T.R.
NİĞDE ÖMER HALİSDEMİR UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF PLANT PRODUCTION AND TECHNOLOGIES

# EVALUATION OF DIRECT ESTABLISHMENT METHODS FOR *ELAEAGNUS ANGUSTIFOLIA*, AN ACTINORHIZAL PLANT IN THE ELAEAGNACEAE WITH AGROFORESTRY AND AGROECOSYSTEM IMPROVEMENT POTENTIAL FOR CENTRAL ANATOLIA, TURKEY

NUR FUNDA TUTAR

November 2021

N. F. TUTAR, 2021

MASTER THESIS

NİĞDE ÖMER HALİSDEMİR UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

T.R.
NİĞDE ÖMER HALİSDEMİR UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF PLANT PRODUCTION AND TECHNOLOGIES

# EVALUATION OF DIRECT ESTABLISHMENT METHODS FOR *ELAEAGNUS ANGUSTIFOLIA*, AN ACTINORHIZAL PLANT IN THE ELAEAGNACEAE WITH AGROFORESTRY AND AGROECOSYSTEM IMPROVEMENT POTENTIAL FOR CENTRAL ANATOLIA, TURKEY

NUR FUNDA TUTAR

Master Thesis

Supervisor

PROF. DR. IAN TIMOTHY RILEY

November 2021

The study titled “Evaluation of Direct Establishment Methods for *Elaeagnus angustifolia*, an Actinorhizal Plant in the Elaeagnaceae with Agroforestry and Agroecosystem Improvement Potential for Central Anatolia, Turkey” and presented by Nur Funda TUTAR under the supervision of Prof. Dr. Ian Timothy RILEY, has been recognised as Master thesis by the jury at the Department of Plant Production and Technologies of Niğde Ömer Halisdemir University, Graduate School of Natural and Applied Sciences.

Head:
Signature

Member:
Signature

Member:
Signature

**CONFIRMATION:**

(This thesis has been found appropriate at the date of …./…./20.... by the jury mentioned above who have been designated by Board of Directors of Graduate School of Natural and Applied Sciences and has been confirmed with the resolution of Board of Directors dated …./…./20.... and numbered ……………)

**/ /2021**
**Prof. Dr. Murat BARUT**
**DIRECTOR**

# THESIS CERTIFICATION

It is certified that I have written this thesis by myself. I further confirm that all information included in this thesis is scientific and is in accordance with the university rules and regulations. Any materials that I have used from external sources as well as help received and all sources used in finalizing this research work and preparing this thesis, all have been acknowledged in the thesis.

Nur Funda TUTAR

# SUMMARY

## EVALUATION OF DIRECT ESTABLISHMENT METHODS FOR *ELAEAGNUS ANGUSTIFOLIA,* AN ACTINORHIZAL PLANT IN THE ELAEAGNACEAE WITH AGROFORESTRY AND AGROECOSYSTEM IMPROVEMENT POTENTIAL FOR CENTRAL ANATOLIA, TURKEY

TUTAR, Nur Funda
Niğde Ömer Halisdemir University
Graduate School of Natural and Applied Sciences
Department of Plant Production and Technologies

Supervisor : Prof. Dr. Ian Timothy RILEY

November 2021, 94 pages

Actinorhizal plants such as *Elaeagnus angustifolia* L. provide vast agroforestry and agroecosystem benefits. However, in Niğde Province, Central Anatolia, natural establishment has not been widely observed. This study aimed at assessing direct establishment methods for *E. angustifolia*. Six experiments were conducted in greenhouse and on the field to evaluate effects of sowing depth, water regimes, backfilling (of the planting hole) depth, hydrophilic gel and soil backfilling types in addition to *Frankia* distribution in Niğde soil. Water regime and sowing depth affected the *E. angustifolia* emergence, root and shoot length and germination rate. The backfilling type, fill quantity and sowing depth also affected the emergence and germination ratio. Hydrophilic gel backfilling enhanced the germination ratios of *E. angustifolia* and 25 mm sowing depth gave higher emergence and plant growth. *Frankia* was found to be distributed across the study site. Winter germination of *E. angustifolia* was low while the furrow application enhanced *E. angustifolia* germination under field conditions. Generally, accessions of the *E. angustifolia* collected in and around the university campus had higher germination and growth compared with the commercial type.

*Keywords: Elaeagnus angustifolia* L., *Frankia,* germination, backfill, hydrophilic gel

# ÖZET

## İÇ ANADOLU'DA TARIMSAL ORMANCILIK VE TARIMSAL EKOSİSTEM GELİŞİM POTANSİYELİ OLAN, ELAEAGNACEAE'DE BİR AKTİNORİZAL BİTKİ OLAN *ELAEAGNUS ANGUSTIFOLIA* İÇİN DOĞRUDAN KURULUŞ YÖNTEMLERİNİN DEĞERLENDİRİLMESİ

TUTAR, Nur Funda
Niğde Ömer Halisdemir Üniversitesi
Fen Bilimleri Enstitüsü
Bitkisel Üretim ve Teknolojileri Anabilim Dalı

Danışman : Prof. Dr. Ian Timothy RILEY

Kasım 2021, 94 sayfa

*Elaeagnus angustifolia* L. gibi aktinorizal bitkiler, geniş tarımsal ormancılık ve tarımsal ekosistem faydaları sağlar. Ancak, İç Anadolu'nun Niğde ilinde doğal yerleşme yaygın olarak gözlenmemiştir. Bu çalışma, *E. angustifolia* için doğrudan yerleşme yöntemlerini değerlendirmeyi amaçlamıştır. Niğde toprağında *Frankia* dağılımına ek olarak ekim derinliği, su rejimleri, dolgu derinliği, hidrofilik jel ve toprak dolgu türlerinin etkilerini değerlendirmek için serada ve tarlada altı deneme yapılmıştır. Sulama rejimi ve ekim derinliği, *E. angustifolia* çıkışını, kök ve sürgün uzunluğunu ve çimlenme oranını etkilemiştir. Dolgu tipi, dolgu miktarı ve ekim derinliği de çıkış ve çimlenme oranını etkilemiştir. Hidrofilik jel dolgu, *E. angustifolia*'nın çimlenme oranını arttırmış ve 25 mm ekim derinliği daha yüksek çıkış ve bitki büyümesi sağlamıştır. *Frankia*'nın çalışma alanı boyunca dağılım gçsterdiği bulunmuştur. Tarla koşullarında *E. angustifolia*'nın kış çimlenmesi düşük olurken, karık uygulaması *E. angustifolia*'nın çimlenmesini arttırmıştır. Genel olarak, üniversite kampüsü ve çevresinden toplanan *E. angustifolia* aksesyonları ticari türe göre daha yüksek çimlenme ve büyüme göstermiştir.

*Anahtar Sözcükler: Elaeagnus angustifolia* L., *Frankia,* çimlenme, dolgu, hidrofilik jel

# ACKNOWLEDGEMENTS

First and foremost, I would first like to thank my supervisor Prof. Dr. Ian Timothy RILEY for his valuable guidance throughout my studies. Without his assistance and dedicated involvement in every step throughout the process, this master thesis would have never been accomplished. You provided me with the tools that I needed to choose the right direction and successfully complete my dissertation. I would like to thank you very much for your support and understanding over these past three years.

I would like to acknowledge my colleagues Eniola OLOWU and Kaddi JAWNEH for their wonderful collaboration and support.

I must express my very profound gratitude to my mother Assoc. Prof. Dr. Filiz KUTLUAY TUTAR, my father Prof. Dr. Erdinç TUTAR and my sisters Dr. Makbule Fulya TUTAR SELÇUK and Dr. Eda Fulden TUTAR ÇÖLGEÇEN for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of this thesis.

Finally, I want to sincerely thank my boyfriend mechanical engineer Halil Ramazan BAŞBÖYÜK for his endless support throughout the entire thesis process and his passionate participation was given to further my research. This accomplishment would not have been possible without them.

Thank you very much.

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# SYMBOLS AND ABBREVIATIONS

| **Symbols** | **Meaning** |
|---|---|
| % | Percentage |
| °C | Degrees celsius |

| **Abbreviations** | **Meaning** |
|---|---|
| µm | Micrometer |
| cm | Centimeter |
| d | Day |
| g | Grams |
| h | Hour |
| HG | Hydrophilic Gel |
| kg | Kilogram |
| L | Liter |
| min | Minute |
| mm | Millimeter |
| ml | Milliliter |
| ppm | Parts Per Million |
| s | Second |

# CHAPTER I

# INTRODUCTION

## 1.1 Introduction

*Elaeagnus angustifolia* L. is an actinorhizal plant that is native to Turkey and well adapted to semi and semi-arid climates. This actinorhizal plant has many benefits and has the potentials to improve the agroforestry and agroecosystem in Central Anatolia. However, the challenge is the establishment by direct methods is not widely reported and no evidence is available in the literature for Central Anatolia, Turkey. Hence this research focused on understanding the uses, optimum germination conditions and establishment methods as well as nodulation.

## 1.2 *Elaeagnus angustifolia*

*E. angustifolia,* a deciduous small arbor or large multi-stemmed shrub, commonly called Russian olive, belongs to the Elaeagnaceae family and originated from Euro-Asian countries (Katz and Shafroth, 2003). It is a species with agroforestry and agroecosystem improvement potentials besides economic and ornamental benefits. Economic and ornamental benefits of the plant include being used as fuel, food and animal feed, medicinal purposes and in the papermaking and furniture industry (Zalesny et al., 2019; Zhang et al., 2018).

Asgarzadeh et al. (2014) determined that the plant is an appropriate landscape species in regions with abiotic (cold and drought stress) conditions. Moreover, as an excellent nitrogen-fixing (Khamzina et al., 2009; Miller and Baker, 1985; Mineau et al., 2011) and saline-alkali tolerance species (Chang et al., 2018; Zhang et al., 2020), it has important roles such as a windbreak, soil and water conservation, revegetation, and afforestation (Katz and Shafroth, 2003; Zhang et al., 2020).

## 1.3 Distribution and Uses of *Elaeagnus angustifolia*

In countries of origin (e.g., China, Turkey and Uzbekistan), several studies have confirmed that the *E. angustifolia* is an appropriate plant species for agroforestry purposes such as afforestation, vegetation restoration and land conservation (Dubovyk et al., 2016; Zhang et al., 2018). In non-native countries such as the USA and Canada, it is established that *E. angustifolia* invades the native vegetative cover and changing nutrient dynamics in surface water bodies (Shafroth et al., 1995; Katz and Shafroth, 2003; Collette and Pither, 2015).

This tree can be found in the Central, Southern and Southeastern Anatolia of Turkey. The plant is well known for its fast-growing and strong lateral roots whose nodules can improve the soil conditions by binding the free nitrogen of the air. The species that can grow in shallow, dry and arid, poor, calcareous and saline soils are very contented in terms of soil demand and effective for erosion control (Göktürk et al., 2009).

## 1.4 Germination and Establishment

The agroforestry uses of *E. angustifolia* have limitations due to germination barriers of *E. angustifolia* seeds. These barriers include factors associated with embryo and seed dormancy. The dormancy stage varies within the same species and individual tree species and the year of the plant (Olson et al., 2004; Ölmez et al., 2007).

There are several methods and techniques to improve the direct establishment of *E. angustifolia*. These include pre-germination treatments like cold and warm scarification to overcome embryo dormancy, floating on hot water, mechanical or chemical scarification and hot aeration for seed dormancy (Olson et al., 2004; Ölmez et al., 2007; Göktürk et al., 2009). It is established that cold scarification of *E. angustifolia* seeds at 1-10°C for more than 60 days overcomes embryo dormancy (Olson et al., 2004).

Additionally, to ensure excellent germination, it is recommended that seeds of *E. angustifolia* can be soaked for 30-31 h in sulphuric acid or 2-chloroethyl phosphonic acid without cold treatment (Olson et al., 2004). Göktürk et al. (2009) found that soaking of *E. angustifolia* seeds in running water at 15°C for 10 days plus cold scarification treatment for 30 days improves the germination rate.

Also, Ölmez et al. (2007) identified that cold scarification treatment for 60 days provided the highest germination percentage for the *E. angustifolia* seeds compared with durations 20 and 40 days. Also, Olson et al. (2004) reported that the sowing depth of *E. angustifolia* seeds should be 13-25 mm without scarification in summer or fall periods or after cold scarification in the spring.

Studies of *E. angustifolia* have focused on seed preconditioning with limited information on direct establishment methods such as organic matter supply, mulching, hydrophilic gel and micro-catchment formation. Also, there is no evidence for the best direct establishment method of *E. angustifolia* in Central Anatolia. Therefore, this study evaluated the direct establishment methods for *E. angustifolia*, an actinorhizal plant in the Elaeagnaceae with agroforestry and agroecosystem improvement potential for Central Anatolia, Turkey.

### 1.5 Aims and Objectives

This research aimed at stimulating revegetation applications in Turkey with direct establishment methods by using *E. angustifolia* for agroecosystem improvement in Central Anatolia. *E. angustifolia*, an actinorhizal plant in the Elaeagnaceae had been identified as a tree with strong potential for revegetation and agroforestry in the Central Anatolia basin. Hence the objectives of this project included:

- Understanding the outcomes for potential uses such as revegetation and agroforestry of *E. angustifolia*.
- Testing different methods for the direct establishment of *E. angustifolia* such as seed sowing depth, organic matter supply, seed preconditioning, mulching, hydrophilic gel and micro-catchment formation.
- The distribution of the nitrogen-fixing symbiont, *Frankia*, in areas with and without existing *E. angustifolia* to confirm if inoculation is needed.

## 1.6 Importance of Research

The semi-arid climate of the Central Anatolia region makes increasing afforestation with *E. angustifolia* important and this will provide a high coping potential against to effects of global warming. Also, *E. angustifolia* tolerates wind, summer heat and saline conditions and is particularly well adapted to semi-arid drought.

Although native to Turkey, it does not appear to be strongly self-propagating and there is limited evidence of volunteer recruitment; mostly it is established by planting seedlings or vegetatively propagated saplings.

Direct establishment methods can be used in dry conditions and semi-arid areas since it has advantages such as relatively low costs, no transportation costs, better root development, better germination rates, very quickly and better survival.

Since there is not enough evidence on the best direct establishment methods of *E. angustifolia* in Central Anatolia, this research will provide new information and data for the best conditions for *E. angustifolia* direct establishment in Central Anatolia.

# CHAPTER II

# REVIEW OF LITERATURE

## 2.1 Description of *Elaeagnus angustifolia* Plant

*Elaeagnus angustifolia* L. (Russian olive) belongs to the family Elaeagnaceae, which includes three genera: *Elaeagnus*, *Shepherdia* and *Hippophae*. It is a small multi-stemmed tree or large shrub. Its wood is ring-porous, with the sap flow concentrated in large xylem canals located in the outermost growth ring (Hultine and Bush, 2011). *E. angustifolia* has a relatively high mean relative growth rate (1.61 mg/g/day), crop growth rate (1.73 $g/m^2$/day), leaf area index (1.65 $m^2/m^2$) and leaf weight ratio (0.24 g/g) compared to other potential agroforestry species tested in Khorezm, Uzbekistan (Khamzina, et al., 2009).

*E. angustifolia* is deciduous with distinctive silvery-green foliage. It produces fragrant yellow flowers in spring or early summer, while the single-seeded fruits ripen in fall. *E. angustifolia* is an actinorhizal species that form a nitrogen-fixing symbiosis with actinomycete bacteria of the genus *Frankia* (Katz and Shafroth 2003). As a result, *E. angustifolia* foliage exhibits high N content and low C:N ratio, compared to native cottonwoods (Abelho and Molles, 2009; DeCant, 2008; Harner et al., 2009; Moline and Poff, 2008; Roggy et al., 2004; Royer et al., 1999; Simons and Seastedt, 1999). *E. angustifolia* plants reach reproductive maturity at approximately 10 years old (Lesica and Miles, 2011).

The flowers appear in early summer and are pollinated by insects. The fruits, single-seeded drupes, ripen in late summer or fall and are dispersed by birds, mammals, gravity and water. Although *E. angustifolia* tends to grow in moist areas of western landscapes (e.g., floodplains and wet meadows), it is not an obligate phreatophyte. Notably, *E. angustifolia* does not require access to groundwater to survive and appears to be more drought tolerant than many native riparian species and non-native tamarisk (Katz and Shafroth, 2003). Field observations indicate that *E. angustifolia* can establish on higher and drier geomorphic surfaces compared to cottonwoods in the USA (Katz et al., 2005) and compared to cottonwoods and tamarisk in the Colorado Plateau, USA

(Reynolds and Cooper, 2010). The root system of the *E. angustifolia* enmeshes the saline soil to a great extent. The seedling has a typical taproot that can extend to a depth of 105 cm. The root of a two-year-old common oak can reach 95 cm in saline soil, the Turkish oak 70 cm, the common hackberry 55 cm and the three years old *E. angustifolia* 302 cm (Bartha and Csiszár, 2008).

### 2.2 Origin and Distribution *Elaeagnus angustifolia*

A nitrogen-fixing tree, *E. angustifolia* is a non-native shrub that is currently one of the most common woody species in western USA riparian ecosystems. It is native to southern Europe and central Asia but was introduced to North America in the late 1800s or early 1900s (Katz and Shafroth 2003). During the twentieth century, it was promoted and planted extensively for wildlife habitat and windbreaks (e.g., Borell, 1971) and it later spread from the afforestation state to becoming naturalized in riparian areas throughout most areas of western North America. In a study of 475 riparian sites in the western USA, Friedman et al. (2005) found that *E. angustifolia* was the fourth most dominantly observed woody riparian species and the fifth most frequent (based on the cover).

Distribution of *E. angustifolia* extends from northern Arizona, New Mexico and Texas northward to the southern Canadian Provinces and from eastern California, Oregon and Washington eastward via all western and midwestern USA and as well have a wide naturalized distribution within western North America (Friedman et al. 2005; Guilbault et al. 2012; Nagler et al. 2011).

On a global scale, the distribution of *E. angustifolia* is linked with cold winter temperatures (Friedman et al. 2005; Guilbault et al. 2012). This relationship is largely a result of chilling requirements for seed germination and bud burst, with low plant performance near the southern distribution limit due to inefficient cooling (Guilbault et al., 2012).

Within its naturalized covered area, it tends to grow along rivers and other areas of the landscape with extra moisture. Nagler et al. (2011) investigated the writings on the dissemination and wealth of *E. angustifolia* within the western USA and found that its plenitude shifted impressively inside attacked stream frameworks. Hamilton et al. (2006) utilized Feature Analyst to conduct a pilot mapping extend of *E. angustifolia* at Salinas Rivulet, Utah, USA but did not investigate natural variables impacting its conveyance.

## 2.3 Climatic and Weather Conditions That Support Growth

Temperatures in Central Anatolia can plummet to -20°C, with higher elevations dropping to -30°C at times. Summers are hot and dry, with daytime temperatures around or above 30°C. The nights are pleasantly cool. *E. angustifolia* is a plant that can be found in southern Europe as well as central and eastern Asia (Hansen 1901, Shishkin 1949, Little 1961). It is found predominantly on the beaches, in riparian areas and other moist habitats within this region (Shishkin 1949, Zhang, 1981).

Drought stress is a typical occurrence in the western USA, even in riparian zones (Albertson and Weaver 1945, Tyree et al. 1994). Drought tolerance is mentioned by several authors as an important aspect of *E. angustifolia* horticultural appeal (Hansen 1901, Deters and Schmitz 1936, Little 1961, Sprackling and Read 1979), but there is comparatively little published research on the subject.

According to literature from the former Soviet Union, the *E. angustifolia* can withstand frosts up to 30°C, but at 20°C, the terminal shoots freeze completely. According to North American writers, the species can withstand temperatures as low as -45°C and as high as 46°C. It plays a significant role in erosion control since it is drought tolerant, salt-tolerant and can withstand harsh winters (Bartha and Csiszár, 2008). According to Walter (2012), its xeromorphic structure can be seen in its large volume root relative to the branch system, powerful osmotic suck and scale-covered leaves. Bartha and Csiszár (2008) stated that the root system of the *E. angustifolia* enmeshes the saline soil to a great extent. The seedling has a typical taproot that can extend to a depth of 105 cm. The root of a two years old common oak can reach 95 cm in saline soil, the Turkish oak 70 cm, the common hackberry 55 cm and the three years old *E. angustifolia* 302 cm.

## 2.4 Economic Benefits of *Elaeagnus angustifolia*

The numerous benefits of *E. angustifolia* drive the continuous use globally (Bartha and Csiszár, 2008). It is an important bee meadow because it blooms profusely, but the sugar content of its nectar is low. However, because its flowers bloom after the black locust, the getting in period is extended and its spicy tasting honey complements the black locust honey (Dodson et al., 2007). The fruit of *E. angustifolia* contains a lot of vitamin C (0.33 mg vitamin C per g of fruit meat). It is eaten as a fruit in Turkey, Iran and Greece and it is also used to make alcoholic beverages (Coman et al., 2020).

According to Desnoues et al. (2014), the primary sugar components (fructose and glucose), as well as the fenolcarbon acids, are responsible for the flavor of fruit (4-hydroxybenzoic acid, coffee acid, ferulic acid, benzoic acid, protocatechuic acid, vanillin acid and the 4-hydroxycinnamic acid). Because of its tasty fruit, the species is frequently cultivated. Because of its diuretic and antipyretic properties, its leaves and blossoms are employed in folk medicine and its fruit is served as a winter snack (Gupta et al., 2014). Lans et al. (2007) asserted that the infusion of the *E. angustifolia* fruit has antiulcerogenic (gastric mucous membrane immunizing) effects.

### 2.5 Agroecosystem Benefits of *Elaeagnus angustifolia*

Alien species may change ecological processes such as disturbance regimes and nutrient cycling (Mack and D'Antonio 1998, D'Antonio et al. 1999; Vitousek et al. 1987). *E. coli* invasion of *E. angustifolia* may have an impact on hydrogeomorphic processes by enhancing floodplain roughness in settings where woody vegetation would otherwise be absent (Tickner et al. 2001). Published data are scarce on the effects of *E. angustifolia* on plant ecosystems. In the USA, Zouhar (2005) described the vegetation types and plant groups where *E. angustifolia* grows in western USA riparian environments.

*E. angustifolia* is found in two types of habitats: the understory of cottonwood-willow gallery forests and monotypic (or near-monotypic) stands on floodplains or in former riparian meadows or wetlands. *E. angustifolia* growth at high densities changes riparian woody plant ecosystems (Katz and Shafroth, 2003). However, little is known about the effects of *E. angustifolia* on herbaceous plant ecosystems. Herbaceous plant communities under *E. angustifolia* in Canyon de Chelly, Arizona, had >60% exotic plant covers, such as Bromus tectorum and Bromus rigidus and 40% native plant cover (Reynolds and Cooper, 2011).

Exotic cover, native cover, weighted wetland indicator and the makeup of plant community altered between controls and treatments, implying that *E. angustifolia* had an impact on plant communities (Katz et al., 2016). *E. angustifolia* is one of the first plant species to arise in the succession of damaged floodplains and riverbanks in North America and it persists even after its sedimentation; it becomes dominant in groups, displacing native poplar species (Stromberg et al., 2007). *E. angustifolia* has nearly three times the growth vigour of the native green ash, according to observations. When compared to native poplar species, it has improved the species' propagation and is less damaged by beavers (Bartha and Csiszár, 2008).

Due to certain adaptive characteristics (drought and salt tolerance, quick germination, regular germination in humid terms of vegetation period, symbiotic nitrogen fixation, fast growth and early fruiting), the *E. angustifolia* can rapidly grow and be established. However, it is regarded as a factor of the most detrimental effect that affects riverside biomes as observed by Cierjacks et al. (2013) in the southwestern USA, according to Shafroth et al. (1995), communities dominated by the *E. angustifolia* are typically more unfriendly to animals than natural vegetation. The willow-beds utilized as nesting places by endangered birds were withdrawn, according to Bartha and Csiszár (2008), due to their proliferation. It uses more water than native tree species, lowering water levels and reducing the habitat of endangered fish species (Rogers et al., 200). Its frequent planting near high-traffic highways may result in a high death rate among the birds who consume its fruits (Spennemann et al., 2000).

## 2.6 Establishment Methods of *Elaeagnus angustifolia*

Like several tree plants, the establishment methods of *E. angustifolia* can be categorized into direct and indirect. Direct methods include direct seeding (e.g., broadcasting, by hand or drilling into the soil) and indirect methods involve nursery stock or transplanting of seedlings and hardwood cuttings.

The common limitation for the establishment of *E. angustifolia* is associated with variation in resources (e.g., nutrients, light and water) and specific disturbance regimes (Daehler 2003; Chytrý et al., 2008; Nagler et al., 2011). However, human activities continuously change the light and moisture availability, yet these two factors are very critical in accelerating the establishment of plants within an ecosystem (Niinemets and Valladares, 2006; González-Muñoz et al., 2011). Some establishment methods of *E. angustifolia* are discussed below.

### 2.6.1 Seed propagation

Generally, *E. angustifolia* seeds possess a hard endocarp with a dormancy stage when mature, thus a period of seed treatment (e.g., cool, moist stratification or scarification) is needed for effective germination (Kris, 2005). It is suggested that to break *E. angustifolia* seed dormancy, seeds should be stored at around 3-5°C (moist conditions) for at least two to three months (Hogue and LaCroix, 1970; Williams and Hanks, 1994). The dormancy is associated with inhibitors that exist in all components of *E. angustifolia* seeds (embryo, endocarp and testa) (Hamilton and Carpenter, 1976; Hogue and LaCroix, 1970; Jinks and Ciccarese, 1997).

Hamilton and Carpenter (1976) showed that the activity of the inhibiting substance (coumarin-like germination inhibitor localized in testa, endocarp and embryo) may be reversed by gibberellic acid and kinetin. While its activity cannot be decreased, an uncharacterized substance that may reverse inhibition can be created under low temperatures (90 days at 5°C). Meanwhile, it was suggested that the inhibitor may be water-soluble because six days of washing before prechilling or warm, moist stratification for four weeks (no prechilling) enhanced germination of *E. angustifolia* seeds (Jinks and Ciccarese, 1997).

Additionally, scarification at 1 to 10°C for almost 1 h, seed cleaning or disinfection, soaking in sulfuric acid for 1 h, notched with a file and treatment with undiluted vitriol for 1 h may enhance germination rates of *E. angustifolia* by breaking seed dormancy (Shafroth et al., 1995; Ölmez et al., 2007; Bartha and Csiszár, 2008; Göktürk et al., 2009). Bartha and Csiszár (2008) identified that treatment of *E. angustifolia* seeds for 1 h with vitriolic ensures 66-98% germination rates.

Ölmez et al. (2007) reported that *E. angustifolia* seeds had the highest germinate rates when soaked in running water at 15°C for 10 days followed by cold stratification for 30 days both within greenhouse (64%) and open field (55%) conditions. It is recommended that *E. angustifolia* seeds should be removed from the fresh fruit and immediately stored at 3°C in a sealed container (Shafroth et al., 1995).

Also, *E. angustifolia* seeds germinate in varying soil types and site conditions (temperature, moisture and light availability). Shafroth et al. (1995) noted that the number of seedlings was higher when *E. angustifolia* seeds were grown under low compared with the high-water table. When González-Muñoz et al. (2011) investigated the effect of sowing *E. angustifolia* seeds under different gradients of light (100, 65, 35 and 7% of full sunlight) blended with varying levels of soil water potential (-0.97, -1.52 and -1.77 MPa), it was found that the effect of light on the seed germination and seedling survival was greater than the effect of soil moisture.

It is recommended to sow *E. angustifolia* seeds at a depth of 13 to 25 mm in the summer or fall deprived of stratification or in the spring conditions after 60 days of cold stratification (Olson 1974; Olson and Barbour, 2008). Olson and Barbour (2008) reported that when sowing, a rate of 200 seeds of *E. angustifolia* or 40 g of cleaned seeds/m should be used, with the expectation of obtaining around 75% functional seedlings (150 functional plants).

*E. angustifolia* can establish under well-established irrigated pasture species such as Kentucky bluegrass and orchard grass owing to its large seed that contain enough food reserves to support emerging root system to infiltrate grass turf (Zouhar, 2005). Even though *E. angustifolia* seeds are much larger than other tree plants, their dispersion by birds, water and animals are also common and animals have been indicated to aid natural scarification of *E. angustifolia* by their digestive chemicals (Bartha and Csiszár, 2008). Shafroth et al. (1995) emphasize the utilization of nurseries for the establishment of *E. angustifolia* because of the complex nature of their seed characteristics.

### 2.6.2 Seedling establishment

Niinemets and Valladares (2006) reported that during the establishment phase of tree plants, seedlings survive under to shade, drought or waterlogging tolerance. However, shade tolerance depends on latitude (Borell, 1971; George, 1953). It was established that seedlings of *E. angustifolia* are not affected by shade but their ability to thrive under conditions of varying soil and moisture is lower compared with fully grown *E. angustifolia* trees (Currier, 1982; Lesica and Miles, 2011). In a study that combined shade and water availability treatments, it was demonstrated that seedling survival of *E. angustifolia* was greater in contrast to cottonwood and tamarisk within all treatment categories, plus low light levels (Reynolds and Cooper, 2010). Shafroth et al. (1995) indicated that the biomass of *E. angustifolia* seedlings planted in the sun was greater than those under shade in a field experiment in USA. The previous researchers concluded that light is not highly critical for the number of seedlings that establish after the growing season.

Also, when tree rings were used, Lesica and Miles (2004) found that growth rates of *E. angustifolia* seedlings accelerated in full sun zones compared with seedlings growing under the nurse cottonwood canopy in USA. It emphasized that the growth rate of both seeds and seedlings is influenced by site characteristics related to temperature, presence of light and moisture. For example, Borell (1971) reported that *E. angustifolia* seed grown under spring conditions will become bushy on average 0.8 m tall in the starting season. Meanwhile, within ideal conditions, nursery seedlings grow to 1.3 m in the first season.

### 2.6.3 Mulching

It is also important to emphasize the indirect establishment methods such as mulching that foster the establishment phase of tree plants. The materials that can be used during mulching can be organic or inorganic and the choice of any type depends on the effectiveness and the cost (Jafari et al., 2012). Examples of successfully proposed mulching materials include (i) organic; pine bark (Blanco-García et al., 2011), eastern redcedar mulch, cypress mulch, hardwood mulch, oak, reddyed mulch, grand eucalyptus mulch (Blanco-García et al., 2011; Maggard et al., 2012), manila turf (Ni et al., 2016), (ii) inorganic; rubber solution (Chaudhry et al., 2004), round gravel, pebbles or polyethylene layer (Ni et al., 2016).

The appropriate use of these materials has benefits comprised of soil moisture conservation, reduction of weed growth, buffering against sudden variation of soil temperatures, etc. While there are many benefits of mulching, some negative effects (harmful to soil quality, retard plant growth, fire hazards, the introduction of exotic plant pathogens) of mulches have been indicated (Ni et al., 2016).

However, studies regarding mulching in the establishment of *E. angustifolia* are lacking, thus providing a strong area for further research. Importantly, studies of mulching in the establishment of trees in unstable landscapes (restoration sites) have shown that mulching improves soil health, thus creating plant density in good health conditions (Chalker-Scott, 2007; Blanco-García et al., 2011). Meanwhile, Blanco-García et al. (2011) noted that the effectiveness of mulching depends on the availability of ideal materials. The application of appropriate mulches positively affects the number and width of leaves, length growth rate and diameter of shoots (Jafari et al., 2012).

### 2.6.4 Nurse plant

Another indirect establishment method is by using nurse plant facilitation (gain from nearby surrounding plants), a common technique during drought periods. Nurse plants such as shrubs can aid the establishment of seedlings by ensuring seedling water potential and decreasing their mortality in drought periods. In addition, the method has been suggested to reduce labor and plant material (Castro et al., 2002).

Additionally, it has been shown that there is improvement in plant survival and growth in zones surrounding nurse plants (Padilla and Pugnaire, 2006). Franco and Nobel (1989) identified that nurse plants accelerate seedling establishment by reducing high temperatures close to the soil surface and offer a microhabitat with high soil nitrogen content. However, there is a significant decrease in seedling growth due to shading and competition for water with the nurse plants.

The impact of the decrease in seedling growth is associated with seedling size and position beneath the nurse plant. Blanco-García et al. (2011) suggested that nurse plant facilitation is possible in areas without ideal existing nurse plants. Gómez-Aparicio et al. (2004) emphasized that shrubs can facilitate the establishment of Mediterranean species and it has positive impacts on reforestation achievement in various ecological sites.

Importantly, Katz et al. (2001) determined that *E. angustifolia* juveniles could establish under an undisturbed herbaceous population compared with other tree plants which need physical disturbance for seedling establishment. Several authors have confirmed that *E. angustifolia* seedlings can grow well within the cottonwood (overstory) and tamarisk (pure stand) population (Campbell and Dick-Peddie, 1964; Knopf and Olson, 1984; Howe and Knopf, 1991). This provides a clear understanding of the ability of seedlings to establish within developed canopy and shade.

### 2.6.5 Vegetative propagation

Other establishment methods for *E. angustifolia* include vegetative propagation techniques such as woody cuttings and micro-propagation (obtaining segment of node from the full-grown plants or induction of shoot formation from the adventitious bud) (Bartha and Csiszár, 2008). Bertrand and Lalonde (1985) reported that micro or invitro propagation can ensure rapid and all-year-round production of loads of axenic and uniform plants in small areas compared with conventional propagation methods.

However, there are insufficient studies for the above methods, this is a need to focus on their effect on the establishment of the plant. Meanwhile, where a compatible *Frankia* strain is lacking, its inoculation is stated to enhance the success of the establishment and early growth of actinorhizal species (Sprent and Parsons, 2000). *Frankia* inoculation is achieved with pure culture, crushed nodules or field soil.

## 2.7 Preferences in Establishment Methods

It is suggested that the preferences in establishment methods would consider the cost-benefit analysis. For example, in most developing countries, mulching with gravels is very common since they are readily available and cheap (Ni et al., 2016). The method of choice should have a significant effect on shoot and root growth which impacts the quality of seedlings. For instance, rainfall cause soil to splash and binds the juvenile leaves of developing *E. angustifolia* seedlings leading to their mortality, thus nursery beds should be mulched to avoid rain splashing (Olson and Barbour, 2008). Additionally, methods that require cheap resources in terms of labor and materials for establishment are highly preferred.

Given that *E. angustifolia* seeds require a period of ripening or treatment, the effect of the treatment method on germination and growth rates of seeds and seedlings should be highly considered (Olson and Barbour, 2008). Also, there is a need to ensure appropriate storage of collected and extracted seeds. Olson (1974) determined that *E. angustifolia* seeds can stay viable for 3 years under ideal storage conditions (sealed containers in between 1 to 10°C) but become unviable with poor storage. *E. angustifolia* seedlings are prone to harm from the above species, especially in areas with high infestation rates of rodents and facultative fungal pathogen (*Tubercularia ulema*) (Katz, 2016). Therefore, sites with history of the above issues are usually avoided.

## 2.8 Nodulation of *Elaeagnus angustifolia*

Similar to all plants that possess nodules and symbiosome, *E. angustifolia* is capable of strongly influencing soil nutrient dynamics in the ecosystems utilizing the same mechanisms such as nitrogen fixation, acceleration of total and available soil nitrogen through high nitrogen inputs and alternation of nitrogen concentration where nitrogen fixation is lacking or insufficient (DeCant, 2008). *E. angustifolia* is a common actinorhizal species that can develop a nitrogen-fixing symbiotic relationship with *Frankia* (actinomycetes bacteria) (Bertrand and Lalonde, 1985; Katz and Shafroth, 2003; Katz, 2016). Because of the ability to establish in areas with poor nitrogen and improvement of the growth rate of neighboring plants, *E. angustifolia* is a common plant of choice for land reclamation (Bertrand and Lalonde, 1985).

Bertrand and Lalonde (1985) determined that after *in vitro* propagation, the *E. angustifolia* retained its nodulation and nitrogen fixation capacity of *E. angustifolia* is associated with soil nitrogen patterns besides, its litter (leave and roots) has a high concentration of nitrogen that can cause variation in nitrogen pools within the ecosystem (Simons and Seastedt, 1999; DeCant, 2008).

## 2.9 *Frankia* Isolation

*Frankia* is a known soil actinobacterial genus capable of establishing nitrogen-fixing root nodules with actinorhizal plants (Benson and Silvester, 1993; Clawson et al., 1998; Ghodhbane-Gtari et al., 2010). All the *Frankia* strains grow slowly, are threadlike, form unique sporangia compared with other free-living actinomycetes and have a growth pattern of at least 20 h (Lie et al., 1984).

The poor success rate associated with the isolation of *Frankia* strains from field-collected root nodules has hindered studies regarding its diversity and distribution. Several techniques have been applied to isolate *Frankia* strains from nodules. These include treatment of nodule tissue with cellulase and pectinase in addition to microdissection, microdissection alone, serial dilution of a crushed nodule and Sephadex fractionation together with sucrose density centrifugation (Benson, 1982). However, most if not all the above methods require special equipment and chemical and labour for manipulation thus leading to a relatively low success rate.

Although in alder actinorhizal root nodules, Benson (1982) identified that a simple technique based on rapid filtration and washing of *Frankia* vesicle clusters using a simple medium comprising mineral salts, casamino acids and sodium pyruvate is effective for *Frankia* isolation. Baker and O'Keefe (1984) reported that a modified sucrose fractionation technique (brief incubation of crushed nodule or soil suspensions in 0.7% phenol before application to a sucrose density gradient) increased the success of isolations from root nodules and permitted the isolation of *Frankia* directly from soil samples. It was observed that phenol incubation decreased the load of contaminating eubacteria and fungi and enhanced the number of *Frankia* developing on the isolation plates. However, just phenol incubation without sucrose fractionation was associated with phenol toxicity causing the death of the organisms (absence of isolated *Frankia*). The previous researchers suggested that the use of selective nitrogen-deficient media is very significant for the isolation of *Frankia* from soils.

Benson (1982) reported that successful isolation techniques should ensure the following: (1) removal of toxic phenolic compounds from the nodule homogenate, (2) separation of endophyte from contaminating microorganisms and (3) the separation of endophyte from contaminating plant tissue. Also, isolation is always hampered by the fact that variation in nutritional requirements is common with *Frankia* strains (Benson and Silvester, 1993). Sprent and Parsons (2000) emphasized that the determination of specific chemical and environmental requirements of the target organism is very important for successful isolation.

## 2.10 *Frankia* Distribution in Turkey

The fact that *E. angustifolia* is a native of some parts of Turkey, it is concluded that the plant can nodulate productively in soils and this is an indication of the presence of a compatible *Frankia* strain. Also, it is established that highly compatible *Frankia* strains are present in soils of sites where actinorhizal species occur naturally or have been grown for an extended period and a possible reason is the co-evolution theory (Lie et al., 1987). Importantly, besides having a symbiotic relationship with actinorhizal plants, several *Frankia* strains can survive for prolonged periods in soil (Smolander and Sarsa, 1990; Sprent and Parsons, 2000). However, due to lack of studies regarding the isolation and characterization of *Frankia* species in *E. angustifolia* in Turkey, it is difficult to understand their distribution.

# CHAPTER III

# MATERIALS & METHODS

## 3.1 General Description

Six experimental research were designed in two broad categories (greenhouse and field) using six accessions of *Elaeagnus angustifolia* L., with varied seedling depth and water regimes, different backfilling (of the planting hole) and soil collected from different locations in Niğde to evaluate the optimum conditions for direct establishment methods.

In the greenhouse, the four experiments conducted were emergence assessment of *E. angustifolia*, seed depth and water regimes assessment, soil backfilling experiment, hydrophilic gel backfilling and *Frankia* assessment in Niğde soil. The two field experiments conducted were backfilling experiment and the pulp experiment.

## 3.2 Plant Materials

In all experiments, six local seed lots of *E. angustifolia* were used in this master thesis project. These seed lots were coded as:
AP16: Collected from near to D330 Aksaray-Niğde highway, 12 December 2018.
AP17: Collected from the campus lake area, 23 December 2018.
AP60: Purchased as commercial from Enderbey Market in Niğde, 15 June 2019.
AP62: Collected from near to D330 Aksaray-Niğde highway, 12 December 2019.
AP63: Collected from the campus lake area, 21 November 2019.
AP64: Collected from kindergarten on campus, 21 November 2019.

All fruits pulps were removed by hand to separate the seeds. Then the seeds and damp sand were mixed with a 1:1 ratio and put in a plastic freezer bag. These bags were stored for at least two months at 4°C for cold stratification pre-treatment before sowing.

### 3.2.1 Seed Characterization

Seed characterization for six seed lots of *E. angustifolia* was done with 20 seeds which were randomly selected from the six seed lots. The length, weight and width of each seed were measured. The seed lots of *E. angustifolia* were used for these experiments when needed.


a b

**Figure 3.1.** Freshly collected *E. angustifolia* (Russian olive) seeds shown in (a) and weighing of cold stratified seeds shown (b)

## 3.3 Plant Culture and Substrates

Niğde soil was collected from near the greenhouse and this soil was used for all greenhouse experiments. Four backfilling components were used: dry sieved Niğde soil (with 850 µm sieve; Loyka, Turkey), perlite (Ultra, Turkey), peat (Klasmann-Deilmann GmbH, Germany) and hydrophilic gel (water storage crystals) (Hortico Organic Lawn Fertiliser, Australia).

## 3.4 Greenhouse and Water

The greenhouse of Niğde Ömer Halisdemir University is a controlled environment with regulated heat, lighting conditions and humidity. The temperature and humidity were measured daily. Plastic pots of 0.9 L were obtained from Çetin Elektro Plastik company

(Turkey) for the greenhouse experiments. Three water regimes were used in greenhouse experiments when it needed: top water, bottom water and no water. For bottom water, 50 ml volumed plastic dishes (purchased from Gross Market, Niğde) were used.

**Figure 3.2.** Greenhouse experimental setup using bottom water

For greenhouse experiments 2 and 3, only bottom water was used. All needed pots were watered two-three times a week with 50 ml (for each pot) to maintain adequate soil moisture.

### 3.5 Depth Measurement

Stainless steel soil corer (Accucore, Australia) with 150 mm in length and a diameter of 10 mm was used for soil coring with different sowing depths both in the greenhouse and on the field. Emergence date data were collected for all experiments. In addition, length of seedling was measured daily for greenhouse experiment 1.

### 3.6 Experimental Design

Four greenhouse and two field experiments were designed with six seed lots of *E. angustifolia,* different sowing depths, water regimes and different types of backfillings. All the treatments were in replicates and laid out in a randomized complete block design for all greenhouse and field experiments.

### 3.6.1 Greenhouse Experiments

The first greenhouse experiment assessed the sowing depth and water regime, the second greenhouse was a soil backfilling experiment, the third greenhouse experiment was designed to assess the hydrophilic gel backfilling on the propagation of *E. angustifolia* and in the fourth greenhouse experiment, the soil was collected from different locations in Niğde to determine the if *Frankia* associated with *E. angustifolia* widely distributed.

### 3.6.1.1 Greenhouse Experiment 1: Sowing Depth and Water Regime

Fruits pulps were removed by hand and mixed with damp sand in a 1:1 ratio. These seeds were stored using a plastic bag for two months in the refrigerator at 4°C for cold stratification pre-treatment. Only AP60 seed lot was used for this experiment and this was set up on 17 September 2019.

A total of 36 pots has six seeds in each filled with Niğde soil collected from near the greenhouse and with different water regimes. There were four sowing depths (25, 50, 100 and 150 mm) and three water regimes (top water, bottom water and no water). All needed pots were watered three times a week with 50 ml (for each pot) to maintain adequate soil moisture.

**Figure 3.3.** Greenhouse experimental setup showing sowing depth and water regime

Seeds were carefully placed at the sowing depth and this was backfilled with dry sieved soil to match the original soil level. The treatments had three replicates laid out in a randomized complete block design.

### 3.6.1.2 Greenhouse Experiment 2: Soil Backfilling

Fruits pulps were removed by hand and mixed with damp sand in a 1:1 ratio. These seeds were stored using a plastic bag for two months in the refrigerator at 4°C for cold stratification pre-treatment. Three seed lots of *E. angustifolia* were used for this experiment: AP16, AP17 and AP60. This soil backfilling experiment was set up on 9 December 2019.

A total of 45 plastic pots with 0.9 L volume were filled with Niğde soil collected from near to the greenhouse and six seeds were planted in each pot. Each of the seed lots was used in 15 pots having only two depths (25 and 50 mm) and three backfills (dry sieved Niğde soil, perlite and peat).

There were five treatments for this experiment. First was 25 mm sowing depth, without cutting the seeds from the three seed lots and backfilled with sieved Niğde soil. Second, 25 mm sowing depth with cut seeds from the three seed lots and backfilled with sieved Niğde soil. Third, 50 mm sowing depth with cut seeds from the three seed lots and backfilled to 20 mm above with sieved Niğde soil (the backfilled soil volume is 2.2 ml). Fourth, 50 mm sowing depth, sowing with cut seeds from the three seed lots and backfilled with perlite. Fifth, 50 mm sowing depth with whole seeds from the three seed lots and backfilled with peat. The treatments were laid out in a randomized complete block design with three replicates.

**Figure 3.4.** Greenhouse experimental setup showing soil backfilling

After sowing seeds, all pots were watered immediately. Only bottom water was used because this water regime was useful for *E. angustifolia* germination as understood from experiment 1. All pots were watered two times a week with 50 ml (for each pot) to maintain adequate soil moisture.

### 3.6.1.3 Greenhouse Experiment 3: Hydrophilic Gel Backfilling

Fruits pulps were removed by hand and mixed with damp sand in a 1:1 ratio. These seeds were stored using a plastic bag for two months in the refrigerator at 4°C for cold stratification pre-treatment. Cold stratified AP16, AP17 and AP60 *E. angustifolia* seed lots were used for this experiment and this was set up on 3 May 2020.

**Figure 3.5.** Greenhouse experimental setup showing the use of hydrophilic gel backfilling

A total of 27 pots of six seeds each was planted per 0.9 L plastic pots filled with Niğde soil were collected from near the greenhouse. The three treatments were 25 mm with full backfilled with the hydrophilic gel-soil mixture, 50 mm partial backfilled with the hydrophilic gel-soil mixture and 50 mm partial backfilled with Niğde soil. The treatments were laid out in a randomized complete block design in three replicates. Only the bottom water regime was used, and all pots were watered twice a week with 50 ml (for each pot).

#### 3.6.1.4 Greenhouse Experiment 4: *Frankia* Distribution

Fruits pulps were removed by hand and mixed with damp sand in a 1:1 ratio. These seeds were stored using a plastic bag for two months in the refrigerator at 4°C for cold stratification pre-treatment. This experiment was conducted in the greenhouse on 15 March 2020 with soils collected from different locations in Niğde.

The soil samples were collected at intervals along a transect across Gümüşler in Niğde, from the wilderness hills to the settlement area, to provide samples representing different types, alludes and proximity to existing *E. angustifolia* trees. The soil sampling was started from the highest altitude (wilderness) point and it was continued to the settlement area of Gümüşler. The soils were sampled from 12 locations; 6 locations were selected from the non-agricultural area and the other 6 locations were selected from the agricultural area. All the plastic pots (0.9 L) to be used were sterilized by spraying 70% ethanol. Three pots of soil were collected from each of the 12 locations. A total of 36 pots containing Niğde soil was collected individually and stored as covered with sterilized plastic bags one by one to avoid cross-contamination. Also, for the control line, the peat in the amount of 6 pots was autoclaved at 121°C for 120 min. Three of these pots were put into the greenhouse experiment and three of them were put in a growth room. These sterilized plastic pots were covered with sterile plastic bags and bottom watered three times a week after seed sowing.

a b

**Figure 3.6.** Growth chamber experimental setup using sterilized seeds shown in (a) and greenhouse experimental setup with sterilized seeds from growth chamber to determine *Frankia* distribution shown in (b)

For this experiment, cold stratified AP17 seed lot was used only. The seeds were sterilized by gently shaking with 70% ethanol for 40 sec for surface sterilization. All these surface-sterilized seeds were used for this experiment. After the transfer of the pots to the greenhouse, sterilized seeds were sowed by pushing gently to seeds into the soil in 50 mm sowing depth with sterilized stainless-steel forceps. The forceps and my hands were sterilized with 70% ethanol for each pot's seed sowing process to prevent cross-contamination. After the seed sowing process, all pots were bottom watered immediately. The experimental design was randomized complete blocks with three replicates. Nodulation was assessed 11 weeks after the starting of the experiment.

### 3.6.2 Field Experiments

Two field experiments conducted were backfilling experiment and the pulp experiment. The backfilling experiment had ten replicates with two seed lots while the pulp experiment had twelve replicates with three seed lots.

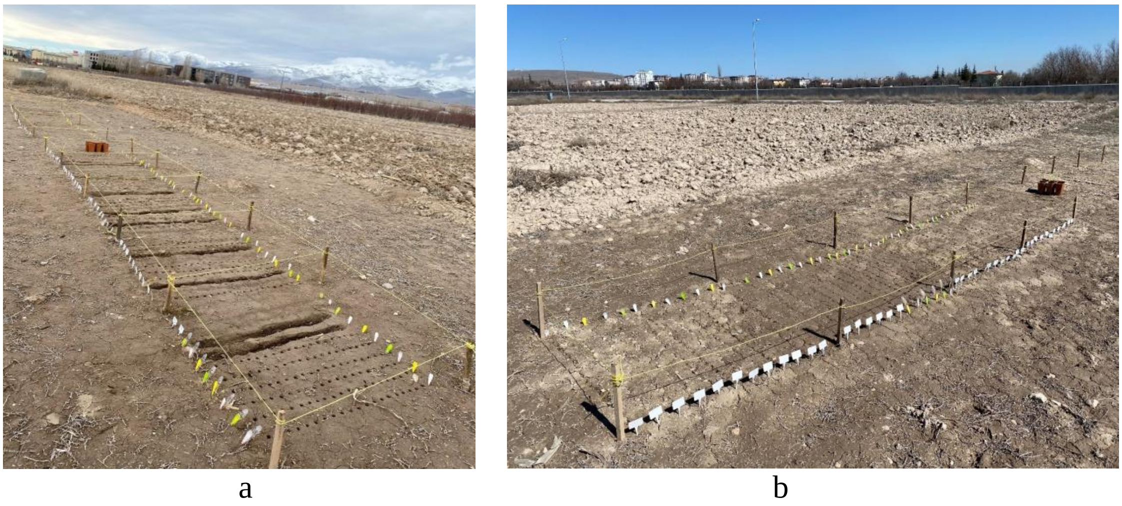
a b

**Figure 3.7.** Front view of field experiment setup shown in (a) and side view of field experiment setup shown in (b)

### 3.6.2.1 Field Experiment 1: Backfilling

Local seeds without cold stratification were sown on the field to allow winter weather conditions to do the seed stratification. The experiment was conducted on 16 January 2020 on a field of 8 m² reserved for the Ayhan Şahenk Faculty of Agricultural Sciences and Technologies. Ten treatment combinations of furrows, depths, seed lots, fill type and depth were set up as shown in Table 3.1.

**Table 3.1.** Treatments of backfilling experiment

| Treatment | Furrow | Depth (mm) | Seed lot | Fill type | Fill depth |
|---|---|---|---|---|---|
| T1 | 0 | 25 | AP62 | Soil | Full |
| T2 | 0 | 25 | AP63 | Soil | Full |
| T3 | 0 | 50 | AP62 | Soil | Partial |
| T4 | 0 | 50 | AP63 | Soil | Partial |
| T5 | 0 | 50 | AP62 | Perlite | Partial |
| T6 | 0 | 50 | AP63 | Perlite | Partial |
| T7 | 0 | 50 | AP62 | Gel | Partial |
| T8 | 0 | 50 | AP63 | Gel | Partial |
| T9 | 1 | 25 | AP62 | Soil | Full |
| T10 | 1 | 25 | AP63 | Soil | Full |

### 3.6.2.2 Field Experiment 2: Pulp Experiment

Local seeds without cold stratification were planted on the field to allow winter weather conditions to do the seed stratification. The experiment was conducted on 19 January 2020 on the field of 10 m² reserved for the Ayhan Şahenk Faculty of Agricultural Sciences and Technologies. Twelve treatments of depths, seed lots, pulp and fill type were set up as shown in Table 3.2.

**Table 3.2.** Treatments of pulp experiment

| Treatment | Depth (mm) | Seed lot | Pulp | Fill depth |
|---|---|---|---|---|
| T1 | 25 | AP62 | 1 | Full |
| T2 | 25 | AP63 | 1 | Full |
| T3 | 25 | AP64 | 1 | Full |
| T4 | 25 | AP62 | 0 | Full |
| T5 | 25 | AP63 | 0 | Full |
| T6 | 25 | AP64 | 0 | Full |
| T7 | 50 | AP62 | 1 | Partial |
| T8 | 50 | AP63 | 1 | Partial |
| T9 | 50 | AP64 | 1 | Partial |
| T10 | 50 | AP62 | 0 | Partial |
| T11 | 50 | AP63 | 0 | Partial |
| T12 | 50 | AP64 | 0 | Partial |

**Figure 3.8.** Picture of field after three months of observation

## 3.7 Assessments

In the four greenhouse experiments, the pots were observed daily for the emergence of seedlings. When a plant emerged, the date was recorded in the spread sheet. In addition, plant lengths, root length. In addition, an assessment of *Frankia's* presence was done in the fourth greenhouse experiment. Similarly, seedling emergence date and plant lengths were assessed in the two field experiments.

## 3.8 Statistical Analysis

The six experiments were subjected to descriptive statistics which were presented in box plots and tables. This was conducted using R Core Team (2021) R: a language and environment for statistical computing (Version 4.0) [Computer software], retrieved from https://crab.r-project.org and the Jamovi project (2021), Jamovi (version 2.0.) [Computer software], retrieved from https://www.jamovi.org.

# CHAPTER IV

# RESULTS

## 4.1 Seed Characterization

The range and mean of the seed length, seed width, fresh seed weight and air-dry weight of the *Elaeagnus angustifolia* L. seeds from AP16, AP17 and AP60 seed lots were found to be similar (Table 4.1).

**Table 4.1.** Characteristics of the seeds of *Elaeagnus angustifolia*

| Seed lot | Length (mm) | Width (mm) | Fresh weight (g) | Airdry weight (g) |
|---|---|---|---|---|
| AP16 | 13.5 | 6.15 | 0.29 | 0.22 |
| AP17 | 14.8 | 5.13 | 0.25 | 0.17 |
| AP60 | 18.6 | 5.17 | 0.33 | 0.30 |
| AP62 | 13.8 | 6.16 | 0.29 | 0.28 |
| AP63 | 13.7 | 4.81 | 0.21 | 0.20 |
| AP64 | 18.8 | 5.37 | 0.37 | 0.35 |

## 4.2 Greenhouse Experiment 1: Sowing Depth and Water Regime

Generally, the highest mean germination of 100% was observed with top water compared to bottom water and no water regimes while the lowest germination of below 50% was seen in the no water regime (Fig. 4.1).

### 4.2.1 Germination

Top water regimes can facilitate germination up to sowing depth of 100 mm while bottom water regime facilitate germinate beyond 100 mm sowing depth of *E. angustifolia*. There was 100% mean germination with top water at sowing depths of 100, 25 and 50 mm (Fig. 4.1). The mean germination with top water at sowing depth of 150 mm was above 60% at sowing depth of 150 mm. In bottom water, the highest mean germination of 100% was seen at a sowing depth of 150 mm while the lowest germination using bottom water was observed at a sowing depth of 50 mm (Fig. 4.1).

Mean germination was generally low for the no water regime (Fig. 4.2). The mean germination for no water was below 50% at sowing depth of 150 mm, below 40% at sowing depth of 10 mm and below 20% at sowing depth of 25 and 50 mm.

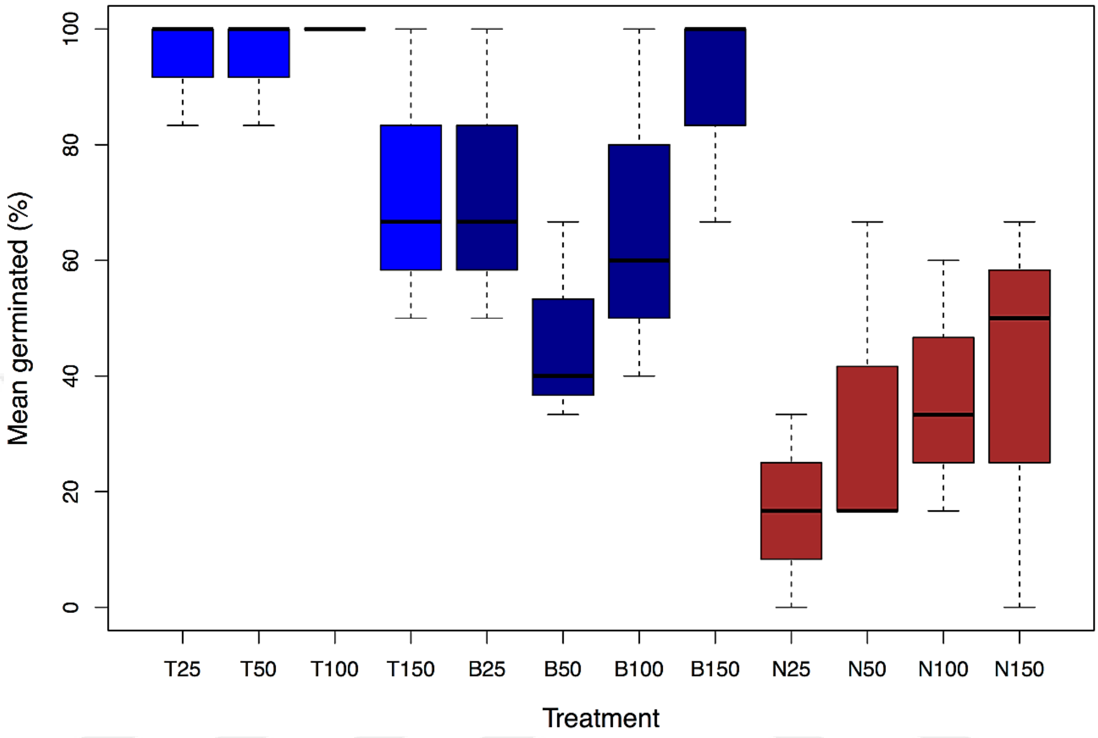

**Figure 4.1.** Mean germination of *Elaeagnus* seeds **(**%) planted in the greenhouse at different sowing depths of 25, 50, 100 and 150 mm using three water regimes: top water, bottom water and no water in 2019. The figure shows mean (-) of the seeds that germinated and 95% confidence intervals above the box plot of the raw data for each treatment. The box plot also shows the median and interquartile range. Treatment codes: T25, Top water with sowing depth of 25 mm, T50, Top water with sowing depth of 50 mm, T100, Top water with sowing depth of 100 mm, T150, Top water with sowing depth of 150 mm, B25, Bottom water with sowing depth of 25 mm, B50, Bottom water with sowing depth of 50 mm, B100; Bottom water with sowing depth of 100 mm, B150, Bottom water with sowing depth of 150 mm, N25, No water with sowing depth of 25 mm, N50, No water with sowing depth of 50 mm, N100, No water with sowing depth of 100 mm, N150, No water with sowing depth of 150 mm

### 4.2.2 Seed rot

There was no rotting with top water at sowing depth of 100 and 150 mm. In addition, bottom water at sowing depth of 25 mm and no water at sowing depth of 25, 50 and 100 mm also did not have any seed rotting (Fig. 4.2). The highest mean rotten of over 60% was observed in no water regime at a sowing depth of 150 mm and there was over 30%

seed rotting when top water was used at a sowing depth of 50 mm. Bottom water at sowing depth of 100 mm had 20% mean rotten (Fig. 4.2).

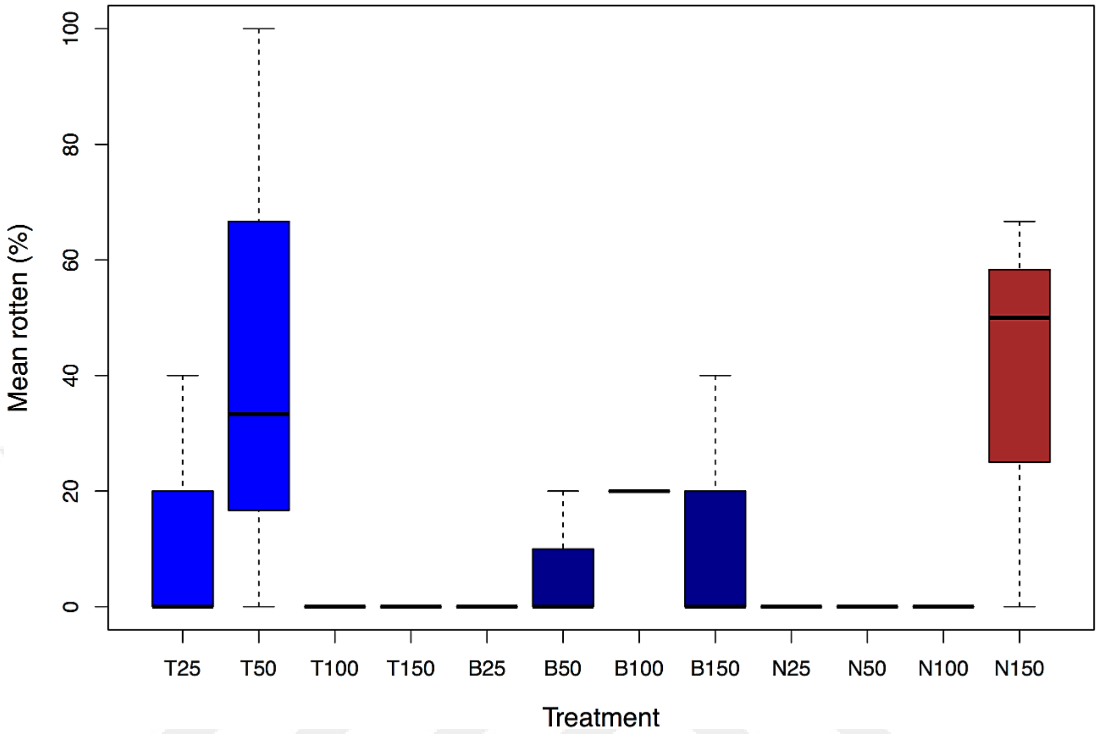

**Figure 4.2.** Mean of *Elaeagnus* seeds that got rotten (%) after sowing in the greenhouse at different sowing depths of 25, 50 and 100 mm using three water regimes: top water, bottom water and no water in 2019. The figure shows the mean (-) of the seeds that rotted and 95% confidence intervals above the box plot of the raw data for each treatment. The box plot also shows the median and interquartile range. Treatment codes: T25, Top water with sowing depth of 25 mm, T50, Top water with sowing depth of 50 mm, T100, Top water with sowing depth of 100 mm, T150, Top water with sowing depth of 150 mm, B25, Bottom water with sowing depth of 25 mm, B50, Bottom water with sowing depth of 50 mm, B100; Bottom water with sowing depth of 100 mm, B150, Bottom water with sowing depth of 150 mm, N25, No water with sowing depth of 25 mm, N50, No water with sowing depth of 50 mm, N100, No water with sowing depth of 100 mm, N150, No water with sowing depth of 150 mm

### 4.2.3 Germination length

The longest mean germination length of over 125 mm was seen with bottom water at a sowing depth of 25 mm (Fig. 4.3). The bottom water at sowing depth of 50 and 100 mm had a mean germinated length below 50 mm and above 50 mm at a sowing depth of 150 mm. The mean germinated length observed for top water at sowing depth of 25 and 100 mm was above 50 mm. It was below 50 mm at sowing depths of 50 and 150 mm. Generally, the shortest mean germinated length was observed in the no water regime at different sowing depths (Fig. 4.3). The mean germination length was less than 50 mm at sowing depths of 50, 100 and 150 mm. There was no germination observed in no water at a sowing depth of 25 mm.

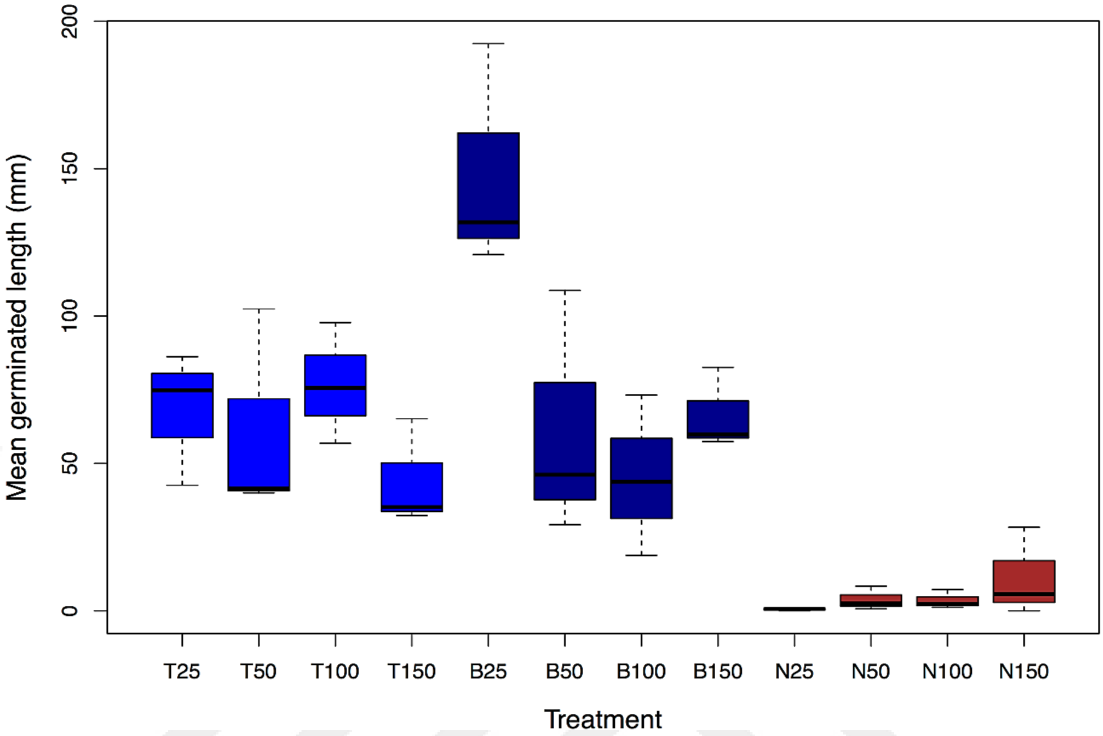

**Figure 4.3.** Mean germinated length of *Elaeagnus* (mm) after sowing at different sowing depths of 25, 50, 100 and 150 mm using three water regimes: top water, bottom water and no water in the greenhouse in 2019. The figure shows mean (-) of the seeds that germinated and 95% confidence intervals above the box plot of the raw data for each treatment. The box plot also shows the median and interquartile range Treatment codes: T25, Top water with sowing depth of 25 mm, T50, Top water with sowing depth of 50 mm, T100, Top water with sowing depth of 100 mm, T150, Top water with sowing depth of 150 mm, B25, Bottom water with sowing depth of 25 mm, B50, Bottom water with sowing depth of 50 mm, B100; Bottom water with sowing depth of 100 mm, B150, Bottom water with sowing depth of 150 mm, N25, No water with sowing depth of 25 mm, N50, No water with sowing depth of 50 mm, N100, No water with sowing depth of 100 mm, N150, No water with sowing depth of 150 mm

### 4.2.4 Emergence

Mean emergence was generally higher in the bottom water regime at different sowing depths (Fig. 4.4). Bottom water with a sowing depth of 25 mm had the highest mean emergence of over 60% while top water at sowing depth of 50 mm had the lowest mean emergence close to 0%. Bottom water at sowing depth of 50 mm, had mean emergence of over 40% and Top water at sowing depth of 25 mm had mean emergence slightly over 20% (Fig. 4.4).

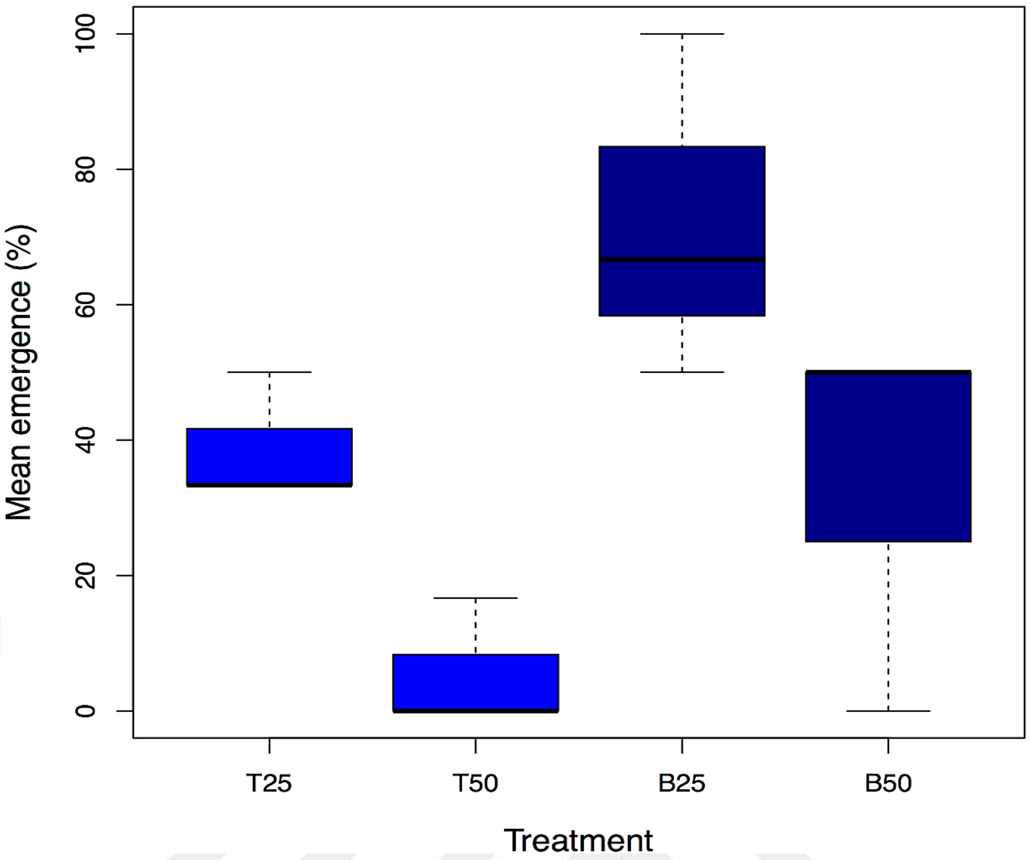

**Figure 4.4.** Mean emergence of *Elaeagnus* seeds (%) at different sowing depths of 25 and 50 mm using top water and bottom water in the greenhouse in 2019**.** The figure shows the mean (-) of the seeds that germinated and 95% confidence intervals above the box plot of the raw data for each treatment. The box plot also shows the median and interquartile range. Treatment codes: T25, Top water with sowing depth of 25 mm, T50, Top water with sowing depth of 50 mm. B25, Bottom water with sowing depth of 25 mm, B50, Bottom water with sowing depth of 50 mm

### 4.2.5 Emergence time

The fastest mean emergence of a little more than 25 days was seen with bottom water at a sowing depth of 25 mm while the slowest emergence time of more than 30 days was observed with top water at a sowing depth of 25 mm with top water. The mean emergence time with bottom water at a sowing depth of 50 mm was also less than 30 days. In contrast, there was no emergence with top water at a sowing depth of 50 mm (Fig. 4.5).

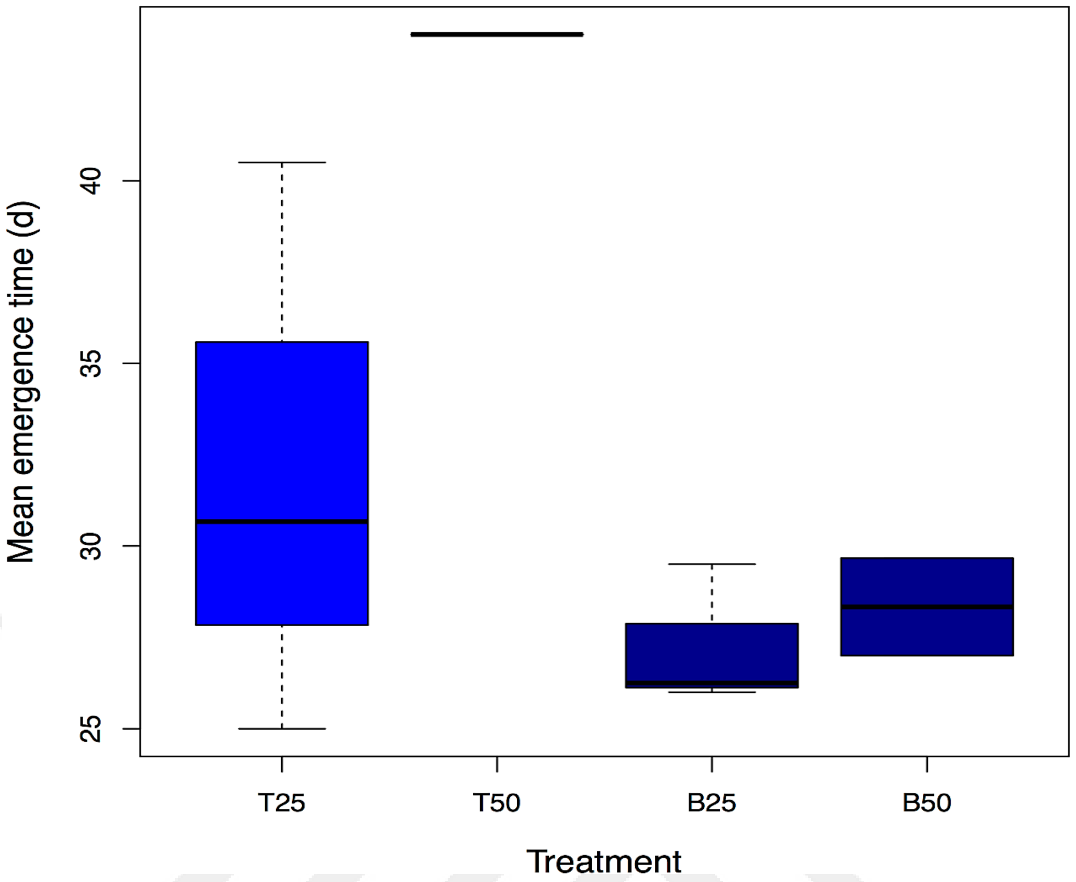

**Figure 4.5.** Mean emergence time of *Elaeagnus* (d) that were treated with top water and bottom water at different sowing depths of 25 and 50 mm in the greenhouse in 2019**.** The figure shows mean (-) of the seeds that germinated and 95% confidence intervals above the box plot of the raw data for each treatment. The box plot also shows the median and interquartile range. Treatment codes: T25, Top water with sowing depth of 25 mm, T50, Top water with sowing depth of 50 mm. B25, Bottom water with sowing depth of 25 mm, B50, Bottom water with sowing depth of 50 mm

### 4.2.6 Shoot length

The highest mean shoot length of more than 60 mm was seen with bottom water at a sowing depth of 25 mm while the lowest mean shoot length of less than 10 mm was observed with top water at a sowing depth of 50 mm. Bottom water at a sowing depth of 50 mm had a mean shoot length of over 50 mm whereas, with top water at a sowing depth of 25 mm, a mean shoot length of over 40 mm was seen (Fig. 4.6).

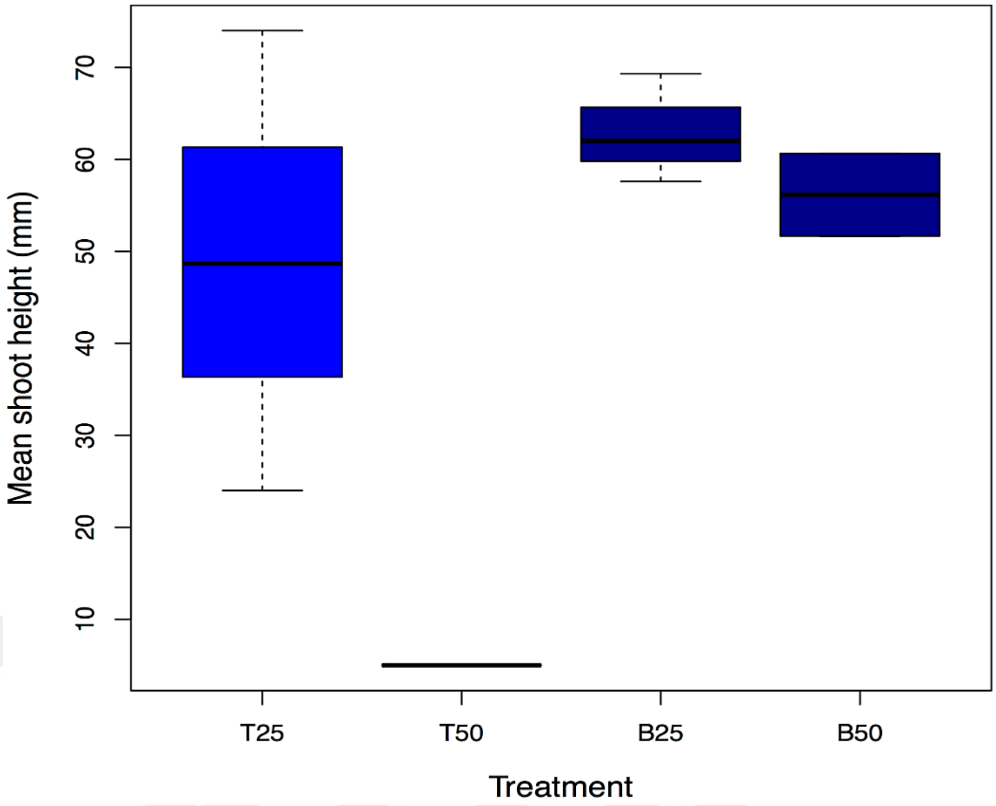

**Figure 4.6.** Mean shoot length of *Elaeagnus* (mm) treated with top water and bottom water at different sowing depths of 25 and 50 mm in the greenhouse in 2019. The figure shows mean (-) of shoot length and 95% confidence intervals above the box plot of the raw data for each treatment. The box plot also shows the median, an interquartile range. Treatment codes: T25, Top water with sowing depth of 25 mm, T50, Top water with sowing depth of 50 mm. B25, Bottom water with sowing depth of 25 mm, B50, Bottom water with sowing depth of 50 mm

### 4.2.7 Shoot growth rate

The highest shoot growth rate of over 2.5 mm/d was seen with both top water at a sowing depth of 25 mm and bottom water at a sowing depth of 25 mm. In contrast, a mean shoot growth rate of 1.0 mm/d was observed in top water at a sowing depth of 50 mm. Bottom water at a sowing depth of 50 mm had a mean shoot growth rate of a little over 2.5 mm/d (Fig. 4.7).

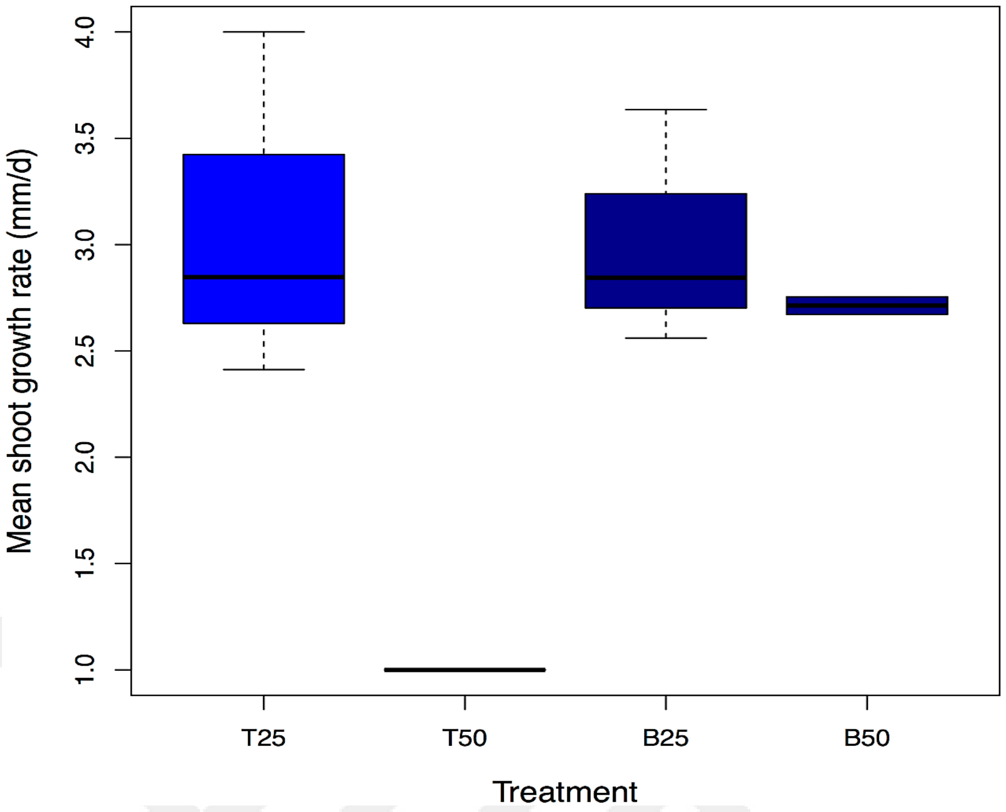

**Figure 4.7.** Mean shoot growth rate of *Elaeagnus* (mm/d) treated with top water and bottom water at different sowing depths of 25 and 50 mm in the greenhouse in 2019. The figure shows mean (-) of shoot growth rate and 95% confidence intervals above the box plot of the raw data for each treatment. The box plot also shows the median and interquartile range. Treatment codes: T25, Top water with sowing depth of 25 mm, T50, Top water with sowing depth of 50 mm. B25, Bottom water with sowing depth of 25 mm, B50, Bottom water with sowing depth of 50 mm

## 4.3 Greenhouse Experiment 2: Soil Backfilling

### 4.3.1 Root length

There was no clear effect of the use of different backfilling on the mean root length of each seed lot. The lowest mean root length was observed in AP17 for all the five treatments used (Fig. 4.8). The highest mean root length of over 90 mm was observed in AP16 using whole seeds backfilled with peat at sowing depth of 50 mm (Fig. 4.8). In addition, AP17 had a mean root length of over 80 mm while AP60 had the lowest mean root length of less than 40 mm with whole seeds backfilled with peat at sowing depth of 50 mm (Fig. 4.8).

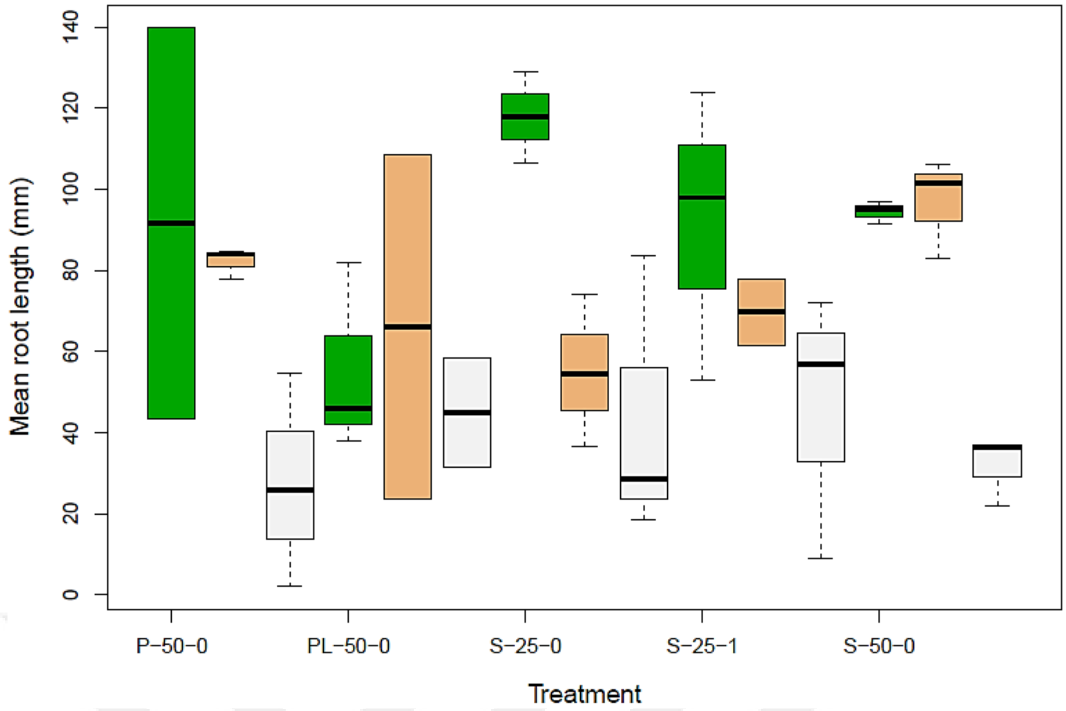

**Figure 4.8.** The mean total root length (mm) of three seed lots AP16, AP17 and AP60 of *Elaeagnus* planted using five treatments of whole seeds backfilled with peat at sowing depth of 50 mm, cut seeds backfilled with perlite at sowing depth of 50 mm, uncut seeds backfilled with Niğde soil at sowing depth of 25 mm, cut seeds backfilled with Niğde soil at sowing depth of 25 mm and cut seeds backfilled with Niğde soil at sowing depth of 50 mm in the greenhouse in 2019. The figure shows means (-) of the root lengths and 95% confidence intervals above the box plot of the raw data for each treatment. The box plot also shows the median and interquartile range. Treatment codes: P-50-0; whole seeds at sowing depth of 50 mm backfilled with peat, PL-50-0; cut seeds at sowing depth of 50 mm backfilled with perlite, S-25-0; uncut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-25-1; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-50-0; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil

The longest mean root length of over 60 mm was seen in AP17 for cut seeds backfilled with perlite and a mean root length of a little higher than 40 mm was observed in both AP16 and AP60 seed lots using cut seeds backfilled with perlite at a sowing depth of 50 mm (Fig. 4.18). The longest mean root length of over 100 mm was observed in AP16 using uncut seeds backfilled with Niğde soil at a sowing depth of 25 mm. AP17 had a mean root length of over 40 mm while AP16 had a mean root length less than 40 mm (Fig. 4.8) using uncut seeds backfilled with Niğde soil at a sowing depth of 25 mm.

In addition, AP16 had the longest mean root length of approximately 100 mm while AP60 had the shortest mean root length of approximately 60 mm using cut seeds backfilled with Niğde soil at a sowing depth of 25 mm. AP17 had a mean root length of over 60 mm also using cut seeds backfilled with Niğde soil at a sowing depth of 25 mm. The longest mean root length of over 100 mm was seen in AP17 using cut seeds backfilled with Niğde soil at a sowing depth of 50 mm while the shortest of less than 40 mm was observed in AP60 (Fig. 4.8). AP16 had mean root length of over 90 mm with cut seeds backfilled with Niğde soil at a sowing depth of 50 mm.

### 4.3.2 Total seedling length

AP60 had the shortest mean total seedling length (mm) for all five treatments (Fig. 4.9). A mean total seedling length of over 100 mm was observed in AP16 and AP17 using whole seeds backfilled with peat at a sowing depth of 50 mm while AP60 had a mean total seedling length of less than 50 mm. AP17 had the longest mean total seedling length of over 50 mm using cut seeds backfilled with perlite at a sowing depth of 50 mm while the mean total seedling length of less than 50 mm was observed in AP16 and AP60. AP16 had the highest total seedling length of approximately 150 mm using uncut seeds backfilled with Niğde soil at a sowing depth of 25 mm (Fig. 4.9).

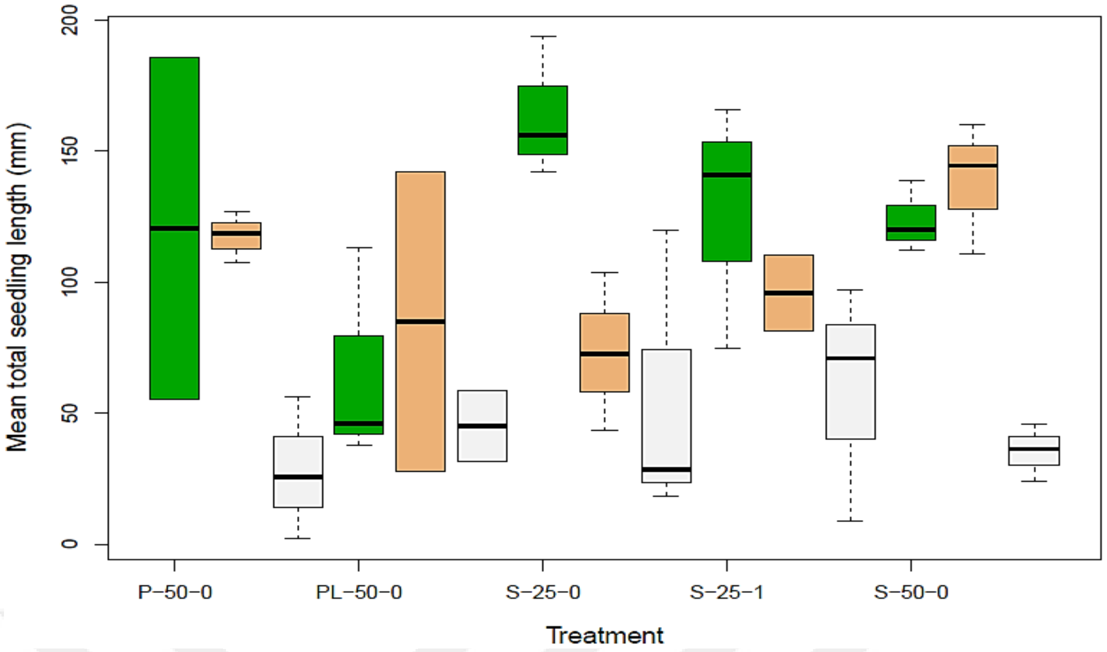

**Figure 4.9.** The mean total seedling length (mm) of three seed lots AP16, AP17 and AP60 of *Elaeagnus* planted using five treatments of whole seeds backfilled with peat at sowing depth of 50 mm, cut seeds backfilled with perlite at sowing depth of 50 mm, uncut seeds backfilled with Niğde soil at sowing depth of 25 mm, cut seeds backfilled with Niğde soil at sowing depth of 25 mm and cut seeds backfilled with Niğde soil at sowing depth of 50 mm in the greenhouse in 2019. The figure shows mean (-) of total seedling length and 95% confidence intervals above the box plot of the raw data for each treatment. The box plot also shows the median and interquartile range. Treatment codes: P-50-0; whole seeds at sowing depth of 50 mm backfilled with peat, PL-50-0; cut seeds at sowing depth of 50 mm backfilled with perlite, S-25-0; uncut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-25-1; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-50-0; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil

A mean total seedling length of over 50 mm was seen in AP17 while AP60 had a mean total seedling length of less than 50 mm also using uncut seeds backfilled with Niğde soil at a sowing depth of 25 mm. Also, AP16 was observed to have the longest mean total seedling length of over 100 mm using cut seeds backfilled with Niğde soil at a sowing depth of 25 mm while AP60 had the shortest mean total seedling length below 90 mm for the same treatment. AP17 had mean total seedling length of over 90 mm also using cut seeds backfilled with Niğde soil at sowing depth of 25 mm. The longest mean total seedling length of over 140 mm was observed in AP17 using cut seeds at sowing depth of 50 mm while the lowest mean total seedling length of below 50 mm was seen in AP60 for the same treatment. AP16 had mean total seedling length of over 100 mm using cut seeds backfilled with Niğde soil at a sowing depth of 50 mm (Fig. 4.9).

### 4.3.3 Root length

The longest mean root length of over 90 mm was observed in AP16 seed lots while the shortest mean root length was seen in AP16 for the five treatments used (Fig. 4.10). In addition, AP17 had a mean root length of approximately 80 mm for the five treatments used.

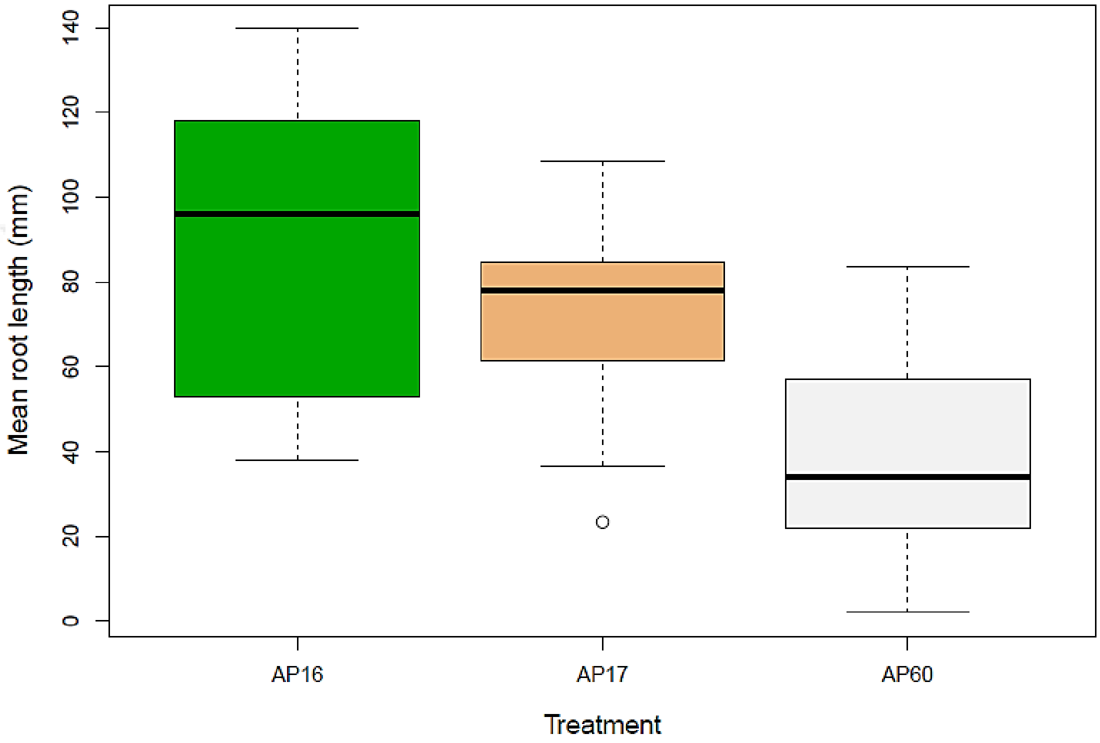

**Figure 4.10.** Mean root length (mm) of three seed lots AP16, AP17 and AP60 of *Elaeagnus* planted in the greenhouse in 2019 cumulatively using five treatments: P-50-0; whole seeds at sowing depth of 50 mm backfilled with peat, PL-50-0; cut seeds at sowing depth of 50 mm backfilled with perlite, S-25-0; uncut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-25-1; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-50-0; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil. The figure shows mean (-) of root length and 95% confidence intervals above the box plot of the raw data for each treatment. The box plot also shows the median, interquartile range and outliers. Codes: AP16 are seed lots collected from matured fruits of a tree growing on the highway in 2018. The fruits were removed from their pericarps to collect the seeds. A tree was randomly selected from the collection grown at the D330 highway (33°36′ N 6°42′ W), AP17 are seed lot seeds collected from a tree grown in the Niğde Ömer Halisdemir University campus in 2018, AP60 is seed lot seeds purchased from Enderbey Market in Niğde as commercial one in 2018

### 4.3.4 Shoot length

AP16 seed lot had the longest mean shoot length compared to the other two seed lots, AP17 and AP60 within the same treatment S-25-0 (uncut seeds at sowing depth of 25 mm and backfilling with Niğde soil) and S-25-1 (cut seeds at sowing depth of 25 mm and backfilled with Niğde soil. The shortest mean shoot length was observed in AP60 for all the five backfillings used. There was no distinct effect of the five-backfilling used on the shoot length of the plant. Cumulatively, for the three accessions, AP17 had the highest mean shoot length of over 30 mm using whole seeds backfilled with peat at a sowing depth of 50 mm while AP60 had the shortest mean shoot length for the same treatment (Fig. 4.11). AP16 had a mean shoot length close to 30 mm using whole seeds backfilled with peat at a sowing depth of 50 mm. AP17 also had the longest mean shoot length of over 10 mm for cut seeds backfilled with perlite at a sowing depth of 50 mm.

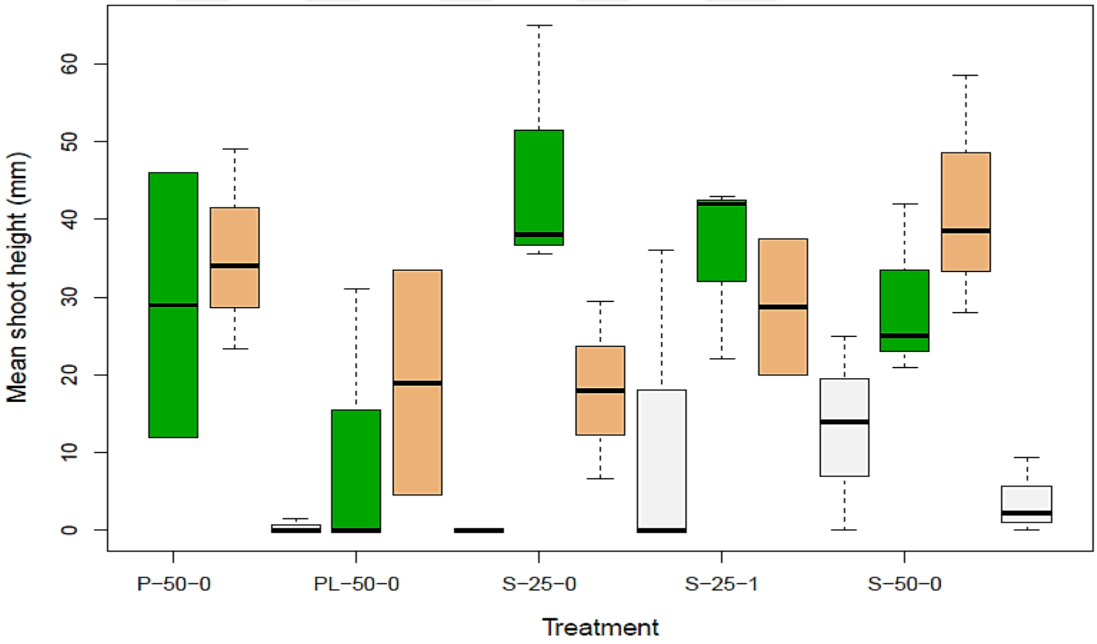

**Figure 4.11.** The mean shoot length (mm) of three seed lots AP16, AP17 and AP60 of *Elaeagnus* planted using five treatments of whole seeds backfilled with peat at sowing depth of 50 mm, cut seeds backfilled with perlite at sowing depth of 50 mm, uncut seeds backfilled with Niğde soil at sowing depth of 25 mm, cut seeds backfilled with Niğde soil at sowing depth of 25 mm and, cut seeds backfilled with Niğde soil at sowing depth of 50 mm. The figure shows means (-) of the total seed length and 95% confidence intervals above the box plot of the raw data for each treatment. Treatment codes: P-50-0; whole seeds at sowing depth of 50 mm backfilled with peat, PL-50-0; cut seeds at sowing depth of 50 mm backfilled with perlite, S-25-0; uncut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-25-1; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-50-0; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of dry Niğde soil

AP16 had the longest mean shoot length of over 40 mm using uncut seeds backfilled with Niğde soil at a sowing depth of 25 mm while AP60 had the shortest mean shoot length of less than 20 mm with the same treatment.

A mean shoot length of approximately 30 mm was seen in seed lot AP17 using uncut seeds backfilled with Niğde soil at a sowing depth of 25 mm. Similarly, AP16 had the longest mean shoot length of 40 mm using cut seeds backfilled with Niğde soil at a sowing depth of 25 mm while the shortest mean shoot length of less than 20 mm was observed in AP60 for the same treatment. AP17 had a mean shoot length of over 30 mm also using cut seeds backfilled with Niğde soil at a sowing depth of 25 mm. In addition, AP17 was seen to have the longest mean shoot length of over 30 mm using cut seeds backfilled with Niğde soil at a sowing depth of 50 mm. In contrast, AP60 was observed to have a mean shoot length of less than 10 mm using the same treatment (Fig 4.11). AP16 was seen to have a mean length lower than 30 mm using cut seeds backfilled with Niğde soil at a sowing depth of 50 mm.

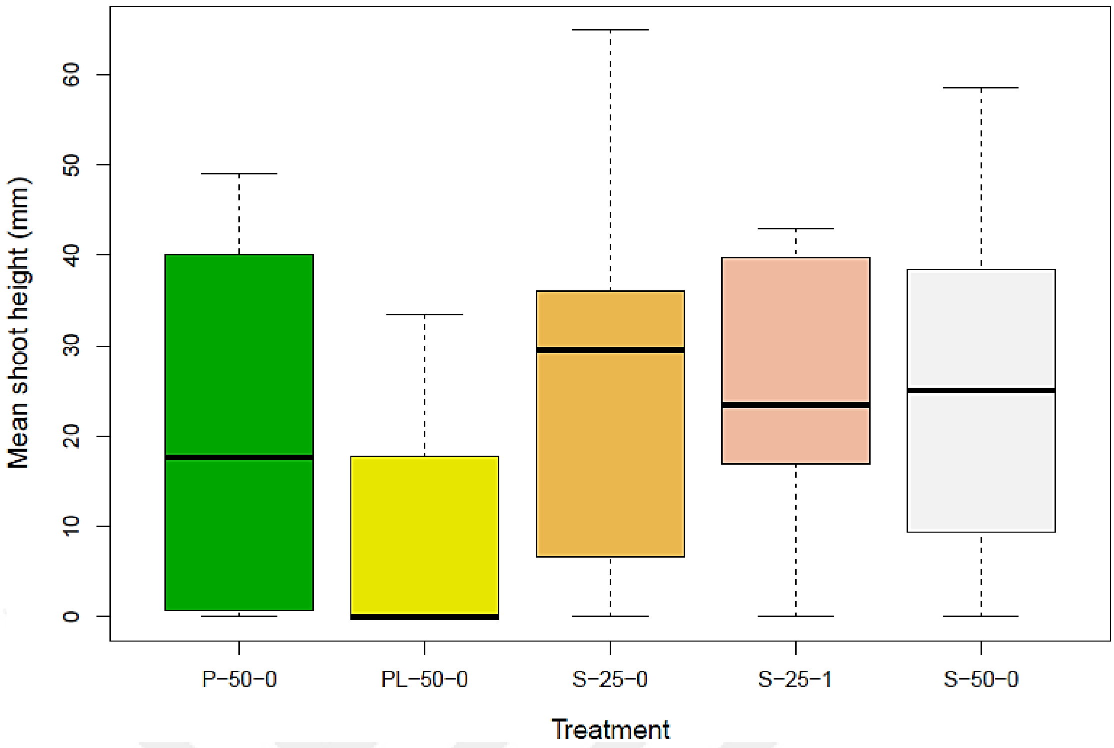

**Figure 4.12.** The mean shoot length (mm) of *Elaeagnus* using five treatments of whole seeds backfilled with peatat sowing depth of 50 mm, cut seeds backfilled with perlite at sowing depth of 50 mm, uncut seeds backfilled with Niğde soil at sowing depth of 25 mm, cut seeds backfilled with Niğde soil at sowing depth of 50 mm in the greenhouse in 2019. The figure shows means (-) of emergence and 95% confidence intervals above the box plot of the raw data for each treatment. The box plot also shows the median and interquartile range. Treatment codes: P-50-0; whole seeds at sowing depth of 50 mm backfilled with peat, PL-50-0; cut seeds at sowing depth of 50 mm backfilled with perlite, S-25-0; uncut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-25-1; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-50-0; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil

The longest mean shoot length of approximately 30 mm was observed in uncut seeds backfilled with Niğde soil at a sowing depth of 25 mm. while the shortest mean shoot length was seen in cut seeds backfilled with perlite at sowing depth of 50 mm (Fig. 4.12). In addition, a mean shoot length of over 20 mm was observed in both cut seeds backfilled with Niğde soil at a sowing depth of 25 mm and cut seeds backfilled with Niğde soil at a sowing depth of 50 mm. Whole seeds backfilled with peat at a sowing depth of 50 mm had a mean shoot length less than 20 mm (Fig. 4.12). Cumulatively, AP16 had the longest mean shoot length of over 30 mm while AP60 had the shortest mean shoot length of close to 0 mm for the five treatments (Fig. 4.13). In addition, AP16 had a mean shoot length of approximately 30 mm cumulatively for the five treatments.

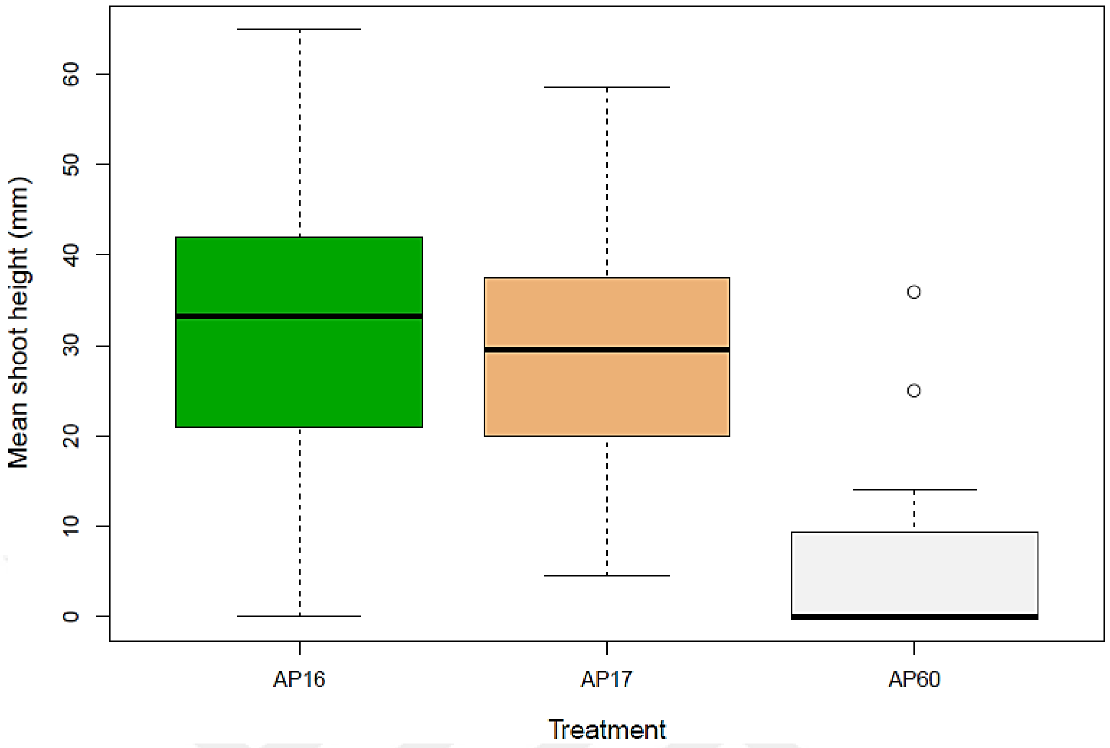

**Figure 4.13.** Mean shoot length (mm) of three seed lots AP16, AP17 and AP60 of *Elaeagnus* that were planted in the greenhouse in 2019 cumulatively using five treatments: P-50-0; whole seeds at sowing depth of 50 mm backfilled with peat, PL-50-0; cut seeds at sowing depth of 50 mm backfilled with perlite, S-25-0; uncut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-25-1; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-50-0; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil. The figure shows means (-) of shoot length and 95% confidence intervals above the box plot of the raw data for each treatment. The box plot also shows the median, interquartile range and outliers. Codes: AP16 are seed lots collected from matured fruits of a tree growing on the highway in 2018. The fruits were removed from their pericarps to collect the seeds. A tree was randomly selected from the collection grown at the D330 highway (33°36′ N 6°42′ W), AP17 are seed lot seeds collected from a tree grown in the Niğde Ömer Halisdemir University campus in 2018, AP60 is seed lot seeds purchased from Enderbey Market in Niğde as commercial one in 2018

### 4.3.5 Emergence time

There was no clear distinction between the mean emergence time of the three seed lots AP16, AP17 and AP60 using whole seeds backfilled with peat at a sowing depth of 50 mm. However, AP60 had the longest mean emergence time of more than 30 days within the same treatment. In addition, the mean emergence time was found to be longest at more than 30 days (Fig. 4.14) for AP17 for cut seeds backfilled with perlite at a sowing depth of 50 mm. In cut seeds backfilled with perlite at a sowing depth of 50 mm, there was also no clear distinction in the mean emergence time among the three seed lots

AP16, AP17 and AP60. AP16 had the longest mean emergence time of more than 30 days while AP17 had the shortest mean emergence time of less than 25 days. Also, AP60 had an emergence time of 25 days (Fig. 4.14).

In cut seeds backfilled with Niğde soil at sowing depth of 25 mm, AP60 had the longest mean emergence time of 35 days. In contrast, AP16 had the shortest mean emergence time of less than 25 days. AP17 had a mean emergence time of 25 days. In uncut seeds backfilled with Niğde soil at sowing depth of 50 mm, AP60 had the longest mean emergence time of more than 25 days while AP16 had the shortest mean emergence time of more than 15 days. AP17 had a mean emergence time of less than 20 days (Fig. 4.14).

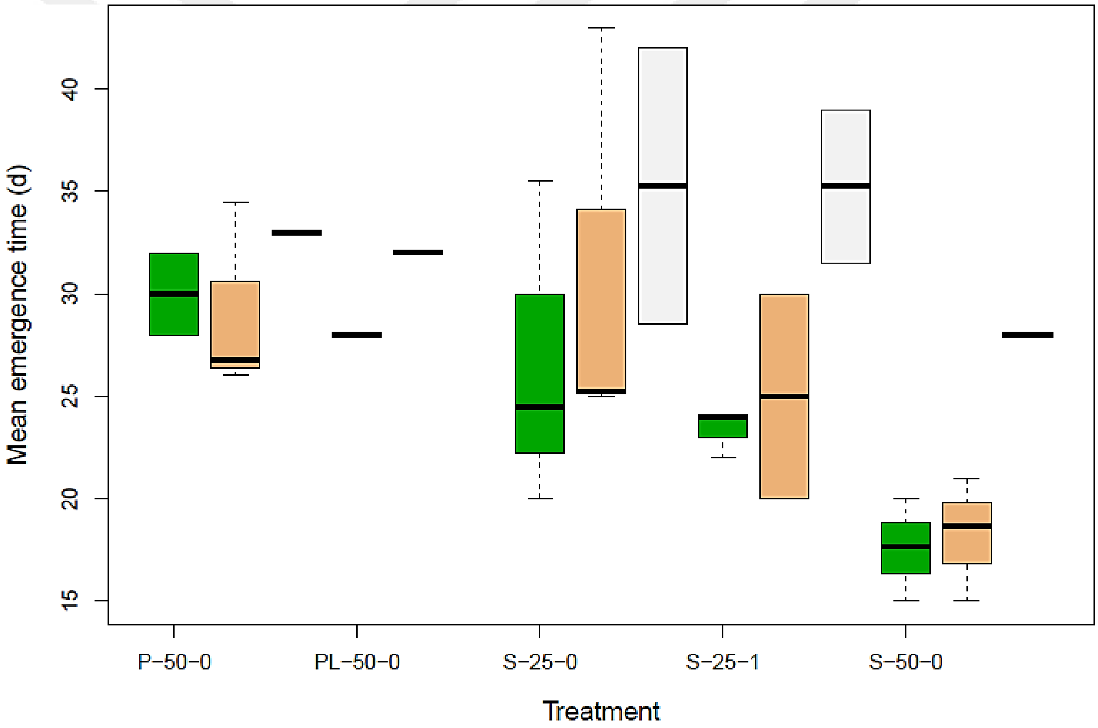

**Figure 4.14.** The mean emergence time (d) of three seed lots AP16, AP17 and AP60 of *Elaeagnus* using five treatments of whole seeds backfilled with peatat sowing depth of 50 mm, cut seeds backfilled with perlite at sowing depth of 50 mm, uncut seeds backfilled with Niğde soil at sowing depth of 25 mm, cut seeds backfilled with Niğde soil at sowing depth of 50 mm in the greenhouse in 2019. The figure shows means (-) of emergence time and 95% confidence intervals above the box plot of the raw data for each treatment. The box plot also shows the median and interquartile range. Treatment codes: P-50-0; whole seeds at sowing depth of 50 mm backfilled with peat, PL-50-0; cut seeds at sowing depth of 50 mm backfilled with perlite, S-25-0; uncut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-25-1; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-50-0; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil

There was no clear difference between the mean emergence time of the five treatments used (Fig. 4.15). Whole seeds backfilled with peat at sowing depth of 50 mm and cut seeds backfilled with perlite at sowing depth of 50 mm both had had mean emergence time of 30 mm. Uncut seeds backfilled with Niğde soil at sowing depth of 25 mm had an emergence time of over 25 days. Cut seeds backfilled with Niğde soil at sowing depth of 50 mm had the shortest emergence time of less than 20 days.

There was no significant difference between the emergence time of the three seed lots AP16, AP17 and AP60 (Fig. 4.16). AP60 seed lot had the longest mean emergence time of more than 30 days. In contrast, AP16 had the shortest mean emergence time of less than 25 days, while AP17 had a mean emergence time of 25 days.

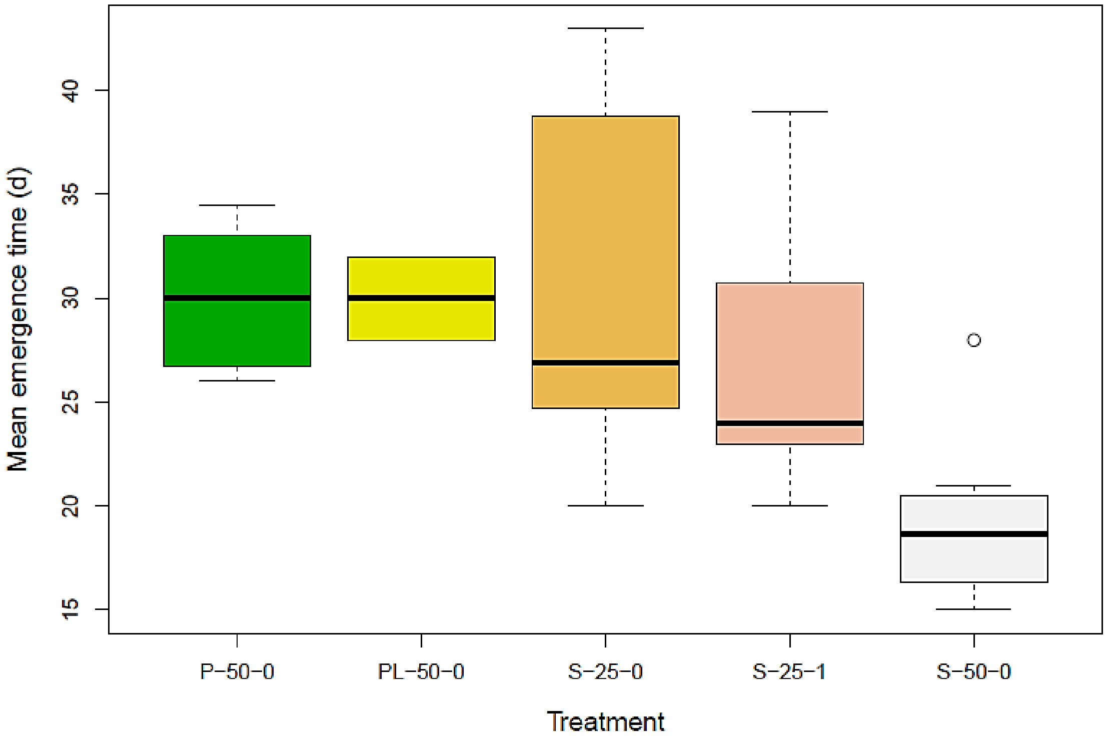

**Figure 4.15.** The mean emergence time (d) of *Elaeagnus* using five treatments of whole seeds backfilled with peatat sowing depth of 50 mm, cut seeds backfilled with perlite at sowing depth of 50 mm, uncut seeds backfilled with Niğde soil at sowing depth of 25 mm, cut seeds backfilled with Niğde soil at sowing depth of 50 mm in the greenhouse in 2019.
The figure shows the mean (-) of emergence time and 95% confidence intervals above the box plot of the raw data for each treatment. The box plot also shows the median, interquartile range and outliers Treatment codes: P-50-0; whole seeds at sowing depth of 50 mm backfilled with peat, PL-50-0; cut seeds at sowing depth of 50 mm backfilled with perlite, S-25-0; uncut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-25-1; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde

soil, S-50-0; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil

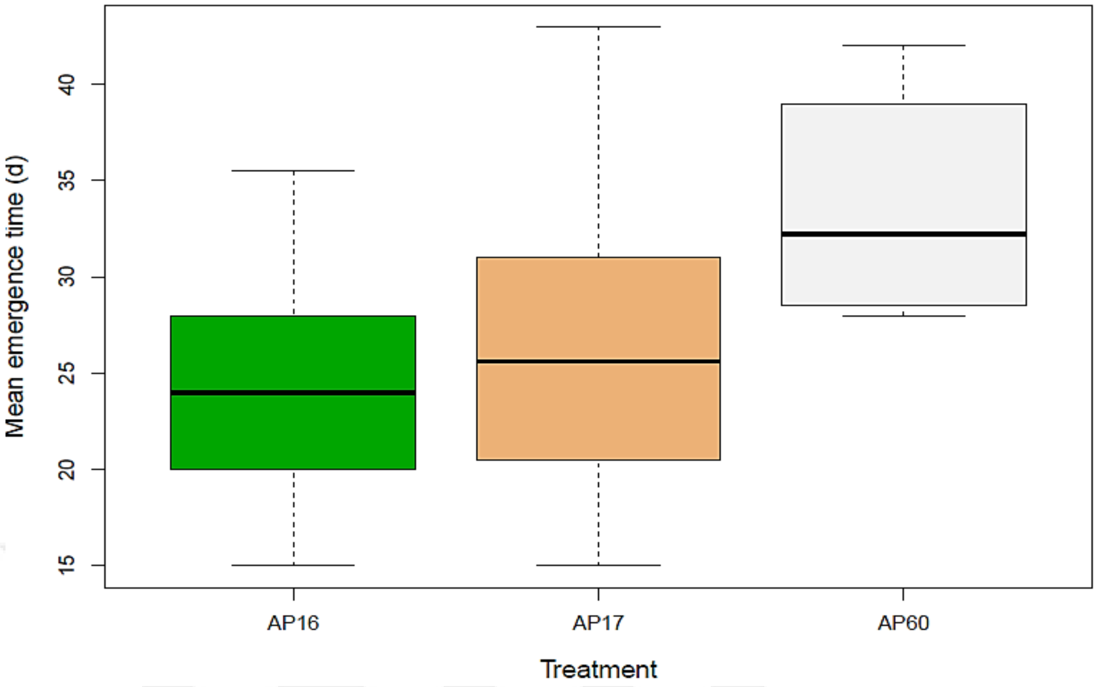

**Figure 4.16.** Mean emergence time (d) of three seed lots AP16, AP17 and AP60 of *Elaeagnus* that were planted in the greenhouse in 2019 cumulatively using five treatments: P-50-0; whole seeds at sowing depth of 50 mm backfilled with peat, PL-50-0; cut seeds at sowing depth of 50 mm backfilled with perlite, S-25-0; uncut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-25-1; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-50-0; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil. The figure shows means (-) of root length and 95% confidence intervals above the box plot of the raw data for each treatment. The box plot also shows the median, interquartile range and outliers. Codes: AP16 are seed lots collected from matured fruits of a tree growing on the highway in 2018. The fruits were removed from their pericarps to collect the seeds. A tree was randomly selected from the collection grown at the D330 highway (33°36′ N 6°42′ W), AP17 are seed lot seeds collected from a tree grown in the Niğde Ömer Halisdemir University campus in 2018, AP60 is seed lot seeds purchased from Enderbey Market in Niğde as commercial one in 2018

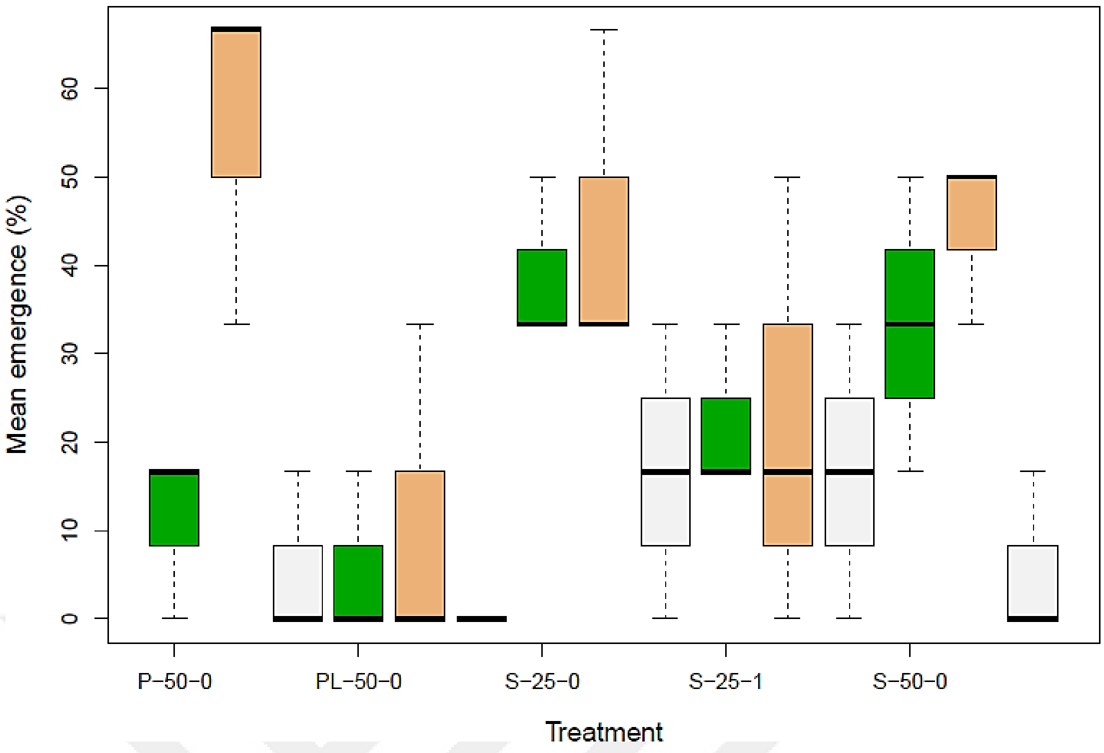

**Figure 4.17.** The mean emergence (%) of three seed lots AP16, AP17 and AP60 of *Elaeagnus* using five treatments of whole seeds backfilled with peat at sowing depth of 50 mm, cut seeds backfilled with perlite at sowing depth of 50 mm, uncut seeds backfilled with Niğde soil at sowing depth of 25 mm, cut seeds backfilled with Niğde soil at sowing depth of 50 mm in the greenhouse in 2019. The figure shows means (-) of emergence and 95% confidence intervals above the box plot of the raw data for each treatment. The box plot also shows the median and interquartile range. Treatment codes: P-50-0; whole seeds at sowing depth of 50 mm backfilled with peat, PL-50-0; cut seeds at sowing depth of 50 mm backfilled with perlite, S-25-0; uncut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-25-1; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-50-0; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil

### 4.3.6 Emergence

AP17 seed lot had the highest mean emergence of over 60% while AP60 had the lowest mean emergence using cut seeds at sowing depth of 50 mm and backfilling with Niğde soil. In addition, AP16 had mean emergence of less than 20% using cut seeds backfilled with Niğde soil at a sowing depth of 50 mm. AP16, AP17 and AP60 had very low emergence using cut seeds at sowing depth of 50 mm, backfilled with perlite. AP16 and AP17 had the highest mean emergence at over 30% with no significant difference using uncut seeds at sowing depth of 25 mm, backfilled with Niğde soil while AP60 had the lowest mean emergence of less than 20%.

There was no significant difference among the mean emergence of the three seed lots AP16, AP17 and AP60. Mean emergence of less than 20% was observed for the three seed lots. The highest mean emergence of 50% was observed in AP16 using S-50-0 (cut seeds at sowing depth of 50 mm, backfilled with Niğde soil) while AP60 had the lowest mean emergence AP17 had mean emergence of over 30% using the same treatment.

The highest mean emergence of over 30% was observed with both uncut seeds at sowing depth of 25 mm, backfilled with Niğde soil and cut seeds at sowing depth of 50 mm backfilled with Niğde soil (Fig. 4.18). In contrast, the lowest mean emergence with several outliers was observed in cut seeds at a sowing depth of 50 mm, backfilled with perlite. Mean emergence of less than 20% was observed both in cut seeds at sowing depth of 25 mm, backfilled with Niğde soil and whole seeds at sowing depth of 50 mm, backfilled with peat. Seed lot AP17 had the highest mean emergence of over 30% while AP60 had the lowest mean emergence. AP16 had a mean emergence of less than 20% (Fig. 4.18).

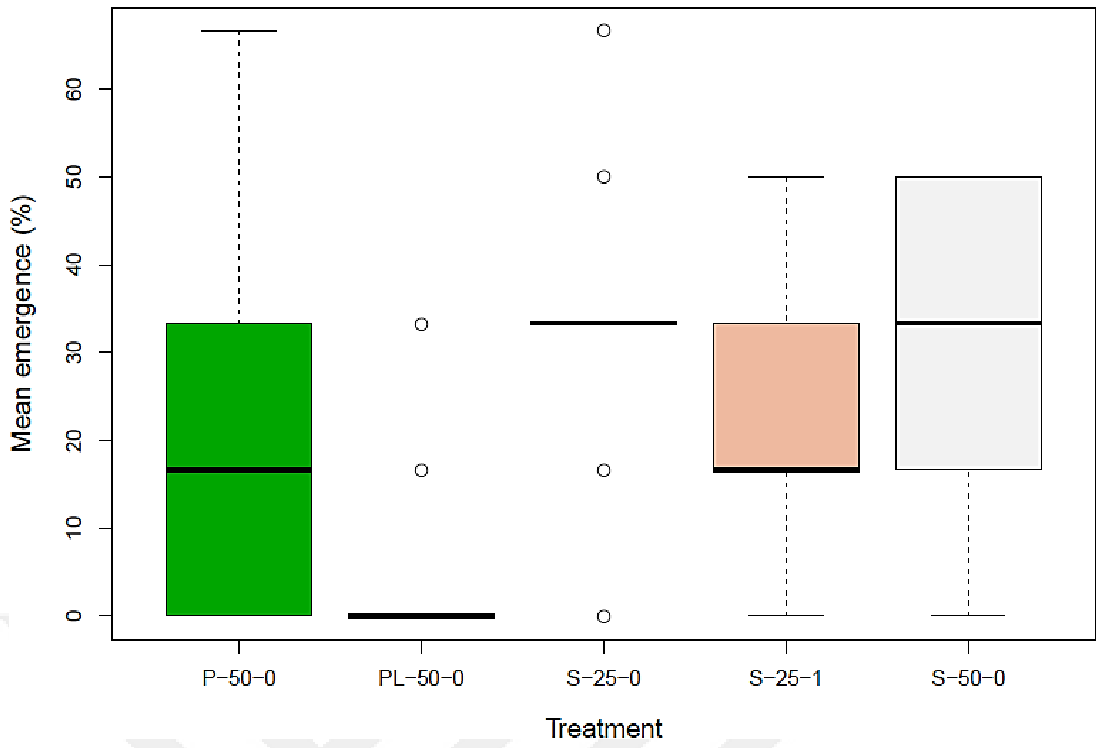

**Figure 4.18.** The mean emergence (%) of *Elaeagnus* using five treatments of whole seeds backfilled with peat at sowing depth of 50 mm, cut seeds backfilled with perlite at sowing depth of 50 mm, uncut seeds backfilled with Niğde soil at sowing depth of 25 mm, cut seeds backfilled with Niğde soil at sowing depth of 50 mm in the greenhouse in 2019. The figure shows means (-) of emergence and 95% confidence intervals above the box plot of the raw data for each treatment. The box plot also shows the median, interquartile range and outliers Treatment codes: P-50-0; whole seeds at sowing depth of 50 mm backfilled with peat, PL-50-0; cut seeds at sowing depth of 50 mm backfilled with perlite, S-25-0; uncut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-25-1; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-50-0; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil

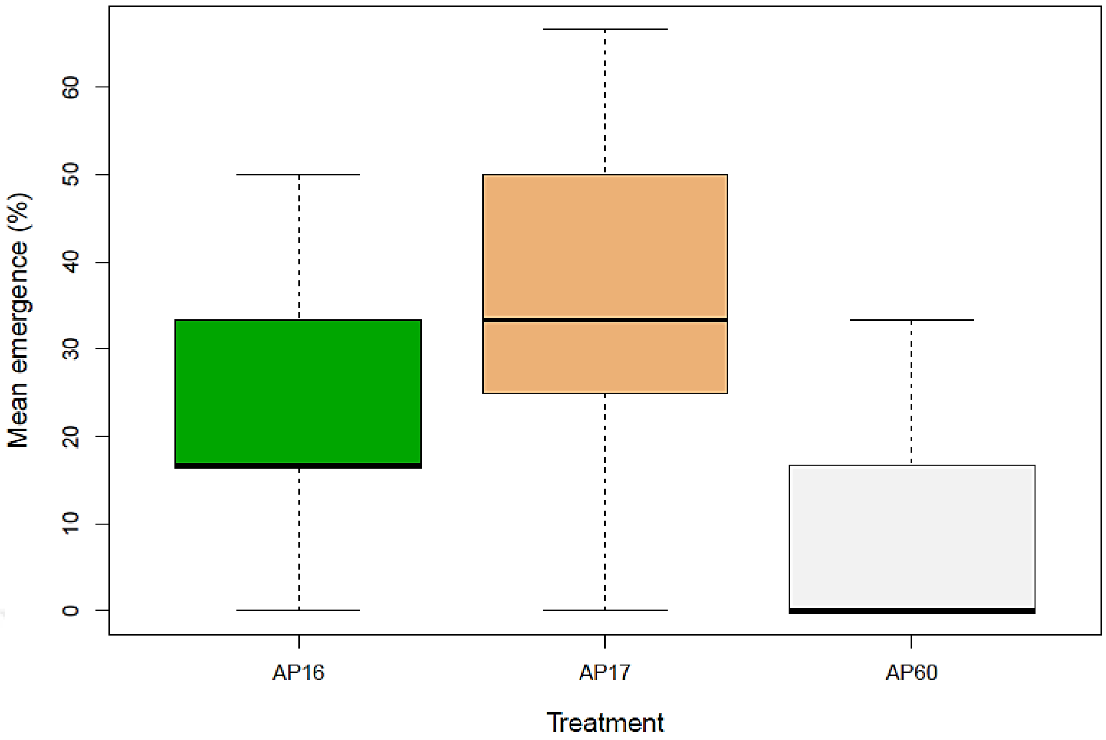

**Figure 4.19.** Mean emergence (%) of three seed lots AP16, AP17 and AP60 of *Elaeagnus* that were planted in the greenhouse in 2019 cumulatively using five treatments: P-50-0; whole seeds at sowing depth of 50 mm backfilled with peat, PL-50-0; cut seeds at sowing depth of 50 mm backfilled with perlite, S-25-0; uncut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-25-1; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-50-0; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil. The figure shows means (-) of root length and 95% confidence intervals above the box plot of the raw data for each treatment. The box plot also shows the median and interquartile range. Codes: AP16 are seed lots collected from matured fruits of a tree growing on the highway in 2018. The fruits were removed from their pericarps to collect the seeds. A tree was randomly selected from the collection grown at the D330 highway (33°36′ N 6°42′ W), AP17 are seed lot seeds collected from a tree grown in the Niğde Ömer Halisdemir University campus in 2018, AP60 is seed lot seeds purchased from Enderbey Market in Niğde as commercial one in 2018

### 4.3.7 Rotting

The highest percentage of rotting of over 10% was observed in AP17 using whole seed at sowing depth of 50 mm backfilled with peat. In contrast, no rotting was observed in AP16 and AP17. In cut seeds backfilled with perlite at a sowing depth of 50 mm, no rotting was observed in the three seed lots AP16, AP17 and AP60. In addition, AP17 was observed to have the highest percentage mean rotting of more than 10% using uncut seeds at sowing depth of 25 mm, backfilled with Niğde soil (Fig. 4.20).

In contrast, no rotting was observed in AP16 and AP60. AP17 also had the highest mean rotten of more than 10% when cut seeds backfilled with Niğde soil at sowing depth of 25 mm were used while AP16 and AP60 had no rots. AP16 was observed to have the highest mean rotten of over 30% only when cut seeds backfilled with Niğde soil at a sowing depth of 50 mm were used. AP17 had a mean rotten of over 10% while in AP60 no rotting was observed using cut seeds backfilled with Niğde soil at a sowing depth of 50 mm (Fig. 4.20). In all the treatments, AP17 was seen to have a percentage mean rotten of more than 10%.

Generally, there was no difference in the mean of the five treatments. There were a few outliers with whole seeds backfilled with peat at a sowing depth of 50 mm and cut seeds backfilled with perlite at a sowing depth of 50 mm. The mean rotting for all the treatments was less than 1% (Fig. 4.21). The highest mean rotting of over 10% was observed in AP17. In contrast, AP16 and AP60 had a very low mean rotting (Fig. 4.22).

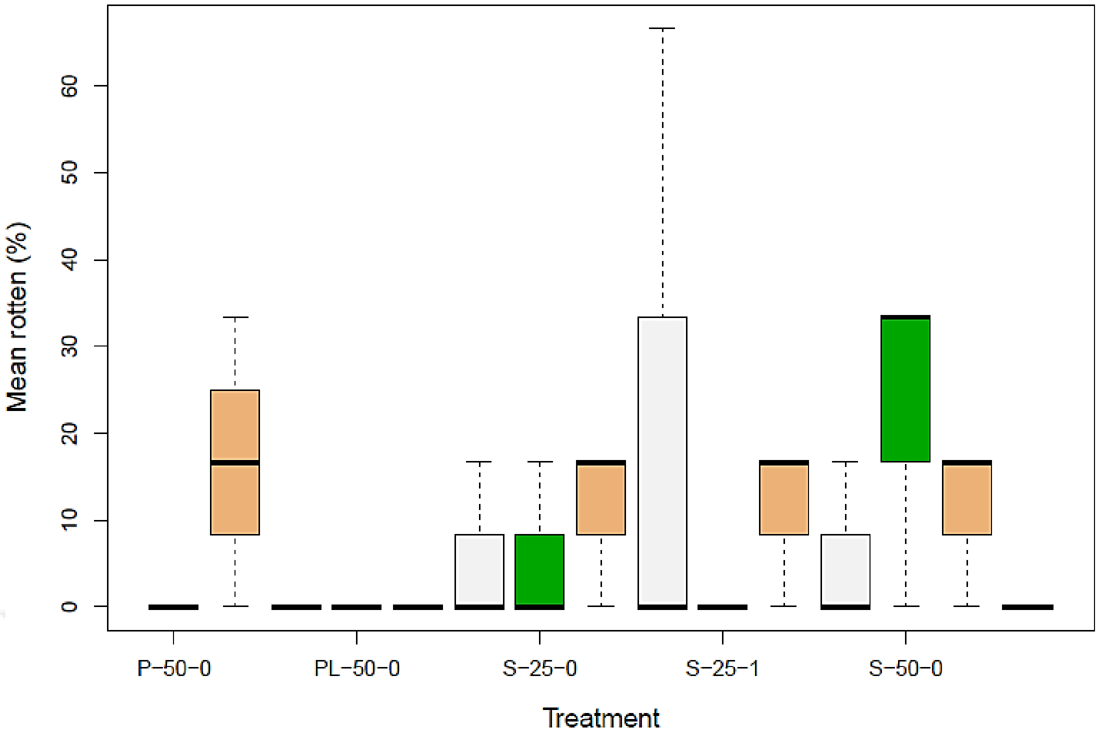

**Figure 4.20.** Mean rotten (%) of three seed lots, AP16, AP17 and AP60 of *Elaeagnus* seeds (cut, uncut and whole) planted at different sowing depths of 25 mm and 50 mm with three backfills of dry sieved Niğde soil, perlite and peat in the greenhouse in 2019. The figure shows mean (-) of the total seed length and 95% confidence intervals above the box plot of the raw data for each treatment. The box plot also shows the median and interquartile range. Treatment codes: P-50-0; whole seeds at sowing depth of 50 mm backfilled with peat, PL-50-0; cut seeds at sowing depth of 50 mm backfilled with perlite, S-25-0; uncut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-25-1; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-50-0; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil

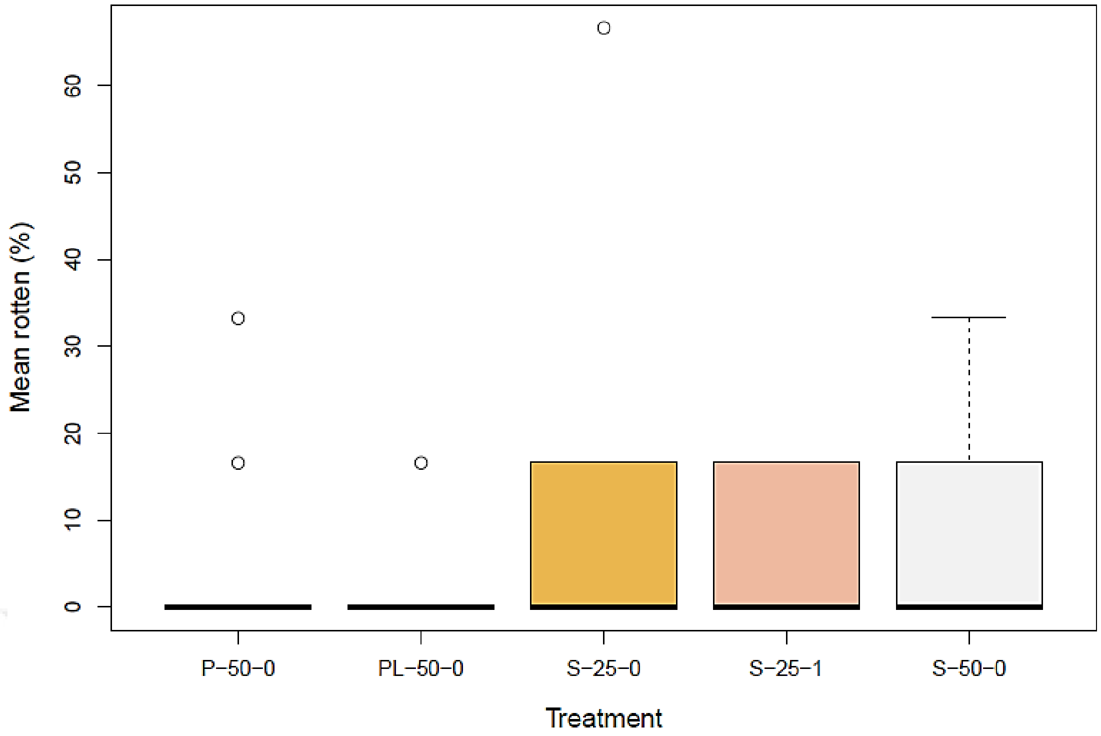

**Figure 4.21.** The mean rotten (%) of *Elaeagnus* using five treatments of whole seeds backfilled with peat at sowing depth of 50 mm, cut seeds backfilled with perlite at sowing depth of 50 mm, uncut seeds backfilled with Niğde soil at sowing depth of 25 mm, cut seeds backfilled with Niğde soil at sowing depth of 50 mm, cut seeds backfilled with Niğde soil at sowing depth of 50 mm in the greenhouse in 2019. The figure shows means (-) of rotten and 95% confidence intervals above the box plot of the raw data for each treatment. The box plot also shows the median, interquartile range and outliers Treatment codes: P-50-0; whole seeds at sowing depth of 50 mm backfilled with peat, PL-50-0; cut seeds at sowing depth of 50 mm backfilled with perlite, S-25-0; uncut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-25-1; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-50-0; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil

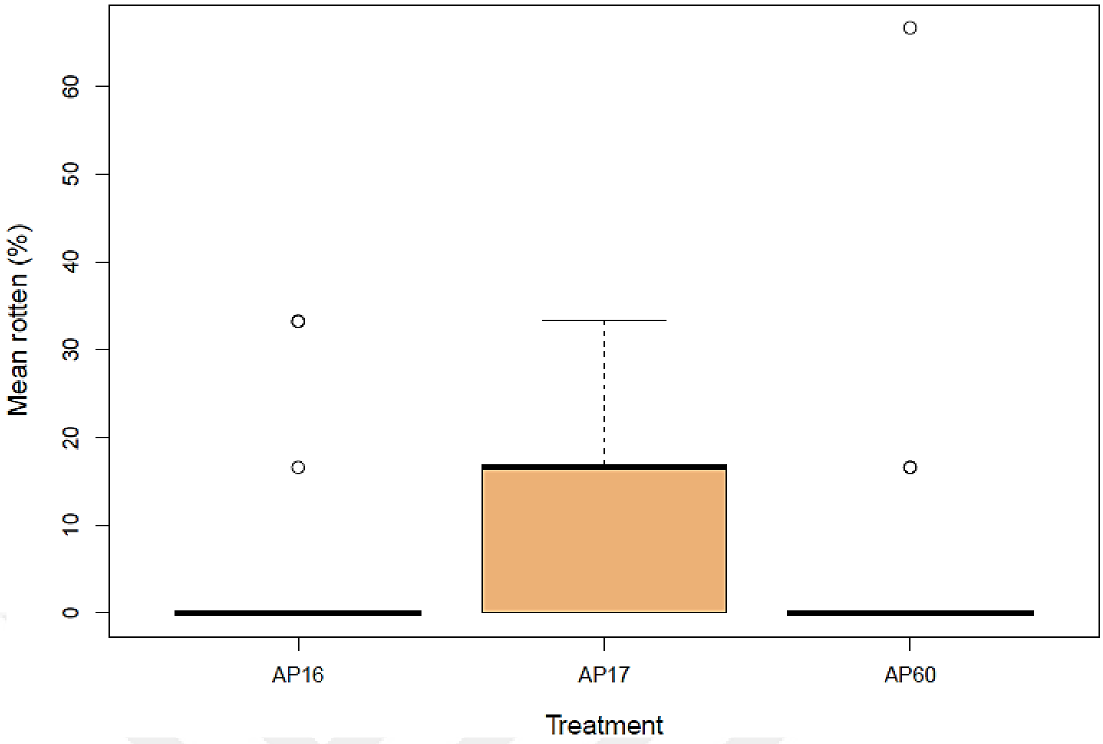

**Figure 4.22.** Mean rotten of three seed lots AP16, AP17 and AP60 of *Elaeagnus* (%) that were planted in the greenhouse in 2019 for the five treatments cumulatively of whole seeds backfilled with peat at sowing depth of 50 mm, cut seeds backfilled with perlite at sowing depth of 50 mm, uncut seeds backfilled with Niğde soil at sowing depth of 25 mm, cut seeds backfilled with Niğde soil at sowing depth of 50 mm, cut seeds backfilled with Niğde soil at sowing depth of 50 mm in the greenhouse in 2019. The figure shows means (-) of rotten seeds and 95% confidence intervals above the box plot of the raw data for each treatment. The box plot also shows the median, interquartile range and outliers. Codes: AP16 are seed lot seeds collected from matured fruits of a tree growing on the highway in 2018. The fruits were removed from their pericarps to collect the seeds. A tree was randomly selected from the collection grown at the D330 highway (33°36′ N 6°42′ W), AP17 seeds were collected from a tree grown in the Niğde Ömer Halisdemir University campus in 2018, AP60 was purchased from Enderbey Market in Niğde as commercial one in 2018

### 4.3.8 Germination

The highest mean germination of over 80% was observed in AP60 using whole seeds backfilled with peat at a sowing depth of 50 mm. In contrast, the lowest mean germination of below 20% was observed in AP16. AP17 had mean germination of over 60%. AP60 had a high mean germination of over 80% in both whole seeds backfilled with peat at sowing depth of 50 mm and cut seeds backfilled with Niğde soil at sowing depth of 50 mm. The same mean germination of over 30% was seen in both seed lots AP17 and AP60 using cut seeds backfilled with perlite at a sowing depth of 50 mm whereas AP16 had the lowest mean germination of over 10% (Fig. 4.23).

AP60 was seen to have the highest mean germination of over 60% using uncut seeds backfilled with Niğde soil at sowing depth of 25 mm while AP16 was observed to have a mean germination of over 30%. Whereas AP17 had mean germination of over 40% for the same treatment. AP17 had the highest mean germination of over 30% using cut seeds backfilled with Niğde soil at a sowing depth of 25 mm. In AP16 and AP60 lower mean germination of below 20% was observed. AP60 had the highest mean germination of over 80% using cut seeds backfilled with Niğde soil at sowing depth of 50 mm while the lowest mean germination of less than 40% was observed in AP16. In AP17 mean germination of over 40% was observed for the same treatment (Fig. 4.23).

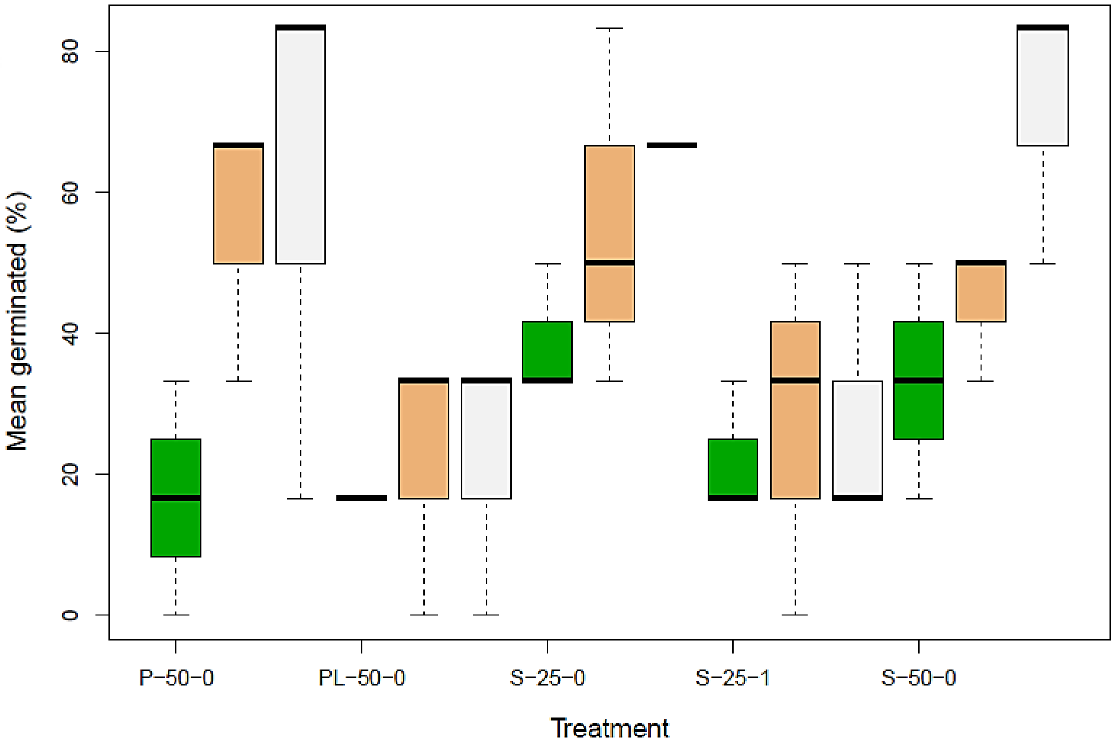

**Figure 4.23.** Mean germinated (%) of three seed lots, AP16, AP17 and AP60 of *Elaeagnus* seeds (cut, uncut and whole) planted at different sowing depths of 25 and 50 mm with three backfills of dry sieved Niğde soil, perlite and peat in the greenhouse in 2019. The figure shows means (-) of the total seed length and 95% confidence intervals above the box plot of the raw data for each treatment. The box plot also shows the median, interquartile range and outliers. Treatment codes: P-50-0; whole seeds at sowing depth of 50 mm backfilled with peat, PL-50-0; cut seeds at sowing depth of 50 mm backfilled with perlite, S-25-0; uncut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-25-1; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-50-0; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil

Uncut seeds backfilled with Niğde soil at sowing depth of 25 mm and cut seeds backfilled with Niğde soil at sowing depth of 50 mm had the highest mean germinated of over 40% (Fig. 4.24). Whole seeds backfilled with peat at a sowing depth of 50 mm had mean germination of over 30%. Also, both cut seeds backfilled with perlite at sowing depth of 50 mm and cut seeds backfilled with Niğde soil at sowing depth of 25 mm had the lowest mean germination of below 20% (Fig. 4.24).

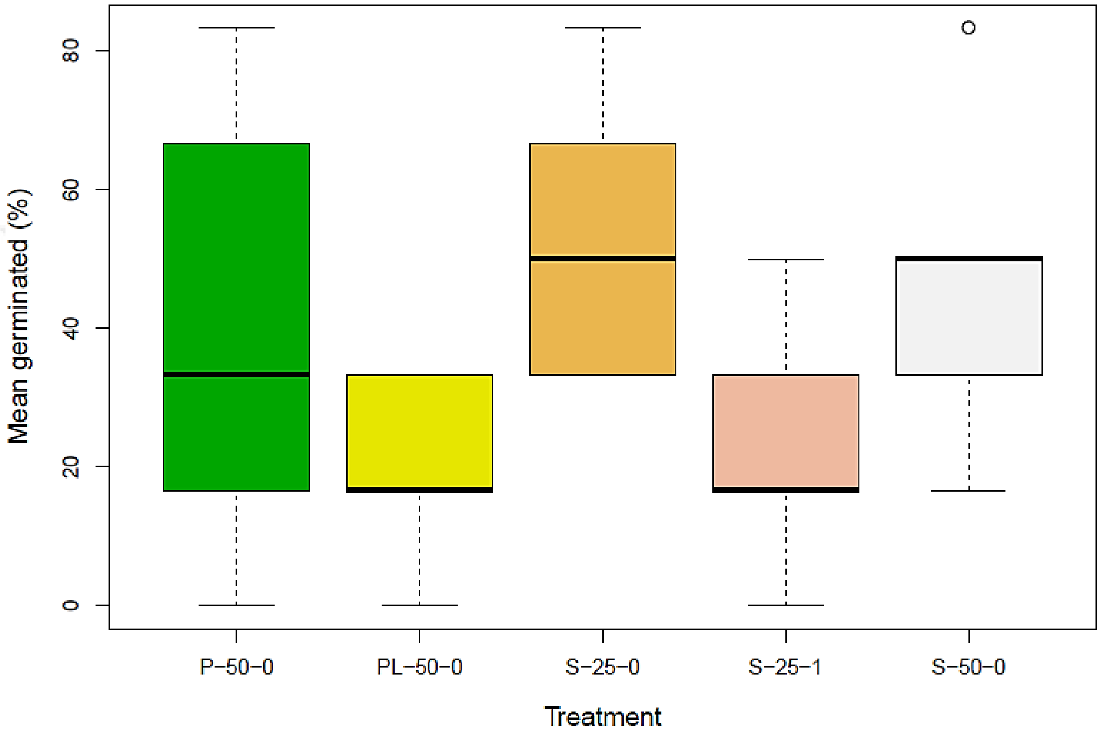

**Figure 4.24.** The mean germinated (%) of *Elaeagnus* using five treatments of whole seeds backfilled with peat at sowing depth of 50 mm, cut seeds backfilled with perlite at sowing depth of 50 mm, uncut seeds backfilled with Niğde soil at sowing depth of 25 mm, cut seeds backfilled with Niğde soil at sowing depth of 50 mm in the greenhouse in 2019. The figure shows means (-) of germinated and 95% confidence intervals above the box plot of the raw data for each treatment. The box plot also shows the median and interquartile range. Treatment codes: P-50-0; whole seeds at sowing depth of 50 mm backfilled with peat, PL-50-0; cut seeds at sowing depth of 50 mm backfilled with perlite, S-25-0; uncut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-25-1; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-50-0; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil

AP60 had the highest mean germination of over 40% for the five treatments used. AP17 had mean germination of below 40% while AP16 had the lowest germination of below 20% for the five treatments used (Fig. 4.25).

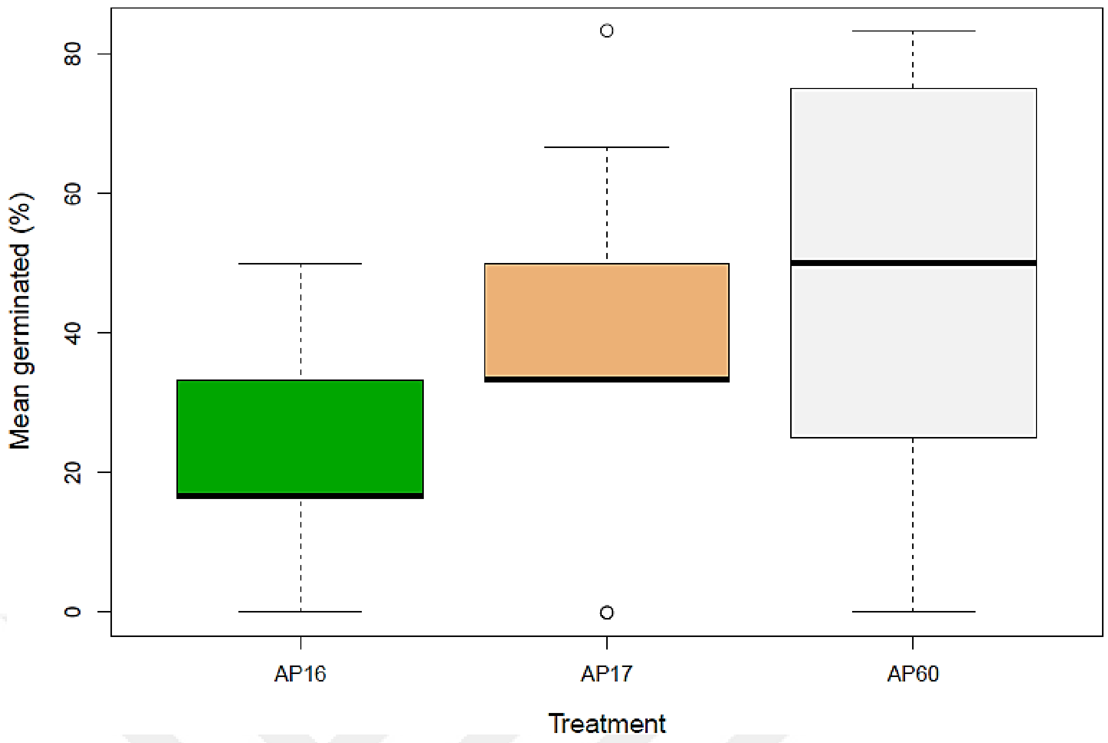

**Figure 4.25.** Mean germinated (%) of three seed lots AP16, AP17 and AP60 of *Elaeagnus* (%) that were planted in the greenhouse in 2019 for the five treatments cumulatively of whole seeds backfilled with peat at sowing depth of 50 mm, cut seeds backfilled with perlite at sowing depth of 50 mm, uncut seeds backfilled with Niğde soil at sowing depth of 25 mm, cut seeds backfilled with Niğde soil at sowing depth of 50 mm, cut seeds backfilled with Niğde soil at sowing depth of 50 mm in the greenhouse in 2019. The figure shows means (-) of rotten seeds and 95% confidence intervals above the box plot of the raw data for each treatment. The box plot also shows the median, interquartile range and outliers. Codes: AP16 are seeds collected from matured fruits of a tree growing on the highway in 2018. The fruits were removed from their pericarps to collect the seeds. A tree was randomly selected from the collection grown at the D330 highway (33°36′ N 6°42′ W), AP17 are seeds collected from a tree grown in the Niğde Ömer Halisdemir University campus in 2018, AP60 are seeds purchased from Enderbey Market in Niğde in 2018

### 4.3.9 Total seedling length

The mean total seedling lengths for the five treatments were not significantly different from each other (Fig. 4.26). Cut seeds backfilled with Niğde soil at sowing depth of 50 mm were observed to have the longest mean seedling length of over 100 mm. Uncut seeds backfilled with Niğde soil at sowing depth of 25 mm were observed to have a mean total seedling length of 100 mm. In addition, cut seeds backfilled with Niğde soil at sowing depth of 25 mm and whole seeds backfilled with peat at sowing depth of 50 mm had mean total seedling length below 100 mm. The shortest total seedling length was seen in cut seeds backfilled with perlite at a sowing depth of 50 mm (Fig. 4.26).

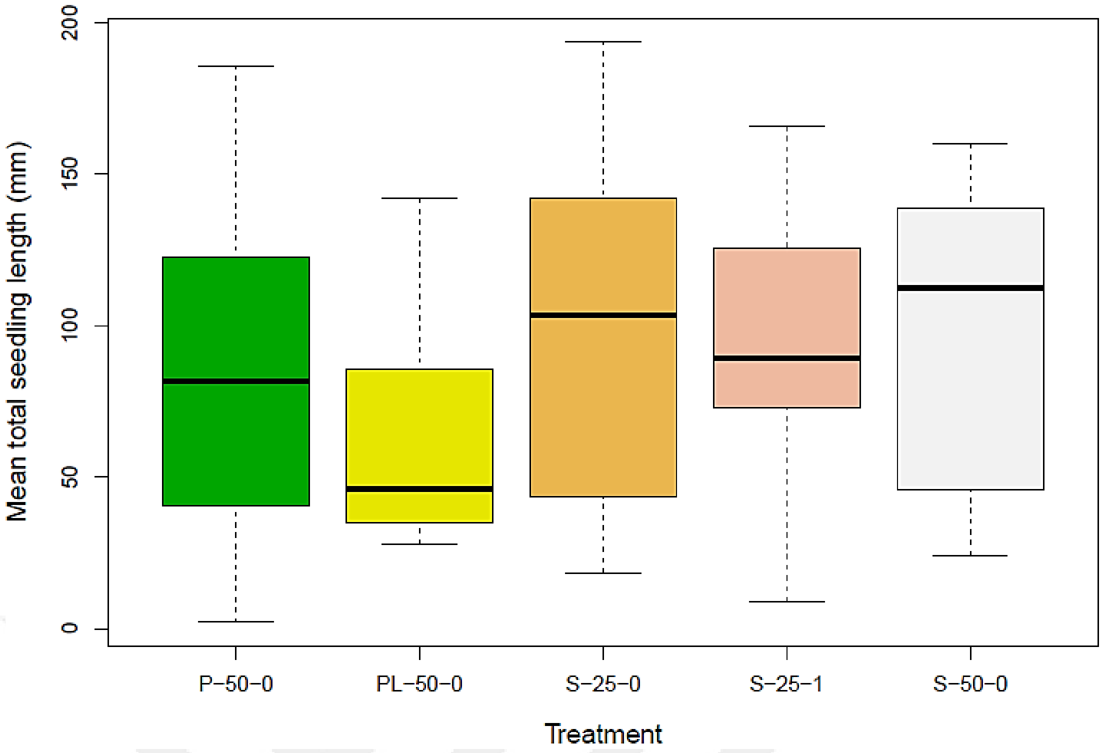

**Figure 4.26.** The mean total seedling length (mm) of *Elaeagnus* using five treatments of whole seeds backfilled with peat at sowing depth of 50 mm, cut seeds backfilled with perlite at sowing depth of 50 mm, uncut seeds backfilled with Niğde soil at sowing depth of 25 mm, cut seeds backfilled with Niğde soil at sowing depth of 50 mm in the greenhouse in 2019. The figure shows means (-) of germinated and 95% confidence intervals above the box plot of the raw data for each treatment. The box plot also shows the median and interquartile range. Treatment codes: P-50-0; whole seeds at sowing depth of 50 mm backfilled with peat, PL-50-0; cut seeds at sowing depth of 50 mm backfilled with perlite, S-25-0; uncut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-25-1; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil, S-50-0; cut seeds at sowing depth of 25 mm, backfilled with 850 µm sizes of fine grains of dry Niğde soil

The mean total seedling length for the three seed lots, AP16, AP17 and AP60 was not significantly different from each other. AP16 had the highest mean total seedling length below 150 mm while AP60 had the shortest total seedling length below 50 mm for the five treatments used (Fig. 4.27). AP17 had the mean total seedling length of over 100 mm for the five treatments used (Fig. 4.27).

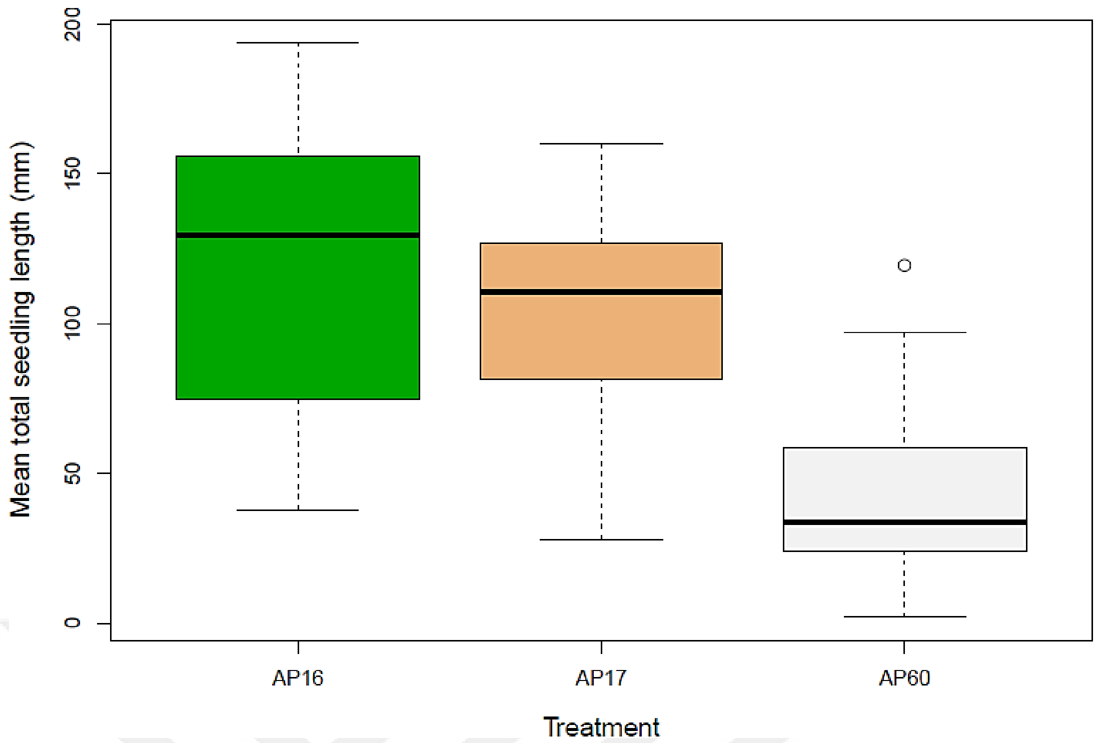

**Figure 4.27.** Mean total seedling length (mm) of three seed lots AP16, AP17 and AP60 of *Elaeagnus* (%) that were planted in the greenhouse in 2019 for the five treatments cumulatively of whole seeds backfilled with peat at sowing depth of 50 mm, cut seeds backfilled with perlite at sowing depth of 50 mm, uncut seeds backfilled with Niğde soil at sowing depth of 25 mm, cut seeds backfilled with Niğde soil at sowing depth of 50 mm, cut seeds backfilled with Niğde soil at sowing depth of 50 mm in the greenhouse in 2019. The figure shows means (-) of total seedling length and 95% confidence intervals above the box plot of the raw data for each treatment. The box plot also shows the median, interquartile range and outliers. Codes: AP16 are seeds collected from matured fruits of a tree growing on the highway in 2018. The fruits were removed from their pericarps to collect the seeds. A tree was randomly selected from the collection grown at the D330 highway (33°36′ N 6°42′ W), AP17 are seeds collected from a tree grown in the Niğde Ömer Halisdemir University campus in 2018, AP60 are seeds purchased from Enderbey Market in Niğde in 2018

## 4.4 Greenhouse Experiment 3: Hydrophilic Gel Backfilling

### 4.4.1 Emergence ratio

The results of the hydrophilic gel backfilling for the seed lots show that there were seed lot emergence of 10 days at 25 mm sowing depth for full backfill for the AP16, AP17 and AP60 while there was no emergence for the full backfill at 50 mm sowing depth for the hydrophilic gel. For the soil full backfill, there was neither emergence at 25 nor 50 mm sowing depth. The total seed length at the 25 mm sowing depth of the hydrophilic gel for the AP16, AP17 and AP60 were respectively 210, 335 and 367 mm.

For the partial backfill, there was no emergence for any of the seed lots at 25 mm sowing depth for the hydrophilic gel or the soil while there was emergence at 50 mm sowing depth for both hydrophilic gel and soil for the AP16, AP17 and AP60. The days to emergence of all the seed lots range from 10 to 18 days with the total seedling length range from 285 to 340 mm.

Nodules were observed in AP17 and AP60 only full backfilling with hydrophilic gel at 25 mm sowing depth. while was observed in the full soil backfill (Table 4.2). Also, there was nodulation for the partial backfill for both hydrophilic gel and soil at 50 mm sowing depth (Table 4.2) which ranges from 2 to 5 nodules.

**Table 4.2.** Results of hydrophilic gel (HG) backfilling experiment

| Seed lot | Quantity | Backfill type | Depth (mm) | Emergence day | Nodules |
|---|---|---|---|---|---|
| AP16 | Full | HG | 25 | 10 | - |
| AP16 | Full | HG | 50 | - | - |
| AP16 | Full | Soil | 25 | - | - |
| AP16 | Full | Soil | 50 | - | - |
| AP16 | Partial | HG | 25 | - | - |
| AP16 | Partial | HG | 50 | 11 | 3 |
| AP16 | Partial | Soil | 25 | - | - |
| AP16 | Partial | Soil | 50 | 11 | 3 |
| AP17 | Full | HG | 25 | 10 | 5 |
| AP17 | Full | HG | 50 | - | - |
| AP17 | Full | Soil | 25 | - | - |
| AP17 | Full | Soil | 50 | - | - |
| AP17 | Partial | HG | 25 | - | - |
| AP17 | Partial | HG | 50 | 8 | 5 |
| AP17 | Partial | Soil | 25 | - | - |
| AP17 | Partial | Soil | 50 | 12 | 3 |
| AP60 | Full | HG | 25 | 10 | 4 |
| AP60 | Full | HG | 50 | - | - |
| AP60 | Full | Soil | 25 | - | - |
| AP60 | Full | Soil | 50 | - | - |
| AP60 | Partial | HG | 25 | - | - |
| AP60 | Partial | HG | 50 | 18 | 2 |
| AP60 | Partial | Soil | 25 | - | - |
| AP60 | Partial | Soil | 50 | 10 | 3 |

This result can be interpreted as that for emergence to occur, seed lots need sowing at 25 mm full backfill for hydrophilic gel or 50 mm sowing depth. For partial backfilling, a seed lot can emerge at a sowing depth of 50 mm for both soil and hydrophilic gel. It also showed that for nodulation to occur a partial backfill at 50 mm sowing depth is recommended. This outcome of the result can be attributed to too much backfilling depth for the seed lot to emerge even if they can germinate.

This may cause the seed lots to rot. In contrast, partial backfilling at 25 mm sowing depth main cause desiccation due to evaporation and high sunlight penetration on the seed lots limiting the emergence for both hydrophilic gel and soil. However, partial backfilling at a sowing depth of 50 mm for both soil and hydrophilic gel can be said to be ideal for the seed lots to emerge with nodulation.

### 4.4.2 Shoot length

The shoot length of the seed lots AP16, AP17 and AP60 did not differ much from each other although, AP60 fully filled with hydrophilic gel had the longest shoot length of the three seed lots (Fig. 4.28).

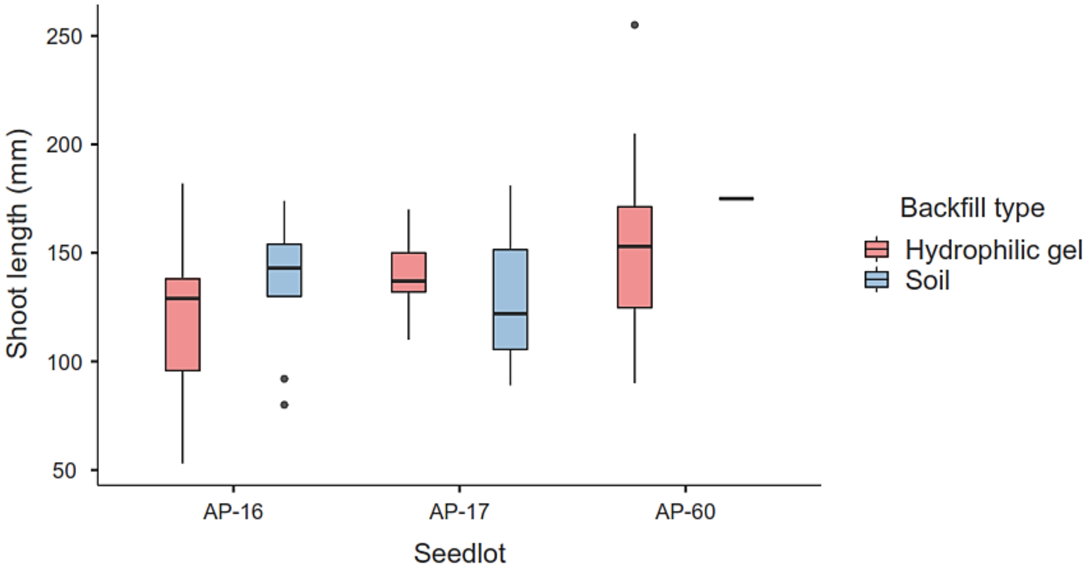

**Figure 4.28.** A Comparison of the effect of fill type on the shoot length AP16, AP17 and AP60. The mean shoot length (mm) of *Elaeagnus* combined for the backfill types: soil and hydrophilic gel. The figure shows means (-) of shoot length and 95% confidence intervals above the box plot of the raw data for each seed lots. The box plot also shows the median and interquartile range

AP60 full quantities of backfill (soil and hydrophilic gel) had the longest shoot length while AP16 had shortest shoot length. Similarly, 25 mm seed depth showed the longest shoot length with AP60 (Fig. 4.29, Fig. 4.30).

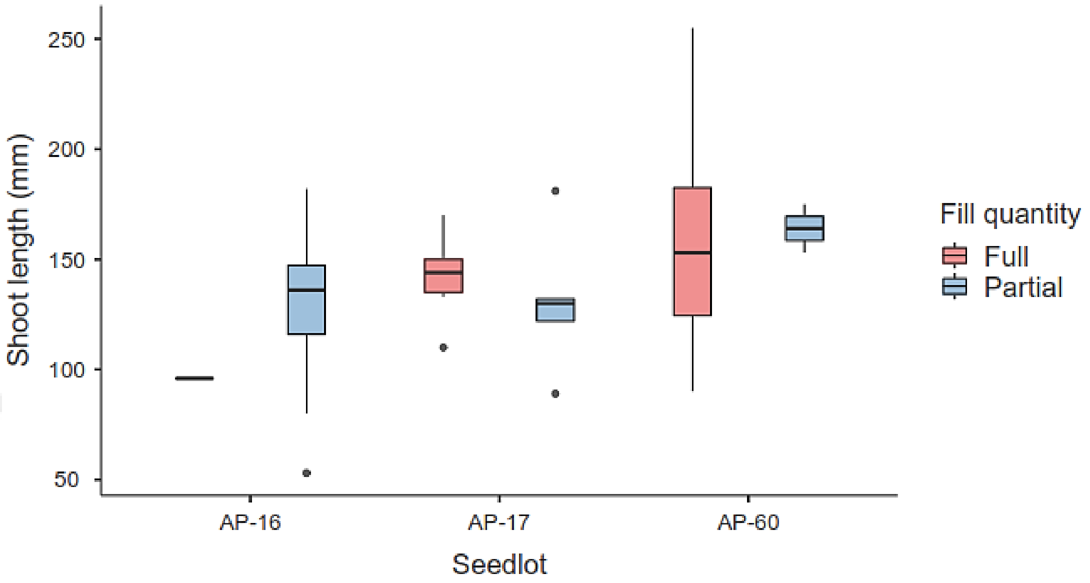

**Figure 4.29.** Comparison of the effect of fill quantity on the shoot length of AP16, AP17 and AP60. The mean shoot length (mm) of *Elaeagnus* combined for the fill quantities: full and partial. The figure shows means (-) of shoot length and 95% confidence intervals above the box plot of the raw data for each seed lots. The box plot also shows the median and interquartile range

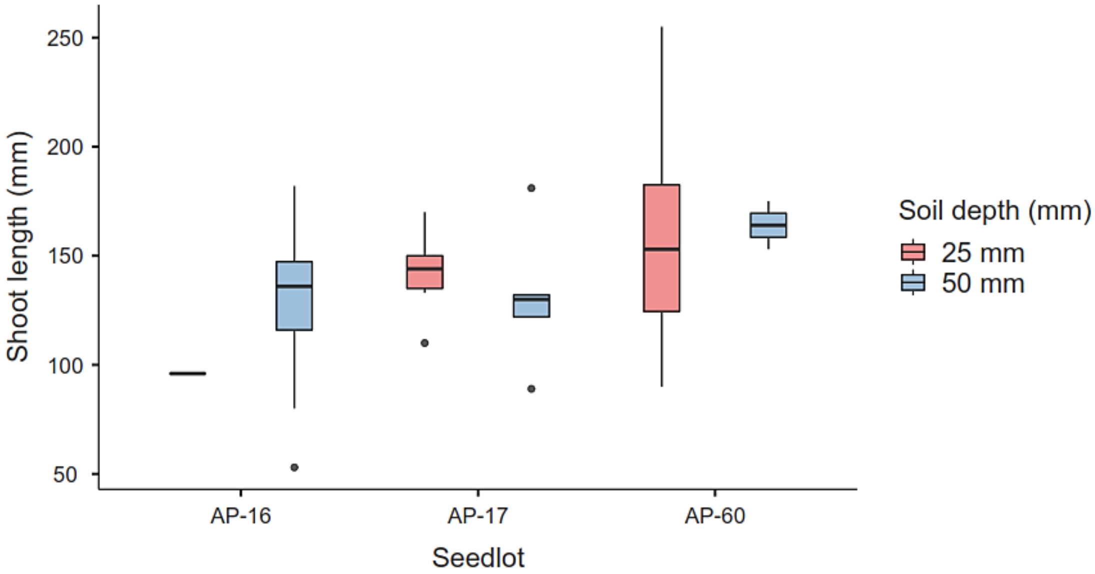

**Figure 4.30.** Comparison of the effect of soil depth on the shoot length of AP16, AP17 and AP60. The mean shoot length (mm) of *Elaeagnus* combined for the soil depth: 25 and 50 mm. The figure shows means (-) of shoot length and 95% confidence intervals above the box plot of the raw data for each seed lots. The box plot also shows the median and interquartile range

### 4.4.3 Root length

AP16 with soil backfill recorded the longest root length among the three seed lots while AP60 with soil backfill had the shortest (Fig. 4.31). In contrast, AP16 with full quantity of backfill had the shortest root length (Fig. 4.32). Similarly, short root length was recorded with AP16 with 25 mm seed depth (Fig. 4.33). This result indicates that seed lot may show different root response depending on backfill type, quantity and soil depth.

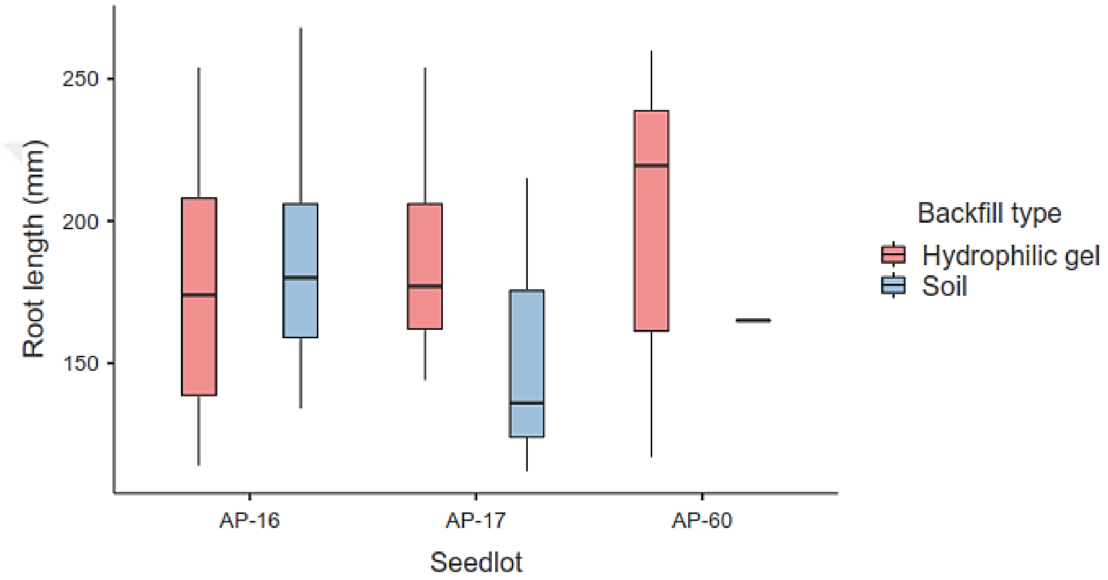

**Figure 4.31.** Comparison of the effect of fill type on the root length of AP16, AP17 and AP60. The mean root length (mm) of *Elaeagnus* combined for the backfill type: hydrophilic gel and soil. The figure shows means (-) of shoot length and 95% confidence intervals above the box plot of the raw data for each seed lots. The box plot also shows the median and interquartile range

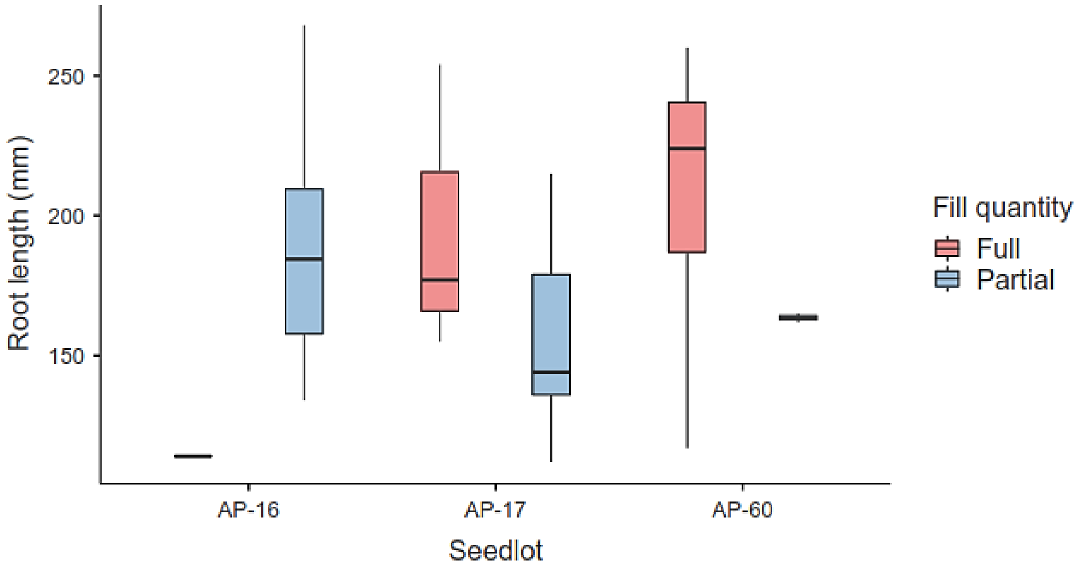

**Figure 4.32.** Comparison of the effect of fill quantity on the root length of AP16, AP17 and AP60. The mean root length (mm) of *Elaeagnus* combined for the fill quantity: full and partial. The figure shows means (-) of shoot length and 95% confidence intervals above the box plot of the raw data for each seed lots. The box plot also shows the median and interquartile range

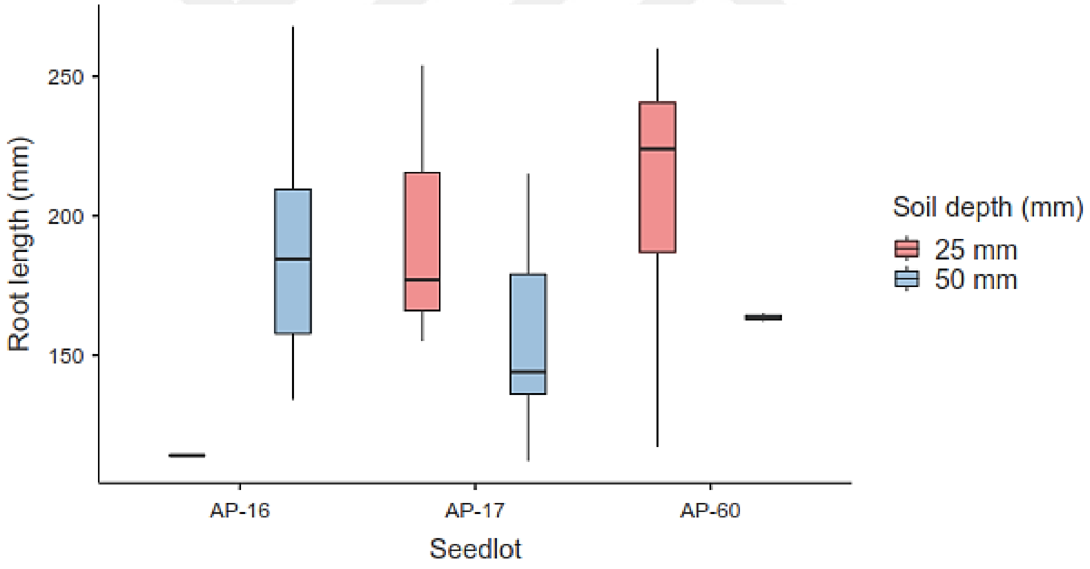

**Figure 4.33.** Comparison of the effect of soil depth on the root length of AP16, AP17 and AP60. The mean root length (mm) of *Elaeagnus* combined for the soil depth: 25 and 50 mm. The figure shows means (-) of shoot length and 95% confidence intervals above the box plot of the raw data for each seed lots. The box plot also shows the median and interquartile range

### 4.5 Greenhouse Experiment 4: *Frankia* Distribution

Nodules were observed in all eleven locations. This indicates the presence of *Frankia* and perhaps a wide distribution of it meaning with Niğde soil, there's no need for

*Frankia* inoculation. The control had no *Frankia* since this was sterilized and it further shows the effectiveness of the sterilization as well as lack of cross-contamination.

The result of the *Frankia* distribution across the study area shows that there were slight differences among the emergence times, shoot length, root length and total length of the seed lots (Table 4.3). The days to emergence range from 5 to 21 days for the 12 sampling locations. For the seed lot growth, shoot length ranges from 156 to 259 mm, root length ranges from 191 to 254 mm while the total length ranges from 368 to 475 mm. This indicates that there was a clear effect between the shoot length, root length and total length for the seed lot with the location. Thus, location has a significant effect on the arial growth and root growth of the seed lots with the location. Apart from one sampling locations, nodules (Fig 4.34) were found to range from 1 to 15.

**Figure 4.34.** *Elaeagnus* nodules observed under the microscope

**Table 4.4.** *Frankia* distribution across the study area

| Soil sampling location | Emergence day | Shoot length (mm) | Root length (mm) | Total length (mm) | Nodules |
|---|---|---|---|---|---|
| Control | 17 | 225 | 215 | 440 | - |
| 1 | 17 | 193 | 222 | 415 | 10 |
| 2 | 16 | 259 | 216 | 475 | - |
| 3 | 15 | 198 | 232 | 430 | 2 |
| 4 | 21 | 161 | 250 | 411 | 3 |
| 5 | 19 | 203 | 254 | 457 | 4 |
| 6 | 22 | 177 | 191 | 368 | 4 |
| 7 | 19 | 192 | 201 | 393 | 11 |
| 8 | 20 | 170 | 227 | 397 | 6 |
| 9 | 19 | 156 | 224 | 380 | 6 |
| 10 | 19 | 202 | 208 | 410 | 4 |
| 11 | 21 | 256 | 211 | 467 | 15 |
| 12 | 18 | 220 | 208 | 428 | 1 |

### 4.6 Field Experiment 1: Backfilling

In this experiment, the days to emergence of the seed lots for the treatments is 115 days whether at 25 or 50 mm sowing depth, full backfill or partial fill. It also shows a very poor emergence rate of which out 100 seeds sowed; the highest number of emergence seedlings were 5. This poor emergence can be attributed to weather conditions at the time of sowing. This experiment was set up during the winter season and by that, the cold stratification affected the emergence time and emergence rate of the seed lot.

**Table 4.5.** Backfilling experimental design

| Treatment | Furrow | Depth (mm) | Seed lot | Fill type | Fill depth | No of seeds | First emergence day | No of emergence |
|---|---|---|---|---|---|---|---|---|
| T1 | 0 | 25 | AP62 | Soil | Full | 100 | 115 | 5 |
| T2 | 0 | 25 | AP63 | Soil | Full | 100 | 115 | 1 |
| T3 | 0 | 50 | AP62 | Soil | Partial | 100 | - | - |
| T4 | 0 | 50 | AP63 | Soil | Partial | 100 | 115 | 2 |
| T5 | 0 | 50 | AP62 | Perlite | Partial | 100 | - | - |
| T6 | 0 | 50 | AP63 | Perlite | Partial | 100 | 115 | 2 |
| T7 | 0 | 50 | AP62 | Gel | Partial | 100 | 115 | 5 |
| T8 | 0 | 50 | AP63 | Gel | Partial | 100 | 115 | 2 |
| T9 | 1 | 25 | AP62 | Soil | Full | 100 | 115 | 1 |
| T10 | 1 | 25 | AP63 | Soil | Full | 100 | 115 | 1 |

Table 4.4 showed that AP62 had a higher germination ratio at 25 mm sowing depth for the furrow than AP63. For the no furrow, there are no differences between the AP62 and AP63. At a sowing depth of 50 mm, the furrow had a higher germination rate comparably while the no furrow had no germination. This can be said that furrow increases the germination rate of the seed lot than the no furrow. Also, there are no differences in germination between the seed lots.

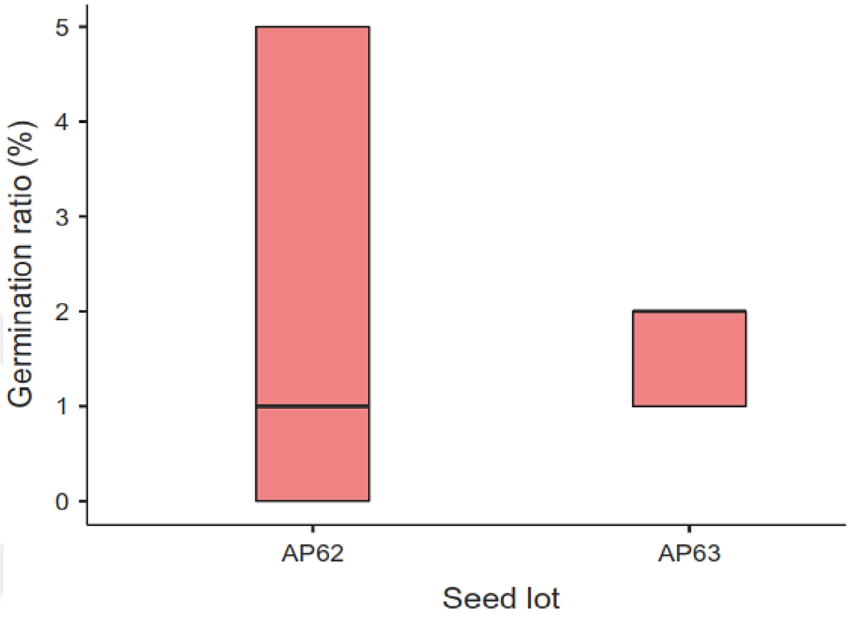

**Figure 4.35.** Germination ratio of AP62 and AP63. The germination ratio (%) of *Elaeagnus* combined for the combined seed lots showed AP62 had better germination. The box plot also shows the median and interquartile range

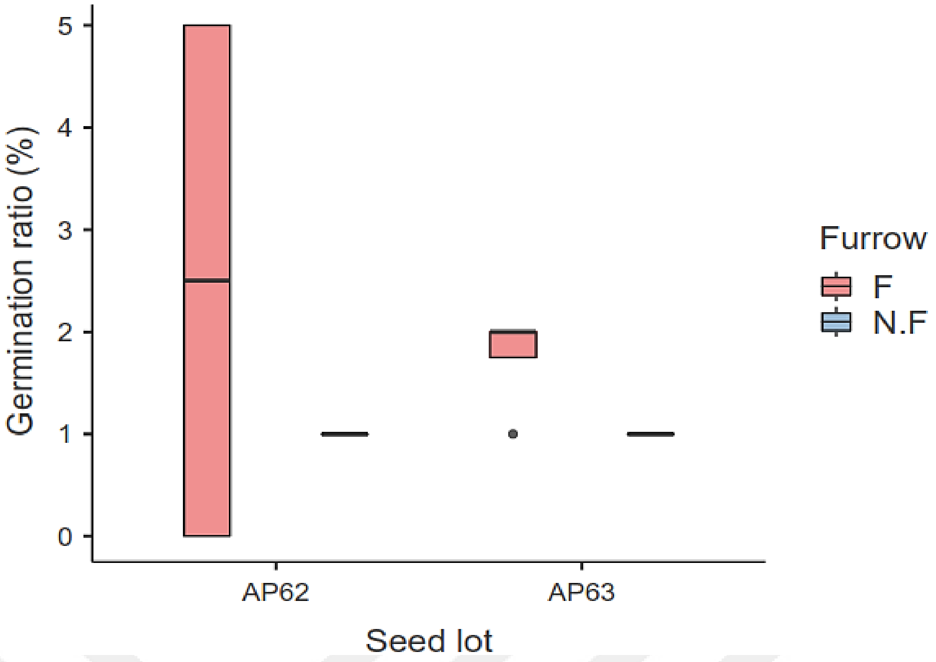

**Figure 4.21.** Effects of furrow on germination ratio for AP62 and AP63. The germination ratio (%) of *Elaeagnus* combined for the field sowing showed AP62 with furrow had better germination. The box plot also shows the median and interquartile range

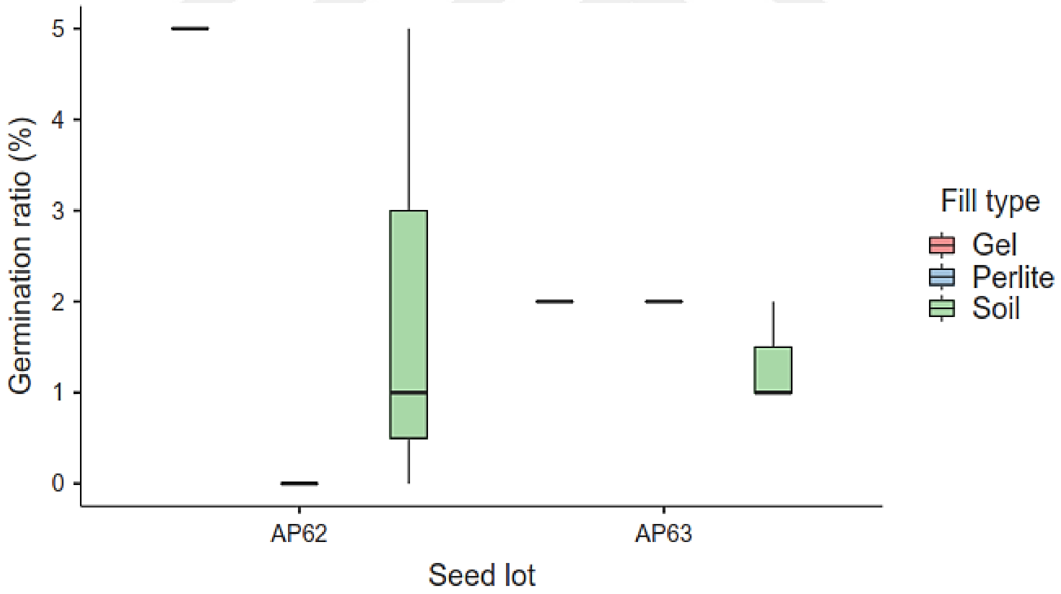

**Figure 4.22.** Effect of fill type on germination ratio of AP62 and AP63. The germination ratio (%) of *Elaeagnus* combined for the fill types: hydrophilic gel, perlite and soil. The box plot also shows the median and interquartile range

There was a significant difference between the germination ratio for the furrow and non-furrow at 25 mm sowing depth between the AP62 and AP63 (Fig. 4.37, Fig. 4.38). At a sowing depth of 50 mm, the gel had a higher germination ratio while there were no differences between the soil and perlite (Fig. 4.37).

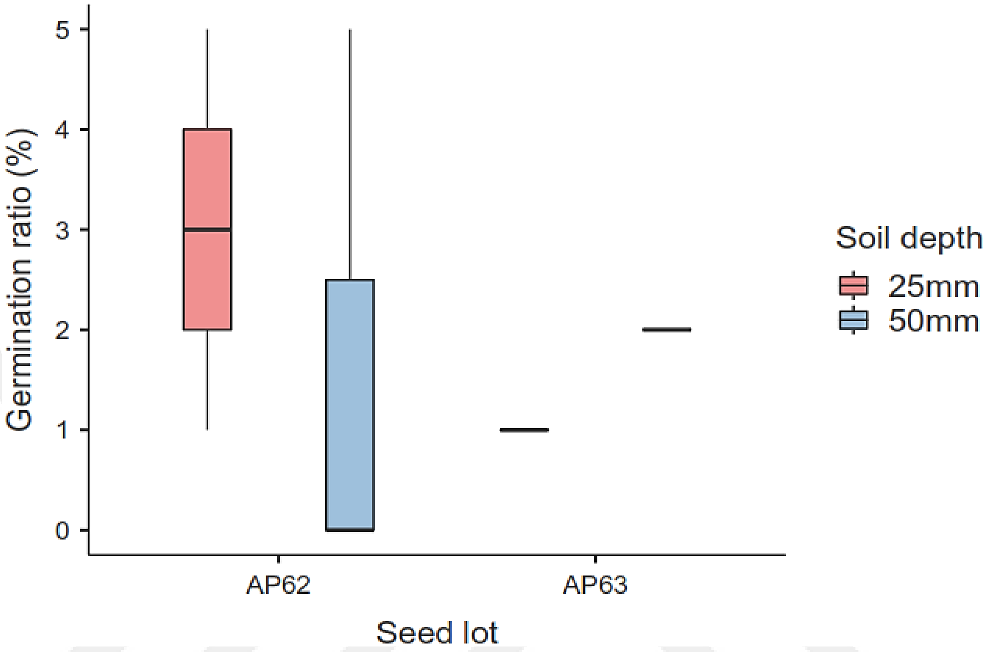

**Figure 4.23.** Effect of soil depth on germination ratio of AP62 and AP63. The germination ratio (%) of *Elaeagnus* for the soil depth: 25 and 50 mm showed that AP62 in both soil depth had higher germination ratio. The box plot also shows the median and interquartile range

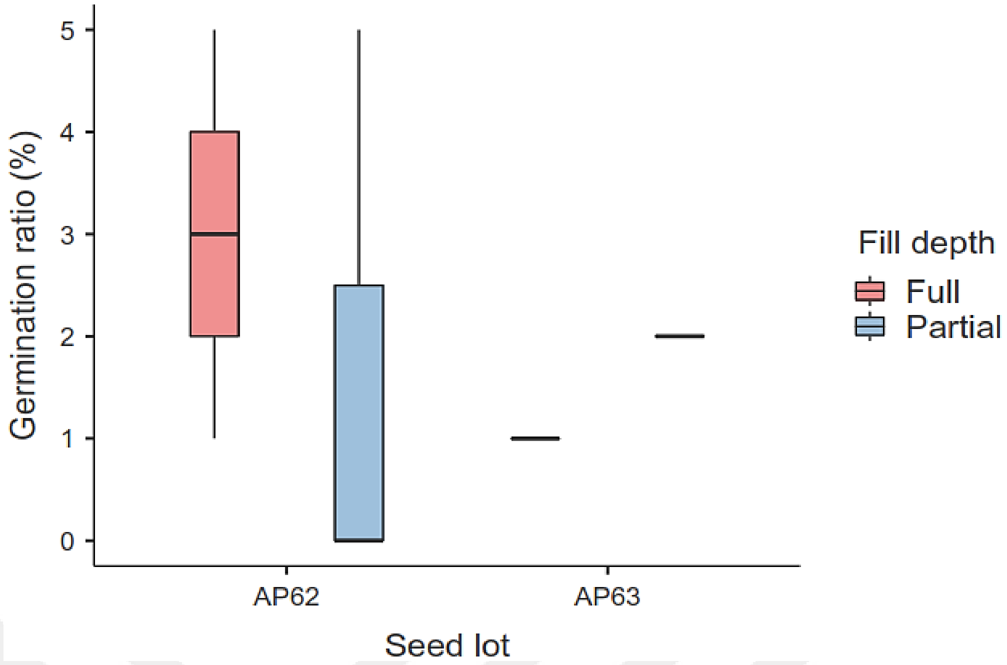

**Figure 4.24.** Effect of fill depth on germination ratio of AP62 and AP63. The germination ratio (%) of *Elaeagnus* for the fill depths: full and partial showed that AP62 had higher germination with both fill depth. The box plot also shows the median and interquartile range

## 4.7 Field Experiment 2: Pulp

Table 4.5 shows the sowing depth, seed lot. Pulp and fill depth does not affect the emergence date. The difference in the number of emergences ranges from 0 to 14 number. This indicated that there are differences in the germination ratio of the seed lots AP62, AP63 and AP64 (Fig. 4.39). Comparing the pulp and no pulp seed lots, pulp increase the rate of AP62, AP63 and AP64 seed lots germination (Fig. 4.40). Also, the results showed that fill type (Fig. 4.41) and sowing depth (Fig. 4.42) significantly affected the rate of AP62, AP63 and AP64 seed lots germination.

**Table 4.6.** Pulp experimental design

| Treatment | Depth (mm) | Seed lot | Pulp | Fill depth | No of seeds | First emergence day | No of emergence |
|---|---|---|---|---|---|---|---|
| T1 | 25 | AP62 | 1 | Full | 80 | 116 | 14 |
| T2 | 25 | AP63 | 1 | Full | 80 | 116 | 8 |
| T3 | 25 | AP64 | 1 | Full | 80 | 116 | 8 |
| T4 | 25 | AP62 | 0 | Full | 80 | 116 | 6 |
| T5 | 25 | AP63 | 0 | Full | 80 | 116 | 3 |
| T6 | 25 | AP64 | 0 | Full | 80 | 116 | 5 |
| T7 | 50 | AP62 | 1 | Partial | 80 | 116 | 2 |
| T8 | 50 | AP63 | 1 | Partial | 80 | - | - |
| T9 | 50 | AP64 | 1 | Partial | 80 | 116 | 4 |
| T10 | 50 | AP62 | 0 | Partial | 80 | - | - |
| T11 | 50 | AP63 | 0 | Partial | 80 | - | - |
| T12 | 50 | AP64 | 0 | Partial | 80 | 116 | 1 |

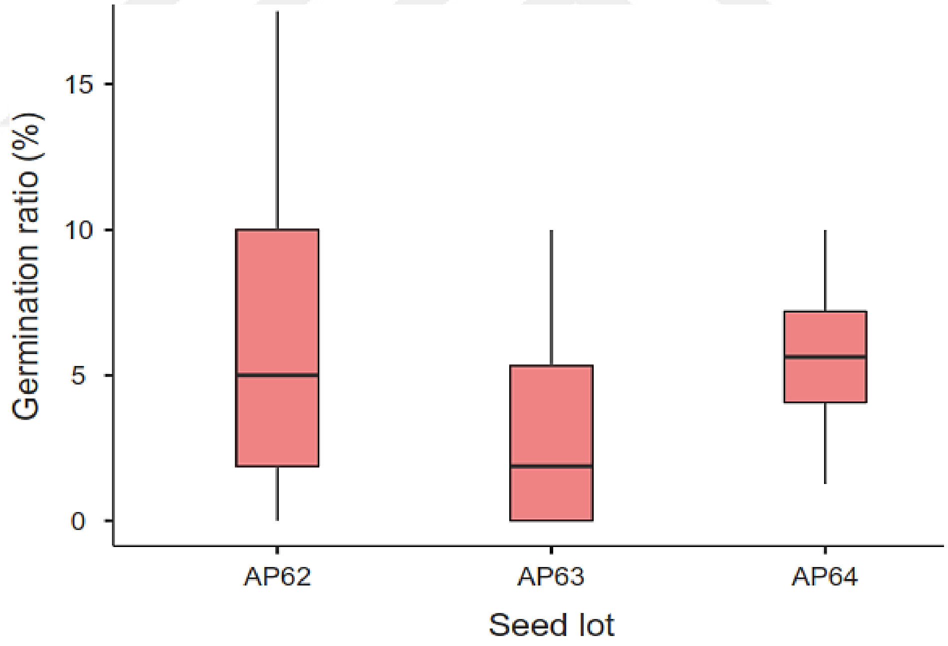

**Figure 4.25.** Germination ratio of AP62, AP63 and AP64. The germination ratio (%) *Elaeagnus* generally was highest in AP62 this field experiment. The box plot also shows the median and interquartile range

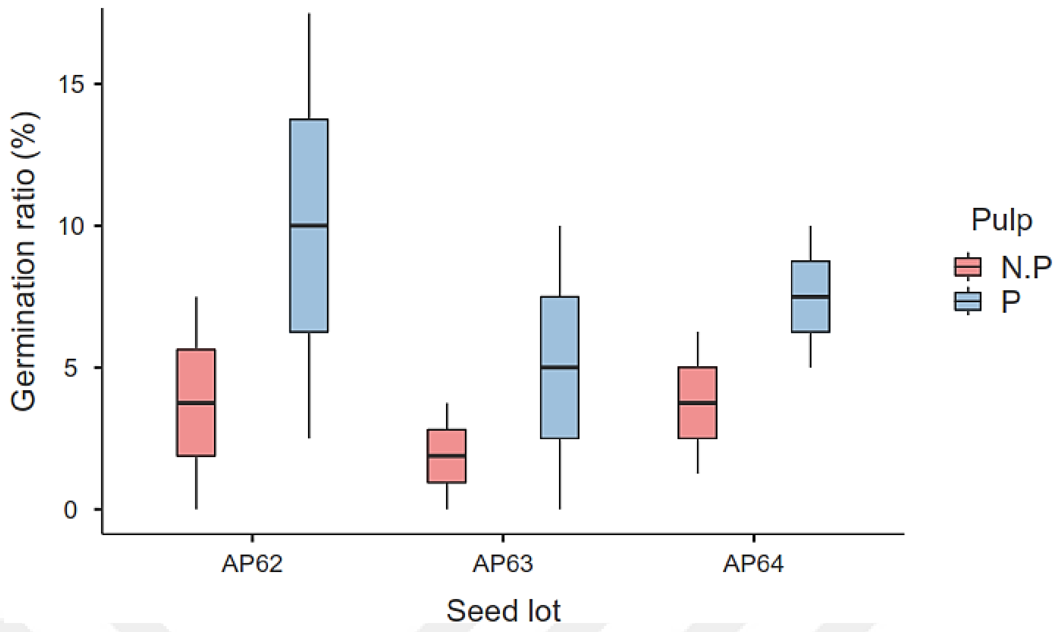

**Figure 4.26.** Effect of pulp on the germination ratio of AP62, AP63 and AP64. The germination ratio of AP62 with pulp had the highest germination ratio. The box plot also shows the median and interquartile range

AP62, AP63 and AP64 had the highest germination ratio with full backfilling depth as compared to seed lots with partial fill depth (Fig. 4.41) with highest germination occurring in AP62 compared with the AP63 and AP64. Soil depth also revealed that 25 mm soil depth generally recorded the highest germination ratio more than the 50 mm soil depth, with the highest germination observed in AP62 more than AP63 and AP64 (Fig. 4.42).

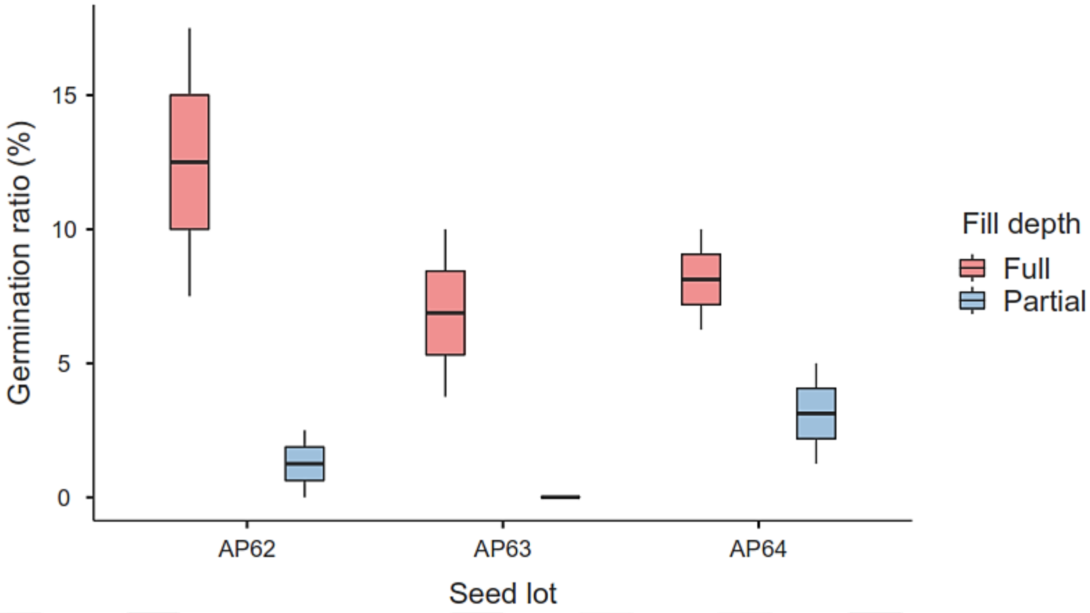

**Figure 4.27.** Effect of fill depth on germination ratio of AP62, AP63 and AP64. The germination ratio of fully filled depth were highest in the three seed lots. The box plot also shows the median and interquartile range

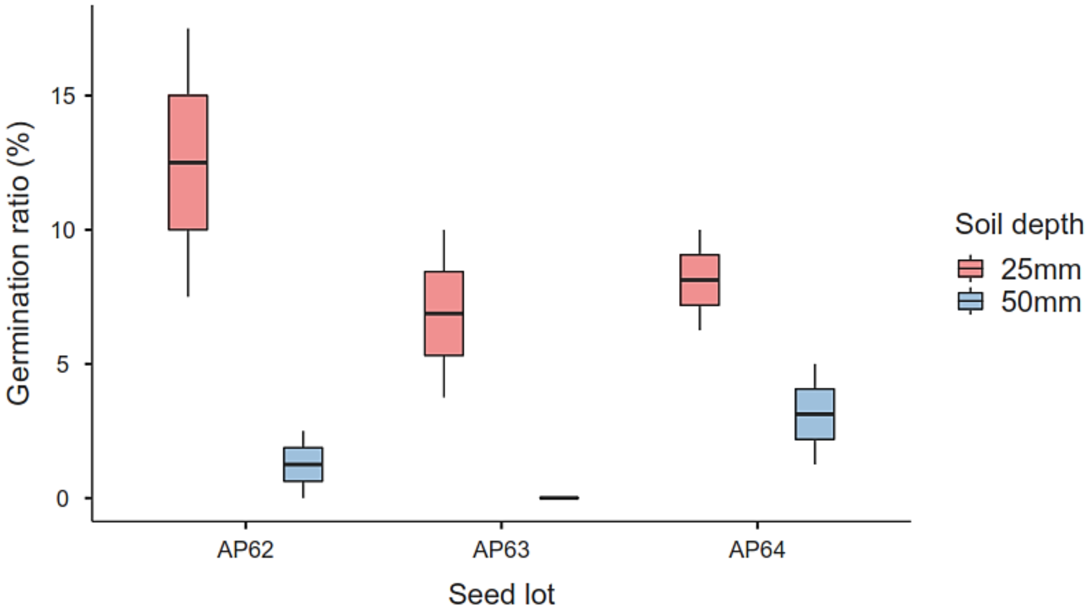

**Figure 4.28.** Effect of soil depth on germination ratio of AP62, AP63 and AP64. The germination ratio was highest for the three seed lots in 25 mm soil depth. The box plot also shows the median and interquartile range

# CHAPTER V

# DISCUSSION

## 5.1 Germination

Factors such as seed viability, sowing depth, soil properties and water availability to the plant are important to the seed emergence time and germination. In this study, sowing depths and water regimes were conducted to assess their effects on the germination rate of *Elaeagnus angustifolia* L. There was 100% mean seed germination was recorded at a sowing depth of 100, 50 and 25 mm for the top water regime (Fig. 4.1) and 150 mm sowing depth for the bottom water regime (Fig. 4.2). However, an above 60% mean seed germination rate was observed for top water at 150 mm sowing depth while there was generally a low mean seed germination rate for the no water regime below 50% at sowing depth of 150 mm, below 40% at sowing depth of 100 mm and below 20% at sowing depth of 25 mm and 50 mm.

The high % mean seed germination rate for the top water regime and the 150 mm for the bottom water can be due to the availability of the optimum moisture for the seeds to germinate. The seeds for top water up till 100 mm sowing depth receives the optimum water for seeds to germinate. Also, for the bottom water regime in the greenhouse, the seeds at 150 mm sowing depth got enough water compared to 25, 50 and 100 mm sowing depths. Also, the water for the bottom water regime travel against gravity while the top water regime travels along with gravity. This could explain the contrast in the germination rates as top water had 100% mean seed germination for 25, 50 and 100 mm sowing depths whereas for the bottom water regime, only 150 mm sowing depth recorded 100% mean seed germination. Also, the very low % mean seed germination of *E. angustifolia* for the no water regime treatment depicts the significant water requirement for optimum seed germination to occur. Though the seed collection or sourcing point was not assessed in this study, the variation in germination rate might be associated with their differential seed response to water.

Research has it that tree plant species respond and adapt differently to water availability and that insufficient moisture below the critical level causes changes in cell structure leading to the death of plants. Shaban (2013) stated that seed germination rate is determined by water availability been an important germination parameter. Fredrick et al., (2019) in a study of the effect of water regimes on the germination and early seedling growth of *Annona muricata* stated that seed germination was significantly affected by water regimes. Asgharipour (2011) revealed that sowing depth affects seed germination and emergence in tree plants which were associated with secondary dormancy induction in the seeds, hardening of the gas exchange sowing depth and the energy stored in the seed. Hybner et al., (2014) on the effect of seed burial depth on seedling emergence and seed viability of *E. angustifolia*.

George (2017) stated that seed germination and emergence rates are reduced with increasing sowing depth, as the seedling emergence increase with deeper sowing depths. The finding of this research agrees with the assertion of George (2017) as a sowing depth of 25 mm recorded the faster mean seed emergence compared to 50 mm. Growth of plant is characterized severally by root and shoot length, leaves number and size among others. The shoot growth of the *E. angustifolia* in this research, bottom water recorded the highest mean shoot length at 25 mm sowing depth followed by sowing depth of 50 mm. whiles the least shoot length was recorded by top water regimes. This is to say that, generally, bottom water in this research is presented as the best water regime for shoot length of the *E. angustifolia*.

It also implies that the different sowing depth influences the shoot length of the plants at a top water regime as revealed by the current research. This corresponding effect for the shoot growth rate of *E. angustifolia* for the top water regimes of 2.5 mm/d at a sowing depth of 25 mm while the lowest shoot growth rate of 1.0 mm/d was recorded for 50 mm sowing depth. On the contrary, it can be said that the bottom water regime does not affect the shoot growth rate as all sowing depths recorded almost the same shoot growth rate of 2.5 mm/d.

## 5.2 Effect of the Soil Backfilling on the Growth and Growth Parameters

It has been hypothesized that soil backfilling influences the growth parameters of plants. In contrast, this research showed that backfilling has no effect on the root length of each seed lot. Among all the different accessions of the *E. angustifolia*, AP17 generally recorded the shortest mean root length while AP16 recorded the highest mean root length occurring at a sowing depth of 50 mm using whole seeds backfılled wıth peat as against all other forms of backfilling and sowing depths.

For the cut seeds backfilled, AP17 recorded the longest root length more than AP16 and AP60. It can be said that the source of soil used in the backfilling and the form of seed lot whether cut seed lot or uncut seed lot have a told on the root length of the *E. angustifolia*. AP16 had the longest root length of about 100 mm in Niğde soil at 25 mm sowing depth for both cut or uncut seed lots as against AP60 accession which recorded the least root length. However, AP16 recorded the highest mean root length of over 100 mm using uncut seeds backfilled with Niğde soil at sowing depth of 25 mm.

The differences in the root length of *E. angustifolia* with either cut or uncut backfilling of the different accessions can be attributed to the difference in the parameters of Niğde soil and the peat-perlite mixture. Hechmi et al. (2012) in a study to investigate the rooting establishment of olive plantation stated that substrates (sand, peat, moss and perlite) and IBA treatment (at 0 and 4000 ppm) had a significant effect on the rooting ability of three olive cultivars (Arbequina, Koroneiki and Picual).

Niğde soil had the highest mean root length in either the cut or uncut seed lots in contrast to Hechmi et al. (2013) who reported perlite to have highest root number and root length of all accessions of olives studied. This disparity can be due to soil physical and chemical parameters and the different agroclimatic location of the studies.

The total seedling was affected by treatment and cultivars in this research. Overall, the mean total seedling length of 140 mm was recorded by AP17 with the highest mean seedling length occurring in AP17 whiles the lowest mean seedling length occurring in AP60. This was found for both cut and uncut seed lots at 50 mm sowing depth using perlite and Niğde soil backfilling at 25 mm sowing depth. For shoot length, though AP16 the mean shoot length as compared to AP17 and AP60, there was no significant effect of the different soil backfilling. This indicates that backfilling has no effect on the shoot length of the *E. angustifolia* plant. Different soil backfilling and sowing depth influenced the emergence time of the *E. angustifolia*. The emergence time ranges from AP17 (25 days) to AP60 (35 days). The differences in the emergence time might be due to seed vigour, viability and their climatic response. For the emergence, there was clear significant difference among the treatment. However, there was no emergence of AP60 while the AP17 had more than 60% emergence.

Some studies have reported that dormancy stage varies within the same species and individual tree species and the year of the plant (Olson et al., 2004; Ölmez et al., 2007). This affects the germination rate of the seed lots collected at difference location. The seeds that failed to germinate later get rotten. In contrast to this claim, there no significant rotten in the present study though some individual cases may record seed lots rotten with some few outliers for both whole seeds backfilled with peat or cut seed backfilled with perlite.

In this experiment, there were differences in percent germination between seed lots and treatments (either cut or whole seed lot and backfilled with or perlite) which ranges from AP16 (collected from near to D330 Aksaray-Niğde highway, 12 December 2018) (20%) to AP60 (purchased as commercial from Enderbey Market in Niğde) (80%). This indicated that, the germination of the seed lot was greatly affected by the sowing depth and seed lot treatment (cut seed or whole seed) and soil type backfilling. The different seed lots germinate differently in different soil type. It was observed that AP60 recorded the highest mean germination when uncut seeds backfilled with Niğde soil at sowing depth of 25 mm. AP60 had the highest mean germination using cut seeds backfilled with Niğde soil at sowing depth of 50 mm. this indicates that uncut seeds backfilled with Niğde soil at sowing depth of 25 mm and cut seeds backfilled with Niğde soil at sowing depth of 50 mm had the highest mean germinated of over.

As revealed in this that irrespective of the seed treatment and soil type, there was no significant difference between in total seedling length. This reflects that after germination the rate of seedling growth was the same and that the seed lots may have similar seed vigour.

### 5.3 Hydrophilic Gel Backfilling

This result can be interpreted as that for emergence to occur, seed lots needs sowed at 25 mm full backfill for hydrophilic gel or 50 mm sowing depth. For partial backfilling, seed lot can emerge at a sowing depth of 50 mm for both soil and hydrophilic gel. It also showed that for nodulation to occur a partial backfill at 50 mm sowing depth is recommended. This outcome of the result can be attributed to too much backfilling depth for the seed lot to emerge even if they are able to germinate. This may cause the seed lots to rot. In contrast, partial backfilling at 25 mm sowing depth main cause desiccation due to evaporation and high sunlight penetration on the seed lots making them not to emerge for both hydrophilic gel and soil. However, partial backfilling at a sowing depth of 50 mm for both soil and hydrophilic gel was best for the seed lots to emerge with nodulation.

The low shoot length of all the seed lots (AP16, AP17 and AP60) for the full backfill of the soil indicates that, there was no emergence of the seed lots for the soil fully filled which can be due to too much soil that accumulated heat beyond the bearable threshold leading to rotten of the seed lots. For emerged seed lots the results can be interpreted that fill type has no significant effect on the shoot length of the seed lots once emergence has occurred (Fig. 4.28). The root lengths of the seed lots have similar response to the partial fill or hydrophilic gel fill type. For the hydrophilic gel fill type, the fully filled of AP16 recorded the lowest which was clearly different from the AP17 and AP60 with the AP60 recording the longest root length followed by the AP17. Contrarily for the partial fill, of the hydrophilic gel fill type, AP16 recorded the longest root length while AP17 and AP60 recording similar root length. This is an indication that different seed lot have response to hydrophilic gel fill type.

## 5.4 Effect of Furrow, Sowing Depth and Fill Type on the Seed Lots Growth and Growth Parameters

Fig. 4. showed that AP62 had a higher germination ratio at 25 mm sowing depth for the furrow than AP63. For the no furrow, there is no difference between the AP62 and AP63. At a sowing depth of 50 mm, furrow had higher germination rate comparably while the no furrow had 0 germination rate. This can be said that furrow clearly increase the germination rate of the seed lot than the no furrow. Also, there is no difference in germination between the seed lot. Indicating that, the seed lots have similar viability rates and seed vigour. This differences in the germination can be explained that, full backfill at 50 mm sowing depth had too much load of soil fill on the seed that prevented

it from germinating while a fully filled at 25 mm sowing depth was optimum for germination to occur. For the partial backfill, 25 mm sowing depth exposes the seeds to heat and evaporation that leads the seed to dry out and get desiccated.

Several research findings stated that, in the tropical and high temperature regions of the temperate climatic zones, shallow sowing depth exposes the seeds or planting materials to evaporation and seed desiccation. This mostly results in no germination leading poor establishment. Several research findings stated that, in the tropical and high temperature regions of the temperate climatic zones, shallow sowing depth exposes the seeds or planting materials to evaporation and seed desiccation. This mostly results in no germination leading poor establishment. The days to emergence of the seed lots for the treatments is 115 days whether at 25 or 50 mm sowing depth, full backfill or partial fill. It also shows a very poor emergence rate of which out 100 seeds sowed; the highest number of emergence seedling was 5. This poor emergence can be attributed to weather conditions at the time of sowing. This experiment was set up during the winter season and by that, the cold stratification affected the emergence time and emergence rate of the seed lot. Fig. 4. showed that AP62 had a higher germination ratio at 25 mm sowing depth for the furrow than AP63. For the no furrow, there is no differences between the AP62 and AP63. At a sowing depth of 50 mm, furrow had higher germination rate comparably while the no furrow had 0 germination rate. This can be said that furrow clearly increase the germination rate of the seed lot than the no furrow. Also, there is no differences in germination between the seed lot. Indicating that, the seed lots have similar viability rates and seed vigour.

### 5.4.1 Effect of Pulp on the Germination and Emergence of Seed Lots

Pulp experiment shows that, apart from T8 (AP63 partial pulp fill), T10 (AP62 partial fill with no pulp and T11 (AP63 partial with pulp) which occurred at 50 mm sowing depth, all other treatment has seed emergence that emerged on 116 days after sowing. This indicated that sowing depth of 50 mm causes seeds not to germinate. The delay in the days to emergence of 116 days after sowing could mainly be blamed on the effect of the cold weather (winter) during the sowing period which affected the degree day energy accumulation of the seeds, this affected the seed variability and the number of emergences. Out of the 80 seed lots for each treatment, only about 17.5% or below seed emerged. Also, though there were low emergences, there was significant variation in the emergence which variably ranged from 1 to 14 (Table 4.7).

Generally, the application of pulp increased the germination of the seed lots irrespective of the sowing depth. Also, sowing depth affect the germination of seed lots. There was greater germination ratio of the seed lots at 25 mm sowing depth whether with or without pulp, which is further enhanced with the application of pulp. It is stated that, pulp helps to enhance the rate of seed germination as it conserves moisture and optimum conditions for the seeds (Guilbault et al., 2012). Indicating that pulp around seeds generally decreased total germination and increased mean time to germination while de-pulped seed germinate faster compared with pulped fruits (Guilbault et al., 2012).

## 5.5 *Frankia* Distribution

As a native plant in Turkey, *E. angustifolia* freely nodulate in Niğde soils and this is an indication of the presence of a compatible *Frankia* strain. Also, it is established that highly compatible *Frankia* strains are present in soils of sites where actinorhizal species occur naturally or have been grown for an extended period and a possible reason is the co-evolution theory (Lie et al., 1987). Several *Frankia* strains can survive for prolonged periods of time in soil (Smolander and Sarsa, 1990; Sprent and Parsons, 2000).

According to greenhouse experiment 4, there is no need for *Frankia* inoculation. Because Niğde soil already has *Frankia* for nodulation. At the control line, *Frankia* was not found, so that's mean the experiment was successful against contamination. In the other treatments, they already have *Frankia* and nodulation observation.

The result of the *Frankia* distribution across the study area shows that, there was a slight difference among the emergence times, shoot length, root length and total length of the seed lots. The days to emergence ranges from 5 to 21 days for the 12 sampling locations. For the seed lot growth, shoot length ranges from 156 to 259 mm, root length ranges from 191 to 254 mm while the total length ranges from 368 to 475 mm. This indicates that there was a clear effect between the shoot length, root length and total length for the seed lot with location. Thus, location has a significant effect on the arial growth and root growth of the seed lots with location.

# CHAPTER VI

# CONCLUSIONS

Maintaining ecological balance is an important consideration for agroforestry and revegetation plants. Actinorhizal plants such as *Elaeagnus angustifolia* have benefits and the potentials to improve agroforestry and agroecosystem. This study was hence conducted to assess the agroecosystem improvement and revegetation potentials of *E. angustifolia* in the Central Anatolia region of Turkey with direct establishment methods.

All six accession of *E. angustifolia* used in this research can be categorized based on the seed characterization into large and small seeds. Top water regimes can facilitate germination up to sowing depth of 100 mm while bottom water regime facilitate germinate beyond 100 mm sowing depth of *E. angustifolia*. Also, when top water regime is appropriate for *E. angustifolia* sowing depth up to 100 mm for maximum germination to occur. However, when sowing is more than 100 mm, bottom sowing depth is appropriate for maximum seed lot germination.

For high percentage emergence, the bottom water regime is the ideal water regime. For maximum shoot length and fast shoot growth rate, bottom water at sowing depth of 25 to 50 mm was found to be optimum. Uncut *E. angustifolia* (small seeds) collected from near to D330 Aksaray-Niğde highway had the longest root length of more than 100 mm which was found in backfilled Niğde soil at a sowing depth of 25 mm while cutting another small seed accession, collected from the campus lake area gave the longest root length of over 100 mm when planted with Niğde soil.

Hydrophilic gel backfilling and furrow increase the seed lot germination and shoot and root growth and length. *Frankia* was found to be widely distributed across away from existing trees. Because *Frankia* was found everywhere around Niğde Province, there is no need to inoculate for nodulation.

Direct sowing of *E. angustifolia* in the field reduces germination and emergence rate. Indirect establishment of *E. angustifolia* using nurse plants and seedlings grown in the greenhouse remains the viable option of *E. angustifolia* establishment in Niğde Province, Turkey.

Direct establishment of *E. angustifolia* is not an ideal propagation method in Niğde soil and this may be attributable to limiting factors such as temperature, water and soil quality. Further studies need to be conducted to understand how the hydrophilic gel backfilling and sowing depth can be optimized for direct seed propagation on the field.

# REFERENCES

Abelho, M. and Molles, M., “Effect of introduced exotic tree litter on consumption patterns of the introduced exotic isopod *Armadillidium vulgare*”, ***European Journal of Soil Biology*** 45, 306-311, 2009.

Asgarzadeh, M., Vahdati, K., Lotfi, M., Arab, M., Babaei, A., Naderi, F. and Rouhani, G., “Plant selection method for urban landscapes of semi-arid cities (a case study of Tehran)”, ***Urban Forestry & Urban Greening*** 13, 450-458, 2014.

Baker, D. and O’Keefe, D., “A modified sucrose fractionation procedure for the isolation of *Frankia*e from actinorhizal root nodules and soil samples. In *Frankia Symbioses*”, ***Springer, Dordrecht***, 23-28, 1984.

Bartha, D. and Csiszár, A., “Russian olive *(Elaeagnus angustifolia* L.). The most important invasive plants in Hungary, Institute of Ecology and Botany”, ***Hungarian Academy of Sciences***, 85-93, 2008.

Benson, D.R., “Isolation of *Frankia* strains from alder actinorhizal root nodules”, ***Applied and Environmental Microbiology*** 44, 461-465, 1982.

Benson, D. R. and Silvester, W.B., “Biology of *Frankia* strains, actinomycete symbionts of actinorhizal plants”, ***Microbiological Reviews*** 57, 293-319, 1993.

Bertrand, L.J. and Lalonde, M., “*In vitro* propagation and nodulation by *Frankia* of actinorhizal Russian Olive (*Elaeagnus angustifolia* L.)”, ***Plant and Soil*** 87, 143-152, 1985.

Blanco-García, A., Sáenz-Romero, C., Martorell, C., Alvarado-Sosa, P. and Lindig-Cisneros, R., “Nurse-plant and mulching effects on three conifer species in a Mexican temperate forest”, ***Ecological Engineering*** 37, 994-998, 2011.

Borell, A.E., “Russian-olive for wildlife and other conservation uses”, ***Leaflet - United States Department of Agriculture Washington: U.S. G.P.O***, 517, 1971.

Campbell, C.J. and Dick-Peddie, W.A., “Comparison of phreatophyte communities on the Rio Grande in New Mexico”, ***Ecology*** 45, 492-502, 1964.

Castro, J., Zamora, R., Hódar, J.A. and Gómez, J.M., “Use of shrubs as nurse plants: a new technique for reforestation in Mediterranean mountains”, ***Restoration Ecology*** 10, 297-305, 2002.

Chang, W., Sui, X., Fan, X.X., Jia, T.T. and Song, F.Q., “Arbuscular mycorrhizal symbiosis modulates antioxidant response and ion distribution in salt stressed *Elaeagnus angustifolia* seedlings”, ***Frontiers in Microbiology*** 9, 652, 2018.

Clawson, M.L., Carú, M. and Benson, D.R., “Diversity of *Frankia* strains in root nodules of plants from the families Elaeagnaceae and Rhamnaceae”, ***Applied and Environmental Microbiology*** 64, 3539-3543, 1998.

Collette, L.K. and Pither, J., “Russian olive biology and ecology and its potential to invade northern North American riparian ecosystems”, ***Invasive Plant Science and Management*** 8, 1-14, 2015.

Chalker-Scott, L., “Impact of mulches on landscape plants and the environment-a review”, ***Journal of Environmental Horticulture*** 25, 239-249, 2007.

Chaudhry, M.R., Malik, A.A. and Sidhu, M., “Mulching impact on moisture conservation-soil properties and plant growth”, ***Pakistan Journal of Water Resources*** 8, 1-8, 2004.

Christensen, E.M., “Naturalization of Russian olive (*Elaeagnus angustifolia* L.) in Utah”, ***American Midland Naturalist***, 133-137, 1963.

Chytrý, M., Maskell, L.C., Pino, J., Pyšek, P., Vilà, M., Font, X. and Smart, S.M., “Habitat invasions by alien plants: a quantitative comparison among Mediterranean, subcontinental, and oceanic regions of Europe", ***Journal of Applied Ecology*** 45, 448-458, 2008.

Cierjacks, A., Kowarik, I., Joshi, J., Hempel, S., Ristow, M., von der Lippe, M. and Weber, E., "Biological flora of the British Isles: Robinia pseudoacacia", ***Journal of ecology*** 101, 1623-1640, 2013.

Currier, P.J., The floodplain vegetation of the Platte River: phytosociology, forest development, and seedling establishment, Ph.D. thesis, Iowa State University, Ames. 1982.

Daehler, C.C., "Performance comparisons of co-occurring native and alien invasive plants: implications for conservation and restoration", ***Annual Review of Ecology, Evolution, and Systematics*** 34, 183-211. 2003.

DeCant, J.P. "Russian olive, *Elaeagnus angustifolia*, alters patterns in soil nitrogen pools along the Rio Grande River, New Mexico", ***USA Wetlands*** 28, 896-904, 2008.

Dubovyk, O.; Landmann, T.; Dietz, A.; Menz, G., "Quantifying the Impacts of Environmental Factors on Vegetation Dynamics over Climatic and Management Gradients of Central Asia", ***Remote Sens.*** 8, 600, 2016.

Franco, A. and Nobel, P.S., "Effect of nurse plants on the microhabitat and growth of cacti", ***The Journal of Ecology***, 870-886, 1989.

George, E.J., "Tree and shrub species for the Northern Great Plains (No. 912)", ***US Department of Agriculture*** 46, 4566, 1951.

Ghodhbane-Gtari, F., Essoussi, I., Chattaoui, M., Chouaia, B., Jaouani, A., Daffonchio, D. and Gtari, M., "Isolation and characterization of non-*Frankia* actinobacteria from root nodules of *Alnus glutinosa*, *Casuarina glauca* and *Elaeagnus angustifolia*", ***Symbiosis*** 50, 51-57, 2010.

Gómez-Aparicio, L., Zamora, R., Gómez, J.M., Hódar, J.A., Castro, J. and Baraza, E., "Applying plant facilitation to forest restoration: a meta- analysis of the use of shrubs as nurse plants", ***Ecological applications*** 14, 1128-1138, 2004.

González-Muñoz, N., Castro-Díez, P and Fierro-Brunnenmeister, N., "Establishment success of coexisting native and exotic trees under an experimental gradient of irradiance and soil moisture", ***Environmental Management*** 48, 764-773, 2011.

Göktürk A., Ölmez Z., Temel F. and Yahyaoğlu Z., "Bazı Önişlemlerin İğde (*Elaeagnus angustifolia* L.) Tohumlarının Çimlenmesi Üzerine Etkileri", ***Turkish Journal of Forestry*** 8(2), 32-41, 2009.

Guilbault, K.R., Brown, C.S., Friedman, J.M. and Shafroth, P.B., "The influence of chilling requirement on the southern distribution limit of exotic Russian olive (*Elaeagnus angustifolia*) in western North America", ***Biological Invasions*** 14, 1711-1724, 2012.

Hamilton, D.F. and Carpenter, P.L., "Regulation of seed dormancy in *Elaeagnus angustifolia* by endogenous growth substances", ***Canadian Journal of Botany*** 54, 1068-1073, 1976.

Harner, M.J., Crenshaw, C.L., Abelho, M., Stursova, M., Shah, J.J.F. and Sinsabaugh, R.L., "Decomposition of leaf litter from a native tree and an actinorhizal invasive across riparian habitats", ***Ecological Applications*** 19, 1135-1146, 2009.

Hechmi, M., Khaled, M., Abed, S., El-Hassen, A., Faiez, R. and M`hamed, A., "Performance of olive cuttings (*Olea europaea* L.) of different cultivars growing in the agro-climatic conditions of Al-Jouf (Saudi Arabia)", ***American Journal of Plant Physiology*** 8, 41-49, 2012.

Hogue, E.J. and LaCroix, L.J., "Seed dormancy of Russian olive (*Elaeagnus angustifolia* L.)", ***Journal of the American Society of Horticultural Science*** 95, 449-52, 1970.

Howe, W.H. and Knopf, F.L., “On the imminent decline of Rio Grande cottonwoods in central New Mexico”, ***The Southwestern Naturalist***, 218-224, 1991.
Hultine, K. R. and Bush, S. E., “Ecohydrological consequences of non-native riparian vegetation in the southwestern United States: A review from an ecophysiological perspective”, ***Water Resources Research*** 47, 2011.

Jafari, M., Haghighi, J.A.P. and Zare, H., “Mulching impact on plant growth and production of rainfed fig orchards under drought conditions”, ***Journal of Food, Agriculture and Environment*** 10, 428-433, 2012.

Jinks, R.L. and Ciccarese, L., “Effects of soaking, washing, and warm pretreatment on the germination of Russian-Olive and Autumn-olive seeds”, ***Tree Planters’ Notes*** 48, 18-23, 1997.

Katz, G.L., Friedman, J.M. and Beatty, S.W., “Effects of physical disturbance and granivory on establishment of native and alien riparian trees in Colorado, USA”, ***Diversity and Distributions*** 7, 1-14, 2001.

Katz, G.L. and Shafroth, P.B., “Biology, ecology, and management *of Elaeagnus angustifolia* L. (Russian olive) in western North America”, ***Wetlands*** 23, 763-777, 2003.

Khamzina, A., Lamers, J.P. and Vlek, P.L., “Nitrogen fixation by *Elaeagnus angustifolia* in the reclamation of degraded croplands of Central Asia”, ***Tree Physiology*** 29, 799-808, 2009.

Knopf, F.L. and Olson, T.E., “Naturalization of Russian-Olive: Implications to Rocky Mountain wildlife”, ***Wildlife Society Bulletin*** 12, 289-298, 1984.

Lesica, P. and Miles. S., “Beavers indirectly enhance the growth of Russian olive and tamarisk along eastern Montana rivers”, ***Western North American Naturalist*** 64, 93-100, 2004.

Lesica, P. and Miles, S., “Natural history and invasion of Russian olive along eastern Montana rivers”, ***Western North American Naturalist***, 1-10, 2011.

Lie, T.A., Göktan, D., Engin, M., Pijnenborg, J. and Anlarsal, E., “Co-evolution of the legume-Rhizobium association”, ***Plant and Soil*** 100, 171-181, 1987.

Lie, T.A., Akkermans, A.D.L. and Van Egeraat, A.W.S.M., “Natural variation in symbiotic nitrogen-fixing *Rhizobium* and *Frankia* spp.”, ***Antonie van Leeuwenhoek*** 50, 489-503, 1984.

Maggard, A.O., Will, R.E., Hennessey, T.C., McKinley, C.R. and Cole, J.C., “Tree-based mulches influence soil properties and plant growth”, ***Journal of Horticulture and Technology*** 22(3), 353-361, 2012.

Miller, I.M. and Baker, D.D., “The initiation, development, and structure of root nodules in *Elaeagnus angustifolia*. (Elaeagnaceae)”, ***Protoplasma*** 128, 107-119, 1985.

Mineau, M.M., Baxter, C.V. and Marcarelli, A.M., “A non-native riparian tree (*Elaeagnus angustifolia*) changes nutrient dynamic in streams”, ***Ecosystems*** 14, 353-365, 2011.

Moline, A. and Poff, N., “Growth of an invertebrate shredder on native (Populus) and non-native (Tamarix, Elaeagnus) leaf litter”, ***Freshwater Biology*** 53(5), 2008.

Nagler, P.L., Glenn, E.P., Jarnevich, C.S. and Shafroth, P.B., “Distribution and abundance of saltcedar and *E. angustifolia* in the western United States”, ***Critical Reviews in Plant Sciences*** 30, 508-523, 2011.

Niinemets, Ü. and Valladares, F., “Tolerance to shade, drought, and waterlogging of temperate northern hemisphere trees and shrubs”, ***Ecological monographs*** 76, 521-547, 2006.

Ni, X., Song, W., Zhang, H., Yang, X. and Wang, L., “Effects of mulching on soil properties and growth of tea olive (*Osmanthus fragrans*)”, ***Plos one*** 11, 2016.

Olson, D.F. and Jill R. Barbour, J.R., “*Elaeagnus* L. In: Bonner, F. T. The woody plant seed manual (No. 727)”, ***Washington, DC: USDA Forest Service***, 484-487, 2008.
Olson D.F.J., “*Elaeagnus* L., *Elaeagnus*. In: Schopmeyer CS, tech. coord. Seeds of woody plants in the United States, Agricultural. Handbook. 450”, ***Washington, DC: USDA Forest Service***, 376-379, 1974.

Ölmez, Z., Temel, F., Göktürk, A. and Yahyaoğlu, Z., “Effect of cold stratification treatments on germination of drought tolerant shrubs seeds”, ***Journal of Environmental Biology*** 28, 447, 2007.

Ölmez, Z., Göktürk, A. and Temel, F., “Effects of some pretreatments on seed germination of nine different drought-tolerant shrubs”, ***Seed Science and Technology*** 35, 75-87, 2007.

Padilla, F.M. and Pugnaire, F.I., “The role of nurse plants in the restoration of degraded environments”, ***Frontiers in Ecology and the Environment*** 4, 196-202, 2006.

R Core Team (2021), R: A language and environment for statistical computing, (Version 4.0) [Computer software]. Retrieved from https://crab.r-project.org, 2021.

Reynolds, L. and Cooper, D., “Environmental tolerance of an invasive riparian tree and its potential for continued spread in the southwestern US”, ***Journal of Vegetation Science*** 21, 733-743, 2010.

Rogers, C.E. and McCarty, J.P., “Climate change and ecosystems of the Mid-Atlantic Region”, ***Climate Research*** 14, 235-244, 2000.

Roggy, J.C., Moiroud, A., Lensi, R., Domenach, A.M., “Estimating N transfers between N2-fixing actinorhizal species and the non-N2-fixing Prunus avium under partially controlled conditions”, ***Biology and Fertility of Soils*** 39 (5), 312-319, 2004.

Royer, T.V., Monaghan, M.T. and Minshall, G.W., “Processing of native and exotic leaf litter in two Idaho (U.S.A.) streams”, ***Hydrobiologia*** 400, 123–128, 1999.

Simons, S.B. and Seastedt, T.R., “Decomposition and nitrogen release from foliage of cottonwood (*Populus deltoides*) and Russian-olive (*Elaeagnus angustifolia*) in a riparian ecosystem”, ***The Southwestern Naturalist*** 44, 256-260, 1999.

Spennemann, D.H. and Allen, L.R., “Feral olives (*Olea europaea*) as future woody weeds in Australia: a review”, ***Australian Journal of Experimental Agriculture*** 40, 889-901, 2000.

Sprent, J.I. and Parsons, R., “Nitrogen fixation in legume and non-legume trees”, ***Field Crops Research*** 65, 183-196, 2000.

Shafroth, P.B., Auble, G.T. and Scott, M.L., “Germination and establishment of the native plains cottonwood (*Populus deltoides* Marshall subsp. *monilifera*) and the exotic Exotic Russian-olive (*Elaeagnus angustifolia* L.)”, ***Conservation Biology***, 1169-1175, 1995.

Shishkin, B. K. and Geranium L., “Flora of the USSR”, ***Izdatel’stvo Akademii Nauk*** 14, 2-63, 1949.

Stromberg, J.C., Beauchamp, V.B., Dixon, M.D., Lite, S.J. and Paradzick, C., “Importance of low- flow and high- flow characteristics to restoration of riparian vegetation along rivers in arid south- western United States”, ***Freshwater Biology*** 52, 651-679, 2007.

Smolander, A. and Sarsa, M.L., “*Frankia* strains of soil under Betula pendula: behaviour in soil and in pure culture” ***Plant and Soil*** 122, 129-136, 1990.

The Jamovi Project (2021), Jamovi. (Version 2.0.) [Computer software]. Retrieved from https://www.jamovi.org, 2021.

Tickner, D., Angold, P., Gurnell, A. and Mountford, J., “Riparian Plant Invasions: Hydrogeomorphological Control and Ecological Impacts. Progress in Physical Geography”, ***Progress in Physical Geography*** 25(1), 22-52, 2001.

Williams, R.D. and Hanks, S.H., “Hardwood nursery guide (No. 473). US Department of Agriculture”, ***Forest Service***, 1994.

Zalesny, R.S., Stange, C.M. and Birr, B.A., “Survival, height growth, and phytoextraction potential of hybrid poplar and Russian Olive (*Elaeagnus angustifolia* L.) established on soils varying in salinity in North Dakota, USA”, ***Forests*** 10, 672, 2019.

Zhang, X., Li, G. and Du, S., “Simulating the potential distribution of *Elaeagnus angustifolia* based on climatic constraints in China”, ***Ecological Engineering***, 113, 27-34, 2018.

Zhang, M., Liu, Y., Han, G., Zhang, Y., Wang, B. and Chen, M., “Salt tolerance mechanisms in trees: research progress”, ***Trees***, 1-14, 2020.

Zouhar, K., “*Elaeagnus angustifolia*. In: Fire Effects Information System, USA Department of Agriculture, Forest Service, Rocky Mountain Research Station”, ***Fire Sciences Laboratory,*** https://www.extension.uidaho.edu/publishing/html/BUL976-Different-Treatment-Options-for-Russian-Olive.aspx, 2005, (date accessed: 05.06.2021).

# CURRICULUM VITAE

…….……. was born on …/…/….. in …….. She completed her primary, secondary, high school and university education in Niğde. She graduated from Niğde Ömer Halisdemir University, department of Agricultural Genetic Engineering in 2018. She graduated from Anadolu University Management of Healthcare Organizations department in 2014 and Anadolu University Economics department in 2018. In 2018, she started her master's degree at Niğde Ömer Halisdemir University, department of Plant Production and Technologies. She started to study at Anadolu University Paralegal Studies department in 2020 and Applied English Translation department at Niğde Ömer Halisdemir University in 2021.