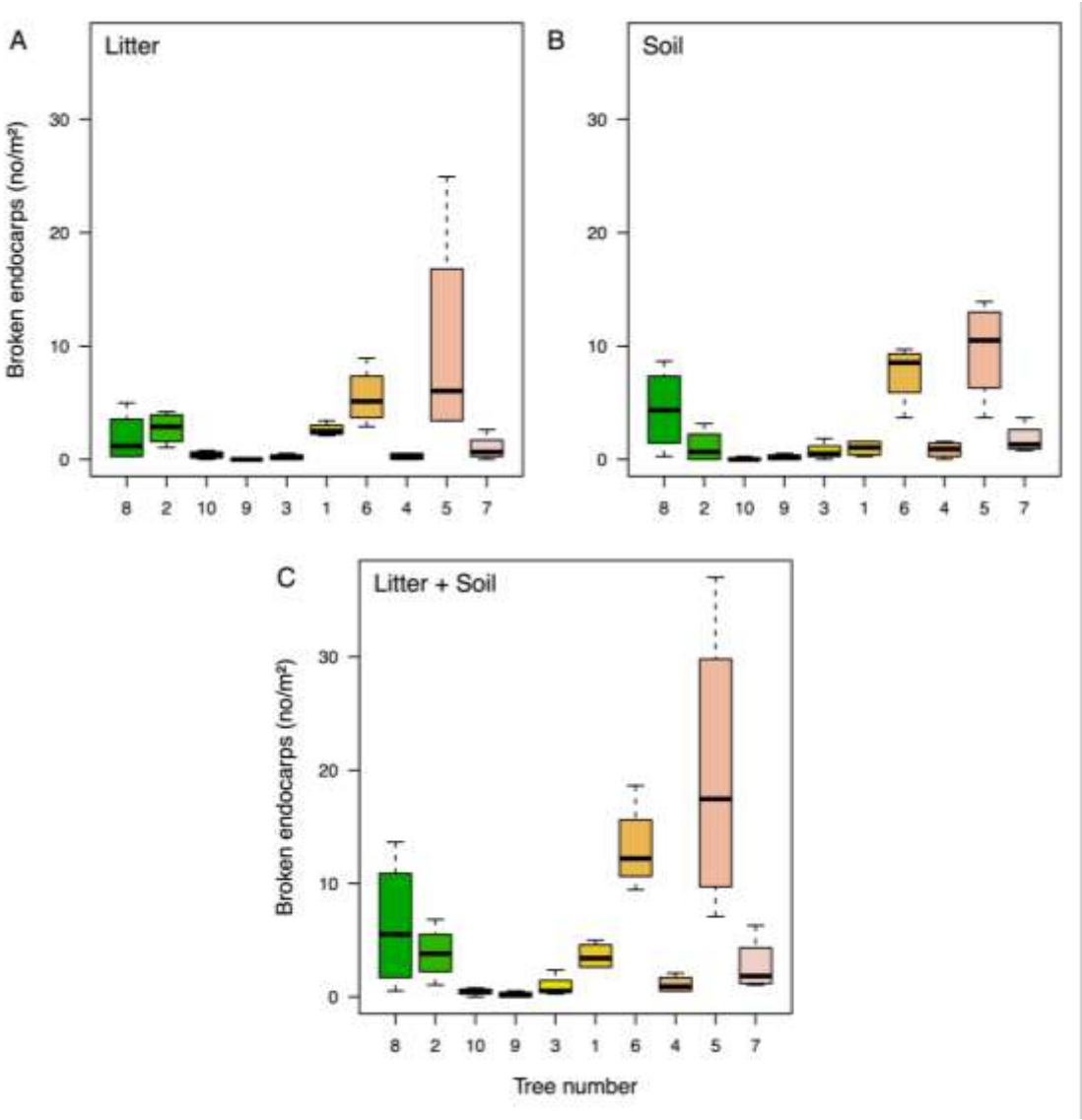

Figure 4.6. Soil seed bank assessment of *E. angustifolia*. A, the number of broken endocarps/m^2 of litter; B, the number of broken endocarps/m^2 of soil; and C, the number of broken endocarps/m^2 of litter + soil.

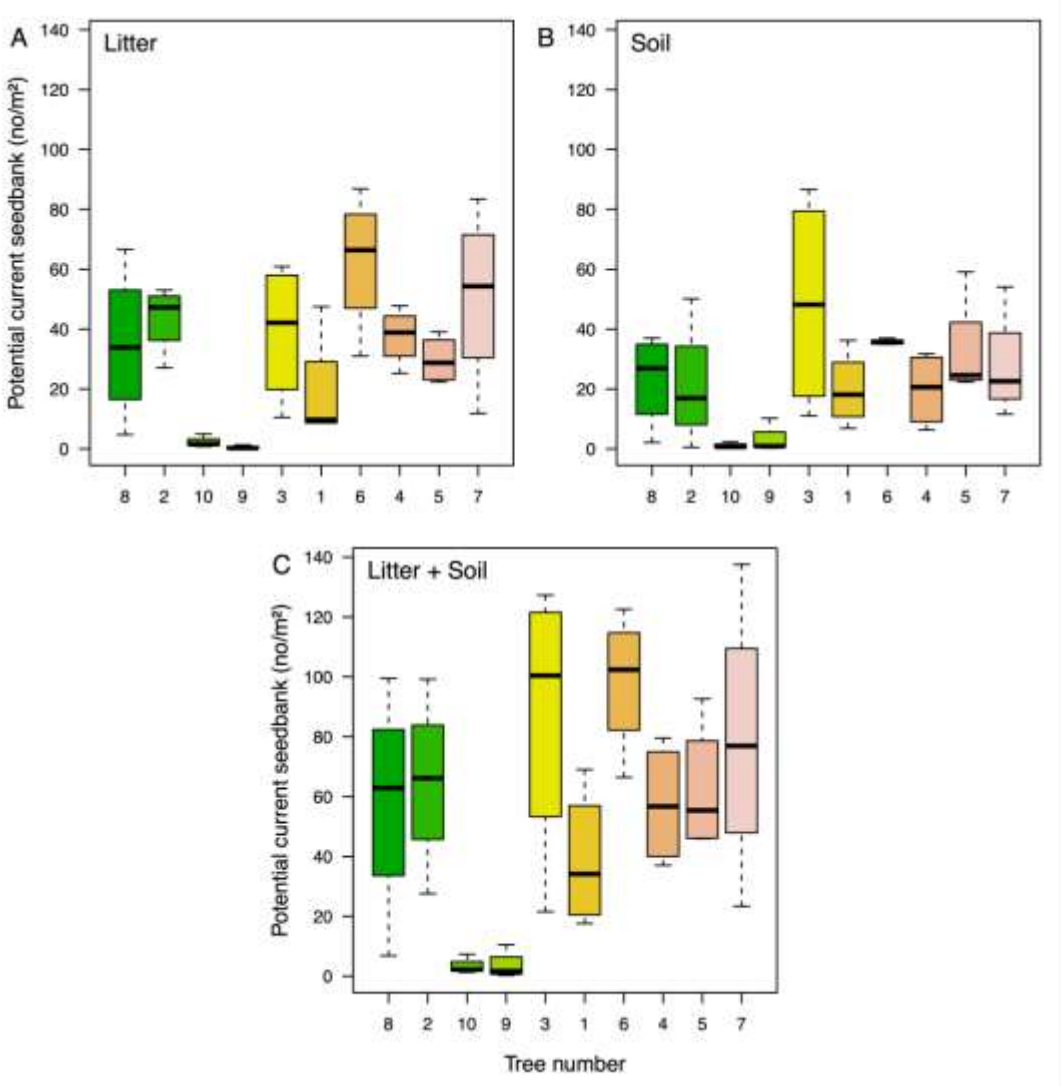

Figure 4.7. Potential current seed bank of *E. angustifolia*. A, the potential current seed bank (number/m^2) of litter; B, the number of broken endocarps/m^2 of soil; and C, the number of broken endocarps/m^2 of litter + soil**.**

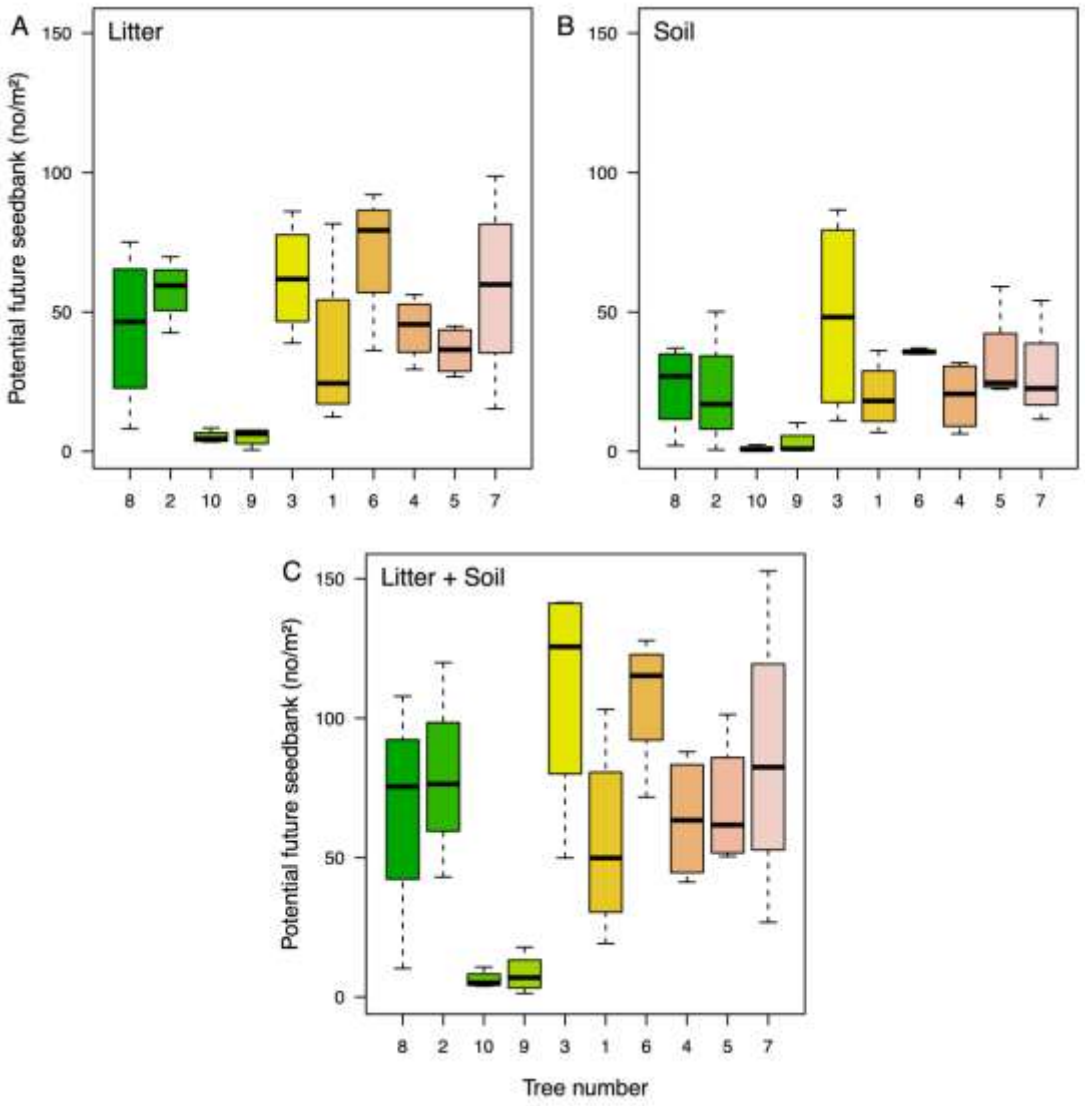

Figure 4.8. Potential future seed bank of *E. angustifolia*. A,the potential future seed bank (number/m^2) of litter; B, the potential future seed bank (number/m^2) of soil; and C, the potential future seed bank (number/m^2) of litter + soil.

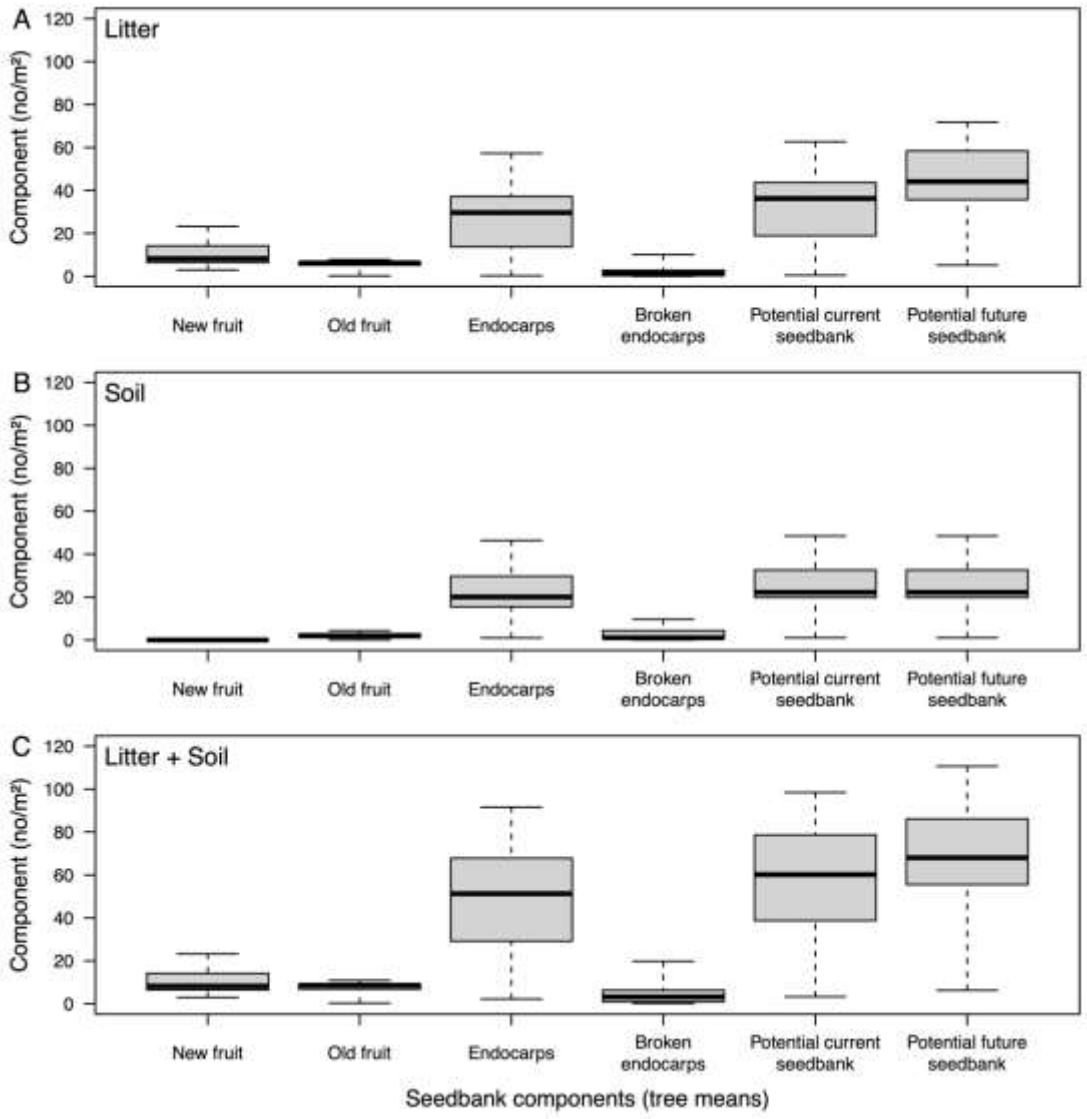

Figure 4.9. Seed bank components of *E. angustifolia*. A, the individual seed bank components (number/m^2) of litter; B, the individual seed bank components (number/m^2) of soil; and C, the individual seed bank components (number/m^2) of litter + soil.

Results from the germination tests showed a greater variability among new fruit, old fruit and whole endocarp. Data from the germination tests showed that new fruits showed the maximum rate of germination from the samples obtained from litter while soil sample do not have any new fruits so there is no germination from soil samples (Figure 4.10). The highest rate of germination was obtained from tree 9 while the tree 1 and tree 10 showed the lowest rate of germination (Figure 4.10).

Data obtained from the germination test of old fruits showed that the germination was significantly different in both soil and litter samples. The rate of germination also varied

in all tree samples collected from different locations. From litter samples, the highest germination rate was obtained from tree 9 and 10 while the lowest was recorded from tree 1 (Figure 4.11). In soil samples the highest germination was observed by tree 5 while the lowest was obtained from tree 9 and tree 10. While comparing the both soil+ litter samples, the highest germination was observed from tree 5 while the lowest was obtained from tree 1.

The germination rate of whole endocarp samples obtained from both soil and litter showed a significant difference (Figure 4.12). Data showed that from litter samples, the highest germination rate was obtained from tree 1 while the lowest rate was observed from tree 9 (Figure 4.12). From soil samples the highest germination was recorded by tree 2 and the lowest was obtained from trees 3, 5 and 6. In comparatively, from both litter and soil samples the highest germination rate was obtained from tree 1 while the lowest germination was recorded by tree 9 and tree 3 (Figure 4.12).

While comparing the germinable ability from all samples (litter and soil) and all kinds (new fruit, old fruit, endocarp), samples from litter has the highest actual future seed bank and actual current seed bank (4.13). Also, in litter samples new fruit showed that maximum germination while old fruits have the lowest rate of germination. In soil samples, new fruits have less contribution while the endocarp has the highest contribution in actual current seed bank and actual future seed bank. Also, from these graphs showed that soil samples have less contribution in actual current seed bank and actual future seed bank (Figure 4.13). While comparing both litter + soil samples, data showed that these samples have highest potential for actual future seed bank.

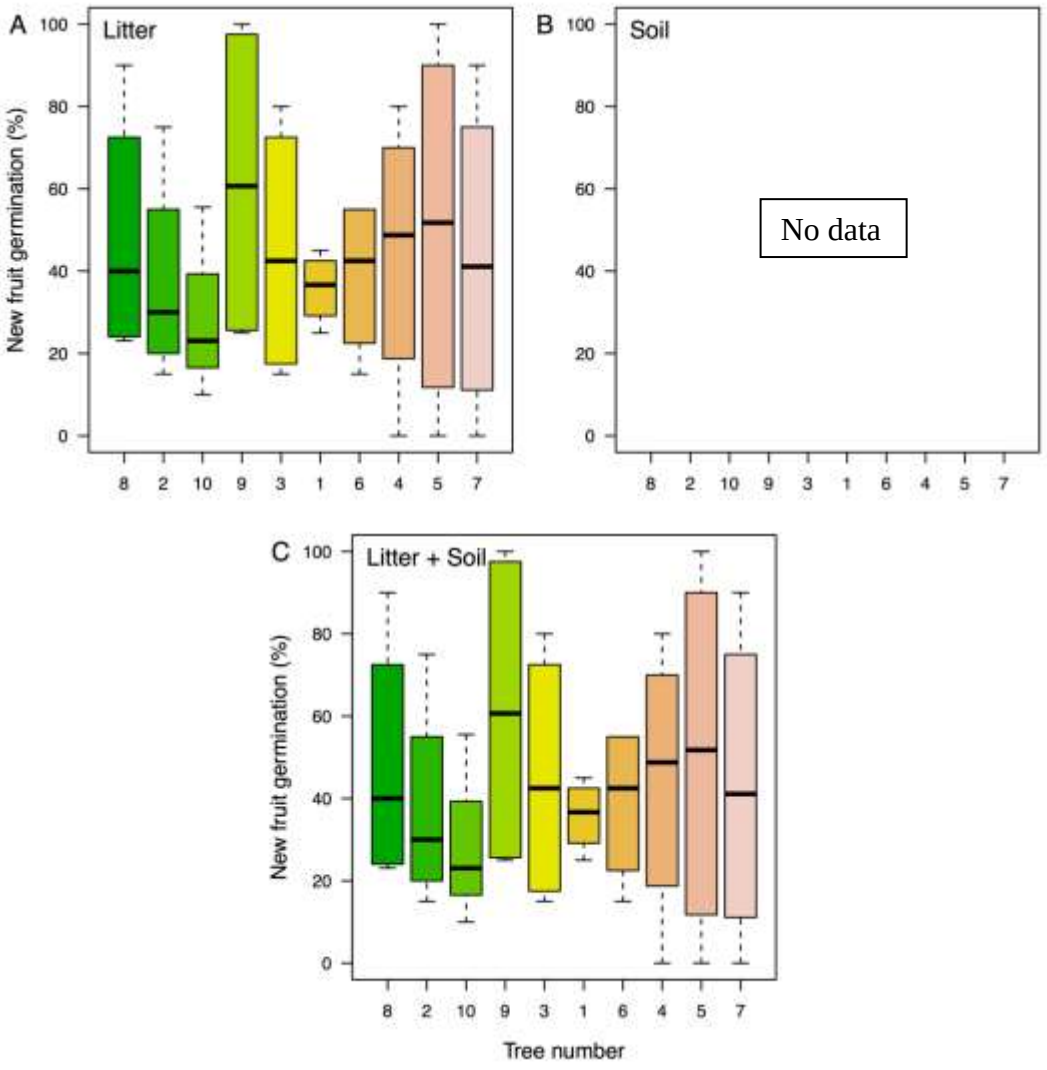

Figure 4.10. Germination of *E. angustifolia*. A, the new fruit germination (%) of litter; B, the new fruit germination (%) of soil; and C, the new fruit germination (%) of litter + soil.

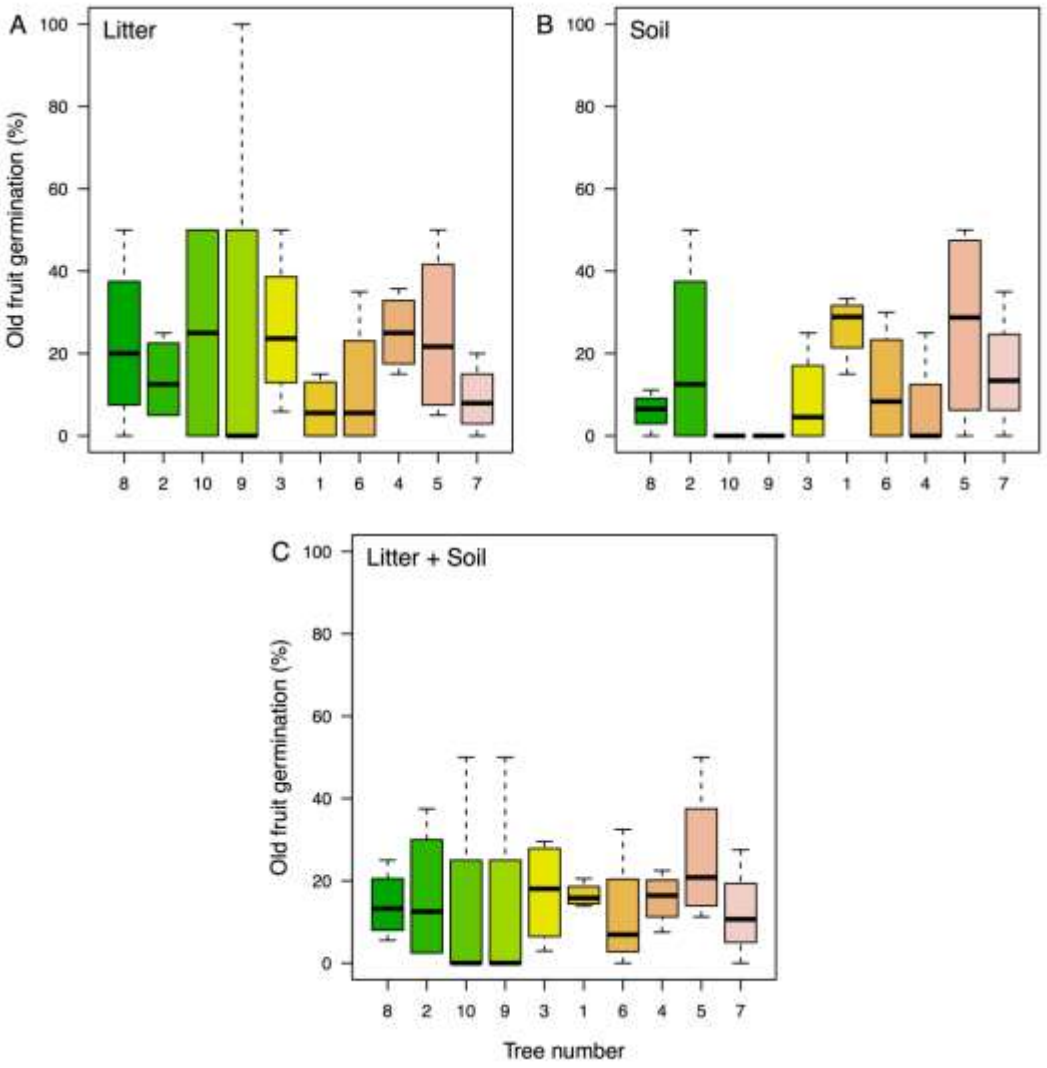

Figure 4.11. Germination of *E. angustifolia*. A, the old fruit germination (%) of litter; B, the old fruit germination (%) of soil; and C, the old fruit germination (%) of litter + soil**.**

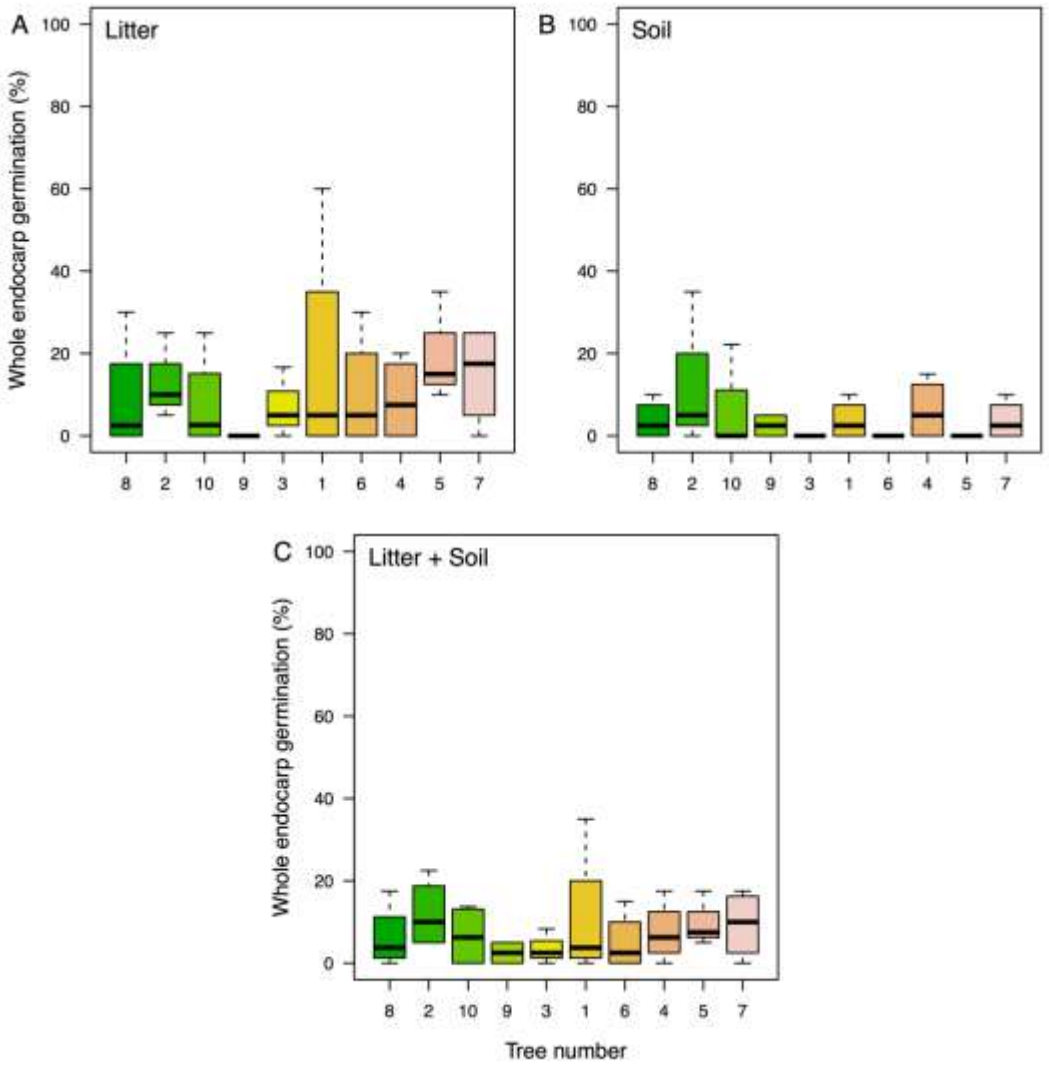

Figure 4:12. Germination of *E. angustifolia*. A, the whole endocarp germination (%) of litter; B, the whole endocarp germination (%) of soil; and C, the whole endocarp germination (%) of litter + soil.

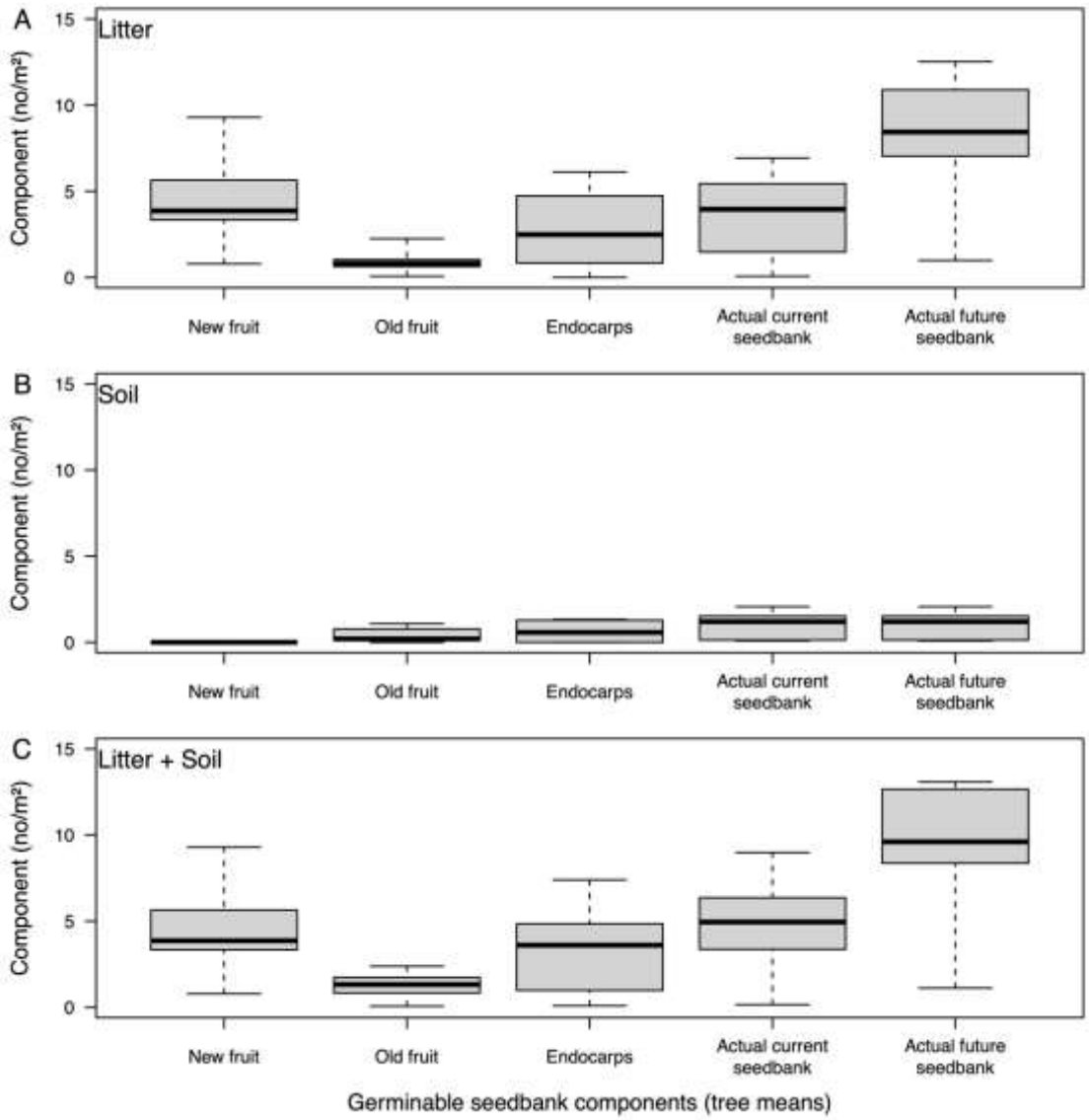

Figure 4.13. Germinable seed bank components of *E. angustifolia*. The germinable seed components (number/m^2) of new fruit, old fruit, endocarps, actual current seedbank and actual future seedbank from litter (A); soil (B) and litter + soil (C) samples.

4.3 Experiment 2 B: Soil seed bank assessment and germination of *H. rhamnoides.*

For determining soil seed bank and germination rate of *H. rhamnoides* a total ten sites were selected. From these ten sites, 15 soil samples were taken. The weight of each sample was approximately 1 kg. Soil seed bank assessment was done by sieving the samples and collecting the number of seeds present in each sample.

Data from soil seed bank experiment showed that all the sites have varied soil seed bank. Total number of seeds obtained from each sample were also varied significantly.

Samples from site 2 had the highest number of seeds as compared with other sites. At all sites, the number of large seeds was higher and small seeds were less in quantity (Figure 4.14).

The germination rate was also varied among all the samples. The large seeds showed the highest rate of germination as compared with small seeds. Also, some sites have more germination rate as compared with the other sites (Figure 4.14).

The germinable seed per kg of soil was also varied from each sample. Samples with large seeds had a greater number of germinable seeds as compared with small seeds that had the lowest numbers of germinable seeds (Figure 4.14).

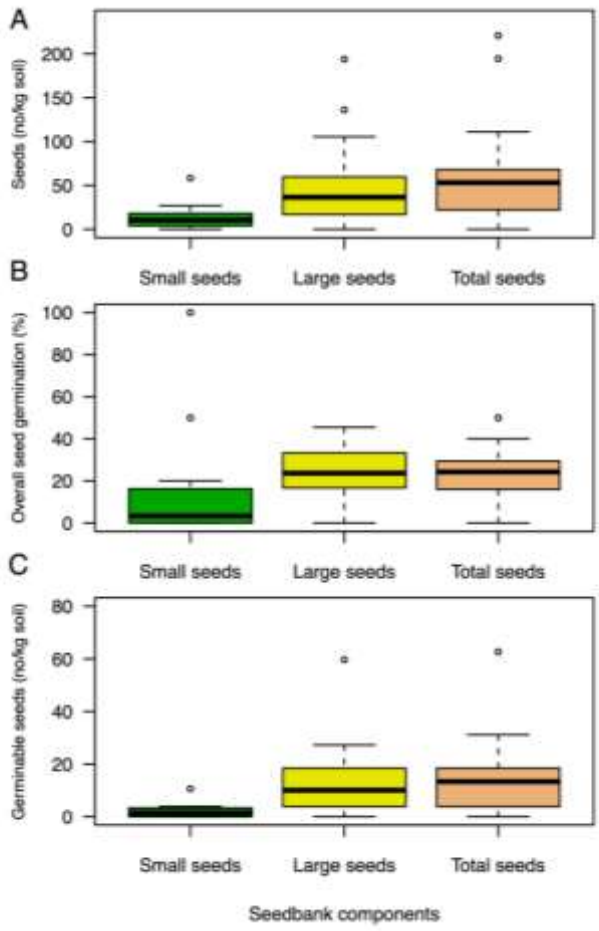

Figure 4.14. Soil seed bank assessment and germination of *Hippophae rhamnoides*. A, the seeds (number/kg soil); B, overall seed germination (%); and C, germinable seeds (number/kg soil) and the total seeds, small seeds and large seeds present in each sample.

4.4 Experiment 3: Watering of natural seedbanks of *E. angustifolia*

For determining that the water is limited factors for the germination of *E. angustifolia*, selected ten trees at different four locations were watered regularly on the area of 1 m^2 along with 1m^2 unwatered areas.

Data obtained from this study showed that the germination rate was differed according to tree number (Figure 4.15). The highest number of seeds was germinated from tree 8 while the lowest was observed from trees 1, 3 and 9.

Results obtained from this study showed that watered trees had the highest rate of germination in all trees at all locations while unwatered trees had lower germination which showed the role of water in the germination of *E. angustifolia*.

Soil temperature also had an important role in germination. At the start of the experiment soil temperature was lower and fewer seeds were germinated while with the increase in soil temperature, the number of germinated seeds was also increased (Figure 4.15).

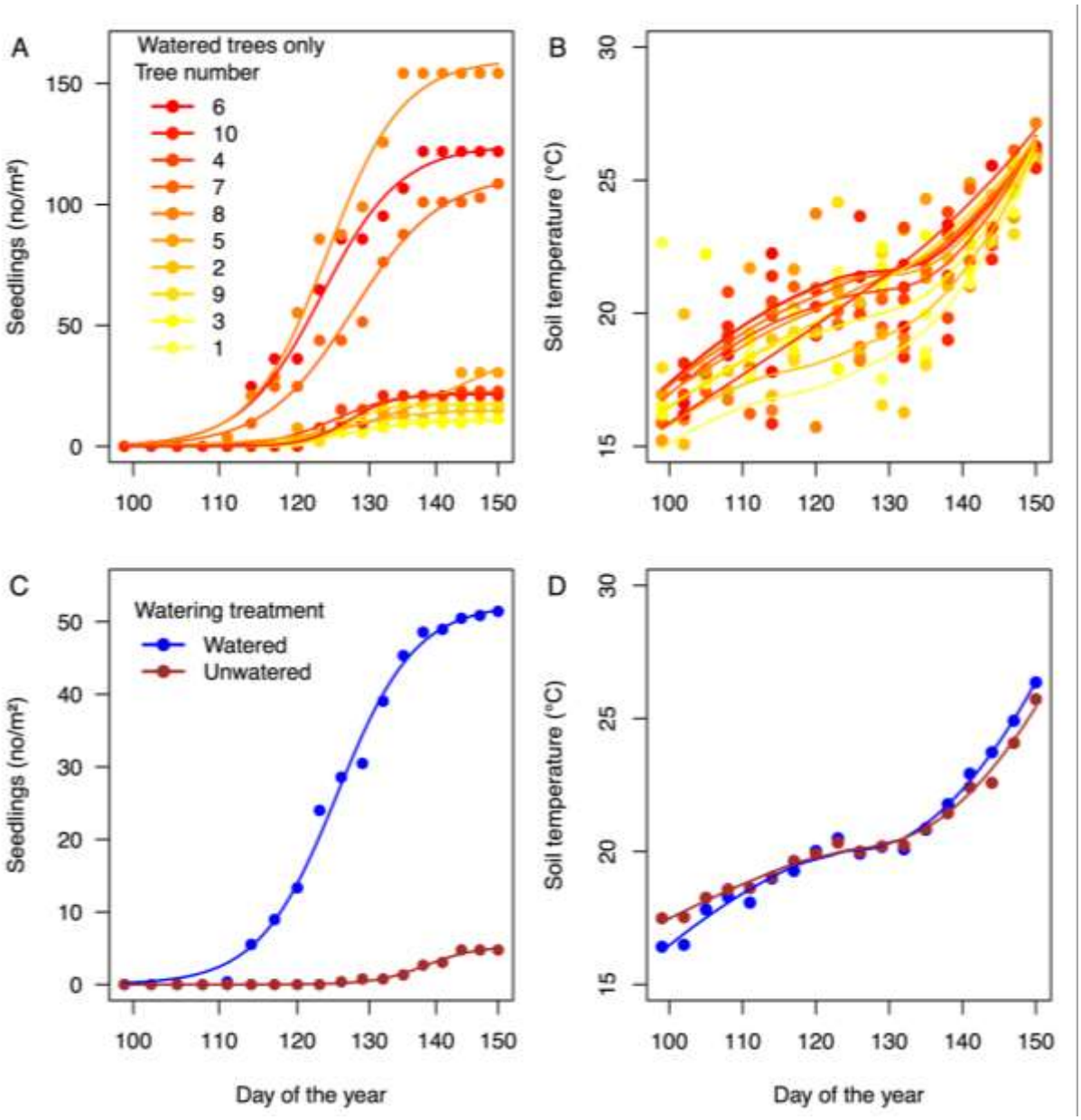

Figure 4.15. Watering of natural seedbanks of *E. angustifolia*. A, the seedlings (number/m^2); B, the soil temperature during whole study; C, the difference between the seedlings (number/m^2) under watered and unwatered conditions and D, rate of germination according to varied soil temperature.

4.5 Experiment 4. Determination of minimum germination temperature for local *E. angustifolia* and *H. rhamnoides*

The minimum germination temperature for *E. angustifolia* and *H. rhamnoides* was determined by placing the seeds in the incubator at different temperature regimes. Data recorded from this experiment showed that in the case of *E. angustifolia* at 10°C no seed

was germinated. Germination was started at 15°C which was considered as the minimum temperature required for its germination, although the maximum number of seeds were germinated at 25°C (Figure 4.16).

For *H. rhamnoides*, germination was started at 10 °C and below this range of temperature no seed was germinated, while the maximum number of seeds was germinated at 25°C (Figure 4.16). The cumulative proportion of germination for *E. angustifolia* and *H. rhamnoides* showed that the minimum temperature for their germination was 10°C (Figure 4.16).

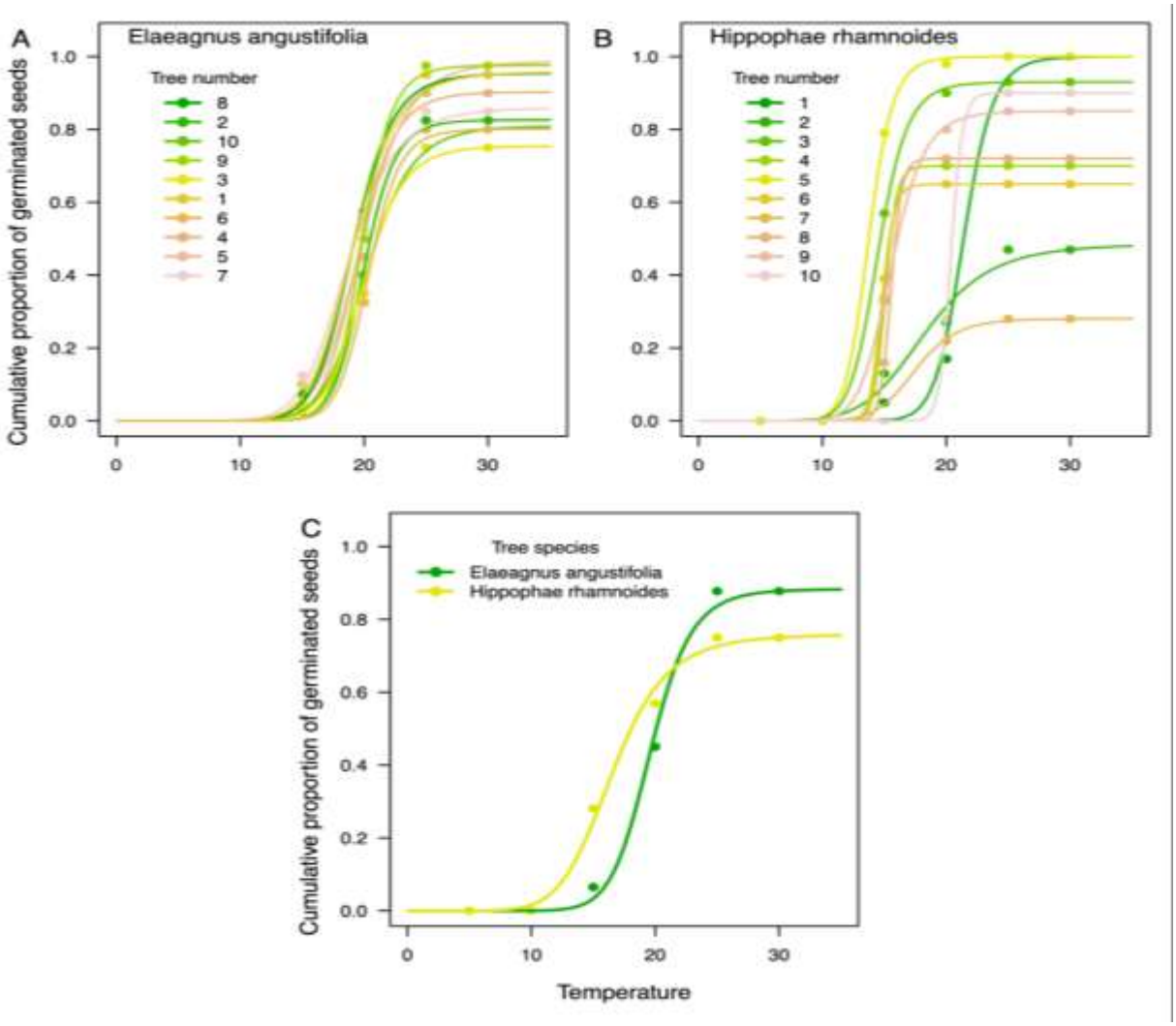

Figure: 4.16. Cumulative proportion of germinated seeds of *E. angustifolia* and *H. rhamnoides*. A, the cumulative proportion of germinated seeds of *E. angustifolia*: B, the cumulative proportion of germinated seeds of *H. rhamnoides*; C, the comparison of *E. angustifolia* and *H. rhamnoides*.

4.6 Experiment 5: Simulated seed bank for *E. angustifolia* and *H. rhamnoides*

The experiment for studying the simulated seed bank in under-watered and unwatered conditions by using three sowing depths (10, 20, and 30 mm) was conducted during the month of December 2021. Watering of the plants was started in April 2022 and data recording was also started.

Data from watered and unwatered treatments showed that *E. angustifolia* plants start germination under both conditions but the germination rate was higher in watered treatments as compared with unwatered treatments (Figure 4.17). In the case of *H. rhamnoides*, germination only occurred in the watered treatment.

Under different sowing depths, *E. angustifolia* plants in both watered and unwatered conditions showed the highest rate of germination at 20 mm sowing depth but for other depths, there were some seeds germinated and the lowest germination rate was observed at 30 mm sowing depth. For *H. rhamnoides*, also the highest germination rate was observed at 20 mm while the lowest was at 30 mm in the watered treatment (Figure 4.17).

Soil temperature was also recorded throughout the experiment and data showed that with the increase in temperature germination rate also started increasing and the highest germination was obtained at 25^{o}C (Figure 4.18).

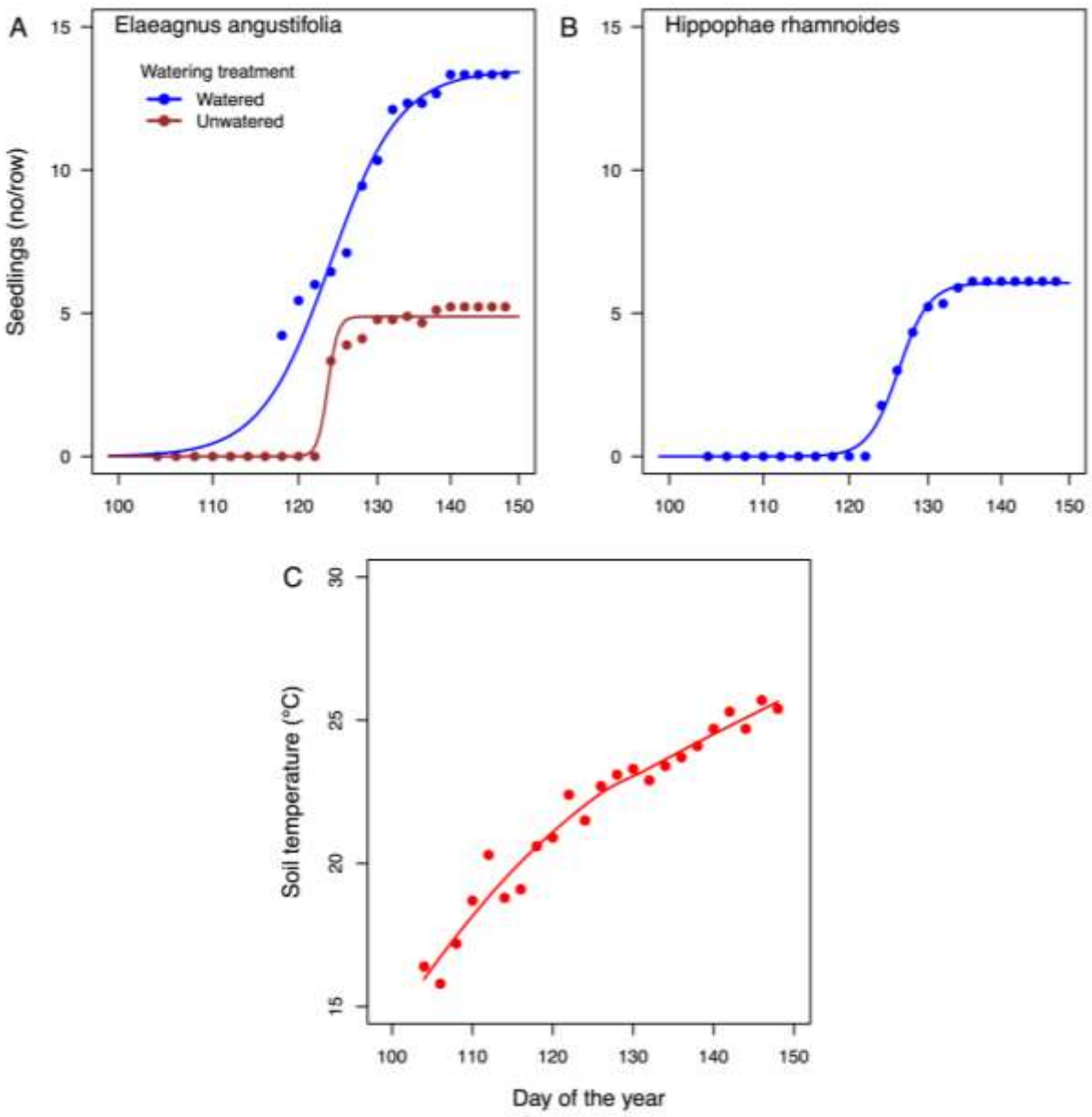

Figure: 4.17. Simulated seed bank for *E. angustifolia* and *H. rhamnoides.* A and B, are the seedlings (number/row) under watered and unwatered conditions of *E. angustifolia* and *H. rhamnoides* respectively and C, the soil temperature.

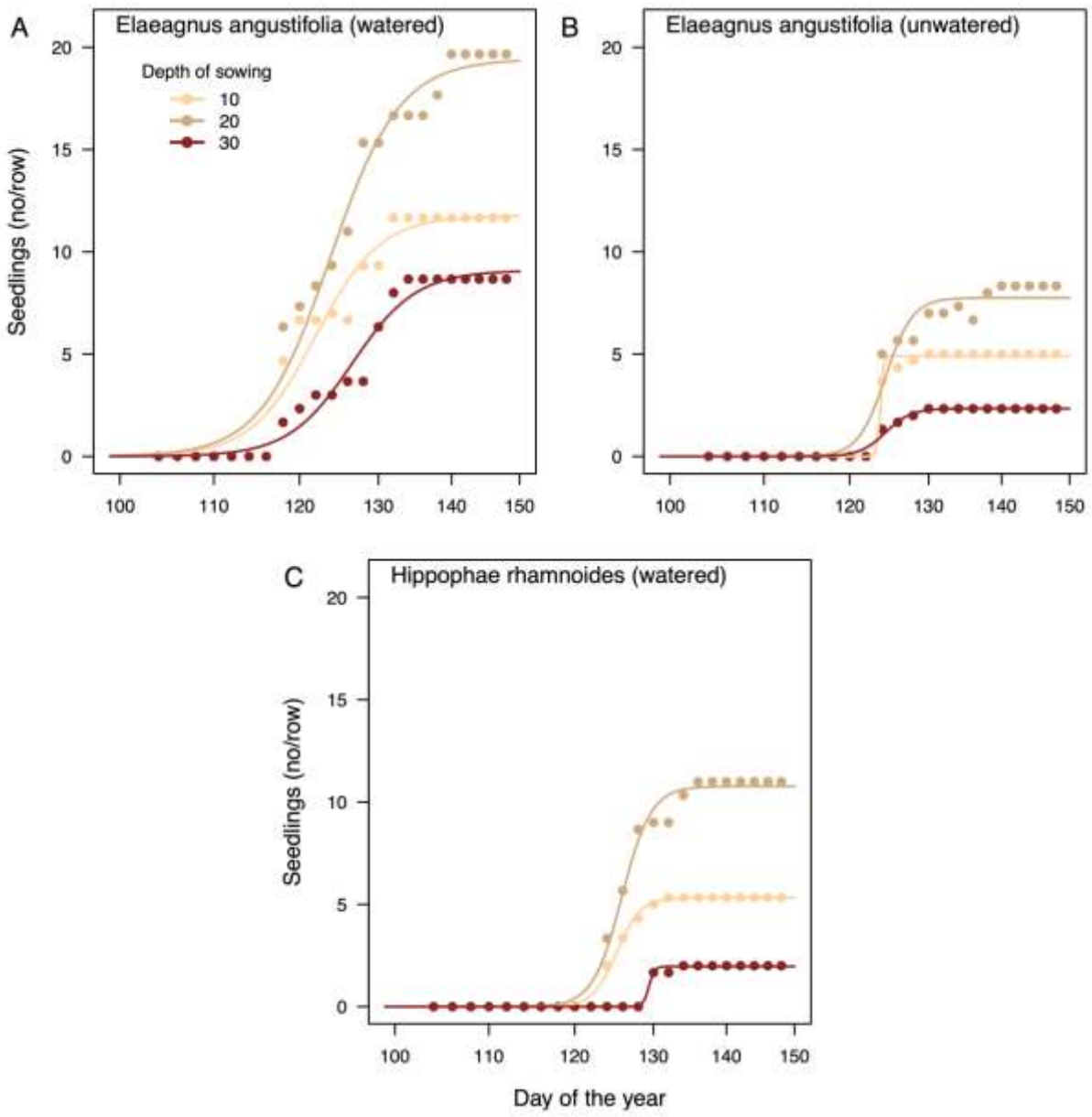

Figure: 4.18. Seedlings (number/row) of *E. angustifolia* and *H. rhamnoides*. A and B, the seedling of *E. angustifolia* under watered and unwatered conditions at three different sowing depths (10, 20, 30 mm) in respect; C, the germination of *H. rhamnoides* under watered conditions at three different sowing depths (10, 20).

CHAPTER V

DISCUSSION

5.1 Fruit and endocarp characterization of *Eleagnus angustifolia*

Morphological characterization such as fruits and seed or endocarp characterization has been widely employed by several researchers for identifying variations among plant genetic resources which is important for efficiency, conservation and sustainability in plant production (Sari, et al., 2021; Solmaz et al., 2007). Seed characterization is also valuable for the identification of plants and their specific properties. Fruit and endocarp characterization of *E. angustifolia* was evaluated for selected ten trees at four different locations. Fruit length, fruit diameter, fruit weight and aspect ratio showed a huge variation among the results at all selected locations and trees (Figure 4.1). Our results showed the variation in fruit length ranging from 10 to 25 mm and these results were in line with Uzun et al. (2015) who observed that the fruit length of *E. angustifolia* accessions taken from Central Anatolian Region Türkiye ranged from 11 to 26 mm. Young and Young (1992) also observed that the fruit length of *E. angustifolia* was 10 to 15 mm and contains a single large-size seed. The fruit diameter of our study ranged between 7 to 16 mm and these results were supported by Uzun et al. (2015) who observed fruit width ranging between 9 to 17 mm among *E. angustifolia* accessions. Akbolat et al. (2008) also reported that the width of *E. angustifolia* fruits varied from 12 to 22 mm. The results of fruit weight from our study (0.5 to 2 g) were supported by Uzun et al. (2015) but lower than the findings of Ersoy et al. (2013) who observed the average fruit weight of 2.90 g of *E. angustifolia*. The reason may be due to the selection of fruits that taken from the local market, but different locations and trees were collected in this study.

The endocarp length, diameter and weight obtained from different selected sites and trees showed a statistically huge variation (Figure 4.2). Results of our study showed that the endocarp length of *E. angustifolia* ranged from 0.5 to 22 mm and these results were supported by Young and Young (1992). The diameter of the endocarp also varied among the locations and selected trees and these results are supported by Katz and Shafroth (2003) in their study on the ecology and biology of *E. angustifolia* in North

America. The main reason for the variation is the difference between sites and maybe *E. angustifolia* population type as in Tukey there are more than 25 populations have been observed in Türkiye (Ozdemir and Kalyoncu, 2011). The statistically significant endocarp weight among all trees and sites hopefully corresponds to the existence of different *E. angustifolia* populations in studied areas.

The overall variations in fruit and endocarp characterization among selected trees and sites were related to the presence of several *E. angustifolia* populations in the selected sites and these results are supported by Ozdemir and Kalyoncu (2011) who conducted a study on the selection of *E. angustifolia* naturally grown in the vicinity of Selcuk University Konya, Türkiye and examined 30 kinds of *E. angustifolia* that have 1.50 to 1.8 g fruit weight, 21.4 to 24.8 mm length and 15.4 to 17.7 mm width with 2.3 to 4.2 g endocarp weight.

5.2 Soil seed bank assessment and germination test of *E. angustifolia*

Assessments of seed banks are thought to be crucial for conservation, restoration and persistence efforts (Chiquoine and Abella, 2018). Results from the seed bank study showed that from both litter and soil samples, number of the new fruits (no./m^2), the number of old fruits (no./m^2), whole endocarps (no./m^2), broken endocarps (no./m^2), potential current seed bank and (no./m^2) and potential future seed bank (no./m^2) were varied according to the tree number and sites (Figure 4.3 - 4.9).

From results, it has been observed that *E. angustifolia* had large current and potential seed bank and these results are in line with Brock et al. (2003) who concluded from their study on seed banks of *E. angustifolia* from invaded riparian habitats in northeastern, Arizona *E. angustifolia* can store a greater quantity of seeds inside the soil as a soil seed bank. The reason for higher seeds is that the upper 15 cm surface of soil mostly contains more fruits and viable seeds and the litter/humus also exhibited some seeds (Mekonnen, 2016). The variation in seed bank also corresponds to the location and site and the reason is that this plant becomes naturalized in a variety of phytocoenoses, including halophytic communities and steppe meadows. Arid climates with limited precipitation characterize the majority of the examined coenopulations

(Abramova et al., 2021). The species can spread swiftly, displacing native species and creating single-species thickets in the process (Pearce and Smith, 2001)

As *E. angustifolia* can grow in a larger variety of places due to the presence of more flesh and a hard seed coat in each seed (Shafroth et al., 1995). It has been observed that the *E. angustifolia* seeds could germinate only when conditions at each specific site are favorably contributing to its capacity to establish in a wider range of floodplain sites (Shafroth et al., 1995). The germination rate varied according to trees number and the results from this experiment correlate with the study of Kiselva and Chindyaeva (2011) on the biology of *E. angustifolia* in the northeastern region where they observed that the ability of seeds from various trees to germinate varied. In seeds from model trees 1, 2, and 3, germination was 23, 68, and 86%, respectively.

5.3 Soil seed bank assessment and germination of *H. rhamnoides.*

Results from the seed bank assessment study showed that collected soil samples have various small and large size seeds and the number of seeds varied according to the tree number and sites (Figure 4.14). As the seeds with just 1% of overall seed production are dispersed across long distances (Cain et al., 2000) so the area near the tees possesses a strong seed bank. *H. rhamnoides* seed bank is mostly the area after landslides due to its ability to grow in such areas as observed by Bolibok et al. (2018). Furthermore, the highest seed bank observed in our study is s supported by Bolibok et al. (2018) as they also found the highest seed numbers from afforested and less developed areas. Also, the Female *H. rhamnoides* start producing fruit between their second and third years of life (Pearson and Rogers, 1962), and they may be able to soon replace males as a secondary source of seeds in the invaded area and provide the soil with a huge soil seed bank.

Hippophae rhamnoides plants are capable to establish and colonize on nutrient-poor, unstable and even sandy soils (Isermann 2008). The germination rate from our research work varied among all the samples and large seeds showed the highest rate of germination as compared with the small seeds (Figure 4.14). Korekar et al. (2013) observed a 99 and 100% of germination rate of H. rhamnoides seeds sown within a year of production and the germination rate starts decreasing with the years.

5.4 Watering of natural seedbanks of *E. angustifolia*

Natural seed banks have a greater role in the dynamic of several plant communities (Fenner, 2017). The role of the natural seed bank in the restoration process of natural vegetation is not consistent and depends on a number of biotic and abiotic factors. Water is considered as the most limited factor in seedling emergence. From our study, the difference between the seedlings (number/m^2) under, watered and unwatered conditions and the rate of germination were varied according to tree sites and tree number (Figure 15). These findings are supported by Ma et al. (2014) who observed from their study on water effects on soil seed bank that water have a huge impact on the seed bank recruitment process and ultimately helps in plant community establishment. Other studies also revealed that the germination and seedling emergence from soil seed banks was determined by the availability of water (Griffith and Forseth, 2003; Johnson, 2004; Liu et al., 2005).

5.5 Determination of minimum germination temperature for local *E. angustifolia* and *H. rhamnoides*

Temperature is the most valuable component in the germination process and has a strong impact on the recruitment process of several plant communities (Grubb, 1977; Grime, 2002; Walck et al., 2011). Also, favorable conditions are important for increasing the survival of seedlings and plant establishment (Fenner and Thompson, 2005; Poschlod et al., 2013). For *E. angustifolia* seeds stratification at 5°C temperature is also required due to the presence of dormancy. From previous studies, it has been proved that prechilling seeds for 10 to 90 days at 1 to 5°C (34 to 41°F) aids in dormancy breakdown. Results of another study showed that for *E. angustifolia*, the minimum effective stratification duration is 9 to 12 weeks (Hogue and LaCroix 1970). Gao et al. (2021) also observed that the germination rate of scarified seeds at 5/15°C was maximum under dark conditions.

Results of the current study showed that the minimum germination temperature for *E. angustifolia* was started at 15°C (Figure 4.16). As this plant is native to temperate regions of Eurasia so this is the main reason for its adoption to lower temperatures (Katz and Shafroth, 2003). Results of another study that evaluated the impact of gibberellin and temperature on the germination rate of *E. angustifolia* showed that seeds started

germination at 15°C while the highest rate of germination was observed at 20°C (Juan, 2017). In our results, the highest germination rate was observed at 25 which is higher than in this study.

For *H. rhamnoides*, germination was started at 15°C which is the minimum temperature for their germination (Figure 4.16). In our study, the optimum temperature where the maximum number of seeds germinated was 25°C. These results were supported by the study that showed that the ideal temperature for the germination of *H. rhamnoides* seed is a range of 20 to 30°C, and tests for germination on stratified seeds in sand flats can be performed in 40 days (Tayier et al., 2006; Busing and Slabaugh, 2008).

5.6 Simulated seed bank for *E. angustifolia* and *H. rhamnoides*

Results from simulated seed banks under the watered and unwatered treatments showed that *E. angustifolia* plants start germination under both conditions but the germination rate was higher in watered treatments as compared with unwatered treatments (Figure 4.17). This study is supported by (Enescu, 2018) who observed that *E. angustifolia* is a multipurpose species and required humid conditions for germination.

Sowing depth is another important factor that determines the growth of *E. angustifolia* and *H. rhamnoides* under natural as well as artificial conditions. George (2017) stated that seed germination and emergence rates are reduced with increasing sowing depth, as the seedling emergence increase with deeper sowing depths.

CHAPTER VI

CONCLUSION

The main objective of the study was to assessed seed biology, soil seed bank, and the possibilities of natural recruitment for *E. angustifolia* and *H. rhamnoides* under the semiarid conditions of Niğde, Türkiye.

From our study on seed banks, both litter and soil samples of E. angustifolia, the number of new fruits (no./m2), the number of old fruits (no.m^2), whole endocarps (no./m2), broken endocarps (no./m2), potential current seed bank and (no./m2) and potential future seed bank (no./m2) were varied according to the trees and sites.

The slight variation in the germination rate between trees of *E. angustifolia* showed little phenotypic difference and *H. rhamnoides* showed more phenotypic difference given the varied germination rate.

Also, there was a big difference between the seedlings (no./m2) under watered and unwatered conditions and the rate of germination varied according to tree sites and trees. Results of the minimum germination temperature for *E. angustifolia* showed that the seed started germination at 15℃ and for *H. rhamnoides*, seed started germination at 10℃ which was considered as the minimum temperature required for germination.

It has been observed that the seeds of *E. angustifolia* and *H. rhamnoides* are present in the soil seed bank but their recruitment was lower in Nigde, Türkiye. This is due to requirement of a specific temperature (15-30 ℃) and water availability to germinate. Before the temperature rise and reach that specific level, the soil moisture contents drop and creates un suitable conditions for the seed's germination. Some seeds germinate due to getting some rainfall but unable to survive due to un availability of water in whole season.

Data for simulated seed bank in under-watered and unwatered conditions by using three sowing depths (10, 20, and 30 mm) showed the highest rate of germination at 20 mm

sowing depth but for other depths, there were some seeds germinated and the lowest germination rate was observed at 30 mm sowing depth.

In general, ecological conditions play an important role in the germination and recruitment of both *E. angustifolia* and *H. rhamnoides*. Seed size had a significant role in the germination of *H. rhamnoides* that is large seeds showed higher germination as compared with smaller seeds. Also, the samples with large seeds had a greater number of germinable seeds. The availability of water is another important factor that determined the germination of seeds. Plots that were irrigated regularly showed higher germination that shows that water is the limiting factor in the natural recruitment of *E. angustifolia*. The water availability to seeds in the seed bank by any source i.e., rainfall, snowfall etc., could be beneficial in the natural recruitment of these plants. Temperature is another important factor for determining the natural recruitment of these plants. At lower and very high temperatures seeds did not germinate. Mostly seeds germinated at 15°C and at 30°C germination rate started decreasing for both *E. angustifolia* and *H. rhamnoides*. Seed depth is another factor that had a significant impact on the germination and natural recruitment of these two species. Seeds present at shallow depth (10 mm) and high depth (30 mm) did not germinated and the highest seeds were germinated at 20 mm depth.

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