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EFFECTS OF WATERING FREQUENCY AND SOIL TYPES ON SEED GERMINATION AND SEEDLING PERFORMANCE OF LESPEDEZA CYRTOBOTRYA AND DIANTHUS BARBATUS

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ABSTRACT

This study was carried out to determine the effect of various watering regimes in different soils on seed germination and seedling establishment for two species (Dianthus Barbatus and Lespedeza cyrtobotrya) The ratio of water used for watering was 16ml water/100g soil. Three watering frequencies (i.e. once daily; once every 2 days; once every 3 days) were applied for two soil types (Leaf mold soil and merchantable soil) for a period of 150 days. The differences in seed germination, seedling survival and seedling growth were monitored in all experimental units. A more regular watering frequency increases soil moisture, and vice versa. The results showed that watering frequency had significant effects on seed germination ($p=0.008$), number of plant leaves ($p=0.05$), with visible but statistically insignificant effects on mean plant height ($p=0.19$) and seedling survival ($p=0.3$) for two plant species. The total height of plant species also varied greatly within treatments. Positive correlations were recorded between germination rate with leaf number ($r=0.79$, $p=0.0001$); plant height with survival rate ($r=0.65$, $p=0.001$); plant height with leaf number ($r=0.77$, $p=0.0001$); survival rate with leaf number ($r=0.66$, $p=0.0001$) etc. Seed germination and aboveground heights of D. barbatus and L. cyrtobotrya decreased at the more regular watering frequency of once daily in both soils. On leaf mold soil, the total height and mean height of L. cyrtobotrya decreased with increased water stress at once every 3 days watering frequency. Also, the total height of D. barbatus decreased with increased water stress at once every 3 days watering frequency on this soil, even though its mean height did not decrease. In contrast on merchantable soil, the total height and mean height of L. cyrtobotrya increased with increased water stress at once every 3 days watering frequency, indicating that this plant is more resistant to stress on this soil. Whereas, the total height and mean height of D. barbatus decreased with increased water stress on this soil. On leaf mold soil, once every 2 days watering was the most suitable watering frequency for both species in terms of achieving best seed germination and seedling establishment. In contrast on merchantable soil, once every 3 days watering frequency was most suitable for L. cyrtobotrya, whereas both once every 2 days and once every 3 days watering frequencies were suitable for D. barbatus on this soil. The results suggest that using the required watering frequency and soil type is imperative to ensure sustainability in plant production and seedling establishment.

1. INTRODUCTION

The choice of plant species and climate condition are imperative considerations for the success of ecological restoration (Andres & Jorda, 2000). Seed germination and seedling growth are critical stages in the life cycle of a plant, and are often subject to high mortality rates (Geraldine and Lisa, 1999). Seed germination and emergence determine the efficient use of the nutrients and water resources available to plants (Gan, 1996; Wojtyla et al. 2016).

Water uptake triggers key biochemical and cellular processes associated with seed germination such as the reactivation of metabolism, resumption of cellular respiration, biogenesis of mitochondria, DNA repair, translation and/or degradation of stored mRNAs, transcription and translation of new mRNAs, and the onset of reserve mobilization (Bentsik and Koornneef, 2008; Nonogaki et al., 2010). Seed germination and seedling growth requirements for plants are vital information needed to facilitate domestication and improvement of their potentials. Seed germination potentials are usually dependent on a combination of environmental conditions (Karssen and Hilhorst, 1992; Carmona and Murdoch, 1995).

Many studies have been carried out and reported on the pretreatment methods of seeds such as soaking seeds in water (Werker, 1980; Yu et al. 2014; Huan et al., 2016), chemical pretreatments such as immersion of seeds in acids (Todd and Duryea, 1993; Edward et al., 2013), soaking seeds in salt solutions (Yu et al. 2014), and treatment with plant growth hormonce, but there is limited information on the adequate watering frequency in different soils to enhance seed germination and seedling growth.

Water is a basic requirement for germination, as it is essential for enzyme activation, breakdown, translocation, and use of reserve storage material (Morad, 2013).

The current research is therefore, designed to investigate the germination of seeds and seedlings growth of *Dianthus barbatus* and *Lesperdeza cyrtobotrya* in response to different watering regimes and soils. *Lespedeza cyrtobotrya* is a deciduous tree of the leguminous family and is distributed in forests at medium and low altitudes. It is a drought-enduring plant, and is highly valued as foliage, green manure crops, or honey resources and also for the prevention of soil erosion (Zhu et al. 2002). *Dianthus barbatus* is a species of Dianthus native to southern Europe and parts of Asia which has become a popular ornamental garden plant. The seeds of *Lespedeza cyrtobotrya* and *Dianthus barbatus* are readily available and widely sold by commercial suppliers. It is hoped that the information gathered would help conservation and domestication of the plant species that could subsequently be used for revegetation of damaged lands.

2. MATERIALS AND METHODS

2.1. SOIL TYPES, SOIL CHARACTERISTICS AND TARGET SPECIES

Two different soils were used for this experiment including: 1. Leaf mold soil consisting of a mixture of fermented coco peat and decomposed leaves; 2. Merchantable soil with weathered granite, consisting of a mixture of fermented coco peat, vermiculite, perlite and nutrient solution. The soils were purchased from commercial suppliers.

Physicochemical analysis of the soils showed that Leaf mold soil had the lowest pH of 5.57. For Electrical conductivity, Leaf mold soil had the highest of 13.86 dS/m, while merchantable soil had 8.12 dS/m. Leaf mold soil had the highest soil organic matter content of 20.175%. For total nitrogen content, Leaf mold soil had 12.71%, and Merchantable soil had 7.72%. Available phosphorus content for Leaf mold soil (204.93 mg/Kg), and Merchantable soil (103.91 mg/Kg). Among the cations, Ca, Mg, K, Na contents were higher in Leaf mold soil.

A woody plant (*Lespedeza cyrtobotrya*), and a herbaceous plant (*Dianthus barbatus*) were used for the experiment.

2.2. EXPERIMENTAL MATERIALS AND EXPERIMENTAL DESIGN

Pots were used as experimental units with the pots placed on a slab in the laboratory. Leaf mold soil was filled in 60 pots and Merchantable soil filled in 60 pots. Mature seeds of *L. cyrtobotrya* and *D. barbatus* were purchased from commercial seed suppliers and used for the experiment. The sample size used for each watering frequency per soil type was 10 pots for each plant species. 10 seeds for each of the plant species were sowed in each pot with each

having 10 replicates per watering frequency. Thus, a total of 1200 seeds (i.e. 600 seeds per plant species) were used in the experiment.

2.3. WATERING OF EXPERIMENTAL POTS

Watering of the pots was done immediately after sowing using spray irrigation to keep the experiment ongoing. Watering was done between 07:00 am and 09:00 am when it was cool in order to reduce evaporative losses. Tap water, drawn fresh as and when required, was used to irrigate the pots. The required amount of water was filled in the spray can and spraying done accordingly. The ratio of water used for watering was 16ml water/100g soil. Three watering regimes each lasting 120 days were applied: (1) Once daily (2) Once every two days (3) Once every three days. The watering regimes were simulations of seasons were rainfall could be frequent, moderate or far apart.

2.4. DATA COLLECTION AND ANALYSIS

The experiment was conducted between the months of February and June. Data were collected on germination rate, survival rate, seedling height, relative growth rate and leaf number. Leaf number was assessed by physical counting. Seedling height was measured with metre rule. The relative growth rates (RGR) were calculated as follows:

$$RGR\ \% = \frac{(Mf/Mi\ \_\ 0.05)}{\Delta T} \times 100$$

Where Mf is the final measurement of the mean stem length, Mi is the initial measurement of the mean stem length, and ΔT is the number of months passed between the measurements (Zammith & Scarano, 2006; Zhong et al., 2009).

Analysis involved a comparison of the measured and calculated parameters of the species by watering regime using two ways ANOVA procedures, with Turkey's Test used ($p<0.05$) to test statistical significance of differences between means. All analyses were conducted using SPSS software (23).

3. RESULTS

3.1. THE EFFECT OF WATERING FREQUENCY ON SEED GERMINATION

The two ways ANOVA showed that watering frequency significantly affected ($p=0.008$) seed germination of all two species. On Merchantable soil, seed germination of *D. barbatus and L. Cyrtobotrya* increased with reduced watering frequency from once daily, to once every 2 days, and to once every 3 days watering (Fig. 1). At a watering frequency of once every 3 days on this soil, *D. barbatus* achieved a germination rate of 90%, and 24.5% for *L. cyrtobotrya*. On Leaf mold soil, seed germination of *D. barbatus* increased with reduced watering frequency (from once daily to once every 2 days), and then decreased as watering frequency reduced further (from once every 2 days to once every 3 days). At a watering frequency of once every 2 days on this soil, *D. barbatus* achieved a germination rate of 67%. Also, on Leaf mold soil, seed germination of *L. cyrtobotrya* increased with reduced watering frequency (from once daily to once every 2 days), and then showed no significant change as watering frequency reduced further (from once every 2 days to once every 3 days), with germination rates of 17% and 18% respectively. Merchantable soil with watering frequency of once every 3 days had the highest germination rate for both species.

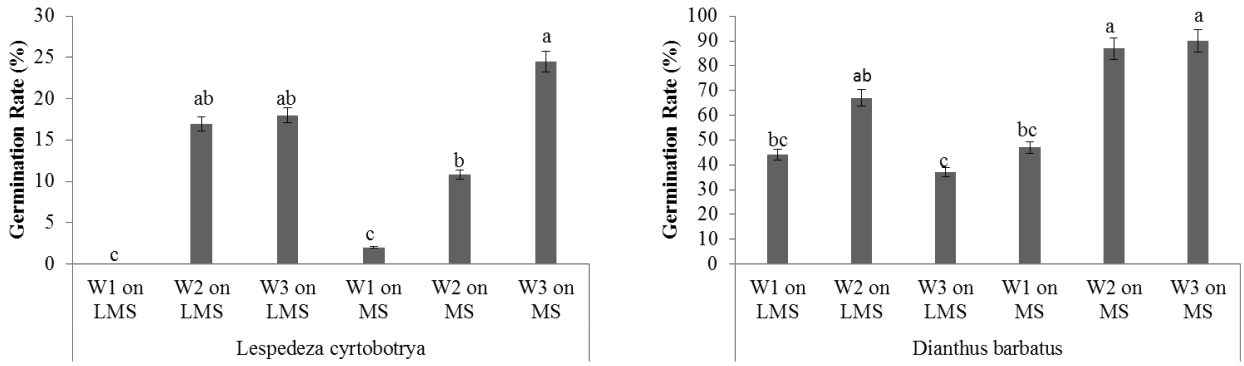

Figure 1: Effects of watering frequency on the seed germination of two species (mean ± SE). Once daily (W1) (control), Once every 2 days (W2), and Once every 3 days (W3) represent the watering frequencies used for leaf mold soil (LMS) and merchantable soil (MS). The letters above the error bar indicates the level of difference among watering frequencies for each soil type at p = 0.05 within each species.

3.2. THE EFFECT OF WATERING FREQUENCY ON SEEDLING SURVIVAL

Watering frequency affected seedling survival of all two species, even though the effect was not significant (p=0.301). On Merchantable soil, seedling survival of *D. barbatus and L. Cyrtobotrya* decreased with reduced watering frequency from once daily, to once every 2 days, and then increased as watering frequency further reduced to once every 3 days (Fig. 2). At a watering frequency of once every 3 days on this soil, *D. barbatus* achieved a survival rate of 61%, and 73% for *L. cyrtobotrya*, whereas at a watering frequency of once daily, *D. barbatus* achieved a survival rate of 73%, and 100% for *L. cyrtobotrya*. On Leaf mold soil, seedling survival of *D. barbatus* decreased with reduced watering frequency (from once daily, to once every 2 days, to once every 3 days). At a watering frequency of once daily on this soil, *D. barbatus* achieved a survival rate of 61%, and 51% at a watering frequency of once every 2 days. Also, on Leaf mold soil, seedling survival of *L. cyrtobotrya* decreased with reduced watering frequency (from once every 2 days to once every 3 days), with survival rates of 41.2% and 28% respectively.

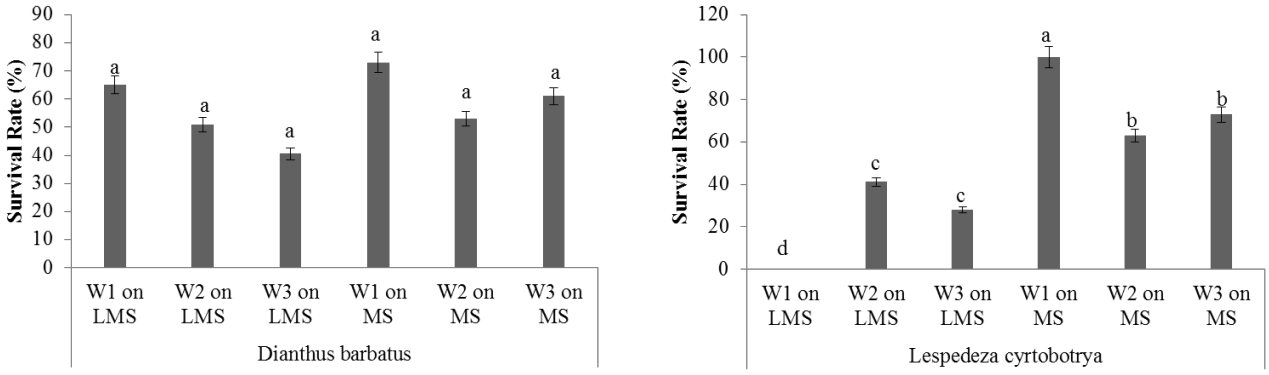

Figure 2: Effects of watering frequency on the seedling survival of two species (mean ± SE). Once daily (W1) (control), Once every 2 days (W2), and Once every 3 days (W3) represent the watering frequencies used for leaf mold soil (LMS) and merchantable soil (MS). The letters above the error bar indicates the level of difference among watering frequencies for each soil type at p = 0.05 within each species.

3.3. SEEDLING LEAF NUMBER

Watering frequency significantly affected (p=0.05) the number of plant leaves for all two species. On Merchantable soil, the number of leaves of *L. Cyrtobotrya* increased with reduced watering frequency from once daily, to once every 2 days, and to once every 3 days watering (Fig. 3), whereas on this same soil the number of leaves for *D. barbatus* increased with decreased watering frequency (from once daily to once every 2 days), and then reduced as watering frequency further decreased to once every 3 days. At a watering frequency of once every 3 days

on this soil, *L. cyrtobotrya* achieved a mean of 17.7 leaves, and 26.5 leaves for *D. barbatus* at watering frequency of once every 2 days. On Leaf mold soil, the number of leaves of *D. barbatus* and *L. cyrtobotrya* increased with reduced watering frequency (from once daily to once every 2 days), and then decreased as watering frequency reduced further (from once every 2 days to once every 3 days). At a watering frequency of once every 2 days on this soil, *D. barbatus* achieved a mean of 24.4 leaves, and 16.1 leaves for *L. cyrtobotrya*.

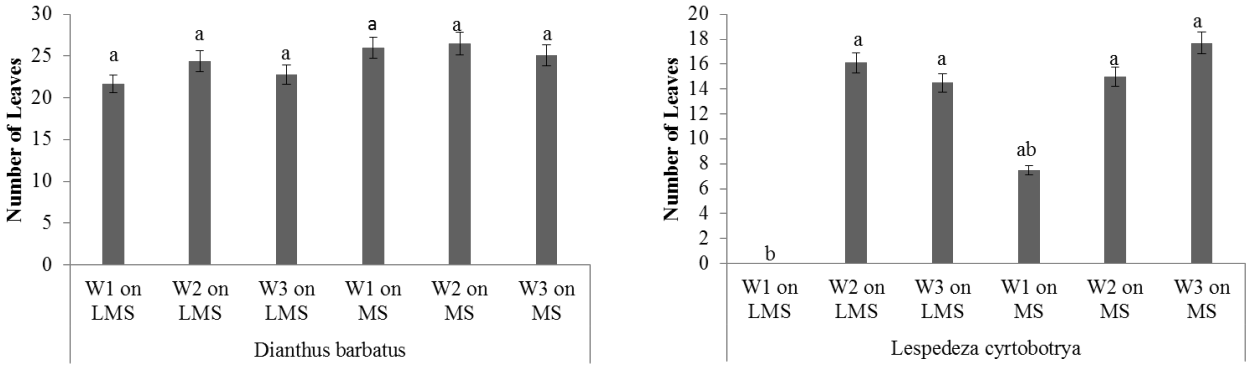

Figure 3: Effects of watering frequency on the number of leaves for two species (mean ± SE). Once daily (W1) (control), Once every 2 days (W2), and Once every 3 days (W3) represent the watering frequencies used for leaf mold soil (LMS) and merchantable soil (MS). The letters above the error bar indicates the level of difference among watering frequencies for each soil type at p = 0.05 within each species.

3.4. THE EFFECT OF WATERING FREQUENCY ON SEEDLING HEIGHT

Watering frequency affected the aboveground height of seedlings for all two species, even though the effect was not significant (p=0.19). On Merchantable soil, seedling height of *L. Cyrtobotrya* increased with increased watering frequency from once daily, to once every 2 days, and to once every 3 days watering (Fig. 4). Also, on this soil, the aboveground height of *D. barbatus* increased as the watering frequency decreased from once daily to once every 2 days, but reduced as watering frequency further decreased to once every 3 days. At a watering frequency of once every 3 days on this soil, *L. cyrtobotrya* achieved mean height of 14.5cm, and 14.1cm for *D. barbatus* at once every 2 days watering frequency. On Leaf mold soil, aboveground height of *D. barbatus* increased with reduced watering frequency. At a watering frequency of once every 2 days on this soil, *D. barbatus* achieved aboveground height of 14.5cm, and 14.9 at a watering frequency of once every 3 days. Also, on Leaf mold soil, the aboveground height of *L. cyrtobotrya* decreased with reduced watering frequency (from once every 2 days to once every 3 days), with mean heights of 13.8cm and 11.5cm respectively.

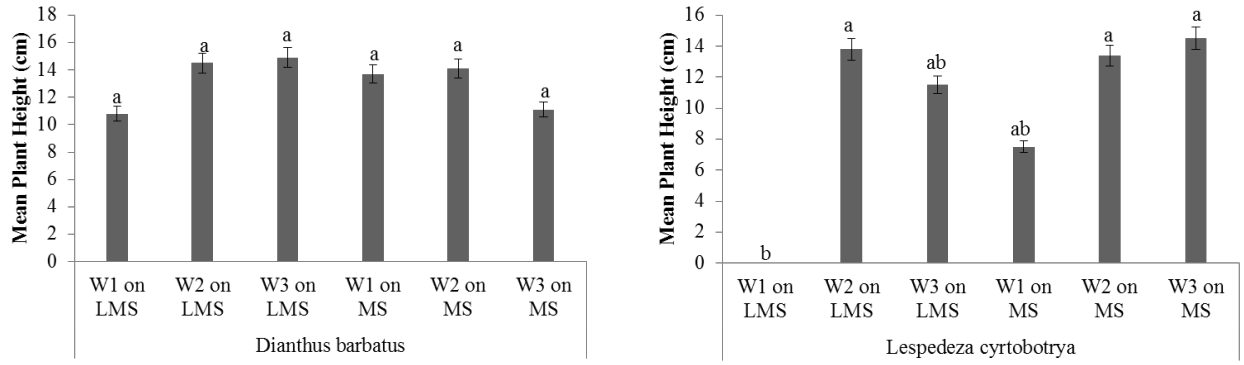

Figure 4: Effects of watering frequency on the seedling height (aboveground height) for two species (mean ± SE). Once daily (W1) (control), Once every 2 days (W2), and Once every 3 days (W3) represent the watering frequencies used for leaf mold soil (LMS) and merchantable soil (MS). The letters above the error bar indicates the level of difference among watering frequencies for each soil type at p = 0.05 within each species.

Watering frequency also affected the total aboveground height of seedlings for all two species. On Merchantable soil, total seedling height of *L. Cyrtobotrya* increased with decreased watering frequency from once daily, to once every 2 days, and to once every 3 days (Fig. 5). Also, on this soil, the total aboveground height of *D. barbatus* increased as the watering frequency decreased from once daily to once every 2 days, but reduced as watering frequency further decreased to once every 3 days. At a watering frequency of once every 3 days on this soil, *L. cyrtobotrya* achieved total height of 231cm, with 651cm and 625cm for *D. barbatus* at once every 2 days and once every 3 days watering frequencies respectively. On Leaf mold soil, the total aboveground height of *D. barbatus* increased with reduced watering frequency (from once daily, to once every 2 days), and then reduced as watering frequency further reduced to once every 3 days. At a watering frequency of once every 2 days on this soil, *D. barbatus* achieved total aboveground height of 449cm. Also, on Leaf mold soil, the total aboveground height of *L. cyrtobotrya* decreased with reduced watering frequency (from once every 2 days to once every 3 days). *L. cyrtobotrya* achieved a total height of 97cm on this soil at the once every 2 days watering frequency.

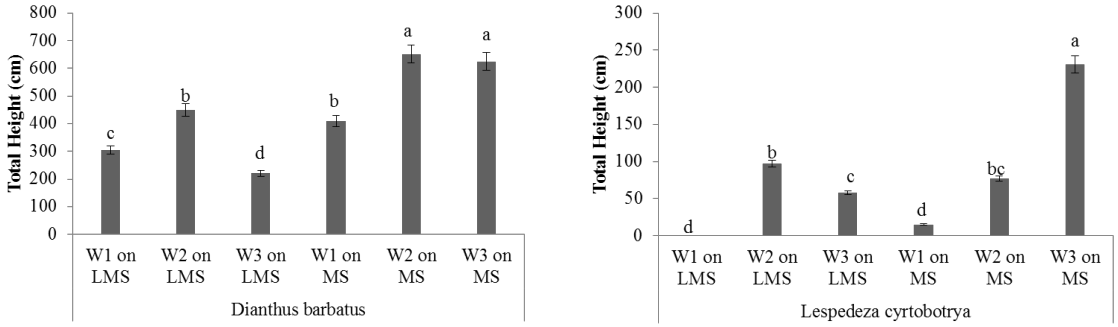

Figure 5: Effects of watering frequency on the total height (aboveground height) for two species. Total height represents the sum of heights for all the plants. Once daily (W1) (control), Once every 2 days (W2), and Once every 3 days (W3) represent the watering frequencies used for leaf mold soil (LMS) and merchantable soil (MS). The letters above the error bar indicates the level of difference among watering frequencies for each soil type at p = 0.05 within each species.

3.5. THE EFFECT OF WATERING FREQUENCY ON SEEDLING RELATIVE GROWTH RATE (RGR)

Watering frequency also affected the Relative Growth Rate (RGR) of seedlings for all two species. On Merchantable soil, RGR of *L. Cyrtobotrya* and *D. barbatus* increased with decreased watering frequency (from once daily, to once every 2 days), and then reduced as watering frequency further decreased to once every 3 days (Fig. 6). At a watering frequency of once every 2 days on this soil, *L. cyrtobotrya* achieved RGR of 94.3%, and 133.2% for *D. barbatus*. On Leaf mold soil, the RGR of *D. barbatus* and *L. Cyrtobotrya* increased with reduced watering frequency. At a watering frequency of once every 3 days on this soil, *D. barbatus* achieved RGR of 172.4%, and 101.5% for *L. cyrtobotrya*.

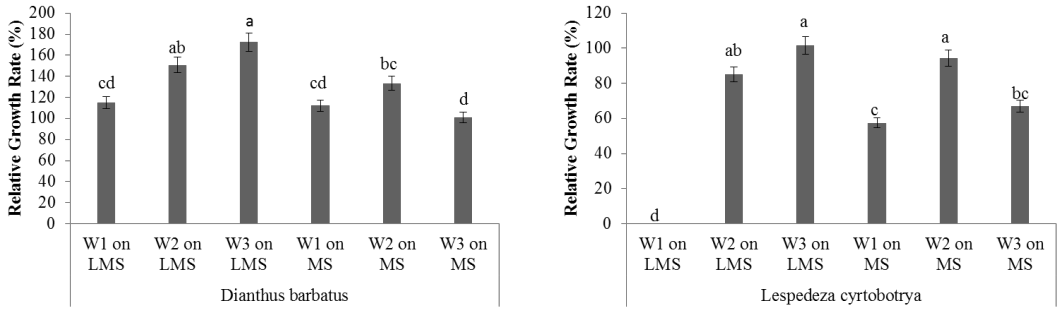

Figure 6: Effects of watering frequency on the relative growth rates (%) of two species (mean ± SE). Once daily (W1) (control), Once every 2 days (W2), and Once every 3 days (W3) represent the watering frequencies used for leaf mold soil (LMS) and merchantable soil (MS). The letters above the error bar indicates the level of difference among watering frequencies for each soil type at p = 0.05 within each species.

4. DISCUSSION

The efficient progression of germination determines the nature of seedling establishment and the proper development of mature plants (Wojtyla et al. 2016). The initial step in germination is the imbibition of water by the various tissues within the seed. This enables the embryo to break through the softened seed coat and renders the seed coat more permeable to gases, so that respiration can proceed (Meyer and Donald, 1952).

The amount of water provided to the soil influence seed germination and seedling establishment (Tylor et al., 1982), but there is limited information on the favorable watering frequency for seed germination and plant growth. The germination and growth of different plant species also vary with respect to soil types and watering frequencies. Stiles (1948) reported that seed germination varies with varieties, as seed coats of different seeds have different absorption capacities of water.

Generally in this study, some measured parameters showed positive correlations with each other such as: germination rate with leaf number (r=0.790**, p=0.000); plant height with survival rate (r=0.651**, p=0.001); plant height with leaf number (r=0.774**, p=0.000); survival rate with leaf number (r=0.657, p=0.000) etc.

This study showed that seed germination and aboveground heights of *D. barbatus* and *L. cyrtobotrya* decreased at the more regular watering frequency of once daily, both in the Leaf mold soil and Merchantable soil. This result is similar to that obtained by Dasberg and Mendel (1971) who reported that at high soil moisture, germination of *Oryzopsis holciformis* decreased. More regular watering of once daily thus provided high soil moisture, which reduced seed germination. This decrease in germination could be as a result of the thickening of the water films around the seeds, which interfered with oxygen diffusion.

On Leaf mold soil, the total height and mean height of *L. cyrtobotrya* decreased with increased water stress at once every 3 days watering frequency. These results agreed with that of Momen et al. (1979); Lutfor and Mesbah (2000); Majnoun et al. (2009) who reported decrease in plant height with more severe soil moisture deficits. Similarly, the total height of *D. barbatus* decreased with increased water stress at once every 3 days watering frequency on this soil, even though its mean height did not decrease. Water stress results in depression of plant height (Nielsen and Nelson, 1998; Shenkut and Brick, 2003; Mannan et al. 2016) especially in soils that easily become dry and hardened as it is the case for Leaf mold soil. Also, reduced soil moisture during the early seedling stage diminishes growth which in turn would result in a reduction of yield (Constable and Hern, 1978). It affects both elongation and expansion growth (Anjum et al., 2003; Shao et al., 2008). Thus, the decrease in plant height on this soil with time could be as a result of the fact that the top soil easily got dried and hardened over time thus reducing the amount of water and dissolved nutrients for uptake by the seedlings.

On Merchantable soil, the total height and mean height of *L. cyrtobotrya* instead increased with increased water stress at once every 3 days watering frequency, indicating that this plant is more resistant to stress on this soil. Contrarily, the total height and mean height of *D. barbatus* decreased with increased water stress at once every 3 days watering frequency on this soil.

Generally, the survival rates of both species reduced in treatments that had higher germination rates especially on Merchantable soil. This could be as a result of the fact that with increased germination, more seedlings were available in the soil, thus resulting to increased competition for soil nutrients and eventual death of some weaker seedlings. However, on leaf mold soil, increased water stress (once every 3 days watering frequency) resulted to lower survival rates of both *L. cyrtobotrya* and *D. barbatus*. Severe water stress may affect photosynthesis, disturb metabolism and finally result to plant death (Jaleel et al., 2008).

This study showed that generally on Leaf mold soil, once every 2 days watering is the suitable watering frequency for many plant species in terms of achieving best seed germination and seedling establishment. Thus, on this soil, high soil moisture (once daily watering frequency) and very low soil moisture (once every 3 days watering frequency) discourages seed germination and seedling establishment.

Contrarily on Merchantable soil, once every 3 days watering is the suitable watering frequency for *L. cyrtobotrya* in terms of achieving best seed germination and seedling establishment, whereas both 2 days and 3 days watering frequencies tend to be suitable for *D. barbatus* on this soil.

Watering frequency is thus an important determinant in soils for seed germination and seedling establishment, as it has a direct contribution to the adequate or required amount of soil moisture.

The results provide enough proof for the selection of species and soils, as well as the determination of the watering frequency, with regards to seed germination and seedling establishment.

5. CONCLUSION

Watering frequency significantly affects seed germination and seedling establishment on different soil types. Generally, once every 2 days watering frequency is appropriate for seed germination and seedling establishment for most species on Leaf mold soil. Thus, more frequent watering (once daily watering frequency) and increased water stress (once every 3 days watering frequency) on this soil discourages seed germination and seedling establishment. Therefore, soils that easily get dried and hardened can be more productive only in conditions of moderate soil moisture. Contrarily, once every 3 days watering frequency is appropriate for seed germination and seedling establishment on Merchantable soil. Both *L. cyrtobotrya* and *D. barbatus* performed well on this soil at the once every 3 days watering frequency. The once every 2 days watering frequency is also adequate for germination and restoration of *D. barbatus* on this soil. This indicates that plants tend to be more resistant on soils that have a good drainage and do not easily become hardened, but have the potential of retaining moisture for a considerable period.

Tree plants (*L. cyrtobotrya*) appeared to perform poorly in conditions of high moisture both on Leaf mold soil and Merchantable, and also in conditions of water stress (once every 3 days watering frequency) on Leaf mold soil. Contrarily, herbaceous plants (*D. barbatus*) performed better than *L. cyrtobotrya* on these conditions.

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CONFLICT OF INTEREST

The author have declared that no competing interests exist.

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