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RESPONSE OF ENDANGERED *DIOSPYROS MESPILIFORMIS* SEED GERMINATION AND SEEDLINGS GROWTH TO SUBSTRATES

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ABSTRACT: Effect of seven Substrates on seed germination and seedlings growth of *Diospyros mespiliformis* (Hochst. ex A.DC.; Ebenaceae), an endangered medicinal plant, under greenhouse conditions, with a view to enhance its propagation and growth. Among the tested Substrates, combination of sand, clay and peat moss 1:1:1(v/v) turned to be the best, as it gave the highest germination percentage, highest germination index and the least mean germination time. With reference to parameters related to seedling growth, also this combination was the best Substrate for cultivation of this species. As the results showed that the medium consisting of sand, clay and peat moss 1:1:1(v/v) (T6) gave highest germination percentage and index (8100 %, 0.67) and least germination time (20.9 days) of *D. mespiliformis*. Also this Substrate significantly enhanced all growth parameters (plant height, stem diameter and relative growth rates. The results revealed that using this combination of Substrate creates good conditions for the production of high quality seedlings within short time for any regeneration and rehabilitation program of this endangered species.

Key words: *Diospyros mespiliformis,* germination, seedling growth, substrate

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INTRODUCTION

The multipurpose tree *D. mespiliformis,* common name swamp ebony, is a large deciduous medicinal plant, indigenous to Africa and Asia [1]. The leaves of this plant have medicinal properties such as, crude leaves extract contains alkaloid, tannins, saponins, glycosides, steroids, flavonoids and terpenoids , the leaf-extract has significant activity against *Escherichia coli, Pesudomonas aeruginosa*, and *Staphylococcus aureus* [2]. However, *D. mespiliformis* medicinally valued plant species is enlisted as endangered in Saudi Arabia [3,4]. Preservation of this wild growing endangered medicinal plant has become necessary, and action plan for its maintenance and sustainable development need to be applied. Propagation and seedling establishment are the basic requirements for sustainable management of endangered species. However, seed germination and seedling establishment, the primary stages of plant life cycle, are most vulnerable to environmental disturbances, and therefore allied with high mortality rates [5]. Studies have shown that factors directly influencing seed germination include soil moisture, soil type and temperature [6]. Seed-soil interface is important for the initial water-uptake required for seed germination [7], and during the emergence stage, when seeds continue to have need of water and oxygen, and exploit their nutrition reserves [8].

Thus, degree of aeration, temperature and water content strongly affect seed germination and seedling emergence by controlling the seed-soil contact, water-holding capacity and aeration of soil. The effect of soil factors on seed germination is based on transmittance of light through the soil, which is determined by particle size, moisture content, particle colour and presence of organic matter [9]; light transmittance decreases with decrease in size of soil particle [10]. According to [11], seed germination is influenced by the type of substrate, oxygen, water, temperature and light. Soil type, moisture content and temperature had a significant effect on seed germination in *Salix variegata* [12]. Germination rate increased with the increase in soil moisture, but decreased in the over-saturated soil. In some current studies, combinations of soil types with different ratios of nutrients have been tested for their suitability towards seed germination [13]. Use of poor planting stock can reduce plantation survival and growth [14]. Generally, growth medium, is the most critical factor determining seedling quality in the nursery, acts as a reservoir of nutrients and moisture [15]. Studies on seed germination and seedlings establishment, become mostly important with reference to threatened species growing naturally in arid environment. This study was undertaken to evaluate the effects of different soil substrates on seed germination and seedling growth of *D. mespiliformis,* a wild endangered tree species, with the intention to produce high quality seedlings for any regeneration program.

MATERIAL AND METHODS

Seeds collection

Seeds of *D. mespiliformis* were collected during seed maturity stage direct from tree stands in Albaha region, Southwest Saudi Arabia, where these species grow naturally. Intact seeds extracted manually were air-dried and kept at room temperature to keep them viable for germination experiments. Different characteristics of seeds are recorded in Table 1. Two experiments were conducted to test the effect of soil media on seed germination and seedling growth performance.

Table1: *D. mespiliformis* seed characteristics

Purity (%)	Number of seeds (per Kg)	Number of seed per fruit	Seed shape	Seed length (mm)	Seed diameter (mm)
87.02±2.35	2509.30± **8.63**	4-6	bean-shaped	14.92±1.27	8.31±0.70

Table 2: Treatments mixture and properties of substrates

Treatment (Substrates)	Combination	Property			
		Grain size (mm)	WHC (mm/m^2)	OM (%)	Color
T1	Sand	0.1-2.0	63-110	0.10	Reddish yellow
T2	Clay	< 0.01	125-192	1.20	Light grey
T3	Sand+Clay(1:1)	0.075-0.10	104-146	0.52	Light reddish brown
T4	Sand+clay(2:1)	0.05-0.10	90-130	0.42	Light brown
T5	Sand+clay(1:2)	0.02-0.10	145-186	2.01	Light yellowish
T6	T3+ peat moss(2:1) (1:1)	0.90-0.14	120-180	4.52	darkish brown
T7	T6+perlite (2: 1)	0.075-0.10	110-185	2.71	Dark brown

Experiment 1: Seed germination on different substrates

A total of 20 seeds (seeds treated by soaked in water for 24 hours, then placed in plastic pot (30×25cm) and seven replicates were used for each treatment. The seven substrates were analyzed in the laboratory of the Soil Department (College of Food and Agricultural Sciences, King Saud University), to obtain the properties of each substrate. Seven Substrate treatments (T1 to T7), consisting of different soil combinations, as described in Table 2 were used. The pots were kept in green house at a constant temperature (25±1°C) under 12/12 h of light and darkness, and irrigated whenever needed during the period of experiment. Germination was monitored and recorded daily. Seeds were considered germinated when plumule had emerged through the media.

Experiment 2: Seedling growth on different substrates

After completion of seed germination experiment, growth performance of seedlings was monitored for six months. Seedlings were measured for total shoot height, collar diameter (using a 30 cm ruler) and seedling stem diameter (using a microcalliper to the nearest 0.01mm).

Data calculation and analysis

The following germination parameters were estimated to evaluate the effect of substrates on germination and seedlings growth:

(1) Germination % (GP) =(germinated seeds/total tested seeds) × 100,

(2) Mean germination time (MGT), estimated according to Scott et al. [16] as;

MGT (in days) = ΣTi Ni / S, where Ti is the number of days from the beginning of experiment, Ni is the number of seeds germinated per day, and S is the total number of seeds germinated.

(3) Germination index according to Esechie, [17] as;

(GI)= (G1 /1) + (G2/2) +......+ (Gx / x), where G is the germination day 1, 2…, and x represents the corresponding day of germination.

(4) Relative growth rate of stem height (HRGR), relative growth rate of stem diameter (DRGR), and seedling vigor index (SVI) were estimated as;

HRGR = $(LnH_2-LnH_1)/\ t_2-t_1,$ according to Ostos et al. [18].

DRGR = $(LnD_2-LnD_1)/\ t_2-t_1,$ according to Dhindwal et al. [19].

SVI = Germination (%) × Seedling total length **[**20]**.** Experiments were carried out in a completely randomized design, the data of seed germination and seedlings growth parameters were subjected to analysis of variance (ANOVA) to determine the level of significance (at 0.05), and means were compared using least significant difference (LSD).

RESULTS

Effect of substrates **on seed germination:**

As evident from figure 1, substrates significantly affected the germination percentage ($P < 0.001$). The combination of sand, clay and peat moss (T6) (in equal proportions), gave the highest GP (81.00. %) followed 66.00%, obtained in substrate consist of sand and clay with peat moss and perlite (**T7**). while the lowest GP (34.00, 35.00 %) was recorded in pure sand and clay substrates (T1 and T2), respectively (fig. 1). Figure 2 also indicates that the substrates significantly ($p < 0.0104$) affected MGT; the shortest MGT (20.90 and 21.8 days) was obtained on substrate consisting of sand, clay and peat moss(T6) and combination of sand, clay, peat moss and perlite (T7) , respectively. In the meantime, these two substrates gave highest germination index (0.67 and 0.58) compared to others. In contrast the longest MGT (37days) was in clay (T2), seeds germinated after 37days, with lowest germination index (0.15) (Fig. 2, 3).

Effect of substrates on seedling growth

Seedling Height (cm): Seedling height was significantly affected by different soil substrates. Mean values in table 3 show that the maximum seedling height (15.20cm) was obtained on substrate consisting of sand, clay and peat moss in equal ratio (T6), followed by seedling height in substrate T7 which consisting of T6+lerlite (3:1), and pure sand substrate (T1). The minimum seedling height (9.54 cm), on the other hand, was recorded in seedlings grown in substrate consisting of sand and clay (1:2). The seedling diameter was significantly affected by different substrates, as in table (3) which shows that the largest diameter (2.71mm) of seedling stem was obtained from substrate consisting of sand, clay and peat moss in equal ratio, followed by 2.58 mm obtained from sand and clay (1:1) substrate. On the contrary, the least seedling diameter (1.82mm) was recorded in sand+clay (1:2) substrate, followed by (1.89 mm) on pure sand (Table 3).

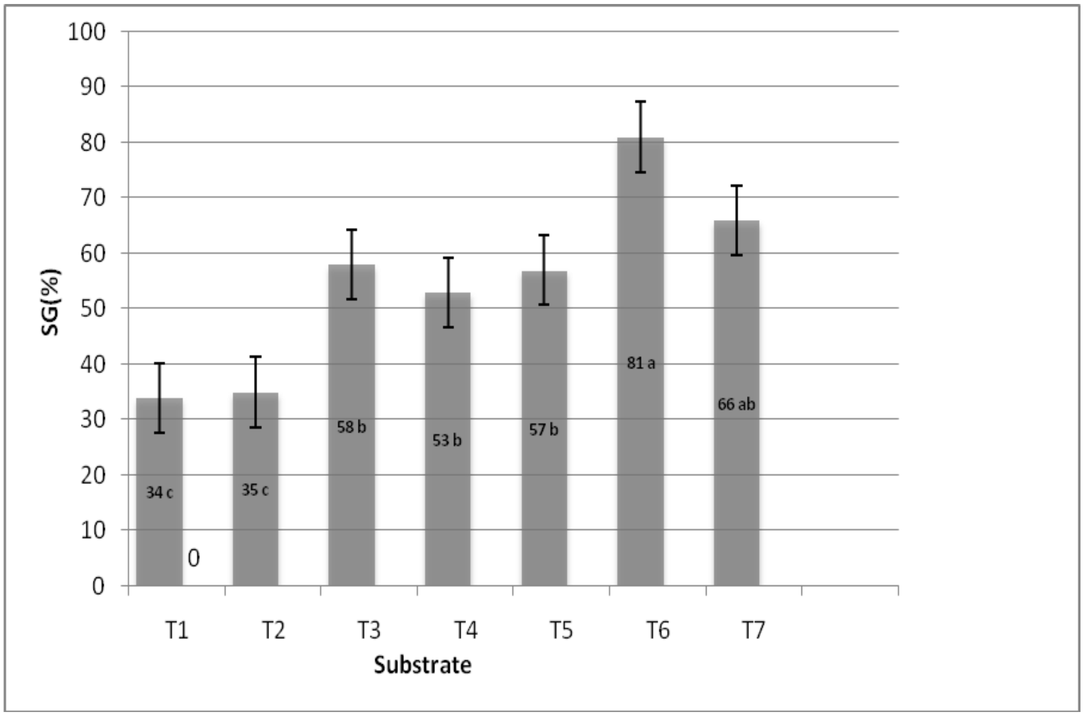

Figure-1. Effect of substrates on seeds germination (SG%), vertical bars on each bar show the standard error of the mean and the bars with the same letter show no significant differences at *P* < 0.001.

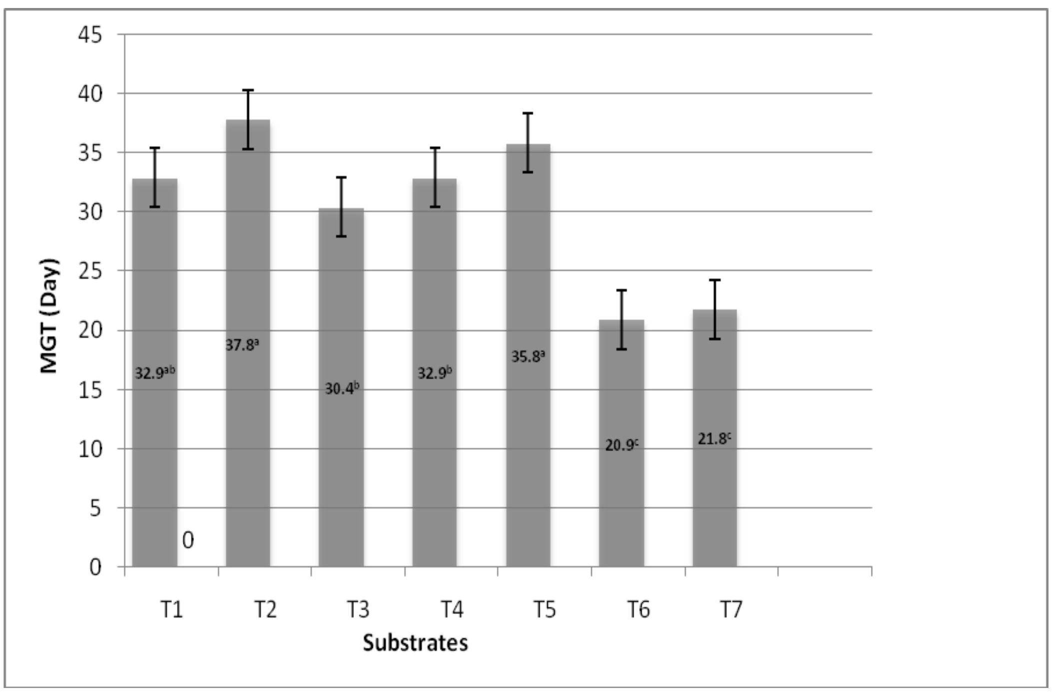

Figure-2. Effect of substrates on seeds mean germination time (MGT)(expressed as days), vertical bars on each bar show the standard error of the mean and the bars with the same letter show no significant differences at *P* < 0.001.

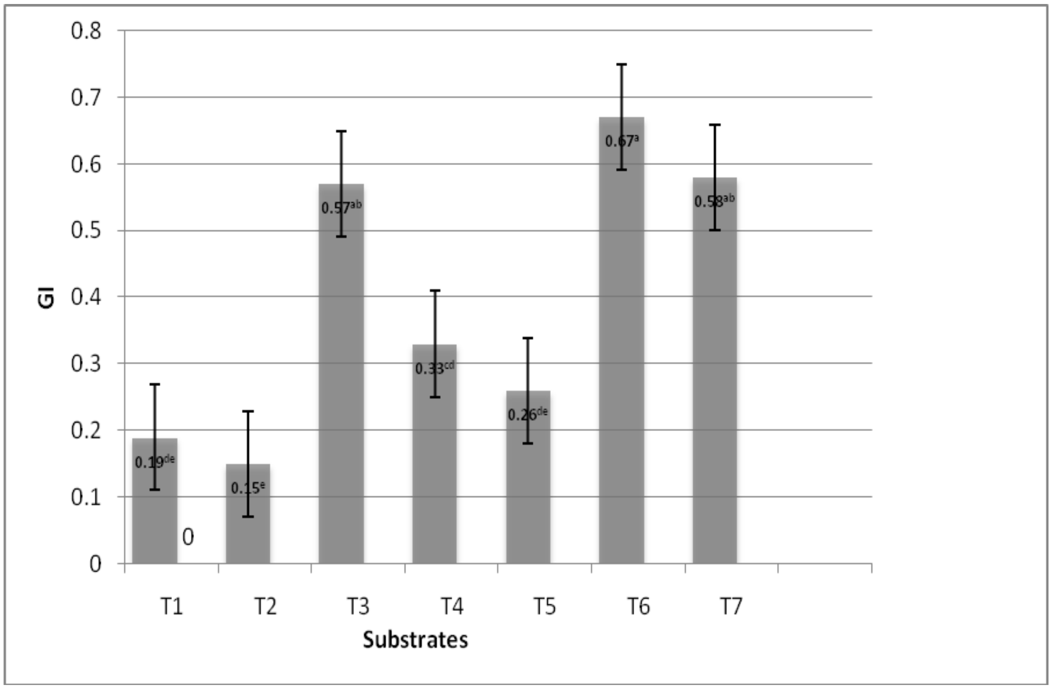

Figure-3. Effect of substrates on germination index(GI) (expressed as days), vertical bars on each bar show the standard error of the mean and the bars with the same letter show no significant differences at *P* < 0.001.

The HRGR was significantly affected by substrates (Table 3) which confirm the trend of seedling height. The substrate consisting of sand, clay and peat moss in equal ratio (T6) and substrate consisting of sand and clay(1:1) gave the maximum HRGR (0.83 and 0.82 cm cm^{-1} $month^{-1}$) respectively, although this values hardly differs from that (0.48 and 0.54 cm cm^{-1} $month^{-1}$) obtained on pure sand and clay. Although the substrate were not significantly affected DRGR, but a similar trend was recorded for DRGR, as that of HRGR. Furthermore, seedling vigor index was significantly affected by the substrate (Table 3). Generally, seedlings grown in substrate consisting of sand, clay and organic matter (peat moss), with or without perlite, had the maximum SVI (8.45 and 6.35 respectively), while substrate of pure clay and sand gave minimum DRGR (2.66 and 3.01) , i.e. T2, gave the least SVI (Table 3).

Table-3: Seedlings growth parameters under effect of different growth substrates

Treatment	H(cm)	D(mm)	HRGR (cm cm^{-1} $month^{-1}$)	DRGR (cm cm^{-1} $month^{-1}$)	SVI
T1	12.84 ^{ab}	1.89 ^{cd}	0.48 ^{c}	0.25 ^{a}	3.01 ^{c}
T2	11.10 ^{b}	1.99 ^{cd}	0.54 ^{c}	0.21 ^{a}	2.66 ^{c}
T3	12.52 ^{ab}	2.58 ^{ab}	0.82 ^{a}	0.30 ^{a}	6.16 ^{b}
T4	11.40 ^{b}	2.17 ^{abcd}	0.64 ^{abc}	0.27 ^{a}	4.90 ^{b}
T5	9.54 ^{b}	1.82 ^{d}	0.45 ^{c}	0.28 ^{a}	3.53 ^{c}
T6	15.20 ^{a}	2.71 ^{a}	0.83 ^{a}	0.32 ^{a}	8.45 ^{a}
T7	13.64 ^{ab}	2.42 ^{abc}	0.74 ^{ab}	0.26 ^{a}	6.35 ^{b}
LSD	3.292	0.593		0.140	1.343
Significant level	< 0.043	< 0.025	< 0.0320	< 0.112	< 0. 001

Means followed by similar letters are not significantly different at 5% level of significance using L.S.D. test. H; seedling height, D; seedling diameter, RGR; relative growth rate and SVI; seedling vigor **index**

DISCUSSION

The Important observation from the results of this study shown impact of the substrate on the germination parameters of *D. mespiliformis* as substrate combine from mixture of sand , clay and peat moss in equal ratios improved germination percentage and index and reduced the germination time this species compared to others substrates. These results are in agreement with the finding of [21] who mentioned that peat represents an ideal growing medium in horticulture due to its physico-chemical characteristics, which are optimal for many plant species and the management of different cultivation systems. Also [22] reported that media improved soil physical and chemical properties, aeration and resulted superior plant growth. The findings of this study was agreed with many other studies such as [23]. Adding peat moss to the germination substrates enhance the germination in terms of percentage and time, the reason for that may be the organic matter, increased water holding capacity as in table1. Many authors reported the roles of organic matter for improving germination. Organic matter has a profound impact on soil physical, chemical and biological properties [24, 25]. Adding peat moss change the soil color to darkish brown and particles size, which increase light transmission. Influence of soil factor on germination of seeds is based on transmittance of light through the soil, which includes particle size, moisture content, particle color and presence of organic matter [9]. When the particles are translucent, as in sand, transmission can increase through the particles; but in dark soil, reduced reflection only leads to increased absorption by the particles [10]. The darker particles considered to absorb the light. Depending on the soil type, moisture content either increases or decreases the light transmittance of the soil. The reduction of germination percentage in pure clay substrate may due to decreasing as stated by [26] the transmission of light decreased when in clay and loam. The nature of substrate has a significant effect on seed germination and seedling growth [27]. Quality of substrate among factors controlling plant growth (28), an ideal planting substratum should be sufficiently porous, and capable of retaining moisture, which is important for seed germination and seedling growth [29]. Mean values given in Table 3 indicate that sandy clay soil with organic matter 'peat moss' (T6) was more effective substrate than others for growth of *D. mespiliformis* in terms of tested growth parameters. These results confirm the findings of many studies such as that organic matter increases soil fertility, seedling survival and growth (30), and seedling quality [31]. Moreover (32) found that combination adding peat moss to sand and clay enhanced growth of *B. aegyptiaca* seedlings in term of height and seedlings vigor index. A good growing medium improves the quality of seedlings [33] by providing sufficient support to the plant, serving as a reservoir for nutrients and water, permitting oxygen diffusion to roots and facilitating gaseous exchange between roots and the atmosphere outside the substratum [34]. The vigorous and fast growth of seedlings on substrate consisting of sand, clay and peat moss in equal ratio may be attributed to better water-holding capacity of this substrate and availability of nutrients, which facilitate plant growth leading to a higher relative growth rate (RGR). In the present study, effect of substrate reflects more prominently in HRGR than in DRGR, and this is a normal response, because seedlings in early growth stages grow more in height than in diameter [13]. The highest relative growth rate obtained in the organic matter treatments shows that organic matters affect the physiological potential of growing plants. Correlation of RGR with nutrient supply is the same as reported previously by [18]. Larger seedlings generally have a larger photosynthetically-active green surface in terms of foliage. The maximum value of seedling vigour index (SVI) obtained in the mixture of sand, clay and organic matter (T6), suggests that the plant experiences rapid development on this substrate [14]. Plants developed in organic matter treatments normally have the maximal values of SVI, as observed in some other species [31]. On the whole, this study reveals that *D. mespiliformis* seedlings have a good potential for survival and growth on the combined substrate containing sand, clay and organic matter in equal ratio.

CONCLUSION

In order to obtain maximum germination percentage of *D. mespiliformis* in a short period, study on seed germination in different substrates was carried out. From our study, best germination substrate for *D. mespiliformis* the highest germination percentage and index within short time were obtained in the substrate combined of sand, clay, peat moss in equal ratios. We concluded that substrate or media applied by adding organic matter to sand and clay in combinations improved seed germination and decrease germination mean time and improve seedlings growth as such. Therefore, this mixed substrate well be recommended for this species as germination and growth substrate in nursery.

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