ABSTRACT

SHALIZI, MOHAMMAD NASIR. Growing Media and Fertilization Effects on Polybag – Raised Camden Whitegum (*Eucalyptus benthamii* Maiden & Cambage) Seedlings Morphology and Drought Hardiness. (Under the direction of Barry Goldfarb.)

Growing media and fertilization are important factors in containerized seedling production systems. In developing countries, seedlings are often produced in polybags filled with mixtures of locally available materials. Seedling growth and quality is affected by the type and amount of these substrates used in the mixture. Differences in seedling growth and quality can also be significantly affected when fertilization is employed during the nursery growing period. In this study, we assessed the effects of five different growing media and two fertilization regimes on nursery growth, seedling morphology and early post-planting response to drought of *Eucalyptus benthamii* Maiden & Cambage seedlings. In the nursery phase, seedlings were raised in 750 ml perforated polybags for 6 months in the greenhouse and in the open. We evaluated the effects of each media by fertilizer treatment combination on morphological attributes during nursery growing period. Fertilized treatments enhanced growth and morphological traits of *E. benthamii* seedlings. Seedlings raised in fertilized media without rice hulls yielded higher growth, root dry mass, shoot dry mass, total dry mass, Dickson quality index (DQI) scores, first order lateral roots (FOLRs) and sturdiness. Root to shoot ratio (R: S ratio) was, however, greater in non-fertilized media that contained rice hulls.

In the drought experiment phase, *E. benthamii* seedlings were randomly sampled from the nursery experiment and were planted in 3-gallons containers and watered for one month. Shoot height and diameter were then measured and the seedlings were covered with plastic to expose them to drought stress. Seedling resistance to drought was evaluated by counting the number of survival days under drought stress. During this period, seedlings grown in non-

fertilized media containing rice hulls survived longer than those in other media. There were not large differences in survival among other media or between fertilized and non-fertilized seedlings. Seedling total size, shoot height, and R:S ratio at the time of planting played a major role in survival. Seedlings with smaller shoot sizes and higher R: S ratios survived longer.

Results from these studies can be used to grow containerized seedlings using fertilizer and growing media from locally available substrates and enhance and alter morphological traits of seedlings during nursery growing periods for planting on drier sites. Seedlings with larger shoot sizes and lower R:S ratios of fertilized media can be planted on more mesic sites or if irrigation is applied after outplanting. On drier sites, seedlings with smaller shoot size, greater root size and, higher R:S ratio is recommended.

Growing Media and Fertilization Effects on Polybag – Raised Camden Whitegum (*Eucalyptus benthamii* Maiden & Cambage) Seedlings Morphology and Drought Hardiness

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INTRODUCTION

During the 1970's, natural forests covered approximately 3% of Afghanistan's total land area (Saba 2001) and 50 to 70 percent of these forests were lost between 1977 and 2002 (UNEP 2003). The FAO Forest Resource Assessment in 2005 also estimated that forest cover has changed from 13090 km^2 in 1990 to 8670 km^2 with a net change of -3.3% per year. Irresponsible human interventions such as fuel wood collection, illegal logging, and over grazing are major drivers of deforestation in Afghanistan (Formoli 1995; Saba 2001; UNEP 2009; Harrington et al. 2012). It is vital to conserve Afghanistan's forests by extending them to their natural boundaries. Reforestation or afforestation provide environmental, social, and economic services such as soil erosion control, improved air quality, wildlife habitat, food and fodder for wildlife and livestock, medicinal plants, timber, and fuelwood (FAO 1989). In order to bring back forest cover, we need to reforest areas previously covered with forests or afforest new regions. Reforestation or afforestation efforts in Afghanistan will require millions of seedlings each year, which will need to be produced in forest nurseries.

Seedlings used for reforestation projects are mainly produced in nurseries of the Department of Forestry, Ministry of Agriculture, Irrigation and Livestock (MAIL). Seedlings produced in these nurseries are mostly bare-root, while polybag seedlings are the only containerized stocks and are produced in smaller numbers (Harrington et al. 2012). Forests are established with these seedlings and planting is typically associated with site preparation (planting hole digging) and water harvesting techniques such as terracing, check dams, and micro catchments (Groninger 2012). The arid climate and uneven distribution of precipitation (Breckle & Rafiqpoor 2010) challenge reforestation or afforestation projects. The newly planted seedlings are hand watered during dry seasons for at least the first two years after planting, which is highly cost ineffective (Groninger 2012).

Successful establishment is related to the survival of planted seedlings. Drought is one of the main limiting factors affecting seedling survival in Afghanistan. Late spring to early fall is the period where seasonal drought dominates the climate, creating harsh conditions for growth and survival of many plants. Drought decreases soil moisture content by evaporation and limits water availability to seedling root systems (Grossnickle 2005). Seedlings close their stomata during drought and photosynthesis is stopped, resulting in seedling mortality due to "carbon starvation" (McDowell et al. 2008).

In addition to adverse climate and site characteristics, poor seedling quality is one of the factors that leads to seedling loss in forest plantings (Haase 2007). High quality seedlings can yield higher survival rates and better growth after planting (Chavasse 1980; Gould & Harrington 2009; Wilson & Jacobs 2006). Seedling quality is influenced by genetics, morphology, and physiology of the seedlings (Davis & Jacobs 2005). Seedling quality can be conceptualized under the target seedling concept. Target seedlings survive at high rates and grow well on particular planting area and, thus, have "fitness for purpose" (Ritchie 1984; Landis et al. 2010). The type of plant material is one of the target seedling concept components

(Landis et al. 2010) that is influenced by several nursery cultural factors and two of the important ones are growing media and mineral nutrition.

The quality of seedlings can be assessed by evaluating morphological and physiological attributes (Chavasse 1980; Johnson & Cline 1991; Grossnickle 2012; Haase 2007; Wilson & Jacobs 2006; Mattsson 1997). Due to a lack of equipment and technology, physiological methods of seedling quality assessment are limited in Afghanistan; however, morphological traits can be analyzed. Shoot height, root collar diameter (RCD), sturdiness, foliage color, bud length, shoot mass, root mass, total mass, the Dickson quality index (DQI), root fibrosity, and root-to-shoot ratio (R:S ratio) are important morphological traits (Chavasse 1980; Thompson 1985; Grossnickle 2012; Johnson & Cline 1991; Dickson et al. 1960).

Shoot height and RCD are important above-ground morphological features of seedlings that can affect growth and survival after planting (Thompson 1985; Dey & Parker 1997). Seedlings with larger shoots often have greater leaf area, which enhances growth through increased photosynthesis. However, larger shoots can also increase transpiration and adversely affect survival on drier sites (Haase 2007). Thompson (1985) suggests RCD as one of the best predictors of survival and growth and has been found to be positively associated with field performance (Haase 2008). The ratio of height to RCD gives the sturdiness value of a seedling. Seedlings with higher sturdiness values are more spindly and can be vulnerable to cold and wind damage (Haase 2007). Root morphological traits such as first order lateral roots (FOLRs; Thompson & Schultz 1995, Dey & Parker 1997) and R:S ratio can also influence the performance of seedlings after planting (Gould & Harrington 2009). R:S ratio is a measure of how much biomass is allocated to roots compared to the shoot (Haase 2007). Seedlings with larger root sizes, higher R:S ratios (Haase 2008), and greater numbers of FOLRs can grow and survive better after planting (Davis & Jacobs 2005).

Seedling morphological traits can be affected by nursery cultural practices (Grossnickle 2012). The nursery location, seed source, soil type, weed control, pest and disease treatments, spacing and density (Chavasse 1980), growing media, fertilization, irrigation, pruning and container size and type are important nursery practices that affect growth and morphology of bare-root and containerized seedlings. In order to improve seedling morphological characteristics, such as larger RCD, lower sturdiness values, more fibrous root systems (Johnson & Cline 1991) and higher R:S ratios, we need to manage root and shoot growth using the best locally available nursery materials. Locally available materials include polyethylene bags (polybags) and, potentially, plastic beverage bottles (Moorman 2013) as containers and media materials as growing media substrates.

Currently, in most developing countries, polybags are commonly used as containers. Polybags with heights of 15 cm to 20 cm and diameters of 12.5 cm are the most common ones used in Afghanistan nurseries (Harrington et al. 2012). Polybags seedling production systems can be a good alternative in terms of reducing overall costs and increasing seedling quality in Afghanistan nurseries (Harrington et al. 2012); however, most of those currently used do not have drainage holes (Nandakumar 1996). We can improve growth of seedlings in polybags by creating bottom and side perforations (Nandakumar 1996) and placing them on wire mesh benches to improve drainage and air pruning (Gera et al. 1996).

The components of growing media used in containerized nurseries can be an important factor in growth and development of seedlings. A good quality growing medium can improve morphological characteristics of seedlings and produce higher quality seedlings in a shorter amount of time than current methods of seedling production. Peat moss, perlite, and vermiculite have replaced soil, sand, and compost in container nurseries in many developed countries. These materials are light and porous, which enhances root growth in containers, and makes it easy to lift and transport seedlings for planting. In less developed countries, the use of peat moss, perlite, and vermiculite as growing media is very expensive and typically not affordable. However, there are locally available materials such as rice hulls, compost, sawdust, and wheat straw that can be incorporated with soil and sand into growing media. In India, organic materials such as wheat straw, rice hulls, coir pith, sawdust, sugar cane waste, tree bark, and water weeds are composted and used in growing media with inorganic materials such as sand, vermiculite, perlite, and pumice (Mohanan & Sharma 2005).

In Afghanistan, soil, sand, and compost are most often used as growing medium ingredients (Harrington et al. 2012). Soil is usually obtained from a nursery bed and sand is obtained from river beds. Sand is used in medium mixtures to increase porosity (Landis et al. 2014). Compost (composted manure) is the only readily available organic material that is commonly used currently as part of growing media in developing countries. It affects chemical and physical properties of growing media, enhancing water holding capacity, increasing porosity, and providing nutrients (Landis et al. 2014). Compost production in Afghan nurseries is not very advanced and mostly produced in smaller volumes within the nursery area (Harrington et al. 2012).

The combination of soil, sand, and compost can provide good growing media for containerized seedlings in Afghanistan. Shrivastava et al. (1998) studied the effects of different growing substrate mixtures on growth of a Eucalyptus hybrid raised in root trainers. Seedlings grown in a mixture of soil, sand, and compost (2:1:2) had larger means for height, RCD and root biomass than the media containing compost, coarse sand and coal pebbles.

Rice hulls could be an alternative medium component with good potential for South Asian countries, including Afghanistan, that grow rice. It has been used as part of growing media in India. Studies have shown that due to its physical characteristics, non-composted rice hulls can improve porosity and drainage in a growing media (Whitcomb 1984) and can substitute for vermiculite and perlite (Miller & Jones 1995). Evans & Gachukia (2004) reported that newly harvested rice hulls had 89% total pore space and 69% porosity, which was greater than perlite (74 – 76% total pore space and 54% porosity).

Studies have shown that rice hulls, incorporated into growing media can have positive effects on seedling growth and development. Tsakaldimi (2006) studied different proportions of rice hulls with peat moss and found that a mixture of 70% peat and 30% rice hulls produced larger shoot height, RCD, shoot dry mass, root dry mass, total dry mass, and DQI of *Pinus halepensis* Miller. seedlings compared to (70:30) peat and perlite, (50:50) peat and rice hulls, 100% kenaf, and (60:20:20) kenaf, peat, and rice hulls mixtures. In another study, Alves et al. (2010) found that a medium mixture of 40% carbonized rice hulls and 60% sludge gave the best results in terms of growth for *Eucalyptus grandis* W.Hill ex Maiden. Rice hulls mixed with vermiculite resulted in greater *Eucalyptus dunnii* Maiden. growth than pure rice hulls or rice hulls mixed with coconut fiber (Kratz & Wendling 2013). Einert & Guidry (1975) found better growth and development of *Juniperus chinensis pfitzerianna* L. in growing media with 1:1 rice hulls and soil and 2:3 rice hulls and hull compost compare to a variety of media mixtures composed of other different proportions of rice hulls and soil or rice hull and hull compost.

Standard mixtures of growing media have not yet been determined for any forest tree species in Afghanistan. Harrington et al. (2012) pointed out that growing media used for polybags in Afghanistan nurseries is a mixture of top soil, sand, and compost with a ratio of 1:2:1 (Medium 4 in this study). In contrast, Dumroese et al. (2008) reported that a common mixture used in Afghanistan is 3:1:2 of top soil, sand and compost (Medium 3 in this study).

Seedling mineral nutrition is also an important factor in seedling production systems (Driessche 1988). Fertilization is a usual practice in many modern nursery operations; however, it is not very common in nurseries in Afghanistan (Harrington et al. 2012). Fertilization enhances seedling growth and development during the nursery growing period and quality of seedlings can be improved by managing type, amount, and time of fertilization (Duryea & Landis 1984). Luis et al. (2009) studied the effects of fertilization on *Pinus canariensis* C.Sm. and found that nursery fertilized seedlings had 90% survival, while survival of non-fertilized seedlings declined to 60%. Oliet et al. (2009) suggested that fertilization influenced nutrient reserves and morphology of seedlings and enhanced resistance to drier conditions. They found that nursery fertilization increased survival rates of *P. halepensis* on the planting site. In their study, Chamshama & Hall (1987) found higher survival rates of *Eucalyptus camaldulensis* Dehnh. seedlings with increasing potassium (K) fertilization. However, with increasing nitrogen (N) fertilizer application, seedlings produced greater numbers of first order lateral roots, greater shoot growth, and stored high N concentrations in the leaves. Stahl et al. (2013) studied the effect of phosphorus (P) on early development of *E. benthamii* and *E. dunnii* seedlings. They found that both species show quadratic growth responses with increased P supply, which ultimately increased total dry mass.

Forest tree seedlings, as other plants, require macro and micro elements in order to grow and survive. N, P and K are three important macro elements that are crucial in the early stage of seedling development. Adequate availability of N in leaves is necessary for photosynthesis that ultimately enhances shoot and root development (Oliet et al. 2009). However, high levels of nutrients can also decrease root growth and reduce survival on drier sites (Harvey & Driessche 1997). Pharis & Kramer (1964) suggested that increased N supply in the nursery yields seedlings with larger shoot systems and decreases drought hardiness of seedlings after planting, which might be due to higher transpiration rates.

In this study, we used *E. benthamii* as a species to test whether different growing media and fertilization treatments would affect growth and morphological attributes. *E. benthamii* is native to New South Wales, Australia (Kole 2011; Brondani et al. 2012) and is widely used in plantation forestry due to its fast growth and adaptability. This species could be cultivated for firewood in the eastern and southwestern provinces of Afghanistan where irrigation water is available.

Objectives. The objectives of this research were to study the effects of five different growing medium types ((soil, sand and compost containing M1 (used in India), M3 & M4 (used in Afghanistan); and rice hulls containing M2 & M5)) and two fertilization regimes on growth and morphological attributes of *E. benthamii* seedlings raised in polyethylene bags. We hypothesized that seedlings in the fertilized treatments would grow larger and have greater morphological responses (e.g., height, RCD, shoot dry mass, root dry mass, total dry mass, sturdiness, DQI, and R:S ratio) compared to seedlings in the non-fertilized treatments. We also hypothesized that morphological attributes of seedlings would vary among the different media and that incorporating rice hulls into media (M2 and M5) would enhance morphological attributes compared to the soil, sand, and compost-containing M1, M3 and M4.

MATERIALS & METHODS

Plant material

Eucalyptus benthamii seeds were sown on October 5, 2013 in a mixture of peat moss and vermiculite in starter trays. Seedlings were transplanted into 750 ml perforated polybags (24 holes with a diameter of 0.5 cm, 16 on the sides and 8 at the bottom of the bag) in mid-November 2013. Seedlings were treated with fungicide three times for damping-off and powdery mildew. Fungicide was first applied a week after germination and two times after transplanting into polybags in November and December, 2013. On Jan 26 to 27, 2014, a frost occurred inside the greenhouse as a result of mechanical problems damaging most of the seedlings and killing 189 seedlings. On Feb 11, all surviving seedlings were pruned to a height of 7 cm in order to stimulate re-sprouting and establish a uniform baseline to evaluate growth after the frost event. Seedlings grew inside the greenhouse from November 2013 to March 2014 and then were moved outside on April 1, 2014.

Fertilization and Irrigation

Seedlings receiving the fertilization treatment were fertilized four times during the growing season. Each of these seedlings received a top dressing of 400 mg in January and March 2014 and 600 mg in April 2014 and May 2014 of 10-10-10 granular lawn and garden fertilizer (total of 2000 mg).

Irrigation varied according to the growing stage. At the beginning, seedlings were irrigated every other day and later the frequency of irrigation was changed based on water holding capacity of media. In order to get a guide for irrigation, an Irrometer® tensiometer – Model MLT was used to measure soil water potential of two seedlings per media mixture. On average it took 6 days after watering for each sample to fall to 90% (-37kPa) of its field capacity. Seedlings were hand watered every 4 to 6 days with a hose and hand nozzle. In order to have a uniform irrigation, at each time of irrigation every seedling was watered for 5 seconds.

Treatments

Treatments were based on growing substrate and fertilization regime. There were five different growing substrates composed of different combinations of sandy loam soil (obtained from nursery bed at Horticulture Field Laboratory), Paveston® Natural Play sand, Black Kow® compost, and parboiled rice hulls (Natures Media Amendments OMRI ™). Parboiled rice hulls are heated to eliminate live weed seeds and microorganisms. Two fertilizer regimes, fertilization and control (no fertilization) were applied to each medium mixture creating ten substrate x fertilization treatment combinations (Table 1.1). The fertilizer used in this research was 10-10-10 Weaver Lawn and garden PLANT FOOD. This fertilizer contained 10% N, 4.3%

P and 8.3% K, 3% Ca and 6% S. Media M3 and M4 are currently used in Afghanistan nurseries (Dumroese et al. 2008; Harrington et al. 2012). M1 is used in India as an alternative growing media (Qaisar et al. 2008). M2 and M5 are two new growing media tested in this study. In M2, rice hulls are used to substitute for the compost in M1 and, in M5, rice hulls are used to substitute for the soil in M3.

Table 1.1. Treatment combinations of substrate types and fertilization regime used to grow seedlings of *E. benthamii*.

Treatment combinations		**Substrates (%)**				**Fertilizer (mg)**
Media	Fertilization	Top soil	Sand	Compost	Rice hull	
M1	*NotFert*	17	33	50	--	0
	Fert					2000
M2	*NotFert*	17	33	--	50	0
	Fert					2000
M3	*NotFert*	50	17	33	--	0
	Fert					2000
M4	*NotFert*	25	50	25	--	0
	Fert					2000
M5	*NotFert*	--	17	33	50	0
	Fert					2000

Experimental design

The study was established as a randomized, complete-block design, with a 2 X 5 factorial arrangement of treatments with 18 blocks. Six seedlings from each treatment were randomly placed in each block (60 seedlings per block surrounded by border seedlings). At the beginning of the study there were 108 samples in each treatment. The total sample size was 1080 seedlings. Later, the total sample size decreased to 889 seedlings, due to mortality. Sample size varied from 105 (highest) in M5-NotFert treatment to 61 (lowest) in M3-Fert treatment. Samples size per treatment per block was 6 for most treatments in most blocks, however, it ranged from 2 (lowest) in M3-Fert to 6 (highest) in most of other treatments.

Measurements

Height and root collar diameter (RCD)

Shoot height (cm) and root collar diameter (RCD, mm) were measured every month from January 2014 to May 2014 both before and after frost damage periods (n = 889). Final shoot height and RCD were measured on all surviving seedlings before destructive sampling on May 25, 2014. Height was measured with a ruler and RCD was measured with a dial caliper.

Root architecture

For all destructively sampled seedlings (n = 533; ranged from 30 to 68), shoot systems were separated from root systems and then root systems were divided into three segments: a) the top segment was the upper 1 – 7 cm, b) the middle segment was 8 – 14 cm from the shoot and c) the bottom segment was 15 – ≥21 cm from the shoot. In each segment, the number of spiraled roots, number of first order lateral roots (FOLRs; only measured on a sub-sample of 204 seedlings from 7 blocks; samples size ranged from 13 to 25 per treatment) and the number of active root tips (measured on a sub-sample of 57 seedlings; sample size ranged from 3 to 6 per treatment) were measured.

Spiraled roots were counted based on visual assessment of lateral roots. We considered a lateral root as spiraled when it touched the edge or bottom of polybag and grew horizontally ¼ of the circumference around the edge of container. FOLRs were counted for lateral roots ≥ 1 mm in diameter. Active root tips are non-suberized white root tips produced on lateral roots.

The number of active roots tips were counted for lateral roots ≥1 mm in diameter. From these measurements, the total number of spiraled roots, total number of FOLRs, and total number of active roots were calculated.

Shoot and root dry biomass

All samples were oven dried at 60^{o}C for 72 hours (n = 533; ranged from 30 to 68 among treatments). Shoot parts and root parts (Root top, middle and bottom segments) biomass were dried separately in paper bags and then weighed to the nearest hundredths of a gram. Shoot dry weight, total root dry weight, and total seedling dry weight (total biomass) were then calculated. R:S ratio was calculated by dividing total root dry mass by shoot dry mass. Sturdiness (Thompson 1985) was calculated by dividing shoot height by RCD and the Dickson quality index (DQI) was calculated using the following equation (Dickson et al. 1960):

$$\mathrm{DQI} = \frac{\text{Total seedling dry weight (g)}}{\frac{\text{Height (cm)}}{\text{RCD (mm)}} + \frac{\text{Shoot dry weight (g)}}{\text{Root dry weight (g)}}}$$

Foliar nutrient analysis

Six samples per treatment (total of 60 samples) were randomly selected, oven dried and ground for foliar nutrient analysis. Due to very small amounts of mass in some treatments, all six samples from each treatment were then combined into one sample. Tissue samples were sent to Waters Agricultural Laboratories, INC. to analyze for concentration (%) of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), boron (B), zinc (Zn), manganese (Mn) and copper (Cu). We used the following equation to then convert concentrations to contents for each nutrient and treatment combination:

$$\text{Nutrient Content} = \frac{\text{Concentration}(\%) \text{ X Total Seedling Biomass (mg)}}{100}$$

Soil and media analysis

Two samples per medium mixture (Table 1.1), plus two samples each of rice hulls, sand and soil were sent to Waters Agricultural Laboratories, INC. for chemical and physical analysis. These samples were analyzed for available ammonium (NH_4), nitrate (NO_3), P, K, Mg, Ca, S, B, Zn, Mn, Fe, and Cu as well as pH, soluble salts, and organic matter. In addition, the soil used as part of the medium mixtures was analyzed for texture as well. Finally, two samples of each medium type (450 cm^3) were selected for analysis of bulk density, particle density, porosity, and gravimetric moisture content. To determine moisture content, samples were watered and weighed after 24 hours. Then samples were dried in an oven at 85^{o}C for 48 hours and re-weighed. The following equations were used to calculate gravimetric moisture content (M.C), bulk density, particle density and porosity:

$$\text{M.C} = \frac{[\text{Wet soil (g)} + \text{Tare (g)}] - [\text{Dry soil (g)} + \text{Tare (g)}]}{[\text{Dry soil (g)} + \text{Tare (g)}] - \text{Tare (g)}}$$

$$\text{Bulk density} = \frac{\text{Mass of solids (g)}}{\text{Volume of cylinder (cm}^3\text{)}}$$

$$\text{Particle density} = \frac{\text{Mass of solids (g)}}{\text{Volume of solids (cm}^3\text{)}}$$

$$\text{Porosity} = \left(1 - \frac{\text{Bulk density (cm}^3}{\text{Particle density (cm}^3}\right) \times 100$$

Growing media and substrate chemical and physical analysis is given in the following table:

Table 1.2. Chemical and physical analysis of five medium mixtures and three components at the start of the experiment used to grow *E. benthamii* seedlings. M1 = soil (17%) : sand (33%) : compost (50%); M2 = soil (17%) : sand (33%) : rice hull (50%); M3 = soil (50%) : sand (17%) : compost (33%), M4 = soil (25%) : sand (50%) : compost (25%), M5 = sand (17%) : compost (33%) : rice hull (50%). O.M = organic matter, Ss = soluble salts, M.C = gravimetric moisture content, B.D = bulk density, P.D = particle density.

Nutrient	M1	M2	M3	M4	M5	Rice hull	Sand	Soil
NH_4 (ppm)	0.2	4.1	4.4	1.4	0.8	3.1	4.0	4.1
NO_3 (ppm)	7.3	0.1	0.3	0.2	0.3	0.2	1.0	82.1
P (ppm)	7.5	11.5	4.0	4.5	28.6	78.4	0.6	0.3
K (ppm)	716.2	113.6	400.1	358.3	578.0	195.6	40.4	41.0
Mg (ppm)	75.3	5.1	72.3	46.4	45.5	12.5	23.6	22.8
Ca (ppm)	187.5	8.9	206.4	139.7	100.4	7.1	166.7	106.5
S (ppm)	289.7	19.8	224.3	181.4	184.3	9.2	184.5	6.0
B (ppm)	0.2	0.1	0.1	0.2	0.2	0.1	0.1	0.1
Zn (ppm)	0.2	0.1	0.1	0.1	0.1	0.2	0.4	1.8
Mn (ppm)	0.3	0.8	0.2	0.1	0.2	4.8	2.8	7.0
Fe (ppm)	0.9	2.7	0.3	0.5	0.7	0.6	0.1	0.1
Cu (ppm)	0.4	0.1	0.1	0.1	0.1	0.1	0.0	0.0
O.M (%)	2.6	2.3	2.7	3.1	8.9	43.8	0.0	0.6
pH	8.0	5.5	7.2	7.4	6.6	4.8	5.6	4.8
Ss (mmohs/cm)	4.4	4.1	3.3	2.5	2.9	622.0	1053.0	974.5
M.C (%)	41.0	30.5	32.4	28.7	73.8	---	---	---
B.D (g/cm^3)	1.1	0.8	1.3	1.3	0.5	---	---	---
P.D (g/cm^3)	1.4	1.3	1.5	1.6	0.7	---	---	---
Porosity (%)	23.4	33.8	10.4	16.9	32.5	---	---	---
Bag weight (g)	972.0	794.0	1016.0	1222.0	670.0	---	---	---

In addition, pH of the media was measured at the outset of the study and during the growing period. Six randomly selected samples per treatment combination were collected from polybags containing seedlings from six blocks for pH data (6 samples/treatment = 60 samples). At each time (January, March, and May 2014), 5 – 10 grams of substrate was taken from each sample, a slurry was made with distilled water and pH was measured with a Hanna meter (Portable pH/EC/TDS/^{o}C meter).

Statistical analysis

Two-way ANOVA and type III sum of squares were used to test main effects of media, fertilizer and their interaction at $\alpha = 0.05$. Linear mixed models were used to analyze the data in GLIMMIX procedure in SAS 9.4© 2014 (SAS Institute Inc., Cary, NC, USA). In the cases of significant interactions, the Tukey-Kramer grouping was used to compare least square means of media type x fertilizer interactions (simple effects) at $\alpha = 0.05$. The effect of fertilizer treatment was tested in each medium as well. All response variables were checked for normality, independence and constant variance assumptions. Residuals of some response variables were non-constant (heteroscedastic) and not normally distributed. Also, the data in these response variables were skewed to the right and had bimodal shapes. Most of response variables were analyzed as normal distribution, only those with non-constant variance were analyzed with gamma distribution. Among response variables, height – May, RCD – April, root top dry weight, shoot dry weight, total root dry weight, total dry weight, R:S ratio had gamma distributions so they were linked with the log (μ) of response. The following linear mixed models were used to test effect of media type, fertilizer level, and their interaction on these variables:

$$Y_{ijkl} = g(\mu) = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \zeta_k + \varepsilon_{ijkl}$$

where;

Y_{ijkl} is the link function $g(\mu)$ for response variables

μ is the overall mean,

α_i is fixed effect of media type; $i = 1,...,5$;

β_j is fixed effect of fertilizer; $j = 1,2$;

$\alpha\beta_{ij}$ is interaction of media type and fertilizer,

ζ_k is random effect of block with expectations $\sim N(0, \sigma^2_b)$; and

ε_{ijkl} is error term with the expectations $\varepsilon_{ijkl} \sim (0, \sigma^2_{\varepsilon})$.

Jan – pH, March – pH, May – pH, height – March, RCD – May, DQI, top FOLRs, mid FOLRs, bottom FOLRs, total FOLRs, top spiraled roots, mid spiraled roots, bottom spiraled roots, total spiraled roots (this variable was transformed using $\sqrt{Y} + \sqrt{Y+1}$ equation prior to analysis in GLMM model), root mid dry weight, root bottom dry weight, sturdiness, top active root tips, mid active root tips, bottom active root tips, and total active root tips response variables had normal distributions so their link function was identity.

RESULTS

Height and RCD

E. benthamii mean seedling height and RCD differed across media and fertilizer treatments. May height and RCD ranged from the smallest (9.5 cm and 1.0 mm, respectively) to the largest (56.4 cm and 7.0 mm, respectively; Tables 1.4 and 1.5). In general, fertilizer resulted in greater height and RCD in each medium type in March, April and May, except in

March, fertilizer did not affect seedling height in M1, M3 and M4, the media composed of soil, sand, and compost (Tables 1.3 & 1 appendix A).

In March, within the fertilized medium types, seedling height was not significantly different among soil, sand, and compost containing M4, M1, M3 and M2, the medium composed of soil, sand, and rice hulls (Table 1.4). The lowest mean height was observed in the M5 medium that contained sand, compost, and rice hulls, which was significantly lower than M4 (Table 1.4). RCD within the fertilized medium types was greater in soil, sand and compost media (M4, M3 and M1; Table 1.6). Within the non-fertilized medium types, M2 produced seedlings with the smallest mean height followed by those in M5 (Table 1.4). RCD of non-fertilized media containing rice hulls (M2 and M5) produced seedlings with smaller RCDs compare to M1, M3, and M4, in fertilized and non-fertilized regimes (Table 1.6).

In May, among the fertilized media, seedlings in the M1, M2, M3, and M4 medium types had the highest mean heights (Table 1.4, Figure 1.1). Those in the fertilized M5 were significantly shorter than those in the M3 and M4 fertilized treatments. RCDs of seedlings were larger in fertilized M1, M2, M3, and M4 than those in M5 (Table 1.5). Seedlings in M1, M3, and M4 had the largest RCDs, although the M1 seedlings were not significantly different from those in the M2 (Table 1.5). All the non-fertilized medium types produced seedlings with lower heights than those in fertilized treatments, with seedlings in M5 the shortest, followed by seedlings in M2 (Table 1.4). Within the non-fertilized medium types, seedlings in the rice hulls containing M2 and M5 treatments had the smallest RCDs (Table 1.5).

Dry weights

Fertilization in all medium types enhanced seedling biomass production and resulted in greater root dry weight (root top, root mid and root bottom sections), shoot dry weight and total seedling dry weights than in non-fertilized medium types (Tables 1.3 & 1 appendix A). Among the fertilized media, soil, sand, and compost-containing media (M1, M3, and M4) were among those with the highest root dry weights, shoot dry weights, and total seedlings dry weights, whereas rice hull-containing media (M2 and M5) were among those with lower root, shoot, and total dry weights (Tables 1.6 & 1.8). Although lower in root, shoot and total dry weight, M2 was not statistically different from M1 and M3 when fertilized. Shoot dry weight of fertilized M2 also did not differ from those in fertilized M4. Under fertilization, roots, shoot, and total dry weight of M5 were significantly lower than M1, M3 and M4, but not than those in M2 (Figure 1.2, Table 1.6 & 1.8). Within the non-fertilized medium types, M1, M3, and M4 resulted in higher root, shoot and total dry weight. Non-fertilized media, M2 and M5 produced seedlings with lowest root, shoot and total seedling dry weights (Figure 1.2, Table 1.6 & 1.8).

Dickson Quality Index (DQI), Sturdiness and R:S ratio

Fertilization significantly affected DQI in most medium types (Table 1.3). Within the fertilized treatments, DQI was higher in soil, sand and compost containing media (M1, M3, and M4); however DQI of M3 was not different from M2. The media containing rice hulls (M2 and M5) produced seedlings with lower DQIs with and without fertilization (Table 1.9).

Sturdiness was affected by fertilization regardless of medium types (Table 1.3). In general, the seedlings in the non-fertilized, rice hull-containing media (M2 and M5) were less sturdy (higher sturdiness ratings, Table 1.9). Within the fertilized medium types, seedlings in the M4 had the lowest sturdiness ratings, but were not significantly different than M1, M2, and M3 (Table 1.9).

Fertilization resulted in lower R:S ratios of seedlings in the two media containing rice hulls (M2 and M5) (Table 1.3), but not in those grown in media containing soil, sand and compost (M1, M3 and M4) (Tables 1.9 & 1 appendix A). Within fertilizer treatments, R:S ratio of M1, M2, M3, M4, and M5 were not significantly different from each other. Within non-fertilized medium types, R:S ratio of rice hulls containing M2 and M5 was significantly higher than M1, M3, and M4 (Table 1.9).

First order lateral roots (FOLRs)

Fertilization significantly affected production of FOLRs in each segment of the root system (root top, mid and bottom) as well as total FOLRs (Table 1.3). In general, most of fertilized medium types produced more lateral roots in the top-section, mid-section, bottom-section, and the entire root system (Tables 1.10 & 1.11). Among the medium types, the rice hull-containing M2 and M5 produced fewer FOLRs than the soil, sand, and compost-containing media, M1, M3, and M4.

Within fertilized medium types, M1, M3, and M4 produced more total FOLRs. Total FOLRs of the fertilized soil, sand, and rice hull medium (M2) was significantly less than M4. Also FOLRs of fertilized sand, compost, and rice hulls medium (M5) was significantly less than M3 and M4 (Table 1.10). Under the no fertilization regime, M1, M3 and M4 produced the highest mean total FOLRs. Non-fertilized M2 and M5 had the fewest total FOLRs (Table 1.10).

Active root tips

The number of active root tips in each root segment (top, mid, and bottom) and the whole root system was significantly affected by fertilization (Table 1.3). Most of the fertilized medium types produced more active root tips than non-fertilized ones (Table 1.12). When fertilized, M5 produced seedlings with the fewest active root tips, although this was not significantly different than seedlings grown in M2 (Table 1.12). Without fertilization, the fewest active root tips were again found in seedlings grown in M2 and M5, although they did not differ significantly from those grown in M3 (Table 1.12).

Root spiraling

Nearly all of the spiraled roots observed (significant interaction) occurred in the bottom portion of the root systems (Table 1.3). In every medium, fertilized seedlings had a higher mean number of spiraled roots than non-fertilized ones (Table 1, appendix A), although the fertilized media did not differ significantly from each other (Table 1.14). In the non-fertilized media, seedlings in the rice hull-containing M2 and M5 treatments had lower numbers of spiraled roots than the other media.

Growing media pH

There were no consistent significant differences in medium pH by treatment (Table 1.3). The pH of the media differed in January and March, but not in May. Fertilizer only affected medium pH in March. The pH differences among the media were relatively small, ranging from 7.55 (M1) to 7.98 (M5) in January and from 7.66 (M4) to 8.03 (M5) in March.

Similarly, fertilized media had a mean pH of 7.64 compared to 8.00 in non-fertilized media in March (Table 1.15).

Substrate chemical analysis

Substrate chemical properties and nutrient levels were determined by the saturated media extract method in Waters Agricultural Laboratories, INC. Compared to Waters Agricultural Laboratories standards, both ammonium and nitrate nitrogen in all five medium types was low. The nutrient analysis of the M1 (soil:sand:compost, 17:33:50) showed an optimum amount of P, but had high concentrations of K. The concentration of Ca was acceptable, but S was very high. Micronutrients, such as B, Zn, Mn and Fe, were in the acceptable range, but Cu was high. Both soluble salts and pH were high as well (Table 1.2). In M2 (soil:sand:rice hulls, 17:33:50), K was in the acceptable range, but P was high. Ca, Mg and S concentrations were low. All micronutrients were in acceptable range. Soluble salts were high and pH was low (Table 1.2). In M3 (soil:sand:compost, 50:17:33), the concentration of K and S were very high. Phosphorus, Ca and Mg concentrations were in acceptable or optimum ranges. Among the micronutrients only Zn was low and others were in acceptable ranges. Soluble salts were relatively high and pH was high as well (Table 1.2). In M4 (soil:sand:compost, 25:50:25), concentrations of K and S were very high. P, Ca and Mg levels were in acceptable ranges. Of the micronutrients, Zn and Mn were low. Soluble salts were optimal, but pH was high (Table 1.2). In M5 (sand:compost:rice hulls, 17:33:50), the amounts of P, K and S were very high. The concentrations of the other nutrients were in acceptable range. Soluble salts, and pH were optimum (Table 1.2).

Of the individual analysis of substrates, rice hulls had high concentrations of P and soluble salts. The N concentration, particularly the nitrate N, was low. Mn was high, but other nutrients were in acceptable ranges. (Table 1.2). Sand had high concentrations of soluble salts and S. The Zn concentration was high as well. Except for Ca and Mn, the concentrations of other nutrients were low (Table 1.2). Soil had high concentrations of soluble salts and Zn and Mn concentrations that were very high. Except for ammonium N, Ca and Cu, other nutrient concentrations were low in this substrate (Table 1.2). Organic matter percent was also determined in the media and their components. Rice hulls had the highest organic matter (43.8%), followed by M5 (8.9%). M1, M2, M3 and M4 all had 2 – 3 % of organic matter. Soil had 0.6% organic matter and sand had no detectable organic matter (Table 1.2).

Substrate physical analysis

Water content. Water content 24 hours after irrigation of each media mixture was determined by the gravimetric method. Water content was highest in M5 (73.8%) followed by M1 (41%), M3 (32.4%), M2 (30.5%) and was lowest in M4 (28.7%) (Table 1.2).

Bulk density. Bulk density was lowest in M5 (0.5 g/cm^3), followed by M2 (0.8 g/cm^3). M1 had bulk density of 1.1 g/cm^3. M3 and M4 had same bulk density (1.3 g/cm^3, Table 1.2).

Particle density. Particle density of all media types ranged from 0.7 to 1.6 g/cm^3. M5 had the lowest particle density (0.7 g/cm^3) followed by M2 (1.3 g/cm^3), M1 (1.4 g/cm^3), M3 (1.5 g/cm^3) and M4 (1.6 g/cm^3, Table 1.2). Particle density increased with the increase in amount of sand and soil in the mixtures of media types.

Porosity. M2 and M5 had the highest porosities among the medium mixtures (M2= 33.8% and M5= 32.5%). The presence of 33% sand in M2, 33% compost in M5 and 50% rice hulls in both of these media led to high porosities. Porosity was lowest in M3 (10.4%), mainly due to the presence of 50% soil in the mixture. M1 had intermediate porosity (23.4%) and M4 had the second lowest porosity (16.9%) among the medium types (Table 1.2).

Foliar nutrient analysis

We compared *E. benthamii* seedlings nutrients concentrations with sufficiency levels reported by Judd et al. (1996) for *Eucalyptus grandis* W.Hill ex Maiden.. In all fertilized and non-fertilized treatments N, P, K, Mg, Ca, B and Zn were in the acceptable range. Although all these nutrients were in acceptable range, M3-NotFert, M4-Fert and M4-NotFert showed higher N concentrations. S concentration was high in all treatments. Mn was low in M1-NotFert, and M4-NotFert treatments. Cu and Fe was low in all treatments (Table 1.17).

We converted concentrations into content to see whether nutrient contents were different based on total size of the plants. Seedlings in all the fertilized treatments had higher nutrient contents than those in non-fertilized ones. Except for Mn, M4-Fert treatment had the highest N, P, K, Ca, Mg, S, B, Zn, Fe, and Cu contents. M1-Fert and M3-Fert treatments had the second and third highest nutrient contents, respectively. Mn was highest in M2-Fert treatment (Table 1.16).

DISCUSSION

Growth variables

Shoot height, RCD, root dry mass, shoot dry mass, total dry mass, R:S ratio, sturdiness and DQI are morphological measures of forest tree seedling growth and quality (Haase 2008). The better the growing conditions, the more seedlings respond by increasing resource allocation toward biomass production. In this study, growth and development of *E. benthamii* seedlings were strongly affected by fertilization in the nursery and generally seedlings responded to fertilization treatments with greater height, RCD, dry mass (root, shoot, and total seedling), sturdiness and DQI; however, fertilization did not consistently alter R:S ratio.

Mineral nutrition is important for biomass production of tree seedlings during the nursery phase. Yongchun & Wenbin (1991) and Zeng et al. (2013) found increased height and RCD growth in fertilized larch and hybrid eucalyptus seedlings during nursery growing period, similar to the results found in this study. Nitrogen, P, and K are three macro elements needed for growth and development of seedlings. We found N (NH_4 & NO_3) as the most limiting nutrient in the medium mixtures prior to fertilization. An adequate supply of N enhances shoot height and RCD growth in nursery seedlings (Mullin & Bowdery 1978; Driessche 1980; Dumroese et al. 2011; Jackson et al. 2012). Ahmadloo et al. (2012) found positive and significant correlations of growth with N, P and K concentrations in *Cupressus arizonica* Greene. seedlings. Although foliar nutrient concentrations in our study were in the acceptable range for all treatments, N, P, and K total contents were higher in fertilized seedlings than in non-fertilized ones. Fertilized seedlings produced larger shoot and root systems, but did not accumulate higher nutrient concentrations in the foliage in M1, M2, and M5. Fertilization did increase N concentration in M3 and M4 medium types. Fertilization may have affected growth through changes in the medium pH as well. Ingram et al. (1993) pointed out that pH of >7.5 can cause fixation of micronutrients in container media. Fertilization slightly decreased the pH in fertilized treatments during the growing period. The lowered pH (from 7.75 – 7.60) in these treatments might have allowed for increased uptake of nutrients and enhanced growth of the fertilized seedlings compared to non-fertilized ones.

Mineral nutrition can enhance quality of seedlings by promoting growth and dry mass accumulation. Enhanced growth of fertilized seedlings led to greater DQIs. DQI is a function of height, RCD, root dry mass, shoot dry mass and total dry mass (Dickson et al. 1960) and is correlated with these morphological attributes of seedlings (Binotto et al. 2010).

Fertilization can also affect the allocation of resources (photosynthates, nutrients and water) toward biomass production within the plant. Results of several studies indicate that shoot growth is enhanced with N fertilization, however, growth and biomass allocation to the root system is not positively affected by N fertilization (Navarro et al. 2006; Wooldridge et al. 2009; Villar-Salvador et al. 2005; Fernandez et al. 2007; Driessche 1980). We observed similar results of higher shoot dry mass than root dry mass in the fertilized treatments. Villar-Salvador et al. (2005) found that N-fertilization increased total plant size of Mediterranean tree species (*Quercus ilex* L., *Quercus suber* L., *Quercus coccifera* L., *Pinus pinea, Juniperus thurifera* L. *and P. halepensis*), but as the N-fertilizer rate increased, shoot growth was promoted and resulting in lower R:S ratios. Oliet et al. (2009) also found that fertilization increased shoot dry mass and decreased R:S ratio of *P. halepensis* seedlings. In our study, although fertilization increased overall growth of *E. benthamii* seedlings, it did not change R:S ratios in M1, M3 and M4. In contrast, non-fertilized M2 and M5 developed seedlings with high R:S ratios due to smaller shoot mass compared to their root masses.

The ratio between height and RCD is an indicator of the sturdiness of a seedling (Haase 2008). The lower the sturdiness value, the sturdier the seedling. Although, fertilized seedlings were taller in height, they also had greater RCDs, which led to lower sturdiness values than non-fertilized seedlings. This contradicts results observed by Navarro et al. 2006, where they found higher sturdiness values for *Abies pinsapo* Boiss. seedlings raised under N fertilization.

The type of growing media also influences the growth response of containerized seedlings growth (Wightman et al. 2001). We observed significant differences among medium types in this study for most of the morphological measurements (e.g., height, RCD, and dry mass). Media types containing rice hulls typically resulted in significantly less growth compared to the non-rice hull treatments. This difference, and others among media types, might be due to the different physical and chemical characteristics of each medium mixtures.

Height, RCD, root dry mass, shoot dry mass, total seedling dry mass and DQI of *E. benthamii* seedlings were high in M1, M3, and M4. These medium types were mixtures of different volumes of soil, sand, and compost. These substrates were composed of organic and inorganic materials (Landis et al. 2014) and may have provided higher amounts of available nutrients. Ahmadloo et al. (2012) found that presence of organic matter in growing media improved growth and development of *C. arizonica* seedlings. They also found high values for shoot dry mass, root dry mass, total dry mass, and DQI of *C. arizonica* seedlings growing in a mixture of 3:1:1:1:1 of loam:sand:bran (hard outer layer of cereal grain):cattle manure:decomposed litter. Seedlings grew better in this medium because of better availability of nutrients (N, P, K, Ca, and Mg) and a lower C/N ratio than other medium types. In our study, nitrogen was a limiting nutrient to the seedlings in all medium types (Table 1.2). Fertilization of these medium types appeared to alleviate the relative shortage of N and elicited greater growth. The greater growth of seedlings in the soil, sand, and compost-containing M1, M3, and M4 without fertilization might be due to moderate physical properties (bulk density, particle density, and porosity) of these media compared to media containing the rice hulls. M3 and M4 are two medium types currently used in Afghanistan. Both of these mixtures showed acceptable results for morphological traits of *E. benthamii* seedlings grown in polybags. Along with M3 and M4, we observed acceptable morphological responses in M1 as well. This medium type was studied by Qaisar et al. (2008) where they found similar results for *C. deodara* seedlings.

Less overall growth was observed in seedlings grown in M2 and M5, compared with the other three media. Both of these medium types contained 50% parboiled rice hulls in their mixtures. Even the presence of 50% soil in M2 and 50% compost in M5 was not sufficient to sustain vigorous growth and biomass production. The rice hulls in M2 and M5 might have decreased growth due to lower medium concentrations of N (NH_4 & NO_3), Mg, Ca, and S. Like other medium types, nutrient concentrations of seedlings in fertilized and non-fertilized M2 and M5 were in the acceptable range; however, without fertilization, nutrient contents in the seedlings were very low. Higher seedling growth and development in fertilized M2 and M5 indicates that there was nutrient deficiency in both medium types under the no fertilization regime. Our nutrient analysis of parboiled rice hulls found unbalanced levels of nutrients (low or high). Low levels of nutrients might have led to deficiency and high levels (P, K, Mn, and soluble salts) might have caused nutrient antagonism or toxicity in these mixtures (Table 1.2).

Some studies suggest rice hulls can be used as part of growing media. Rice hulls might be better if used in composted form. Einert & Guidry (1975) found good growth of juniper seedlings growing in media containing 50% composted rice hulls and 50% soil and 40%

composted rice hulls and 60 % compost compared to media containing parboiled rice hulls. Dueitt (1994) reported that both composted and fresh rice hulls can be used as a substitute for vermiculite, but with increasing proportions in the mixture, it could result in a decreased plant growth due to its lower nutritional value, as was found in our study. Rice hulls can be used instead of perlite (Tsakaldimi 2006) or other materials to improve physical properties of media. In his study, Tsakaldimi (2006) found greater *P. halepensis* growth in a medium mixture containing 3 peat: 1 rice hulls compared to 3 peat: 1 perlite. Gomez & Robbins (2010) studied the effect of rice hull volume in growing media on growth of spirea (Spirea x bumalda L.) and found that as percentage (0, 20, 40, 60, 80 and 100%) of rice hulls increased, growth of spirea (*Spirea x bumalda* L.) decreased.

Larger R:S ratios were observed in M2 and M5 treatments, under the no fertilization regime. Ericsson (1995) pointed out that greater R:S ratios can be due to lower availability of nutrients. Seedlings grown without fertilization might have experienced a lack of nutrients (especially N), which led to relatively greater root system development and limited shoot growth. Sturdiness values on the other hand were higher in both M2 and M5. Seedlings grown in these two medium types produced lower RCDs compare to their heights, which led to higher height-diameter ratios and less sturdy seedlings.

Root architecture

The number of FOLRs is an important measure of seedling root morphology and can be a measure of seedling quality (Dey & Parker 1997, Kozlowski et al. 1973). It can affect seedling field growth and performance (Davis & Jacobs 2005). In this study, the number of FOLRs increased through fertilization regardless of medium type. Chamshama & Hall (1987) also observed more FOLRs with N fertilization of *E. camaldulensis* seedlings during the nursery phase. Among the medium types, M4, M3, and M1 produced more total numbers of FOLRs, with and without fertilization, than the other two medium types, probably as a result of greater overall growth.

Fertilization also increased production of active root tips by enhancing seedling growth. Larger seedlings produced larger and more fibrous root systems. Thompson (1985) pointed out that the number of active root tips can be greater in seedlings with high fibrosity and FOLRs. Jacobs et al. (2003) found that active root tip production in Douglas-fir seedlings increased with supply of controlled release fertilizer. We observed more active root tips in fertilized medium types compared to non-fertilized ones. The number of active root tips decreased from the root top to the bottom segment. The higher number of active root tips in the root top segment might be due to more availability of water, nutrients and aeration. Alternatively it could be due to the developmental progression of growth of the root systems.

The larger number of spiraled roots for fertilized seedlings may be the result of the associated larger root systems with fertilization. Most of the observed root spiraling occurred at the bottom of the polybag. This is similar to the findings of Dumroese & Wenny (1997) who observed root spiraling of ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) seedlings at the bottom of polybags. No root spiraling was observed at root top and mid segments of root systems for our study.

Differences in numbers of FOLRs, active root tips, and root spiraling among media might also be the result of different physical or chemical properties of growing media (Table 1.2). Physical properties such as medium moisture content, bulk density and porosity might have affected root development among these three medium types. For instance, presence of sand in M4 might have improved physical properties of the mixture such as aeration and fostered better root development, increasing the number of FOLRs and active root tips production, but also leading to the greatest amount of root spiraling.

The number of FOLRs, active root tips, and root spiraling was affected by root and total seedling size. Ruehle & Kormanik (1986) found positive correlations between Northern red oak seedlings' growth variables (height, RCD, root and shoot dry mass) and FOLRs. We observed more FOLRs, active root tips, and root spiraling in bigger seedlings with larger root systems. Although it is better to have higher number FOLRs and active root tips, root spiraling is considered a negative characteristic. A balance is required between the number of FOLRs, active root tips, and root spiraling, which necessitates further research. Seedling nutrition management might be one of the ways where we can manipulate root growth and create a balance between FOLRs, active root tips and root spiraling.

CONCLUSIONS

The main results of this study are that fertilization enhanced seedling morphological attributes of *E. benthamii* seedlings raised in five medium types. The inclusion of 50% rice hulls in the medium mixtures reduced overall seedling growth. Although lower than some non-fertilized treatments, R:S ratio did not decrease substantially with fertilization. Non-fertilized treatments on the other hand, produced smaller seedlings in size and they were less sturdy. Fertilized seedlings produced more first order later roots and more active root tips.

Supply of nutrients can enhance seedlings growth and quality. Nutrition management is very important in the nursery. Fertilizer type, amount, rate of application, physical and chemical properties of soil or media are all important factors that need to be considered during fertilization. Because Afghanistan is an arid country, seedlings with high R:S ratios could survive better after planting in the field. While an abundant supply of nutrients, especially N, can promote shoot growth, it might also decrease root growth and yield seedlings with lower R:S ratios. This might cause poor field survival so nutrition management needs to be balanced.

Soil, sand, and compost-containing media (M1, M3 and M4) can be used as acceptable growing media in Afghanistan or other developing countries. These medium types can support growth and development of seedlings and perform even better when fertilizers are applied. Likewise, substrates used in these mixtures are readily available and are cheap. Parboiled rice hulls can also be used as part of the mixture only if fertilizers are applied.

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TABLES & FIGURES

Table 1.3. Analyses of variance showing P – values from testing the effects of Media, Fertilizer and the Media X Fertilizer interaction ($\alpha = 0.05$) on growing media pH, growth and morphological attributes of *E. benthamii* seedlings (RCD = root collar diameter, FOLR = first order lateral roots, DWT = dry weight, R:S ratio = root-to-shoot ratio).

Variable	Media	Fertilizer	Media*Fertilizer	Variable	Media	Fertilizer	Media*Fertilizer
Height-March (cm)	<.0001	<.0001	<.0001	*Root Mid Spiral*	0.46	0.15	----
RCD-March (mm)	<.0001	<.0001	0.009	*Root Bottom Spiral*	<.0001	<.0001	<.0001
Height-April (cm)	<.0001	<.0001	<.0001	*Root Total Spiral*	<.0001	<.0001	<.0001
RCD-April (mm)	<.0001	<.0001	<.0001	*Root Top DWt (g)*	<.0001	<.0001	<.0001
Height-May (cm)	<.0001	<.0001	<.0001	*Root Mid DWt (g)*	<.0001	<.0001	----
RCD-May (mm)	<.0001	<.0001	<.0001	*Root Bottom DWt (g)*	0.005	<.0001	0.001
Root Top FOLRs	<.0001	<.0001	0.009	*Total Root DWt (g)*	<.0001	<.0001	<.0001
Root Mid FOLRs	<.0001	<.0001	----†	*Shoot DWt (g)*	<.0001	<.0001	<.0001
Root Bottom FOLRs	0.042	<.0001	----	*Total DWt (g)*	<.0001	<.0001	<.0001
Total FOLRs	<.0001	<.0001	0.003	*R:S Ratio*	<.0001	<.0001	<.0001
Top Active Root Tips	<.0001	<.0001	0.004	*Dickson Quality Index*	<.0001	<.0001	<.0001
Mid Active Root Tips	0.019	<.0001	----	*Sturdiness*	<.0001	<.0001	0.030
Bottom Active Root Tips	0.079	0.015	----	*pH-Jan*	<.0001	0.053	----
Total Active Root Tips	<.0001	<.0001	0.010	*pH-March*	0.008	<.0001	----
Root Top Spiral	0.56	0.43	----	*pH-May*	0.081	0.066	----

† Media X Fertilizer interaction was not significant at $\alpha = 0.05$ so that a reduced model was used.

Table 1.4. Least square means, standard errors (S.E.) and number of observations (N) of Media X Fertilizer treatment combinations for height growth of *E. benthamii* seedlings in March, April and May, 2014. Superscript letters within a column with the same letter are not significantly different using Tukey-Kramer multiple comparison test at $\alpha = 0.05$.

Treatments	March			April			May		
	Height (cm)	**S.E**	**N**	**Height (cm)**	**S.E**	**N**	**Height (cm)**	**S.E**	**N**
M1 - Fert	14.4^{ab}	±0.67	89	37.1^{a}	±1.44	89	51.5^{ab}	±2.66	89
M2 - Fert	13.0^{ab}	±0.71	78	37.4^{a}	±1.51	78	49.7^{ab}	±2.66	78
M3 - Fert	14.1^{ab}	±0.79	61	38.7^{a}	±1.65	61	53.8^{a}	±3.12	61
M4 - Fert	14.9^{a}	±0.71	77	41.9^{a}	±1.51	77	56.4^{a}	±3.03	77
M5 - Fert	10.4^{b}	±0.65	96	30.1^{b}	±1.41	96	44.4^{bc}	±2.24	96
M1 - NotFert	12.7^{ab}	±0.64	100	29.0^{b}	±1.40	100	37.9^{cd}	±1.89	100
M2 - NotFert	3.3^{d}	±0.65	96	6.3^{c}	±1.41	96	9.5^{f}	±0.48	95
M3 - NotFert	11.9^{ab}	±0.65	96	26.9^{b}	±1.41	96	35.8^{d}	±1.81	95
M4 - NotFert	12.4^{ab}	±0.66	94	27.7^{b}	±1.42	94	36.3^{d}	±1.84	94
M5 - NotFert	5.2^{c}	±0.63	105	9.9^{c}	±1.38	105	13.2^{e}	±0.65	105

Table 1.5. Least square means, standard errors (S.E.) and number of observations (N) of Media X Fertilizer treatment combinations for RCD (root collar diameter) growth of *E. benthamii* seedlings in March, April and May, 2014. Superscript letters within a column with the same letter are not significantly different using Tukey-Kramer multiple comparison test at $\alpha = 0.05$.

Treatments	March			April			May		
	RCD (mm)	S.E	N	RCD (mm)	S.E	N	RCD (mm)	S.E	N
M1 - Fert	2.3^{ab}	±0.06	89	4.5^{a}	±0.18	89	6.2^{ab}	±0.65	89
M2 - Fert	1.3^{d}	±0.06	78	3.7^{b}	±0.16	78	5.4^{b}	±0.62	78
M3 - Fert	2.4^{a}	±0.07	61	4.7^{a}	±0.23	61	6.7^{a}	±0.87	61
M4 - Fert	2.4^{a}	±0.06	77	4.9^{a}	±0.21	77	7.0^{a}	±0.80	77
M5 - Fert	1.1^{d}	±0.06	96	3.0^{c}	±0.12	96	4.2^{c}	±0.43	96
M1 - NotFert	2.0^{c}	±0.06	100	3.0^{c}	±0.12	100	3.8^{c}	±0.38	100
M2 - NotFert	0.6^{e}	±0.06	96	0.8^{e}	±0.03	96	1.0^{d}	±0.10	95
M3 - NotFert	1.9^{c}	±0.06	96	2.9^{c}	±0.11	96	3.6^{c}	±0.37	95
M4 - NotFert	2.1^{bc}	±0.06	94	3.0^{c}	±0.12	94	3.8^{c}	±0.39	94
M5 - NotFert	0.8^{e}	±0.06	105	1.0^{d}	±0.04	105	1.1^{d}	±0.11	105

Table 1.6. Least square means, standard errors (S.E.) and number of observations (N) of Media X Fertilizer treatment combinations for root dry weight (DWt = dry weight) of *E. benthamii* seedlings. Superscript letters within a column with the same letter are not significantly different using Tukey-Kramer multiple comparison test at $\alpha = 0.05$.

Treatments	Root Top DWt (g)			Root Mid DWt (g)			Root Bottom DWt (g)			Total Root DWt (g)		
	Mean	**S.E**	**N**	**Mean**	**S.E**	**N**	**Mean**	**S.E**	**N**	**Mean**	**S.E**	**N**
M1 - Fert	5.16^{ab}	±0.60	53	0.43	±0.04	53	0.21^{ab}	±0.03	53	5.80^{ab}	±0.67	53
M2 - Fert	3.19^{bc}	±0.41	42	0.24	±0.04	42	0.29^{a}	±0.03	42	3.70^{bc}	±0.47	42
M3- Fert	4.32^{ab}	±0.64	30	0.42	±0.05	30	0.16^{bc}	±0.03	30	4.88^{ab}	±0.72	30
M4 - Fert	6.40^{a}	±0.83	41	0.38	±0.04	41	0.16^{bc}	±0.03	41	6.92^{a}	±0.89	41
M5 - Fert	2.54^{c}	±0.28	60	0.17	±0.03	60	0.12^{bcd}	±0.02	60	2.83^{c}	±0.31	60
M1 - NotFert	1.50^{d}	±0.16	64	0.14	±0.03	64	0.05^{cde}	±0.02	64	1.68^{d}	±0.18	64
M2 - NotFert	0.06^{e}	±0.01	59	0.01	±0.03	59	0.00^{e}	±0.02	59	0.07^{e}	±0.01	59
M3 - NotFert	1.26^{d}	±0.14	58	0.08	±0.03	58	0.02^{e}	±0.02	58	1.36^{d}	±0.15	58
M4 - NotFert	1.54^{d}	±0.17	58	0.11	±0.03	58	0.05^{cde}	±0.03	58	1.70^{d}	±0.19	58
M5 - NotFert	0.09^{e}	±0.01	68	0.01	±0.03	68	0.01^{e}	±0.02	68	0.11^{e}	±0.01	68

Table 1.7. Least square means, standard errors (S.E.) and number of observations (N) of Media and Fertilizer predictor variables for root mid dry weight (DWt = dry weight). Superscript letters within a column with the same letter are not significantly different using Tukey-Kramer multiple comparison test at α = 0.05.

Media	Root Mid DWt		
	Mean (g)	**S.E**	**N**
M1	0.28^{a}	±0.03	117
M2	0.13^{b}	±0.03	101
M3	0.24^{a}	±0.03	88
M4	0.24^{a}	±0.03	99
M5	0.09^{b}	±0.02	128
Fertilizer	**Mean (g)**	**S.E**	**N**
Fert	0.32^{a}	±0.02	226
Notfert	0.07^{b}	±0.02	307

Table 1.8. Least square means, standard errors (S.E.) and number of observations (N) of Media X Fertilizer treatment combinations for shoot and total dry weight (DWt = dry weight) of *E. benthamii* seedlings. Superscript letters within a column with the same letter are not significantly different using Tukey-Kramer multiple comparison test at α = 0.05.

Treatments	**Shoot DWt**			**Total DWt**		
	Mean (g)	**S.E**	**N**	**Mean (g)**	**S.E**	**N**
M1 - Fert	7.19^{a}	±0.79	53	13.08^{ab}	±1.47	53
M2 - Fert	5.24^{ab}	±0.64	42	9.00^{bc}	±1.11	42
M3- Fert	6.71^{a}	±0.94	30	11.67^{ab}	±1.67	30
M4 - Fert	8.31^{a}	±1.02	41	15.34^{a}	±1.91	41
M5 - Fert	3.44^{b}	±0.36	60	6.31^{c}	±0.67	60
M1 - NotFert	2.13^{c}	±0.22	64	3.84^{d}	±0.40	64
M2 - NotFert	0.06^{e}	±0.01	59	0.13^{e}	±0.01	59
M3 - NotFert	1.82^{c}	±0.19	58	3.21^{d}	±0.35	58
M4 - NotFert	2.01^{c}	±0.21	58	3.73^{d}	±0.40	58
M5 - NotFert	0.11^{d}	±0.01	68	0.22^{e}	±0.02	68

Table 1.9. Least square means, standard errors (S.E.) and number of observations (N) of Media X Fertilizer treatment combinations for R:S ratio (root-to-shoot ratio), sturdiness and DQI (Dickson quality index) of *E. benthamii* seedlings. Superscript letters within a column with the same letter are not significantly different using Tukey-Kramer multiple comparison test at α = 0.05.

Treatments	R:S Ratio			Sturdiness			Dickson Quality Index		
	Mean	**S.E**	**N**	**Mean**	**S.E**	**N**	**Mean**	**S.E**	**N**
M1 - Fert	0.76^{cd}	±0.03	53	9.6^{def}	±0.61	53	1.6^{ab}	±0.12	53
M2 - Fert	0.71^{cd}	±0.03	42	9.9^{cdef}	±0.66	42	0.9^{dc}	±0.13	42
M3- Fert	0.71^{cd}	±0.04	30	9.0^{ef}	±0.74	30	1.3^{bc}	±0.15	30
M4 - Fert	0.80^{cd}	±0.04	41	8.7^{f}	±0.66	41	2.0^{a}	±0.13	41
M5 - Fert	0.83^{c}	±0.03	60	11.0^{bcde}	±0.58	60	0.6^{de}	±0.11	60
M1 - NotFert	0.75^{cd}	±0.03	64	11.5^{bc}	±0.57	64	0.3^{ef}	±0.11	64
M2 - NotFert	1.43^{a}	±0.06	59	14.5^{a}	±0.59	59	0.01^{f}	±0.11	59
M3 - NotFert	0.68^{d}	±0.03	58	11.9^{bc}	±0.59	58	0.3^{ef}	±0.11	58
M4 - NotFert	0.82^{c}	±0.03	58	10.8^{cdef}	±0.59	58	0.3^{ef}	±0.11	58
M5 - NotFert	1.08^{b}	±0.04	68	12.9^{ab}	±0.56	68	0.01^{f}	±0.10	68

Table 1.10. Least square means, standard errors (S.E.) and number of observations (N) of Media X Fertilizer treatment combinations for number of FOLRs (first order lateral roots) of *E. benthamii* seedlings. Superscript letters within a column with the same letter are not significantly different using Tukey-Kramer multiple comparison test at α = 0.05.

Treatments	Root Top FOLRs			Root Mid FOLRs			Root Bottom FOLRs			Total FOLRs		
	Mean	**S.E**	**N**	**Mean**	**S.E**	**N**	**Mean**	**S.E**	**N**	**Mean**	**S.E**	**N**
M1 - Fert	49.3^{bc}	±4.1	20	12.2	±1.3	20	5.6	±1.0	20	67.1^{bc}	±7.2	20
M2 - Fert	47.7^{bc}	±4.4	16	10.2	±1.4	16	9.0	±1.1	16	66.9^{bcd}	±7.6	16
M3- Fert	57.3^{ab}	±4.8	13	13.7	±1.6	13	8.2	±1.3	13	79.3^{ab}	±10.0	13
M4 - Fert	66.7^{a}	±4.3	17	13.4	±1.4	17	10.2	±1.1	17	90.4^{a}	±10.3	17
M5 - Fert	42.8^{bc}	±4.0	23	8.5	±1.2	23	6.8	±1.0	23	58.2^{cde}	±5.9	23
M1 - NotFert	35.9^{c}	±3.9	25	8.6	±1.2	25	2.9	±0.9	25	47.4^{e}	±4.6	25
M2 - NotFert	11.8^{d}	±4.1	20	2.2	±1.3	20	0.4	±1.0	20	14.3^{f}	±1.6	20
M3 - NotFert	38.7^{c}	±4.0	23	6.9	±1.2	23	2.4	±1.0	23	48.0^{de}	±4.9	23
M4 - NotFert	42.6^{bc}	±4.0	22	8.0	±1.3	22	3.4	±1.0	22	54.1^{cde}	±5.7	22
M5 - NotFert	14.9^{d}	±3.9	25	2.7	±1.2	25	0.6	±0.9	25	18.2^{f}	±1.7	25

Table 1.11. Least square means, standard errors (S.E.) and number of observations (N) of Media and Fertilizer predictor variables for root mid and root bottom number of FOLRs (first order lateral roots) of *E. benthamii* seedlings. Superscript letters within a column with the same letter are not significantly different using Tukey-Kramer multiple comparison test at $\alpha = 0.05$.

Media	Root Mid FOLRs			Root Bottom FOLRs		
	Mean (#)	**S.E**	**N**	**Mean (#)**	**S.E**	**N**
M1	10.5^{a}	±0.97	45	4.4^{ab}	±0.69	45
M2	6.1^{b}	±1.05	36	4.5^{ab}	±0.77	36
M3	10.1^{a}	±1.05	36	5.3^{ab}	±0.78	36
M4	10.7^{a}	±1.02	39	6.7^{a}	±0.74	39
M5	5.6^{b}	±0.95	48	3.7^{b}	±0.67	48
Fertilizer	**Mean (#)**	**S.E**	**N**	**Mean (#)**	**S.E**	**N**
Fert	11.5^{a}	±0.80	89	7.9^{a}	±0.50	89
Notfert	5.7^{b}	±0.76	115	2.0^{b}	±0.43	115

Table 1.12. Least square means, standard errors (S.E.) and number of observations (N) of Media X Fertilizer treatment combinations for number of active root tips of *E. benthamii* seedlings. Superscript letters within a column with the same letter are not significantly different using Tukey-Kramer multiple comparison test at α = 0.05.

Treatments	Top Active Roots			Mid Active Roots			Bottom Active Roots			Total Active Roots		
	Mean	**S.E**	**N**	**Mean**	**S.E**	**N**	**Mean**	**S.E**	**N**	**Mean**	**S.E**	**N**
M1 - Fert	28.3^{ab}	±2.26	6	3.3	±0.66	6	0.3	±0.53	6	32.0^{ab}	±2.54	6
M2 - Fert	20.3^{bcd}	±2.77	4	1.3	±0.81	4	2.5	±0.65	4	24.0^{bc}	±3.12	4
M3- Fert	39.3^{a}	±3.19	3	2.3	±0.94	3	1.7	±0.75	3	43.3^{a}	±3.60	3
M4 - Fert	26.5^{ab}	±2.77	4	3.0	±0.81	4	2.8	±0.65	4	32.3^{ab}	±3.12	4
M5 - Fert	13.0^{de}	±2.09	7	1.6	±0.61	7	0.6	±0.49	7	15.1^{cd}	±2.36	7
M1 - NotFert	16.3^{cd}	±2.09	7	0.4	±0.61	7	0.3	±0.49	7	17.0^{c}	±2.36	7
M2 - NotFert	4.3^{e}	±2.26	6	0.0	±0.00	6	0.0	±0.00	6	4.3^{d}	±2.54	6
M3 - NotFert	14.0^{de}	±2.26	6	0.5	±0.66	6	1.2	±0.53	6	15.7^{cd}	±2.54	6
M4 - NotFert	20.2^{bcd}	±2.26	6	1.8	±0.66	6	0.8	±0.53	6	22.8^{bc}	±2.54	6
M5 - NotFert	5.8^{e}	±1.96	8	0.1	±0.57	8	0.4	±0.46	8	6.3^{d}	±2.20	8

Table 1.13. Least square means, standard errors (S.E.) and number of observations (N) of Media and Fertilizer predictor variables for root mid and root bottom active root tips. Superscript letters within a column with the same letter are not significantly different using Tukey-Kramer multiple comparison test at α = 0.05.

Media	Mid Active Root Tips			Bottom Active Root Tips		
	Mean (#)	S.E	N	Mean (#)	S.E	N
M1	1.8^{ab}	±0.44	13	0.3^{a}	±0.37	13
M2	0.7^{b}	±0.51	10	1.1^{a}	±0.43	10
M3	1.4^{ab}	±0.54	9	1.5^{a}	±0.45	9
M4	2.5^{a}	±0.51	10	1.7^{a}	±0.43	10
M5	0.9^{b}	±0.41	15	0.5^{a}	±0.35	15
Fertilizer	**Mean**	**S.E**	**N**	**Mean**	**S.E**	**N**
Fert	2.3^{a}	±0.33	24	1.5^{a}	±0.28	24
Notfert	0.6^{b}	±0.28	33	0.6^{b}	±0.23	33

Table 1.14. Least square means, standard errors (S.E.) and number of observations (N) of Media X Fertilizer treatment combinations for number of spiraled roots of *E. benthamii* seedlings. Superscript letters within a column with the same letter are not significantly different using Tukey-Kramer multiple comparison test at $\alpha = 0.05$.

Treatments	Root Top Spiral			Root Mid Spiral			Root Bottom Spiral			Total Spiral		
	Mean	**S.E**	**N**	**Mean**	**S.E**	**N**	**Mean**	**S.E**	**N**	**Mean**	**S.E**	**N**
M1 - Fert	0.2	±0.1	53	0.0	±0.1	53	6.3^{a}	±0.5	53	6.3^{a}	±0.5	53
M2 - Fert	0.1	±0.1	42	0.0	±0.1	42	4.8^{a}	±0.6	42	4.8^{a}	±0.4	42
M3- Fert	0.0	±0.1	30	0.0	±0.0	30	7.2^{a}	±0.7	30	7.0^{a}	±0.6	30
M4 - Fert	0.0	±0.1	41	0.0	±0.1	41	7.5^{a}	±0.6	41	7.4^{a}	±0.6	41
M5 - Fert	0.0	±0.0	60	0.2	±0.1	60	6.5^{a}	±0.5	60	6.6^{a}	±0.5	60
M1 - NotFert	0.0	±0.0	64	0.0	±0.1	64	2.7^{b}	±0.5	64	2.7^{b}	±0.2	64
M2 - NotFert	0.0	±0.0	59	0.0	±0.1	59	0.0^{c}	±0.5	59	0.0^{c}	±0.0	59
M3 - NotFert	0.0	±0.0	58	0.0	±0.1	58	2.1^{b}	±0.5	58	2.1^{b}	±0.2	58
M4 - NotFert	0.1	±0.0	58	0.0	±0.0	58	2.2^{b}	±0.5	58	2.2^{b}	±0.2	58
M5 - NotFert	0.0	±0.0	68	0.0	±0.0	68	0.1^{c}	±0.5	68	0.1^{c}	±0.0	68

Table 1.15. Least square means, standard errors (S.E.) and number of observations (N) of Media and Fertilizer predictor varibles for growing media pH measured in January, March and May 2014. Superscript letters within a column with the same letter are not significantly different using Tukey-Kramer multiple comparison test at α = 0.05.

Media	**January**			**March**			**May**		
	pH	**S.E**	**N**	**pH**	**S.E**	**N**	**pH**	**S.E**	**N**
M1	7.55^{c}	±0.04	12	7.83^{ab}	±0.07	12	7.67^{ab}	±0.09	12
M2	7.79^{b}	±0.06	12	7.84^{ab}	±0.12	12	7.52^{b}	±0.10	12
M3	7.59^{c}	±0.03	12	7.73^{b}	±0.09	12	7.68^{ab}	±0.07	12
M4	7.64^{bc}	±0.04	12	7.66^{b}	±0.10	12	7.64^{ab}	±0.07	12
M5	7.98^{a}	±0.07	12	8.03^{a}	±0.04	12	7.75^{a}	±0.06	12
Fertilizer	**pH**	**S.E**	**N**	**pH**	**S.E**	**N**	**pH**	**S.E**	**N**
NotFert	7.67^{a}	±0.04	30	8.00^{a}	±0.03	30	7.70^{a}	±0.05	30
Fert	7.75^{a}	±0.04	30	7.64^{b}	±0.06	30	7.60^{a}	±0.05	30

Table 1.16. Nutrient contents (mg) of five months old *E. benthamii* seedlings grown in six media with and without additional fertilizer.

Treatments	N	P	K	Mg	Ca	S	B	Zn	Mn	Fe	Cu
M1-Fert	137.6	15.7	99.6	36.7	96.9	27.5	0.85	0.29	1.48	0.46	0.01
M2-Fert	96.8	10.0	59.7	24.4	47.1	10.9	0.42	0.20	9.84	0.36	0.01
M3-Fert	128.5	13.9	93.8	34.7	92.6	25.5	0.97	0.24	1.83	0.53	0.01
M4-Fert	213.3	21.6	140.7	43.3	120.6	34.0	1.11	0.42	2.47	0.74	0.03
M5-Fert	67.1	6.5	41.3	16.8	43.2	7.7	0.37	0.13	0.89	0.20	0.01
M1-NotFert	46.6	4.7	23.9	14.9	34.9	7.8	0.18	0.10	0.24	0.13	0.01
M2-NotFert	1.8	0.2	1.0	0.5	1.2	0.3	0.01	0.00	0.06	0.01	0.00
M3-NotFert	53.8	4.2	18.6	11.1	35.9	4.9	0.15	0.08	0.32	0.17	0.01
M4-NotFert	49.4	5.3	22.2	13.9	41.1	5.3	0.17	0.09	0.25	0.14	0.01
M5-NotFert	2.8	0.3	1.4	0.9	2.5	0.4	0.01	0.01	0.04	0.01	0.00

Table 1.17. Macro nutrients (%) and micronutrients (ppm) concentrations of leaves of six months old *E. benthamii* seedlings grown in five media with and without additional fertilizer.

Treatments	N	P	K	Mg	Ca	S	B	Zn	Mn	Fe	Cu
M1-Fert	1.05	0.12	0.76	0.28	0.74	0.21	65	22	113	35	1
M2-Fert	1.07	0.11	0.66	0.27	0.52	0.12	46	22	1087	40	1
M3-Fert	1.11	0.12	0.81	0.3	0.8	0.22	84	21	158	46	1
M4-Fert	1.38	0.14	0.91	0.28	0.78	0.22	72	27	160	48	2
M5-Fert	1.04	0.1	0.64	0.26	0.67	0.12	57	20	138	31	1
M1-NotFert	1.19	0.12	0.61	0.38	0.89	0.2	47	25	61	34	3
M2-NotFert	1.26	0.16	0.74	0.38	0.85	0.21	39	31	394	57	1
M3-NotFert	1.65	0.13	0.57	0.34	1.1	0.15	47	24	99	53	2
M4-NotFert	1.31	0.14	0.59	0.37	1.09	0.14	45	25	67	37	2
M5-NotFert	1.17	0.12	0.58	0.37	1.05	0.15	44	29	147	35	3

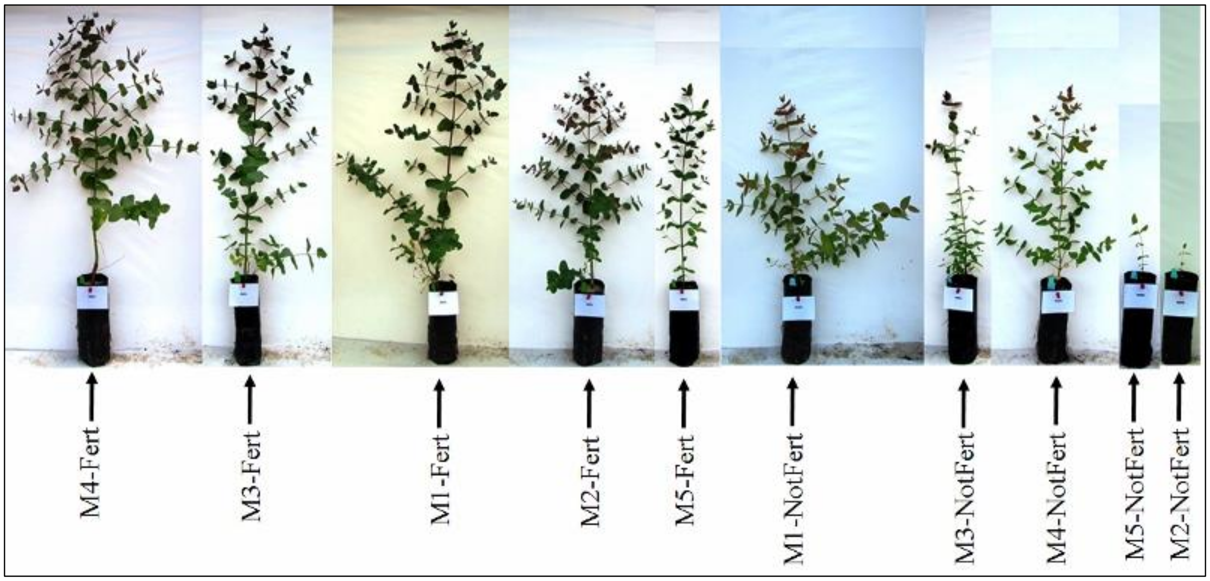

Figure 1.1. Final height of *E. benthamii* seedlings grown in five media types and two fertilization regimes. M1 = soil (17%) : sand (33%) : compost (50%), M2 = soil (17%) : sand (33%) : rice hull (50%), M3 = soil (50%) : sand (17%) : compost (33%), M4 = soil (25%) : sand (50%) : compost (25%), M5 = sand (17%) : compost (33%) : rice hull (50%).

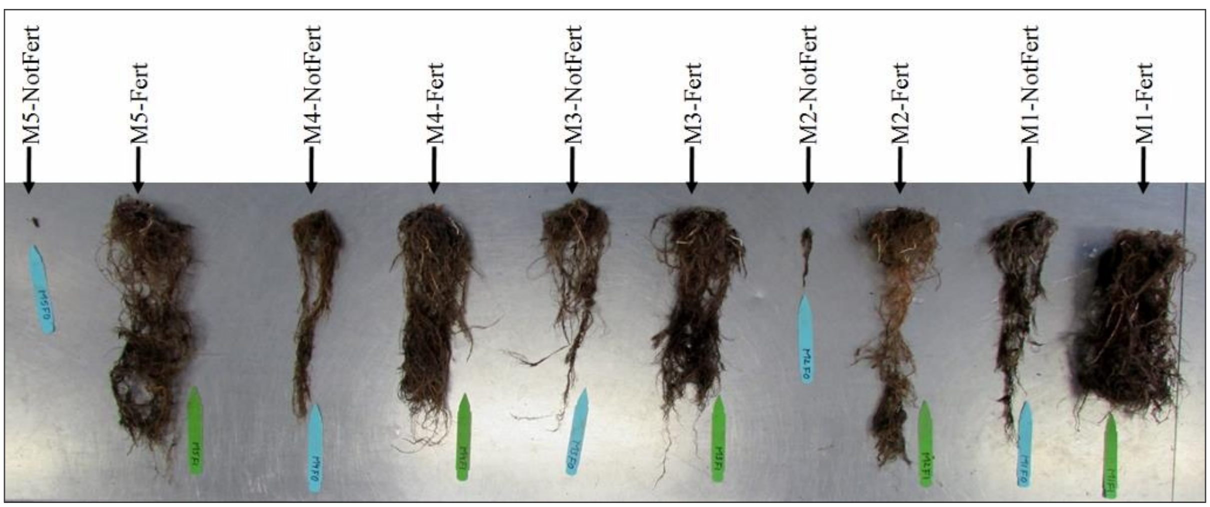

Figure 1.2. Six months old *E. benthamii* seedlings root system grown in five media types and two fertilization regimes. M1 = soil (17%) : sand (33%) : compost (50%), M2 = soil (17%) : sand (33%) : rice hull (50%), M3 = soil (50%) : sand (17%) : compost (33%), M4 = soil (25%) : sand (50%) : compost (25%), M5 = sand (17%) : compost (33%) : rice hull (50%).

CHAPTER II: Growing Media and Fertilization Effects on Early Post – Planting Growth and Survival of *Eucalyptus benthamii* Maiden & Cambage Seedlings under Drought Stress

INTRODUCTION

In reforestation projects, drought is one of the environmental stresses that can challenge survival of newly planted trees in arid regions (Grossnickle 2012). Under droughty conditions, survival of seedlings is affected by inadequate soil water (Harper et al. 2009) and in newly planted seedlings, water stress can be higher due to weak root contact with soil particles (Burdett 1990). Also, at high levels of plant moisture stress, seedling growth is slowed due to lower stomatal conductance and reduced photosynthesis (Haase 2008). In order to resist water stress, a seedling needs to be morphologically and physiologically capable of coping with drought stress on a planting site (Slot & Poorter 2007).

Higher quality seedlings increase both survival and growth on planting sites (Gould & Harrington 2009; Wilson & Jacobs 2006). Morphology and physiology are two important factors that affect seedling quality (Davis & Jacobs 2005) and survival and growth on site. Jacobs et al. (2005) pointed out that hardwood seedling morphological attributes in the nursery are positively correlated with first-year field height and root collar diameter (RCD) growth. Tsakaldimi et al. (2012) observed high positive correlations between initial morphology and field survival in Mediterranean species (*Pinus halepensis* Miller, *Q. ilex, Q. coccifera, Ceratonia silqua* L. *and Pistacia lentiscus* L.), two years after planting; however, the significance of the correlations differed among species and was variable among morphological attributes.

Studies show that RCD is an important predictor of seedling performance in the field (Johnson & Cline 1991; Mexal & Landis 1990). It can indicate root system size, sturdiness, and resistance to drought (Mexal & Landis 1990). Shoot height of seedlings in the nursery is not a clear indicator of field survival given variable results from past research. Sharma et al. (2007) observed a significant and negative relationship between nursery final height and field survival of *Pinus radiata* D.Don seedlings. Thompson (1985) pointed out that height has an unpredictable effect on survival of planted seedlings on dry sites. Others indicate no correlation between nursery height and field survival (Pawsey 1972; Mullin & Svaton 1972).

Root to shoot (R:S) is another morphological attribute that can affect survival rates after planting (Lamhamedi et al. 1997). R:S ratio is the ratio between the mass of roots as water absorbing sites and shoot mass as transpirational sites (Haase 2008). Seedlings with lower R:S ratio have shown to maintain higher survival rates in mesic climates (Barnett 1984; Mexal & Landis 1990). Seedlings with larger shoot sizes can grow better due to greater photosynthetic capacity if there is adequate moisture, but it also results in higher transpiration and can reduce survival on dry sites (Haase 2008). A lack of balance between leaf area and root system can lead to moisture stress (Baldwin & Barney 1976). In order to have better survival and growth it is important to produce seedlings with balanced root system and shoot sizes (Haase 2008). Seedlings with larger root system sizes, higher root system fibrosity, and smaller shoots (greater R:S ratio) might be preferable for the arid conditions as found in Afghanistan. Sands (1984) suggested that seedlings with larger root systems increase the likelihood of survival due to their higher water absorption surface area. Greater root size increases the ability of newly planted seedlings to avoid drought (Grossnickle 2012).

Physiological attributes such as xylem water potential can indicate water stress in newly planted seedlings. Water potential in a plant indicates to what extent tissues are hydrated (Burdett 1990). The lowest amount of water stress occurs at pre-dawn, where water potential in plant tissue reaches its highest state (Ritchie & Landis 2010). The water potential of a seedling can be measured using the pressure chamber technique (Cleary & Zaerr 1980). Seedlings with larger shoot sizes and lower R:S ratios would be expected to have more negative xylem water potentials due to higher transpirational rates if exposed to dry conditions.

Nursery practices, such as fertilization and selection of growing media, can influence the morphological and physiological status of containerized seedlings and, therefore, survival and growth of seedlings after planting (Grossnickle 2012). Previous research on the effects of nursery fertilization on survival of planted seedlings have yielded contradictory results. Villar-Salvador et al. (2013), Villar-Salvador et al. 2004, and Oliet et al. 2009 observed higher survival of *Q. coccifera*, *Q. ilex* L., and *P. halepensis* fertilized seedlings planted in the dry Mediterranean region. Trubat et al. (2010) found that nursery fertilization affected growth and morphology of *Quercus suber* L. in the nursery, but did not affect field performance after planting. Pharis & Kramer (1964) observed reduced field survival of loblolly pine seedlings fertilized with high levels of N during nursery growth. A higher supply of N in the nursery can enhance shoot growth, but can increase water stress due to greater transpiration if planted on dry sites (Duryea & Landis 1984).

Raising seedlings of *E. benthamii* under two fertilization regimes and five growing media resulted in varying morphology (Chapter 1). Those under the fertilization regime and in several of the media types had greater overall growth. This study tested the effects of these fertilization regimes and medium types and the resulting seedling morphological attributes on survival in droughty conditions.

Objectives: The objective of this study was to evaluate the effects of seedling morphology resulting from five alternative growing media types and two fertilization regimes on drought hardiness and survival of early post-planted *E. benthamii* seedlings. We hypothesized that seedlings with larger root systems, smaller shoot size and higher R:S will result in greater survival. In addition, we tested which morphological attributes of seedlings were correlated with survival.

MATERIALS & METHODS

Layout and sample size

The drought hardiness test was established on the container pad at the Horticultural Field Laboratory of North Carolina State University, using 355 *E. benthamii* seedlings from the nursery experiment (Chapter 1). There were five media (M1, M2, M3, M4 & M5) and two fertilizer levels (Fert & NotFert) creating ten media X fertilizer treatment combinations (For details see Table 1.1, Chapter 1). Seedlings from each of the ten medium x fertilizer treatment combinations were randomly assigned to a randomized complete block design, with 12 blocks. Each block comprised 30 or 29 seedlings; three seedlings per treatment combination per block, except that the M3–Fert treatment only had two seedlings per block in some blocks, because of the lower number of seedlings remaining from the nursery experiment. The other nine treatment combinations had 36 seedlings and M3–Fert had 31 seedlings represented in the experiment. Each block was surrounded by 26 border seedlings that also came from the nursery experiment. Seedlings were transplanted into 3-gallon containers filled with sandy loam soil and irrigated for 30 days. After the 30-day period, irrigation was discontinued and a plastic cover was placed above the seedlings to prevent rainfall and expose the seedlings to drought stress.

Measurements

Growth and mortality

After the 30-day irrigation period, height and RCD were measured. We used nursery May height and RCD (initial) and height and RCD (final) measured after 30-day period to determine absolute and relative height and RCD growth. The following equations were used to calculate absolute and relative growth:

$$Absolute\ Growth = Final - Initial$$

$$Relative\ Growth = \frac{Final - Initial}{Initial} \times 100$$

Once the drought stress treatment began, the trees were visually assessed every 48 hours for permanent wilting and desiccation and the number of survival days (SurvDays) until death was recorded.

Plant water potential

Six seedlings per treatment were designated for shoot, pre-dawn water potential measurement (total of 60 seedlings). Pre-dawn xylem water potential was measured three times: on the first day of drought stress (MPa 1), on the 16th day of drought stress (MPa 16) and on the 25th day of drought stress (MPa 25). At each sampling time, two seedlings per treatment combination were randomly selected for water potential measurement. Because *E. benthamii* leaves do not have petioles of sufficient size to be measured using the pressure chamber, stems (3 – 5 cm in length) from the top of the seedling were collected for water potential measurement. In some treatments, seedlings were very small and had only the main stem available for measurement, whereas in other treatments, side shoots were measured. Pre-dawn shoot xylem water potential was measured with a pressure chamber (Cleary & Zaerr 1980) at 2:00 am.

Statistical Analysis

Two-way ANOVA and type III sum of squares were used to test main effects of media, fertilizer and their interaction at $\alpha = 0.05$. Linear mixed models were used to analyze the data in GLIMMIX procedure in SAS 9.4© 2014 (SAS Institute Inc., Cary, NC, USA). In the cases of significant interactions, the Tukey-Kramer grouping was used to compare least square means of media type x fertilizer interactions (simple effects) at $\alpha = 0.05$. The effect of fertilizer treatment was tested in each medium as well. Residuals of all response variables were independent, constant, and normally distributed. The following linear mixed models were used to test effect of media type, fertilizer level, and their interaction on absolute growth, relative growth, and number of seedlings survival days (SurvDays) of *E. benthamii* seedlings under drought stress.

$$Y_{ijkl} = g(\mu) = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \tau_k + \varepsilon_{ijkl}$$

where;

Y_{ijkl} is the link function $g(\mu)$ for response variables

μ is the intercept, also the overall mean

α_i is fixed effect of media type; i = 1,...,5;

β_j is fixed effect of fertilizer; j = 1,2;

$\alpha\beta_{ij}$ is interaction of media type and fertilizer,

τ_k is random effect of block ~ $N(0, \sigma^2_b)$; and

ε_{ijkl} is error term $\varepsilon_{ijkl} \sim (0, \sigma^2_\varepsilon)$.

Simple and multiple linear regression were used to test relationships between SurvDays and morphological (initial and final height and RCD of seedlings) and physiological (xylem water potential) attributes of seedlings. Multiple linear regression was used to test the relationship between initial height, initial RCD, final height, final RCD, and SurvDays. The data met linear regression assumptions of independence, normality, and equal distribution of variances and the mean of the response variable was accurately modeled by linear function of the predictor variables. The following multiple linear regression model was used to test whether seedling initial height, initial RCD, final height, and final RCD affected SurvDays:

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \varepsilon_i$$

where;

Y_i is SurvDays of i^{th} tree

β_0 is the intercept

β_1 is the slope associated with X_1 (Initial height)

β_2 is the slope associated with X_2 (Initial RCD)

β_3 is the slope associated with X_3 (Final height)

β_4 is the slope associated with X_4 (Final RCD)

ε_i is the error term $\varepsilon_i \sim N(0, \sigma^2_\varepsilon)$.

In order to determine the best model for the response variable, five different regression model selection methods ((Adjusted-R^2, forward selection, backward elimination, stepwise selection, and SBC (Schwarz Bayesian Criteria)) were used to predict the effects of initial and final height and RCD on SurvDays of seedlings. There was a collinearity problem in the full model. Three out of four predictor variables had VIFs (variation inflation factors) greater than 10. The best model selected to predict SurvDays under drought stress is as follow:

$$Y_i = \beta_0 + \beta_1 X_1 + \varepsilon_i$$

where;

Y_i is SurvDays under drought stress of i^{th} tree

β_0 is the intercept

β_1 is the slope associated with X_1 (Initial height)

ε_i is the error term $\varepsilon_i \sim N(0, \sigma^2_\varepsilon)$

Correlation coefficients were derived between the drought experiment response variable SurvDays and seedling morphological and physiological attributes from nursery experiment and drought test experiments. Simple linear regression was used to test the relationships between the dependent variable (SurvDays) and xylem water potential separately at each time. The data met linear regression independence, equal distribution and normality of residuals assumptions. The following linear regression model was used to test whether pre-dawn xylem water potential (Xylem (ψ)-Day1, Xylem (ψ)-Day16, Xylem (ψ)-Day25) affected SurvDays.

$$Y_i = \beta_0 + \beta_1 X_1 + \mathcal{E}_i$$

where;

Y_i is SurvDays of i^{th} tree

β_0 is the intercept

β_1 is the slope associated with X_1 (Xylem (ψ)-Day1, Xylem (ψ)-Day16 or Xylem (ψ)-Day25)

$\mathcal{E}_i$ is the error term $\mathcal{E}_i \sim N(0, \sigma^2_{\mathcal{E}})$.

RESULTS

Height and RCD growth

The effect of fertilizer was significant for relative height growth (Table 2.1). Relative height growth was lower in all fertilized medium types (Table 2.2). Within non-fertilized medium types, seedlings grown previously in the two media that contained rice hulls (M2 and

M5) had the highest relative height growth (Table 2.2). Absolute height growth, on the other hand, was low in seedlings grown in non-fertilized M2, and M5 (Table 2.3). Fertilized M5 and non-fertilized M1, M3 and M4 treatments showed high absolute growth (Table 2.3).

The relative RCD growth was greatest in seedlings previously grown in non-fertilized M2 and M5 compared to all the other seedlings (Table 2.2). Absolute RCD growth, however, remained higher in most of the fertilized medium types (Table 2.3), with the non-fertilized M2 and M5 exhibiting the least amount of absolute growth (Table 2.3).

Seedling survival under drought stress

There were significant ($\alpha = 0.05$) medium, fertilizer and medium X fertilizer treatment interaction effects on the number of survival days, with an overall mean range of 33 days to 51 days (Tables 2.1 & 2.2).Seedlings in non-fertilized M2 and M5 treatments survived the longest under drought stress, although those in non-fertilized M5 not significantly different than those in fertilized M3 (Table 2.2). Seedlings in the M4 medium type performed relatively poorly under drought stress, both with and without nursery fertilization. Seedlings under these treatments had the highest growth and biomass accumulation during the nursery phase (Table 2.2).

Survival rates of *E. benthamii* seedlings over time also varied among treatments. There was no change in survival in the first week of drought stress. However, during week two and three there was a noticeable decline in survival of seedlings in all treatments except non-fertilized M2 and M5 (Figure 2.1). Non-fertilized M2 and M5 treatments maintained their survival rate at 95 to 100% through week five, whereas survival of other treatments at that point was lower than 80% (Figure 2.1). In weeks 5 to 7, fertilized M3 and M1 treatments showed some resistance to drought stress and kept their survival 60 – 70%. At the same point, survival of non-fertilized M2 and M5 treatments was between 70 and 95% (Figure 2.1). Both fertilized and non-fertilized M4 treatments showed a more rapid decline in survival than the other treatments. Seedlings in both of these treatments were completely dead at the end of week eight. By the end of week 10, survival in all treatments was 0% except non-fertilized M2 and M5 which was 10 – 15% (Figure 2.1).

Effect of initial and final height and RCD on SurvDays

In order to determine whether seedling initial height, initial RCD, final height, and final RCD affected SurvDays of *E. benthamii* seedlings, a multiple linear regression analysis was conducted. Five model selection methods were used to determine the best model. A summary of all five model selection methods is given in Table 1, Appendix B. The Height-nursery predictor variable was selected by all of the model selection methods to predict SurvDays (Table 1, Appendix B). The relationship between initial height and SurvDays was negative and significant (Table 2.4a). It indicates that increasing nursery shoot height at the time of planting may likely result in seedlings surviving fewer days under drought stress.

Effect of drought stress on pre-dawn xylem water potential

Xylem water potential decreased in all treatments as the number of days under drought stress increased (Figure 2.2), with the exception of the non-fertilized M2 and M5 treatments. Xylem water potential of non-fertilized M2 and M5 treatments did not change at any of the three measurement times. In other treatments, however, water potential decreased dramatically after day 16 (Figure 2.2). Xylem water potential was lowest in fertilized M1 and non-fertilized

M3 treatments in day 25 (Figure 2.2). The correlation between pre-dawn xylem water potential and SurvDays was positive, but only significant in Day 16 of the drought stress period (Table 2.5). Simple linear regression was used to test the relationship of xylem water potential with SurvDays. Xylem water potential measured at day 16 of drought stress showed a significant positive linear relationship with number of survival days (Table 2.4c, Figure 2.3).

Correlation between seedling morphology and SurvDays

Most seedling pre-planting (nursery) and post-planting morphological attributes were negatively correlated with SurvDays. Initial and final shoot heights and RCDs were negatively correlated with SurvDays (Table 2.5). Other variables, such as Total FOLRs, were also negatively correlated. R:S ratio, sturdiness, and xylem water potential (Mpa16) were the only variables that had significant and positive correlations with SurvDays (Table 2.5). Total seedling size (total dry weight) had a negative logarithmic relationship with SurvDays. (Figure 2.4).

DISCUSSION

Height and RCD

Higher relative height and RCD growth in seedlings previously grown in non-fertilized media containing rice hulls, M2 and M5, might be due to availability of water and nutrients during 30-days of growth in the pots filled with sandy loam soil compared to the more limiting media during the nursery phase. Because these seedlings had the higher R:S ratios after the nursery they could have responded to the additional water and nutrients by putting on relatively more shoot growth. Although relative growth was higher in seedlings raised in these media, their absolute growth was still lower compared to seedlings grown in the media containing soil, sand and compost (M1, M3, and M4). Nursery fertilization and use of soil, sand, and compost led to greater *E. benthamii* heights and RCDs in M1, M3, and M4 which might led to greater absolute height and RCD growth in these treatments. Nursery fertilization increased height and RCD growth of *P. halepensis* (Puertolas et al. 2003; Oliet et al. 2009) and Douglas-fir seedlings planted on site (Driessche 1980). Dey & Parker (1997) found that red oak seedlings with larger initial RCDs grew bigger than seedlings with lower RCDs.

Seedling survival under drought stress

After implementation of the drought treatment, we observed different survival of seedlings among the fertilizer and medium treatments. Seedlings survived for fewer days when previously grown in media M1, M3 and M4, both with and without fertilization. These medium types produced larger seedlings than those grown in M2 and M5. In these two media, which contained rice hulls, seedlings grown without fertilization survived longer than seedlings grown with fertilizer. Seedlings in non-fertilized M2 and M5 had very little growth (height and RCD) during the nursery and were small at the time of planting. Trubat et al. (2011) found that non-fertilized *Q. coccifera* seedlings had higher survival on a dry site. Chamshama & Hall (1987) observed that *Eucalyptus camaldulensis* seedlings, fertilized with N during the nursery growing period, had lower survival in response to a drought treatment in a greenhouse test.

We found a negative linear relationship between nursery seedling initial height and the number of survival days in the drought test. Seedlings with larger shoot sizes can have better height growth and survival on better sites (Villar-Salvador 2004); however they can have decreased survival on drier sites (Tuttle et al. 1987; McTague & Tinus 1996). Larger shoot size increases vulnerability of newly planted seedlings to mortality due to increased transpiration and water loss (Grossnickle 2005). Tuttle et al. (1987) observed that height of loblolly pine seedlings was positively correlated with survival on good sites; however it was negatively correlated on adverse sites. This indicates that, under drought stress, seedlings with larger shoot systems transpire water more quickly because of greater leaf area (Burdett 1990). In this study, seedlings were planted in 3-gallon pots filled with sandy loam soil and only had access to water stored in soil within the pots. Once the drought stress period began, larger seedlings likely used and transpired water quickly due to their larger shoot height and greater leaf area. Smaller seedlings, those from the non-fertilized M2 and M5 treatments, survived the drought longer due to very small shoot size and lower leaf area. Stewart & Bernier (1995) pointed out that smaller seedlings survive better on droughty sites than larger seedlings.

The pre-dawn xylem water potential measurement results supported our findings that transpirational stress negatively affected seedling survival. Xylem water potential in larger seedlings dropped quickly by day 16 of the drought period, indicating that these seedlings had depleted the supply of water from the soil within the pot and were experiencing water stress. Jobidon et al. (1998) studied effect of containerized black spruce (*Picea mariana* Mill.) seedling initial size on water stress at a planting site. They found greater water stress and lower xylem water potentials in seedlings with larger initial sizes during the first three growing seasons. Pre-dawn xylem water potential of non-fertilized M2 and M5 seedlings did not change substantially during the entire stress period. Seedlings in both of these treatments had lower leaf area allowing them to transpire less water compared to seedlings with larger shoot systems.

In addition to overall size, R:S ratio is a morphological indicator that may affect survival of newly planted seedlings. Chamshama & Hall (1987) pointed out that R:S ratio can be used to predict field survival of *E. camaldulensis* seedlings. An increase in R:S ratio of containerized seedlings increases survival on drier sites (Zida et al. 2008). We observed high R:S ratios in non-fertilized M2 and M5 during the nursery phase. Although seedlings in both of these treatments were very small in size, they had larger root sizes compared to their shoots and there was a positive, significant correlation between R:S ratio and days of survival. It is important to mention that seedlings were planted in pots so root systems of the larger seedlings were limited to the soil volume inside the container. These seedlings might have survived longer if they had been planted in soil in the field, if there had been adequate moisture immediately after planting and they had an opportunity to expand their root systems.

CONCLUSIONS

The response of *E. benthamii* seedlings to induced drought was affected by seedling total size that resulted by growing them in five growing media with and without fertilization. Larger seedlings survived fewer days than smaller ones. The response of seedlings to drought stress was also related with total seedling size. The very small seedlings that resulted from growth in the non-fertilized M2 and M5 and largest seedlings grown in the fertilized M4 medium might be too extreme for good performance following planting. Larger seedlings with greater shoot size and lower R:S ratios can be planted on areas where seasonal drought and soil water stress is not an issue, but might not be good candidate for drier sites such as in Afghanistan. Very small seedlings might be more resistant to water stress, but could be more sensitive to competition from weeds and might also result in low survival. Seedlings sizes similar to the ones produced by the fertilized M1 and M3 might be optimal for planting on drier sites. These seedlings were intermediate in size and might have acceptable levels of transpiration and yet might be large enough to withstand weed competition. Because arid climates dominate most parts of Afghanistan, there are many sites where it will be important to plant seedlings with larger roots, smaller shoots, and higher R:S ratios. Further research must be conducted to further define the relationship between seedling morphology and survival on drier sites.

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TABLES & FIGURES

Table 2.1. Type III sum of squares of F statistics and P – values from Analyses of Variance testing effects of Media, Fertilizer and Media * Fertilizer ($\alpha = 0.05$) on relative height growth, relative RCD growth, absolute height growth, absolute RCD growth and SurvDays (number of survival days) of *E. benthamii* seedlings grown for 30 days under irrigation and then subjected to drought. RCD = root collar diameter, RG = relative growth, AG = absolute growth.

Variable	**Media**		**Fertilizer**		**Media * Fertilizer**	
	F-Value	Pr > F	F-Value	Pr > F	F-Value	Pr > F
Height (cm)-RG	27.8	<.0001	206.4	<.0001	15.0	<.0001
RCD (mm)-RG	21.7	<.0001	66.1	<.0001	4.19	0.003
Height (cm)-AG	10.4	<.0001	0.04	0.85	30.2	<.0001
RCD (mm)-AG	16.0	<.0001	185.9	<.0001	20.4	<.0001
No of Survival Days	13.8	<.0001	9.2	0.003	14.0	<.0001

Table 2.2. Least square means, standard errors (S.E.) and the number of observations (N) of Media X Fertilizer treatment combinations for relative height growth (Height-RG), relative RCD growth (RCD-RG), and SurvDays (number of survival days). Superscript letters within a column with the same letter are not significantly different using Tukey-Kramer multiple comparison test at $\alpha = 0.05$.

Treatments	Height-RG (%)			RCD-RG (%)			No of Survival Days		
	Mean	S.E	N	Mean	S.E	N	Mean	S.E	N
M1 - Fert	38.1^{d}	±5.02	36	63.7^{b}	±8.99	36	39.8^{cd}	±2.81	36
M2 - Fert	42.2^{d}	±5.02	36	89.7^{b}	±8.89	36	37.1^{cd}	±2.81	36
M3- Fert	37.5^{d}	±5.39	31	61.6^{b}	±9.43	31	42.1^{bc}	±2.90	31
M4 - Fert	47.6^{d}	±5.02	36	62.8^{b}	±8.89	36	33.1^{d}	±2.81	36
M5 - Fert	59.6^{cd}	±5.09	36	90.1^{b}	±8.99	36	38.1^{cd}	±2.81	36
M1 - NotFert	64.2^{c}	±5.02	36	83.4^{b}	±8.99	36	35.3^{cd}	±2.81	36
M2 - NotFert	132.0^{a}	±5.02	36	160.4^{a}	±8.99	36	51.4^{a}	±2.81	36
M3 - NotFert	67.0^{c}	±5.02	36	84.5^{b}	±8.89	36	36.2^{bc}	±2.81	36
M4 - NotFert	67.5^{c}	±5.02	36	93.9^{b}	±8.89	36	33.9^{d}	±2.81	36
M5 - NotFert	106.4^{b}	±5.09	35	150.1^{a}	±9.09	35	50.4^{ab}	±2.84	35

Table 2.3. Least square means, standard errors (S.E.) and the number of observations (N) of Media X Fertilizer treatment combinations for aboslute height growth (Height-AG) and absolute RCD growth (RCD-AG). Superscript letters within a column with the same letter are not significantly different using Tukey-Kramer multiple comparison test at $\alpha = 0.05$.

Treatments	Height-AG (cm)	S.E	N	RCD-AG (mm)	S.E	N
M1-Fert	23.3^{bc}	±1.16	36	4.8^{ab}	±0.22	36
M2-Fert	24.4^{ab}	±1.16	36	5.1^{a}	±0.22	36
M3-Fert	22.5^{bc}	±1.23	31	4.4^{ab}	±0.23	31
M4-Fert	23.1^{bc}	±1.16	36	4.7^{ab}	±0.22	36
M5-Fert	28.7^{a}	±1.17	35	4.3^{bc}	±0.22	35
M1-Notfert	28.4^{a}	±1.16	36	3.7^{c}	±0.22	36
M2-Notfert	16.7^{d}	±1.16	36	1.7^{d}	±0.22	36
M3-Notfert	28.6^{a}	±1.16	36	3.7^{c}	±0.22	36
M4-Notfert	28.3^{a}	±1.16	36	4.0^{bc}	±0.22	36
M5-Notfert	19.4^{dc}	±1.17	36	2.1^{d}	±0.22	36

Table 2.4. Parameter estimates testing relationship between explanatory variables a) Initial height (seedlings nursery final height), b) xylem water potential – Day 1, c) xylem water potential – Day 16, d) xylem water potential – Day 25 and response variable SurvDays (number of survival days) at α = 0.05.

	Variable	Parameter Estimate	S.E	t Value	Pr > |t|
a)	*Intercept*	51.32	1.88	27.3	<.0001
	Initial height	-0.25	0.04	-6.7	<.0001
b)	*Intercept*	39.8	15.13	2.6	0.02
	Xylem (ψ)-Day1	25.1	45.74	0.6	0.59
c)	*Intercept*	50.9	3.33	15.3	<.0001
	Xylem (ψ)-Day16	52.31	13.39	3.9	0.001
d)	*Intercept*	48.4	4.75	10.2	<.0001
	Xylem (ψ)-Day25	3.0	2.70	1.1	0.29

Table 2.5. Pearson correlation coefficients of SurvDays (number of survival days) with important nursery and drought test morphological and physiological variables. N = 10, Prob > |r| under H0: Rho = 0. * P < 0.05; **< 0.01. For Final height, Final RCD, MPa (Day 1), MPa (Day 16) and MPa (Day 25) variables actual n were used for correlation coefficients. DQI = Dickson quality index, R:S ratio = root-to-shoot ratio, Mpa = xylem water potential.

Variable	**r**	**Variable**	**r**
Height (March)	-0.85**	*Shoot dry weight*	-0.49
Initial height	-0.74*	*Root dry weight*	-0.49
Final height	-0.33**	*Total dry weight*	-0.49
RCD (March)	-0.70*	*R:S ratio*	0.82**
Initial RCD	-0.64*	*Sturdiness*	0.67*
Final RCD	-0.28**	*DQI*	-0.43
Total FOLRs	-0.71*	*Mpa (Day 1)*	0.13
Total Spiral	-0.50	*Mpa (Day 16)*	0.66**
Total Active Root Tips	-0.48	*Mpa (Day 25)*	0.25

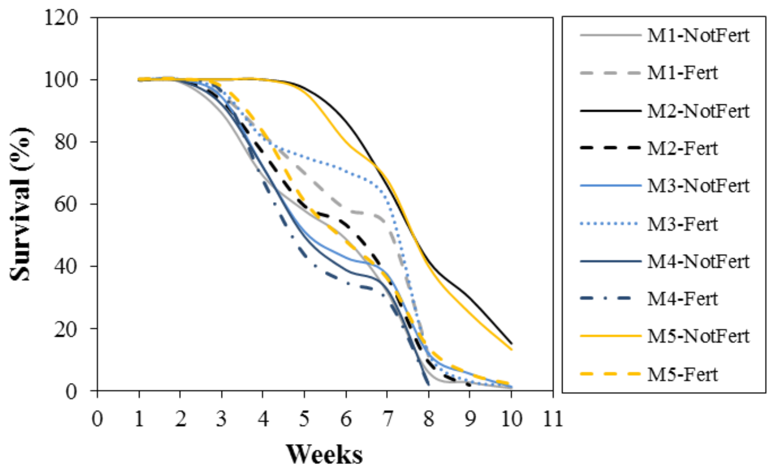

Figure 2.1. Line plot of seedlings survival from beginning of drought stress period until complete death. Each line represents one Media X Fertilizer treatment combination. Solid lines represent non – fertilized treatments and dashed lines represent fertilized treatments.

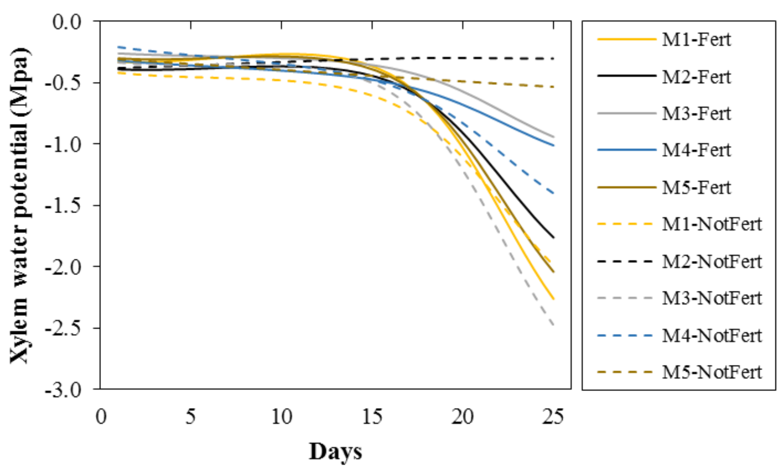

Figure 2.2. Pre-dawn xylem water potential (MPa) of Media X Fertilizer measured in day 1, day 16 and day 25 of drought stress period. M1 = soil (17%) : sand (33%) : compost (50%), M2 = soil (17%) : sand (33%) : rice hulls (50%), M3 = soil (50%) : sand (17%) : compost (33%), M4 = soil (25%) : sand (50%) : compost (25%), M5 = sand (17%) : compost (33%) : rice hull (50%). Fert = fertilized and Notfert = not fertilized.

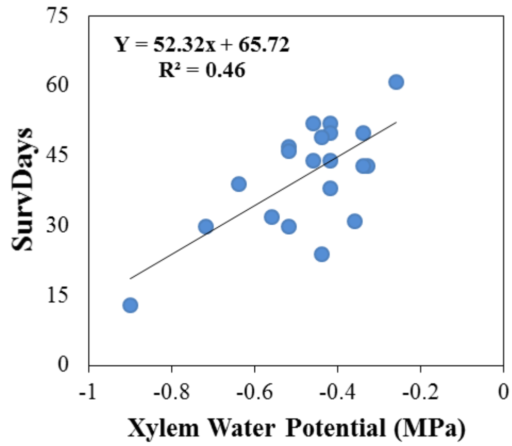

Figure 2.3. Scatter plot of response variable SurvDays (number of survival days) vs. pre-dawn xylem water potential measured at day 16 of drought stress period.

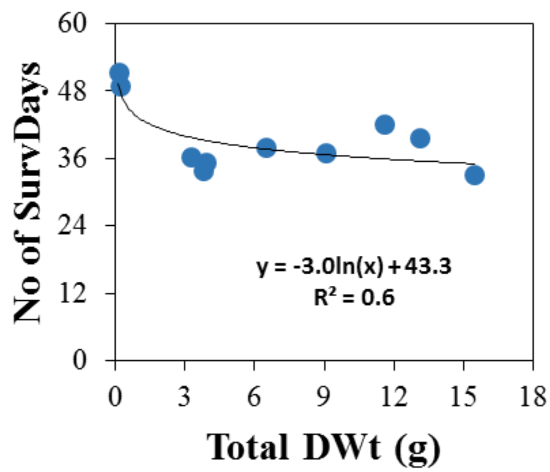

Figure 2.4. Relationship between *E. benthamii* seedling post planting survival under drought stress (measured as number survival of days) and nursery total dry weight.

GENERAL CONCLUSIONS

Growth and morphology of *Eucalyptus benthamii* seedlings during the nursery phase was affected by both growing media and fertilization. Medium 3 and Medium 4 are currently used in Afghanistan. Both of these medium types along with Medium 1 resulted in better seedling growth during the nursery phase. Growth of seedlings was very low in Medium 2 and Medium 5, which contained rice hulls. Fertilization enhanced growth of *E. benthamii* seedlings in all medium types. Fertilized seedlings had greater shoot height; RCD; root, shoot and total mass, and DQI and lower height - diameter ratios. . R:S ratio was higher in non-fertilized Medium 2 and Medium 5.

Medium 1, Medium 3 and Medium 4 can be used as acceptable media to grow containerized seedlings in Afghanistan. These three medium types are composed of soil, sand and compost, which provide some nutrients and can support growth even without the application of fertilizers. Medium 2 and Medium 5 contained 50% parboiled rice hulls. Although the presence of higher amounts of rice hulls can improve physical properties of the growing media, it may also lead to nutrient deficiency. In our study, this was somewhat alleviated by fertilization. Further research is needed to evaluate the effects of different percentages of parboiled rice hulls in a growing media on growth and development of seedlings. In addition, composted rice hulls might be a better medium component that parboiled rice hulls.

Fertilization can be an important part of nursery practices. We observed a significant effect of fertilization on growth and morphological traits of seedlings in the nursery phase. We need to include fertilization in Afghanistan nursery practices. It can affect growth and development, morphological and physiological attributes of seedlings. However, nutrition management is very important during the nursery growing period. The type, amount, timing and method of application of fertilization is very important and needs to be carefully managed in order to yield target seedlings. In Afghanistan, due to the arid climate, we want seedlings with large root sizes, small shoot sizes and high R:S ratios. We need to understand the effect of each macro and micro element on plant growth. If we carefully manage nutrition, we can produce seedlings with ideal root sizes, shoot sizes and R: S ratios. We need to conduct more research on nutrition management and its effects on morphology and physiology of seedlings.

Our focus on seedlings growth and quality in the nursery is to produce ideal seedlings for outplanting. One of the major challenges that seedlings face soon after planting is water stress. Water stress is common in arid environments such as Afghanistan. We tested the effect of different seedling morphologies produced in varying media and fertilizer regimes on *E. benthamii* seedling survival during simulated post-planting drought stress. Under drought stress, larger seedlings with greater shoot sizes and lower R: S ratios survived fewer days than seedlings with smaller shoot sizes and higher R: S ratios. This indicates that we need to focus on targets for shoot size, root size, total size, and R: S ratio of seedlings during the nursery phase. Through growing media and fertilizer management we can enhance root growth and slow shoot growth during nursery phase. In order to do that, we need to conduct more research and evaluate seedling responses to different amounts of nutrients and assess morphology and field performance.

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APPENDICES

APPENDIX A: Tables & figures of chapter I

Table 1. Analyses of variance showing P – values testing effect of fertilizer (α = 0.05) in each medium on growth and morphological attributes of *E. benthamii* seedlings for response variables measured in nursery experiment. Only those variables with a significant interaction between fertilizer and medium were tested. RCD = root collar diameter, FOLR = first order lateral roots, DWT = dry weight, R:S ratio = root-to-shoot ratio.

Variables	Media 1	Media 2	Media 3	Media 4	Media 5
Height-March (cm)	0.729	<.0001	0.114	0.062	<.0001
RCD-March (mm)	<.0001	<.0001	<.0001	<.0001	<.0001
Height-April (cm)	<.0001	<.0001	<.0001)	<.0001	<.0001
RCD-April (mm)	<.0001	<.0001	<.0001)	<.0001	<.0001
Height-May (cm)	<.0001	<.0001	<.0001	<.0001	<.0001
RCD-May (mm)	<.0001	<.0001	<.0001	<.0001	<.0001
Root Top FOLRs	0.003	<.0001	0.0003	<.0001	<.0001
Total FOLRs	0.0006	<.0001	<.0001	<.0001	<.0001
Top Active Root Tips	0.0003	<.0001	<.0001	0.083	0.015
Total Active Root Tips	<.0001	<.0001	<.0001	0.024	0.008
Root Bottom Spiral	<.0001	<.0001	<.0001	<.0001	<.0001
Root Total Spiral	<.0001	<.0001	<.0001	<.0001	<.0001
Root Top DWt (g)	<.0001	<.0001	<.0001	<.0001	<.0001
Root Bottom DWt (g)	<.0001	<.0001	0.001	0.004	0.0002
Total Root DWt (g)	<.0001	<.0001	<.0001	<.0001	<.0001
Shoot DWt (g)	<.0001	<.0001	<.0001	<.0001	<.0001
Total DWt (g)	<.0001	<.0001	<.0001	<.0001	<.0001
R:S Ratio	0.910	<.0001	0.478	0.656	<.0001
Dickson Quality Index	<.0001	<.0001	<.0001)	<.0001	<.0001
Sturdiness	0.003	<.0001	0.0003	0.004	0.003

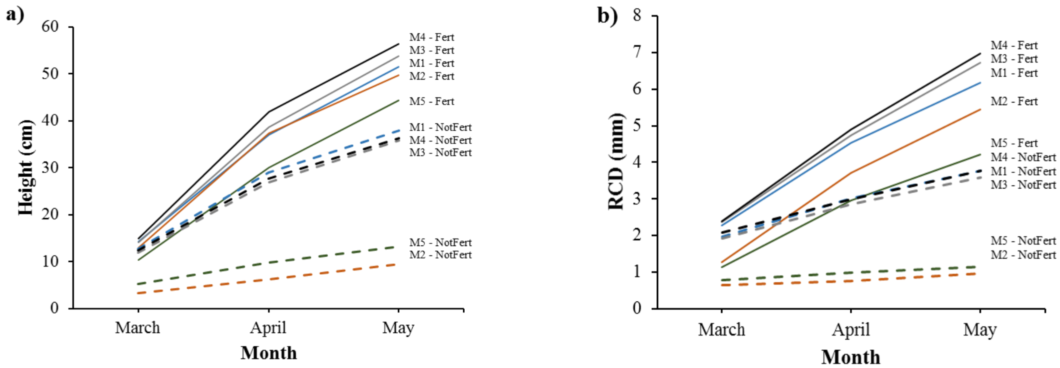

Figure 1. a) Height and b) root collar diameter ((RCD) of seedlings grown in10 medium X fertilizer treatment combinations in March, April and May 2104. M1 = soil (17%) : sand (33%) : compost (50%), M2 = soil (17%) : sand (33%) : rice hull (50%), M3 = soil (50%) : sand (17%) : compost (33%), M4 = soil (25%) : sand (50%) : compost (25%), M5 = sand (17%) : compost (33%) : rice hull (50%). Fert = fertilization (2000 mg/seedling) and NotFert = no fertilization (0 mg/seedling).

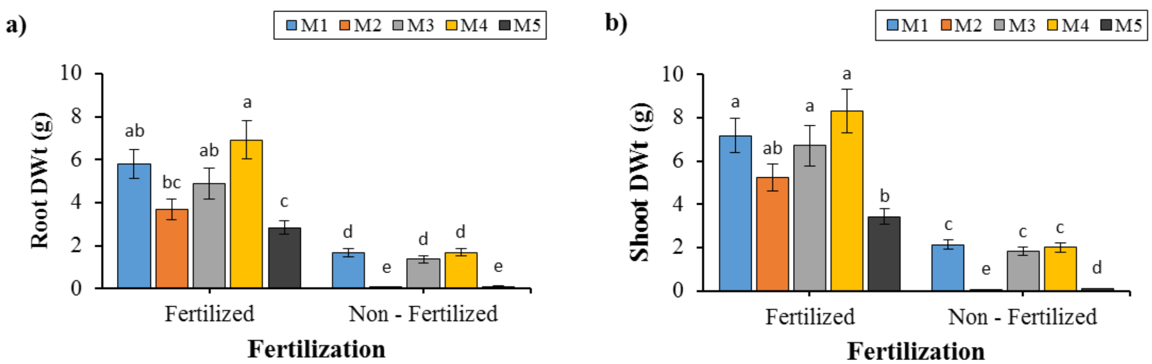

Figure 2. a) Mean total root dry weight and b) total shoot dry weight of *E. benthamii* seedling grown in 10 medium X fertilizer treatment combinations. M1 = soil (17%) : sand (33%) : compost (50%), M2 = soil (17%) : sand (33%) : rice hull (50%), M3 = soil (50%) : sand (17%) : compost (33%), M4 = soil (25%) : sand (50%) : compost (25%), M5 = sand (17%) : compost (33%) : rice hull (50%). Lines on each bar represents standard errors of the mean. Letters on top of each bar are Tukey-Kramer multiple range test significance values for least square means. Bars with the same letter are not significantly differet at $\alpha = 0.05$.

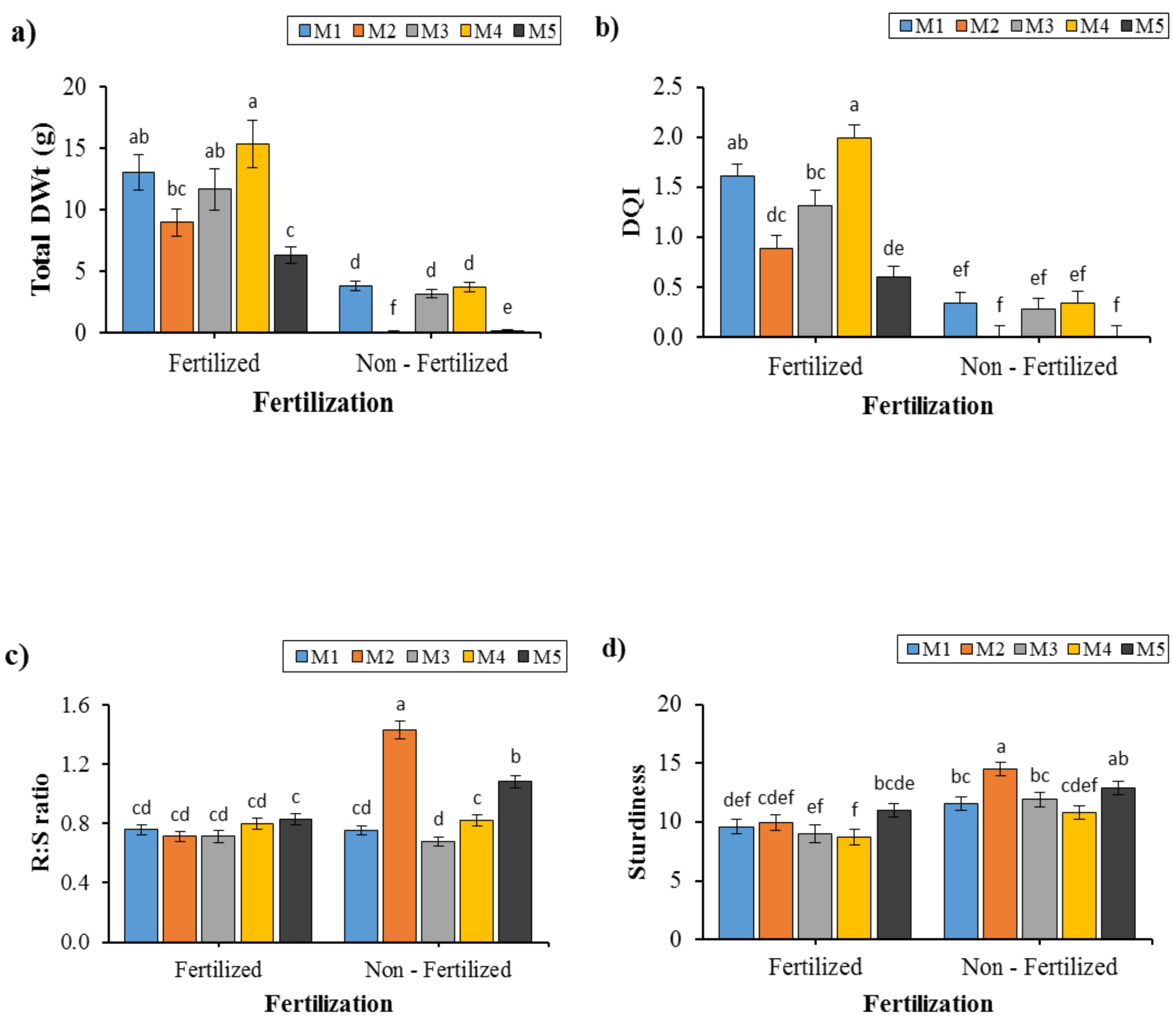

Figure 3. a) Mean total dry weight, b) Dickson quality index (DQI), c) R:S ratio and d) sturdiness of *E. benthamii* seedlings grown in 10 medium X fertilizer treatment combinations. M1 = soil (17%) : sand (33%) : compost (50%), M2 = soil (17%) : sand (33%) : rice hull (50%), M3 = soil (50%) : sand (17%) : compost (33%), M4 = soil (25%) : sand (50%) : compost (25%), M5 = sand (17%) : compost (33%) : rice hull (50%). Lines on each bar represents standard errors of the mean. Letters on top of each bar are Tukey-Kramer multiple range test significance values for least square means. Bars with the same letter are not significantly differet at α = 0.05.

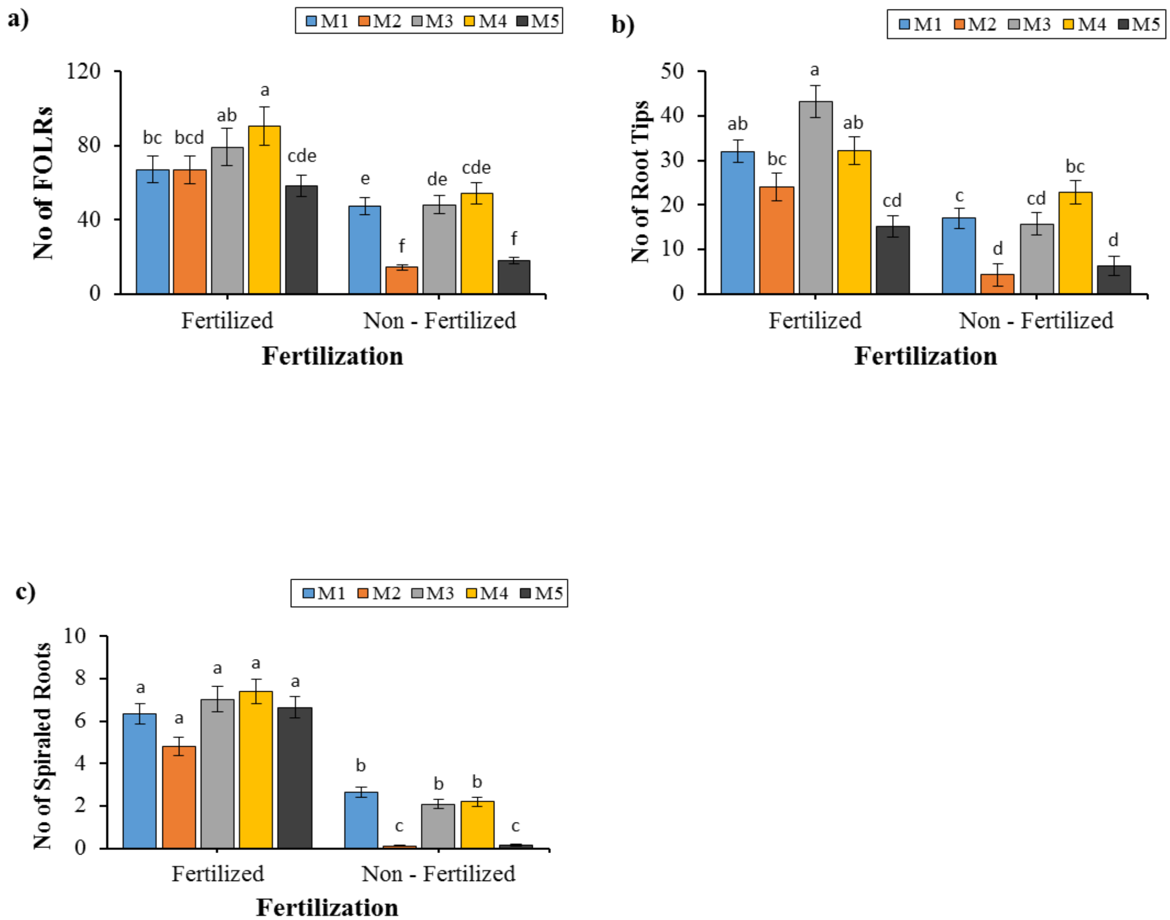

Figure 4. a) Mean total number of first order lateral roots (FOLRs), b) total active root tips (unsuberized) and, c) total number of spiraled roots of *E. benthamii* seedlings grown in 10 medium X fertilizer treatment combinations. M1 = soil (17%) : sand (33%) : compost (50%), M2 = soil (17%) : sand (33%) : rice hull (50%), M3 = soil (50%) : sand (17%) : compost (33%), M4 = soil (25%) : sand (50%) : compost (25%), M5 = sand (17%) : compost (33%) : rice hull (50%). Lines on each bar represents standard errors of the mean. Letters on top of each bar are Tukey-Kramer multiple range test significance values for least square means. Bars with the same letter are not significantly differet at α = 0.05.

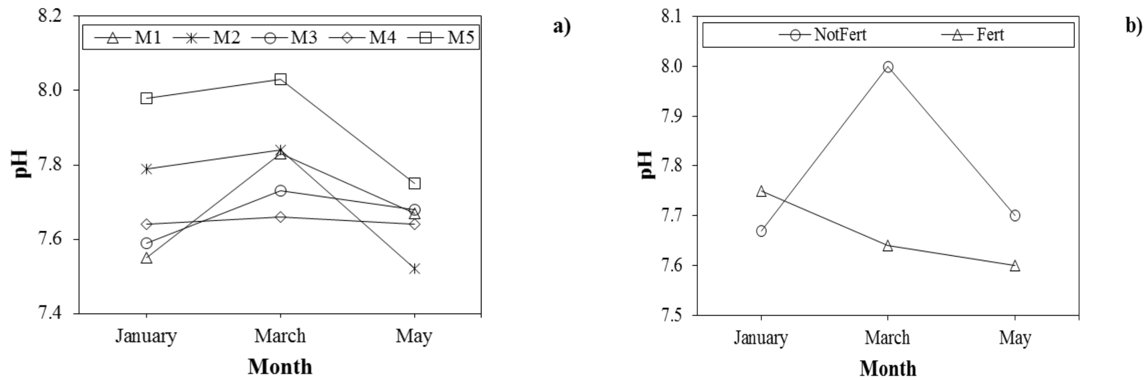

Figure 5. a) Mean pH change in medium types and b) pH change in fertilized and non-fertilized medium types measured in January, March and May 2014. M1 = soil (17%) : sand (33%) : compost (50%), M2 = soil (17%) : sand (33%) : rice hull (50%), M3 = soil (50%) : sand (17%) : compost (33%), M4 = soil (25%) : sand (50%) : compost (25%), M5 = sand (17%) : compost (33%) : rice hull (50%).

APPENDIX B: Tables & figures of chapter II

Table 1. Type III sum of squares of F – statistics (P – values) from Analyses of Variance testing effect of Fertilizer within each medium (α = 0.05) on relative height growth, relative root collar diameter growth and SurvDays (number of survival days) of *E. benthamii* seedlings grown for 30 days under irrigation and then subjected to drought. RG = relative growth.

Media	**Height (cm)-RG**	**RCD (mm)-RG**	**No of Survival Days**
M1	14.6 (0.0002)	3.1 (0.080)	3.2 (0.075)
M2	172.3 (<.0001)	40.2 (<.0001)	33.1 (<.0001)
M3	17.3 (<.0001)	4.0 (0.047)	5.2 (0.023)
M4	19.1 (<.0001)	7.9 (0.005)	0.1 (0.762)
M5	45.4 (<.0001)	28.1 (<.0001)	23.8 (<.0001)

Table 2. Summary of variables in full model and variables selected by five different model selection methods to predict SurvDays (number of survival days) of *E. benthamii* seedlings. Ht_init = initial height, rcd_init = initial RCD, Ht_final = final height and rcd_final = final RCD.

Selection Method	‡No of Parameters	Variables Selected	R^2	Adjusted-R^2	SBC
Full model	4	Ht_init, Rcd_init, Ht_final, Rcd_final	0.118	0.107	-
Adjusted-R^2	2	Ht_init, Rcd_init	0.117	0.112	1843.9
Forward selection	2	Ht_init, Rcd_init	0.117	0.112	1843.9
Backward elimination	1	Ht_init	0.113	0.111	1839.6
Stepwise selection	1	Ht_init	0.113	0.111	1839.6
SBC	1	Ht_init	0.113	0.111	1839.6

‡ Number of parameters in the table does not include intercept.

Table 3. Parameter estimates of full model variables associated with t –test statistics and P – value (α = 0.05).

Variable	Parameter Estimate	S.E	t Value	Pr > |t|	VIF
Intercept	52.33	2.68	19.5	<.0001	0
Initial height	-0.28	0.14	-2.0	0.04	14.0
Initial RCD	0.32	0.77	0.4	0.67	9.2
Final height	-0.05	0.11	-0.4	0.67	11.2
Final RCD	0.23	0.64	0.4	0.72	12.1

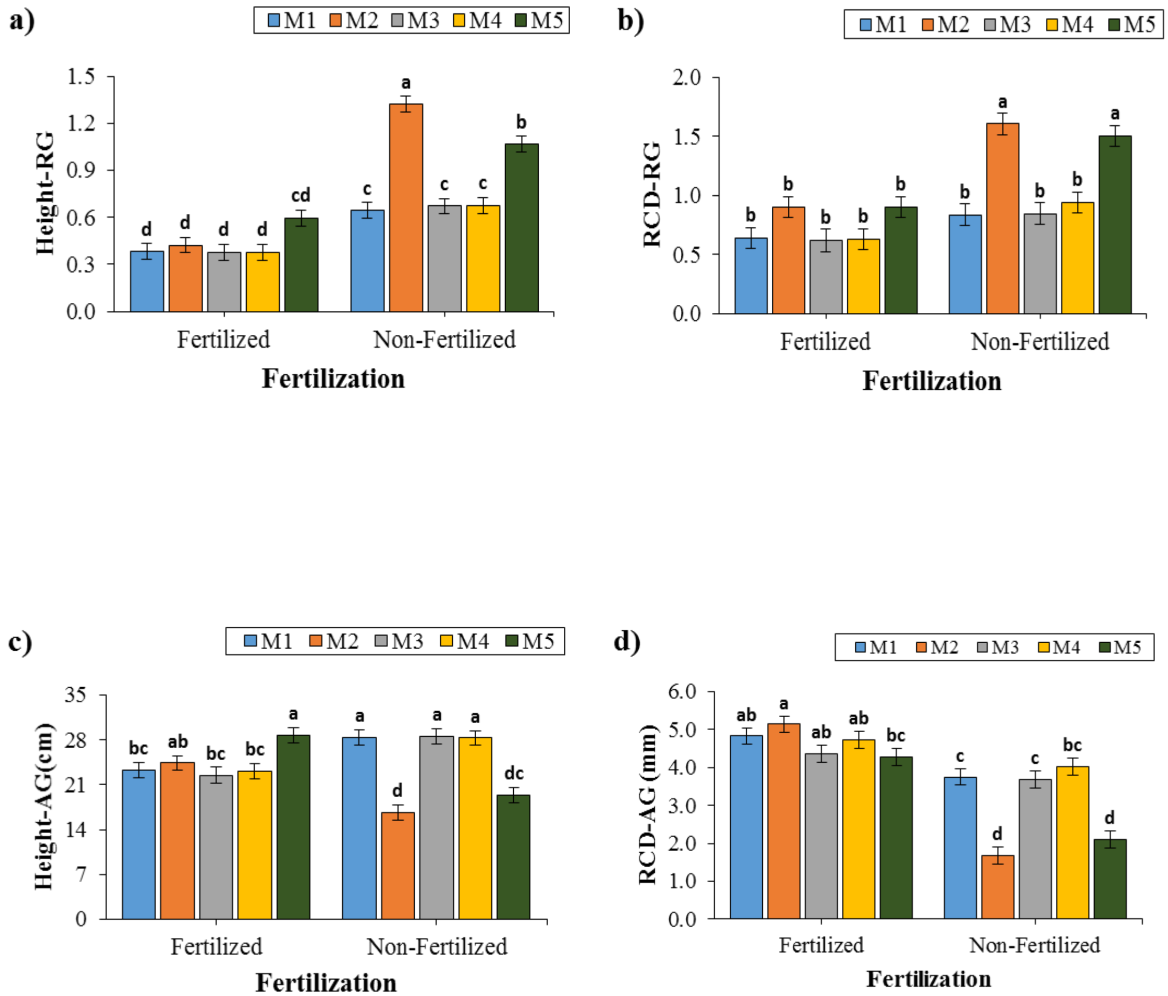

Figure 1. a) Relative height growth, b) relative RCD growth, c) absolute height growth, and d) absolute RCD growth of *E. benthamii* seedlings grown in 10 medium X fertilizer treatment combinations. M1 = soil (17%) : sand (33%) : compost (50%), M2 = soil (17%) : sand (33%) : rice hull (50%), M3 = soil (50%) : sand (17%) : compost (33%), M4 = soil (25%) : sand (50%) : compost (25%), M5 = sand (17%) : compost (33%) : rice hull (50%). Lines on each bar represents standard errors of the mean. Letters on top of each bar are Tukey-Kramer multiple range test significance values for least square means. Bars with the same letter are not significantly differet at α = 0.05. RG = relative growth, AG = absolute growth.

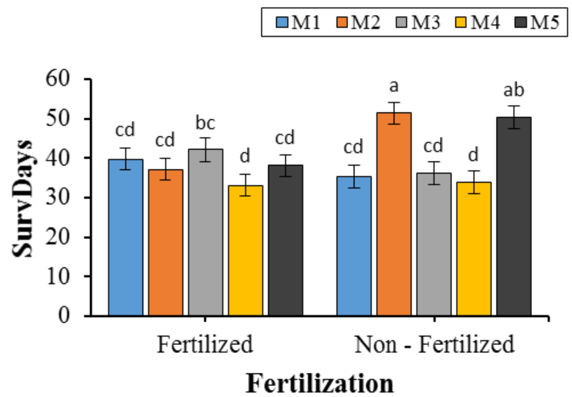

Figure 2. Mean SurvDays (number of survival days) of *E. benthamii* seedlings grown in 10 medium X fertilizer treatment combinations. M1 = soil (17%) : sand (33%) : compost (50%), M2 = soil (17%) : sand (33%) : rice hull (50%), M3 = soil (50%) : sand (17%) : compost (33%), M4 = soil (25%) : sand (50%) : compost (25%), M5 = sand (17%) : compost (33%) : rice hull (50%). Lines on each bar represents standard errors of the mean. Letters on top of each bar are Tukey-Kramer multiple range test significance values for least square means. Bars with the same letter are not significantly differet at $\alpha = 0.05$.