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Original Research Article

In vivo and *In vitro* Seed Germination of an Endemic Rhododendron Species (*Rhododendron arboreum subsp. zeylanicum*) in Sri Lanka

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Abstract: *Rhododendron arboreum subsp. zeylanicum* is an endemic woody plant found in Sri Lanka. Although it is a vulnerable plant and has ornamental value. Rhododendron species naturally grow from seeds and regeneration rate is low. The vegetative propagation of most woody rhododendrons is slow. The investigation of seed germination is important for the conservation of this species. The first experiment was carried out to study the effect of growing media (Unsterilized native soil, native soil: coir dust (1:1), native soil: leaf mould (1:1), coir dust: sand (1:1), native soil: sand (1:1), and clay soil) and to compare the impact on two geographical locations (*Peradeniya* and *Hakgala*) for *in vivo* seed germination. The second experiment was carried out to investigate the effect of different culture media (Anderson (ADW), autoclaved distilled water + agar, ½ strength Murashige and Skoog (MS), and full-strength MS) with two different sterilization methods (15% Clorox with few drops of Teepol for 15 minutes and 20% Clorox with few drops of Teepol for 10 minutes) on *in vitro* seed germination. The germination percentage was significantly affected by the location and growing media. The highest germination percentage was recorded in coir dust: sand medium (1:1). *Hakgala* is better than *Peradeniya* for *in vivo* seed germination. In the *in vitro* seed germination, the lowest germination and the highest contamination percentages were recorded in the full-strength MS medium. The highest root length was recorded in the seeds treated with ADW + agar. And the highest plant height was recorded in the seeds treated in ADW + agar with 20% Clorox and few drops of Teepol for 10 minutes.

Keywords: Growing media, *Rhododendron arboreum subsp. zeylanicum*, Seed germination, Sterilization.

INTRODUCTION

Rhododendron is the biggest woody plant genus in the family Ericaceae. Rhododendron L. mostly distributed in Asia and they are completely absent from both South America and Africa [1]. Rhododendron L. consists of more than a thousand species and 50% of Rhododendron species are cultivated around the world [2]. Due to the high demand for Rhododendrons, 28000 hybrids are already developed [1]. Rhododendron L. is a popular plant genus with a long and rich horticultural history for its beautiful and magnificent flowers and evergreen foliages [3 & 4]. In addition to their ornamental value, the many Rhododendron species are used for medicinal purposes, to produce different food items like jam, wine, tea, and also as firewood, and timber [3]. Most of the Rhododendron species are commercially cultivated as ornamental plants in landscaping in various parts of the world due to their flower richness and colour.

Rhododendron arboreum subsp. zeylanicum known as *Maha Rath Mal* is an endemic flowering plant in Sri Lanka [5] and also it is the official flower of the Central Province, Sri Lanka. It is sub specie of *Rhododendron arboreum*. It is found at an elevation beyond 1500m above mean sea levels [6]. This evergreen plant grows up to 2 or 3 feet in open plains and the same is growing up to 10 or 15 feet in the forest. The growth rate of rhododendron is low [7]. It can survive in the cold regions of the mountains.

Rhododendron arboreum subsp. zeylanicum is a threatened species and currently, it belongs to the category of vulnerable according to the national red list 2012 [8]. Before disappearing this beautiful flowering

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plant from the earth, conservation and restoration programs are needed. Furthermore, Rhododendrons naturally grow from seeds, but their natural regeneration rate is low [9]. The rate of vegetative propagation of many Rhododendrons is very slow [3]. Although the capsules of Rhododendron produce hundreds of tiny seeds but seeds are too small to easily germinate in the soil because of low reserves of nutrients and the low number of viable seeds [10].

The propagation from seed is a prerequisite for establishing effective and efficient conservation practices [11]. Seed plays a crucial role within the regeneration of tree species either by natural or artificial means [12]. Seed germination is a key in the plant life cycle [13] and it directly affects plant reproductive success. Therefore, the identification of seed germination requirements is more useful for its conservation programs. Many factors affect germination. The environmental condition such as elevation effect and growing medium are important factors that can directly affect the success of seed germination. The studying of seed germination behaviour along environmental gradients such as altitudes [14] and selection of a suitable growing medium for seed germination are more useful for both ex-situ and in-situ conservation programs of *Rhododendron arboreum subsp. zeylanicum*.

Plant tissue culture is an ideal technique for plant genetic conservation. *In vitro* seed germination is the starting point and important stage for plant micro-propagation [15], and also it has been widely used on many plants to obtain seedlings from the seeds which are fail to germinate using conventional methods [16]. *In vitro* seed germination includes two main steps as sterilization protocol of seeds and selection of a proper culture medium. The use of proper sterilization method and culture medium leads to saving time, explants, and effort [17]. Also, it helps to overcome the difficulties encountered in *in vivo* propagation technique. Therefore, it is important to find out the suitable culture media and sterilization protocol for in vitro seed germination.

The present study aimed to investigate the best *in vivo* and *in vitro* seed germination procedure for *Rhododendron arboreum subsp. zeylanicum*. Therefore, the present experiments were carried out to compare two locations and establish a suitable growing medium for *in vivo* seed germination and determine a suitable sterilization protocol and identify an optimum culture medium for in vitro seed germination.

MATERIALS AND METHODS

Mature capsules of *Rhododendron arboreum subsp. zeylanicum* were collected from the Botanic Gardens *Hakgala* and *Nuwara-Eli*ya (Plate 1a), Sri Lanka. The capsules were placed in the paper bag and kept in the shade at room temperature until they split (Plate 1b). The capsules were dried within 1 to 2 weeks. The seeds collected from the dehisced capsules were put into a glass bottle and sealed. The sealed glass bottle was placed in a refrigerator condition until used. The viability test was done using water before the seeds were used for experiments (Plate 1c and 1d). A container of water was taken and the seeds were placed inside. The seeds were soaked in water for 15 minutes. The seeds that sink were selected as viable seeds.

Plate-1: Seed collection and selection of *Rhododendron arboreum subsp. zeylanicum* (a) The mature capsules were collected; (b) The capsules split downward from the top; (c) The viability test using water; (d) The small size seeds

***In vivo* seed germination**

Experiment 01: Determination of a suitable growing medium and the comparison of two locations for *in vivo* seed germination

Two different locations were compared to figure out the best location for *in vivo* seed germination. Therefore, this experiment was conducted in Royal Botanic Gardens *Peradeniya*, Sri Lanka and Botanic Gardens *Hakgala*, Sri Lanka. The geographical data and mean monthly temperature of the two locations are given in Tables (1) and (2) respectively.

Table-1: Geographical data of two locations

	Royal Botanic Gardens *Peradeniya*	**Botanic Gardens *Hakgala***
Latitude & Longitude	7.26851- 80.59663	6.9262- 80.82159
Agro ecological zone	WM_2	WU_3
Elevation	460 m	1670 m
Mean annual rainfall	1900 mm– 2500 mm	2300 mm
Mean annual temperature	$18^0C – 30^0C$	$3.4^0C - 27.8^0C$

Table-2: Mean monthly temperature of two locations

			Katugastota	***Nuwara- Eliya***
Mean monthly temperature (^0C)	January	Maximum	28.6	19.4
		Minimum	20.4	11.7
		Difference	8.2	7.7
	February	Maximum	30.3	21.2
		Minimum	19.6	9.6
		Difference	10.7	11.6
	March	Maximum	30.2	23.1
		Minimum	19.2	11.1
		Difference	11	12

(**Sources:** Department of Meteorology, 2021)

Note: The nearest meteorological station to *Hakgala* and *Peradeniya* are located at *Nuwara-Eliya* and *Katugastota* respectively.

Following six different growing media were tested in the above-mentioned locations to find out the best growing medium and the optimum location for *in vivo* seed germination.

1. Unsterilized native soil (Control) – (T_0)
2. Native soil: coir dust (1:1) – (T_1)
3. Native soil: leaf mould (1:1) – (T_3)
4. Coir dust: sand (1:1) – (T_4)
5. Native soil: sand (1:1) – (T_5)
6. Clay soil – (T_6)

Sand and native soils were sieved using 5 mm mesh. Sand, coir dust, native soil, leaf mould, and clay soil were autoclaved. The growing media were prepared according to different ratios (Table 3). Soil pH was measured using a soil pH meter (Table 3). The 10 cm diameter black colour pots were taken. The pots were filled with growing media. The watering was done with autoclaved water. The 25 seeds were sowed in a pot. The pots were covered with polythene. They were kept under greenhouses conditions (60% shade with 80% relative humidity).

Table-3: Ratios and pH of the growing media

	Growing medium	Soil pH
T_0	Unsterilized native soil	4.25
T_1	Native soil: coir dust (1:1)	5.39
T_2	Native soil: leaf mould (1:1)	5.45
T_3	Coir dust: sand (1:1)	6.37
T_4	Native soil: sand (1:1)	4.23
T_5	Clay soil	5.64

The treatments were arranged as a two-factor factorial experiment in a Completely Randomized Design (CRD) with 12 treatments. Each treatment was contained 3 replicates. A single replicate was a pot consisted of 25 seeds. The experimental design is given in Table (4).

Table-4: Experimental design of *in vivo* seed germination

Location (L)	Growing medium (T)	Treatment
Peradeniya (L_0)	Unsterilized native soil- (T_0)	L_0T_0
	Native soil: coir dust (1:1)- (T_1)	L_0T_1
	Native soil: leaf mould (1:1)- (T_2)	L_0T_2
	Coir dust: sand (1:1)- (T_3)	L_0T_3
	Native soil: sand (1:1)- (T_4)	L_0T_4
	Clay soil- (T_5)	L_0T_5
Hakgala (L_1)	Unsterilized native soil- (T_0)	L_1T_0
	Native soil: coir dust (1:1)- (T_1)	L_1T_1
	Native soil: leaf mould (1:1)- (T_2)	L_1T_2
	Coir dust: sand (1:1)- (T_3)	L_1T_3
	Native soil: sand (1:1)- (T_4)	L_1T_4
	Clay soil- (T_5)	L_1T_5

***In vitro* seed germination**

Experiment 02: The optimization of a sterilization protocol and identification of an optimum culture medium for *in vitro* seed germination

This experiment was conducted at the tissue culture unit at the Royal Botanic Gardens, *Peradeniya*, Sri Lanka.

Four different culture media compositions (Table 5) were tested to find out the optimum culture medium for *in vitro* seed germination.

1. Anderson medium (ADW) – (M_0) (Control)
2. Autoclaved distilled water + agar medium – (M_1)
3. Half strength MS (Murashige and Skoog) medium – (M_2)
4. Full strength MS (Murashige and Skoog) – (M_3)

Table-5: Composition of different culture media

		Culture medium			
	Components	M_0	M_1	M_2	M_3
Macronutrients (mgL^{-1})	NH_4NO_3	400		825	1650
	KNO_3	480		950	1900
	$CaCl_2.2H_2O$	332.2		220	440
	$MgSO_4.7H_2O$	180.7		185	370
	KH_2PO_4			85	170
	NaH_2PO_4	330.6			
Micronutrients (mgL^{-1})	H_3BO_3	6.2		3.1	6.2
	$MnSO_4.2H_2O$	16.9		11.15	22.3
	$ZnSO_4.7H_2O$	8.6		4.3	8.6
	KI	0.3		0.415	0.83
	$NaMoO_4.2H_2O$	0.25		0.125	0.25
	$CuSO_4.5H_2O$	0.025		0.0125	0.025
	$CoCl_2.2H_2O$	0.025		0.0125	0.025
Iron compound (mgL^{-1})	Na_2EDTA	74.5		18.65	37.3
	$FeSO_4.5H_2O$	55.7		13.9	27.8
Amino acids & vitamins (mgL^{-1})	Glycine			1	2
	Nicotinic acid			0.25	0.5
	Pyridoxal-HCl			0.25	0.5
	Thiamine			0.05	0.1
pH		5.7- 5.8	7	5.7- 5.8	5.7- 5.8
Agar (W/V)%		0.7	0.7	0.7	0.7
Sucrose (W/V)%		2	0	1	2

(**Sources**: 18, 19, 20 and 21)

All the media, water, tools, and glassware were sterilized by autoclaving at 121^oC for 20 minutes under 1.2-1.3 kg/cm^3 pressure. The tools were dipped in 70 % (v/v) ethanol and exposed to flame, before culturing. The entire surface of the laminar flow hood was wiped with 70 % ethanol and kept the UV light on 20 minutes and followed by 20 minutes under the airflow.

Two different sterilization protocols were tested to figure out the optimum sterilization method for *in vitro* seed germination.

Sterilization protocol 1 - S_0 (Control) – The seeds were washed with running tap water for 30 seconds. Then seeds were sterilized in 15% Clorox

(commercially prepared NaOCl) solution with few drops of Teepol (detergent) for 15 minutes. Subsequently, seeds were rinsed 3-5 times thoroughly with autoclaved distilled water. In sterilization protocol 2 - S_1 – the seeds were washed with running tap water for 30 seconds. Then seeds were sterilized in 20% Clorox solution with few drops of Teepol for 10 minutes. Subsequently, seeds were rinsed 3-5 times thoroughly with autoclaved distilled water.

Rhododendron seeds were sterilized using either with previously described S_0 or S_1 methods, and the seeds were kept for 10 to 20 minutes within the laminar airflow cabinet for air drying. Then the seeds were transferred into a different culture media.

The treatments were arranged as a two-factor factorial experiment in a Completely Randomized Design (CRD) with 8 treatments. Each treatment was contained 10 replicates. A single replicate consisted of 30 seeds in one culture bottle. The experimental design is given in Table (6).

Table-6: Experimental design of *in vitro* seed germination

Sterilization protocol	Culture medium	Treatments
S_0 – 15% Clorox + few drops of Teepol (detergent) for 15 minutes	M_0	S_0M_0
	M_1	S_0M_1
	M_2	S_0M_2
	M_3	S_0M_3
S_1 – 20% Clorox + few drops of Teepol for 10 minutes	M_0	S_1M_0
	M_1	S_1M_1
	M_2	S_1M_2
	M_3	S_1M_3

The seed which was shown the visible radical protrusion counted as a germinated seed. Data were analyzed with two-factor factorial ANOVA using the statistical analysis software R with 95% confidence level. Mean separation was done by using Least Significant Difference (LSD).

Germination percentage, root length, plant height, and number of days taken to initial germination were recorded for *in vivo* seed germination. At 70 days after sowing, 20 seedlings from each treatment were sampled and the root length and plant height were recorded manually with a scale. The *in vivo* germination percentage was recorded 70 days after sowing using the following equation.

$$\text{Germination Percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds used for germination}} \times 100$$

Germination percentage, root length, plant height, contamination percentage (using below-mentioned equation), and the number of days taken to initial germination was recorded for *in vitro* seed germination. The germination percentage was recorded 30 days after culturing. At 30 days after culturing, 20 seedlings from each treatment were sampled. The root length and plant height were recorded manually with a scale.

$$\text{Contamination Percentage} = \frac{\text{Number of seeds contaminated}}{\text{Total number of seeds used for germination}} \times 100$$

RESULTS AND DISCUSSION

Experiment 1: *In vivo* seed germination

The following results showed the efficiency of the *in vivo* seed germination by using two different locations and six different growing media.

The number of days taken to initial germination

Germination was initiated within 4 weeks under *in vivo* conditions. The average number of days taken to initial germination in *Hakgala* was 24.83 ± 1.49 (mean ± SD). In *Peradeniya*, it was 24.3 ± 1.4. Hence, the number of days taken to initial germination was not significantly different between the two locations (Figure 1a). As shown in Figure (1b), it was not significantly different among growing media tested. The interaction between growing media and location also had no significant effect on the number of days to initial germination (Figure 1c).

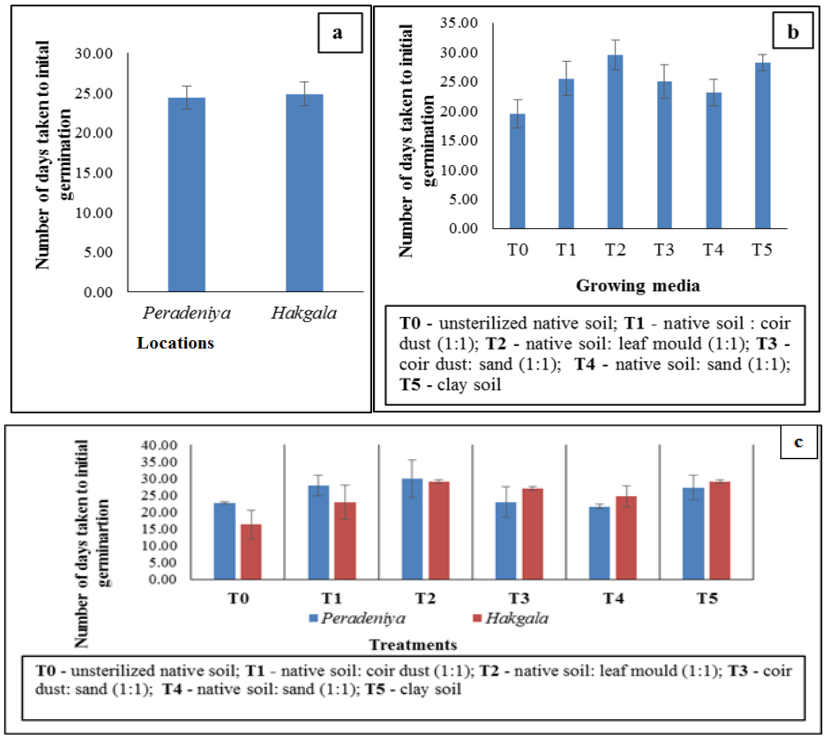


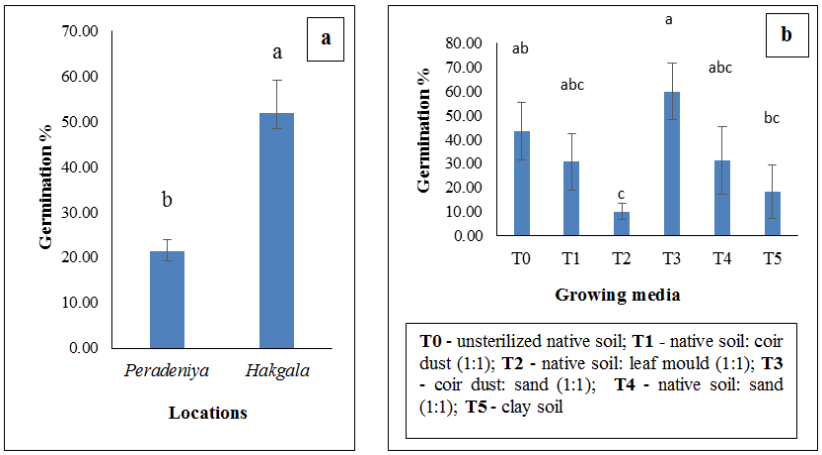
Fig-1: Effect of locations and growing media on number of days taken to initial germination (a) The effect of the location; (b) The effect of the growing media; (c) The interaction between locations and growing media.

Note: The results are represented as average ± SD of three replicates, n = 25 (n = total number of seeds per replicate) (p value <0.05).

Although mean germination time for *Rhododendron arboreum subsp. zeylanicum* was 4 weeks in this experiment. Previous studies have reported that the mean germination time of Rhododendrons was 3 to 12 weeks and also it depended on Rhododendron species [3 & 10]. Further, alternating temperature (day and night temperature difference) results in faster germination [22]. When compare *Peradeniya* with *Hakgala*, the mean monthly day-night temperature difference was closely equal. It could be the reason for the mean germination time was not significantly different between the two locations.

Germination percentage

The location and growing media had a significant effect on seed germination. However, the interaction between the locations and growing media had no significant effect on germination percentage (Figure 2). The germination percentage in *Peradeniya* and *Hakgala* was 21.44% ± 2.44 and 51.78% ± 7.32 respectively. In both locations, the highest germination was recorded in coir dust: sand media (1:1). Although germination percentage in coir dust: sand media (1:1) was 90.67% ± 7.42 in *Hakgala*, in *Peradeniya*, it was only 29.33% ± 2.67. The lowest germination percentage was recorded in native soil: leaf mould media (1:1). According to the above results, germination percentage was highest in *Hakgala* and coir dust: sand medium (1:1) was the best medium compared to the other media tested.



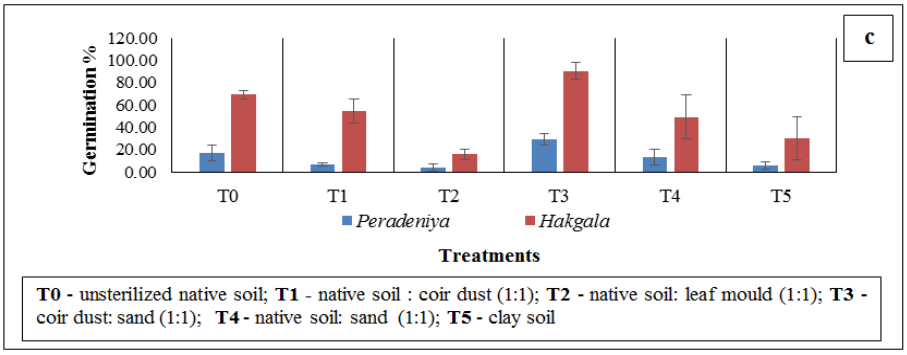


Fig-2: Effect of the location and growing media on germination percentage (a) The effect of the location; (b) The effect of the growing media; (c) The interaction between locations and growing media.

Note: The results represented are average ± SD of three replicates, n = 25 (n = total number of seeds per replicate) and different letters indicate significant differences (p value <0.05).

Many researchers suggested that peat moss was the best germination medium for Rhododendrons [23, 24 & 25]. Since peat moss is not readily available in Sri Lanka, it was necessary to select a good alternative media to peat moss. The coir dust medium is good alternative to peat moss [26, 27, 28 & 29] mainly due to, both peat moss and coir dust show similar organic matter content (>90%) and mineral content (<10%), bulk density, as well as water-holding capacity. pH and electrical conductivity, are higher in coir dust than peat moss [30]. The coir dust: sand (1:1) medium which had 6.37 pH, contained good aeration and good drainage. According to previous literature, Rhododendron seeds germination was successful in acidic media [4, 31, 32 & 33]. The optimum pH range was 5.2 – 6.5 for Rhododendron [34] and also Rhododendron seed germination was successful in the media that had higher aeration and drainage [35]. The above-mentioned information could be the reasons for the highest germination percentage that was recorded in the coir dust: sand medium (1:1).

Although this experiment was conducted under the greenhouse condition, the elevation, temperature, photoperiod, and light intensity were different with the locations. The elevation positively affects germination ability in Rhododendrons [36]. Verma, [37] has mentioned that seeds of Rhododendrons could be collected during the last 3 weeks of October. *Rhododendron arboreum subsp. zeylanicum* seeds can be collected from October to January months and December and January are the coolest months in Sri Lanka. Alpine species have physiological dormancy and cold stratification is needed to break their dormancy [22]. Therefore, *Rhododendron arboreum subsp. zeylanicum* needs a cool climate to break dormancy.

In this experiment, the highest germination was recorded in *Hakgala* which has a cooler climate (mean annual temperature 3.4^0C - 27.8^0C). The mean monthly maximum temperature of January to March was higher than 25^0C in *Peradeniya*. The previous researcher was suggested that germination percentage was increasing with increasing temperature up to 25^0C and the temperature above 25^0C was unfavourable for Rhododendron seed germination [37]. As well as [11] has mentioned that *Rhododendron prostistum va. gigenteum* seeds reach their highest germination at a relatively a low-temperature range like 15^0C to 20^0C. According to [39], 20^0C was the best temperature for *Rhododendron calopytum* seed germination. This may be the reason that germination percentage was lower in *Peradeniya* compared to *Hakgala*.

The root length

The root length was not significantly different between locations but the interaction between the growing media and locations significantly affected root length (Figure 3b). In *Peradeniya* and *Hakgala* native soil: coir dust medium (1:1) showed the highest significant effect on root length. The root length was significantly affected by growing media (Figure 3a). Both native soil: coir dust media (1:1) and coir dust: sand media (1:1) were significant than other growing media (Figure 3). Both above-mentioned growing media contained coir dust. The media had high porosity due to coir dust. It could be the reason for longest roots appeared in both native soils: coir dust media (1:1) coir dust: sand media (1:1).

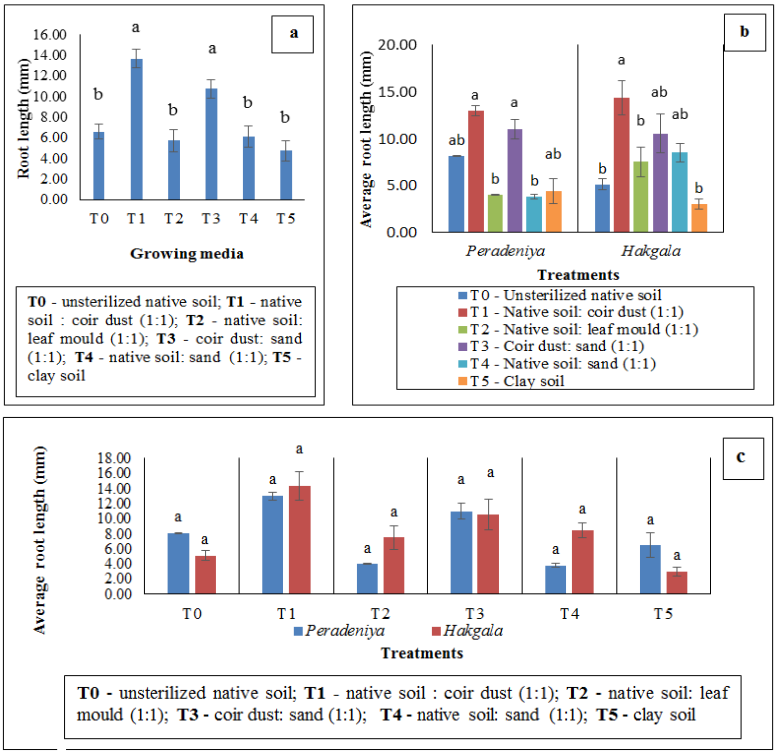


Fig-3: The effect of locations and growing media on the root length (a) The effect of the growing medium; (b) The average root length between two locations when changed the growing medium; (c) The average root length among growing media when changed the location.

Note: The results are represented as average ± SD of three replicates, n = 25 (n = total number of seeds per replicate) and different letters indicate significant differences (p value <0.05).

The plant height

The plant height was significantly different between the two locations (Figure 4a). But had no significant effect among the growing media tested (Figure 4b). The interaction between growing media and location also had no significant effect on the plant height (Figure 4c).

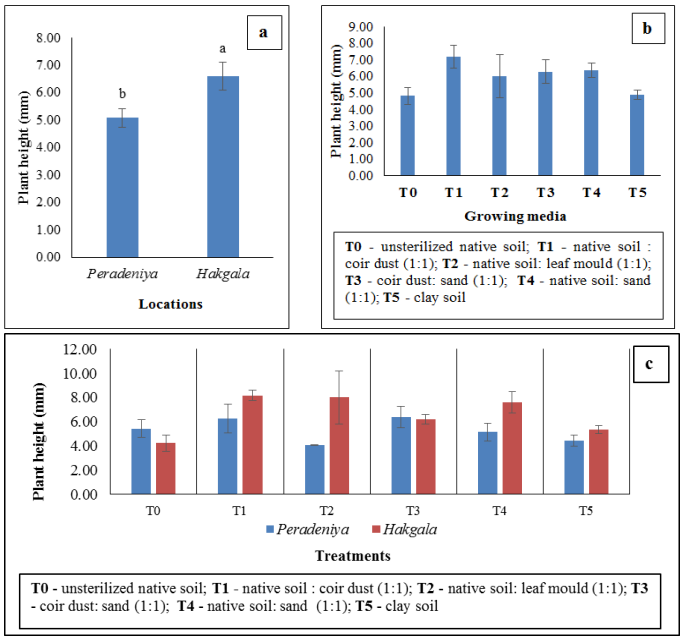


Fig-4: Effect of the location and growing media on plant height (a) The effect of the location; (b) The effect of the growing media (c) The plant height in two locations grown in different growing media.

Note: The results are represented as average ± SD of three replicates, n = 25 (n = total number of seeds per replicate) and different letters indicate significant differences (p value <0.05).

Experiment 2: *In vitro* seed germination

The following results showed the efficiency of the *in vitro* seed germination of *Rhododendron arboreum subsp. zeylanicum* by using two different sterilization protocols and four different culture media.

Number of days taken to initial germination

The number of days taken to initial germination was not significantly different between sterilization protocols but it was significantly different among culture media. The highest number of days (20.77 days ± 0.59) taken to germinate was observed in the full strength MS medium. Other media had no significant effect on mean germination days. The interaction between sterilization protocol and growing media had no significant effect on the mean germination days (Figure 5).

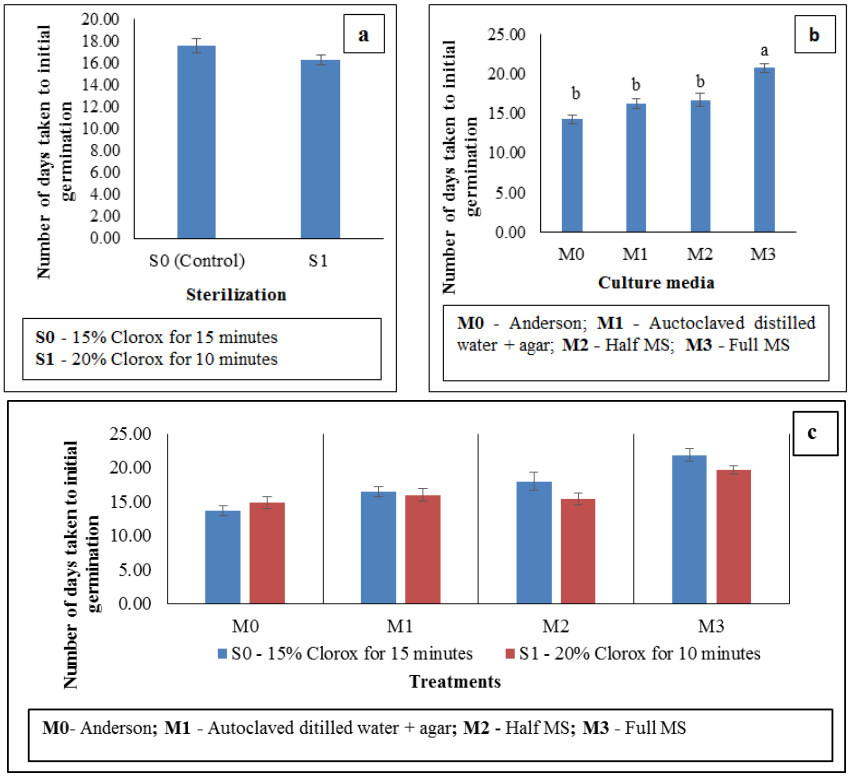


Fig-5: The effect of sterilization protocol and culture media on the number of days taken to initial germination (a) The effect of the sterilization protocol; (b) The effect of the culture media; (c) The interaction between sterilization protocol and culture media.

Note: The results are represented are average ± SD of ten replicates, n = 30 (n = total number of seeds per replicate) and different letters indicate significant differences (p value <0.05).

Germination percentage

The germination percentage was not significantly different between sterilization protocols but it was significantly different among culture media (Figure 6a and 6b). The lowest germination percentage (23.01 ± 3.16) was observed in the full-strength MS medium (Plate 2). Other media had no significant effect on germination percentage. The interaction between sterilization protocol and growing media also had no significant effect on germination percentage (Figure 6c).

The lowest germination percentage was recorded in full-strength MS medium which had 2% sucrose and the highest nutrients composition than other media. [16] reported that higher nutrients in MS medium might be excessive to minute young seeds and it suppressed both germination and seedling development. It could be the reason to declining of germination percentage on full-strength MS medium. [3] have reported that different Rhododendron species required different culture conditions like media compositions for successful seed germination.

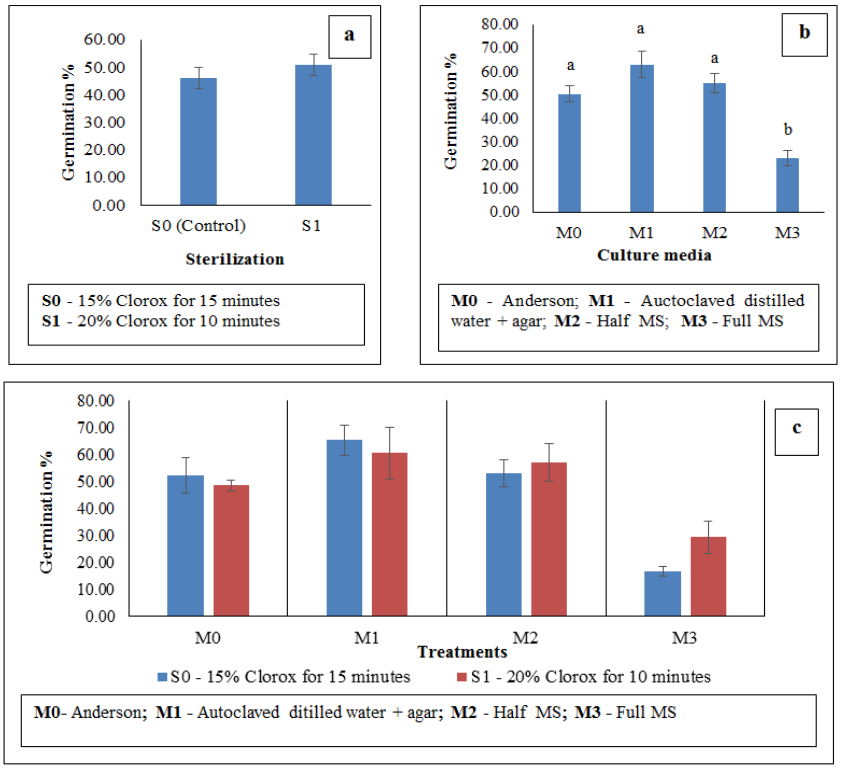


Fig-6: The effect of sterilization protocol and culture media on germination percentage (a) The effect of the sterilization protocol; (b) The effect of the culture media; (c) The interaction between sterilization protocol and culture media.
Note: The results are represented as average ± SD of ten replicates, n = 30 (n = total number of seeds per replicate) (p value <0.05).

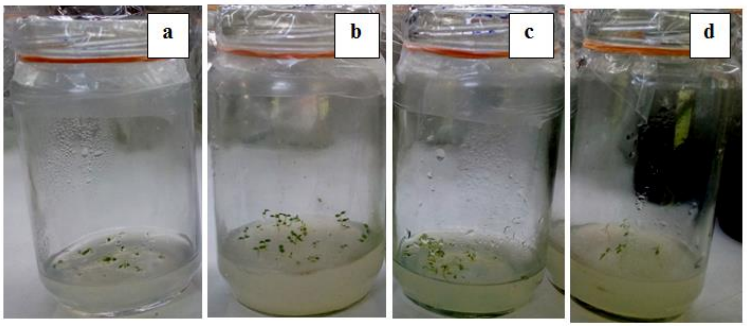


Plate-2: Effect of different culture media on germination percentage (a) Effect of Anderson medium; (b) Effect of autoclaved distilled water + agar; (c) Effect of half-strength MS medium; (d) Effect of full-strength MS medium.

The root length

The root length was not significantly different (Figure 7a) between sterilization protocols but the root length was significantly different (Figure 7b) among culture media. The longest roots were observed in the autoclaved distilled water + agar medium (7.69 mm ± 0.31) (Plate 3b). The shortest roots were observed in the Anderson medium (1.45 mm ± 0.12) (Plate 3a). The interaction between sterilization protocol and growing media had no significant effect on root length (Figure 7c).

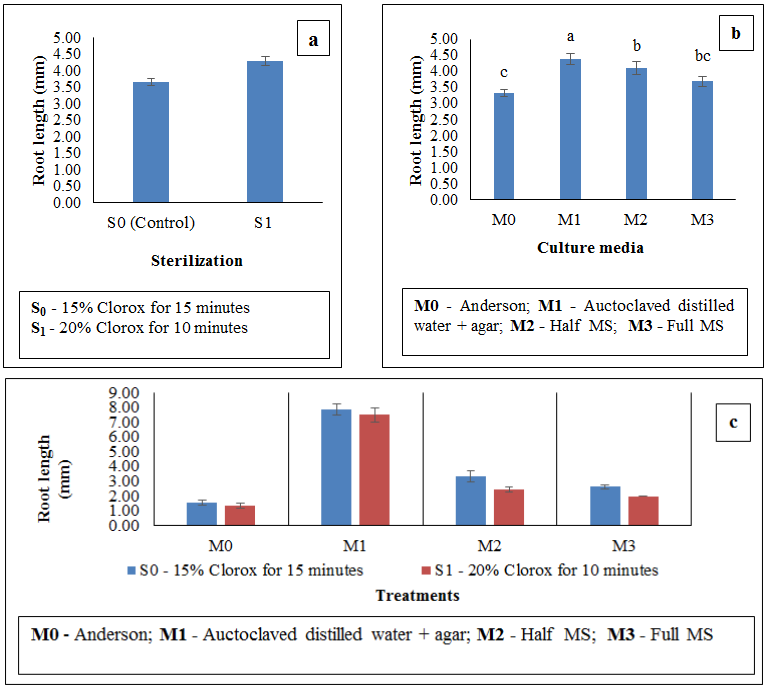


Fig-5: The effect of sterilization protocol and culture media on the root length (a) The effect of the sterilization protocol; (b) The effect of the culture medium; (c) The interaction between sterilization protocol and treatments

Note: The results are represented as average ± SD of ten replicates, n = 30 (n = total number of seeds per replicate) and different letters indicate significant differences (p value <0.05).

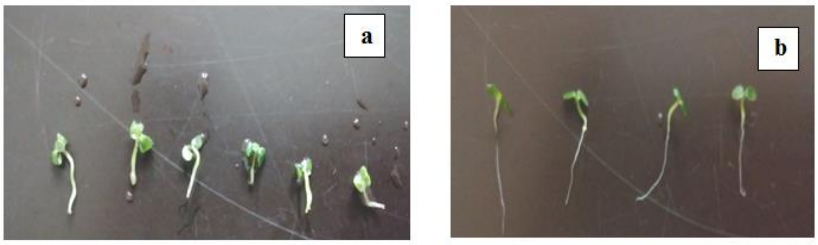
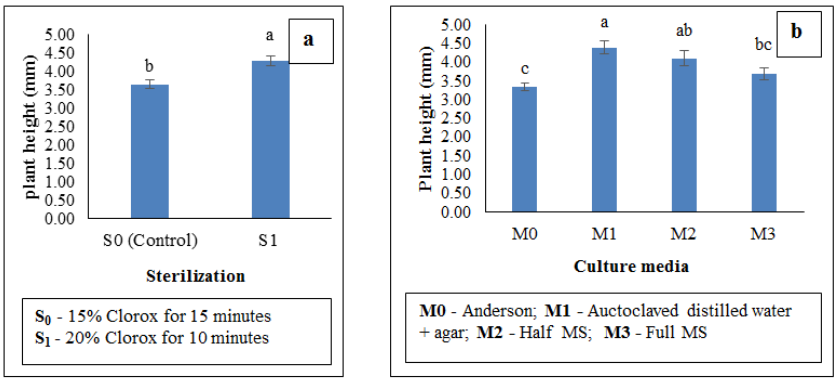


Plate-3: Effect of culture media on root length (a) The shortest roots were observed in Anderson medium (ADW); (b) The longest roots were observed in autoclaved distilled water + agar medium.

Plant height

The plant height was significantly different between the sterilization protocol (Figure 8a) and among culture media (Figure 8b). The highest plant height was 4.99 mm ± 0.45 and it was recorded in the S_1 sterilization protocol. Among culture media, autoclaved distilled water + agar medium had the highest plant height as 4.39 mm ± 0.18. The interaction between sterilization protocol and growing media had a significant effect on germination percentage (Figure 8c and 8d).

Note: The value represented are average ± SD (p value <0.05)



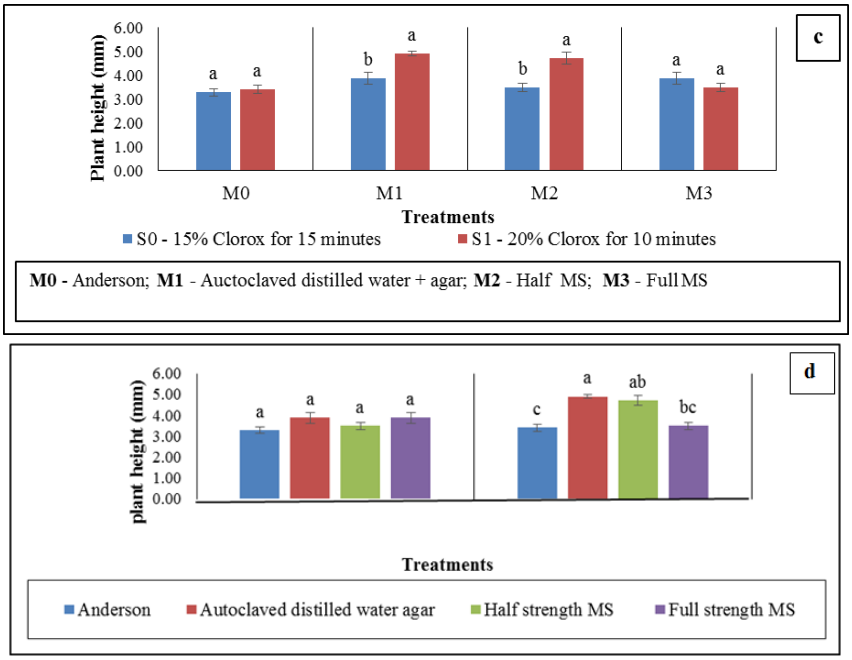


Fig-6: The effect of sterilization protocol and culture media on plant height (a) The effect of the sterilization protocol; (b) The effect of the culture media; (c) The plant height between sterilization protocols when changed the culture media; (d) The plant height among culture media when changed the sterilization protocol.

Note: The results are represented as average ± SD of ten replicates, n = 30 (n = total number of seeds per replicate) and different letters indicate significant differences (p value <0.05).

S0 – 15% Clorox for 15 minutes S1 - 20% Clorox for

Anderson media is mostly utilized for Rhododendron seed germination. But in this experiment, the plant height and the root length were highest in autoclaved distilled water + agar medium which had the lowest level of minerals and zero amount of sucrose. The presence of sucrose and more minerals in culture media had also shown negative effect on both seed germination and the development of *Althaea officinalis* seedlings [13]. Similar results also were mentioned by [16] for *Kalmia latifolia* L.–Therefore, these experimental results also agreed with the reported literature.

20% of Clorox with few drops of Teepol for 15 minutes was given the highest plant height than 15% of Clorox with few drops of Teepol for 10 minutes. A similar finding has been recorded by previous literature as an example, the positive effect in seed germination, seedling growth, and development of *Capsicum frutescens* L. in different concentration of sodium hypochlorite [40].

The contamination percentage

The contamination percentage was not significantly different between sterilization protocols but it was significantly different among culture media. The highest contamination percentage (30.91% ± 9.11) was recorded in the full-strength MS medium. The interaction between sterilization protocol and growing media had no significant effect on contamination percentage (Figure 9).

Both sterilization protocols had no significant effect on the contamination percentage. The active ingredient of Clorox is sodium hypochlorite (NaOCl). When NaOCl dilutes with water, the hypochlorite irons could direct the formation of HClO and that was affected bacterial activity by damaging its DNA [17].

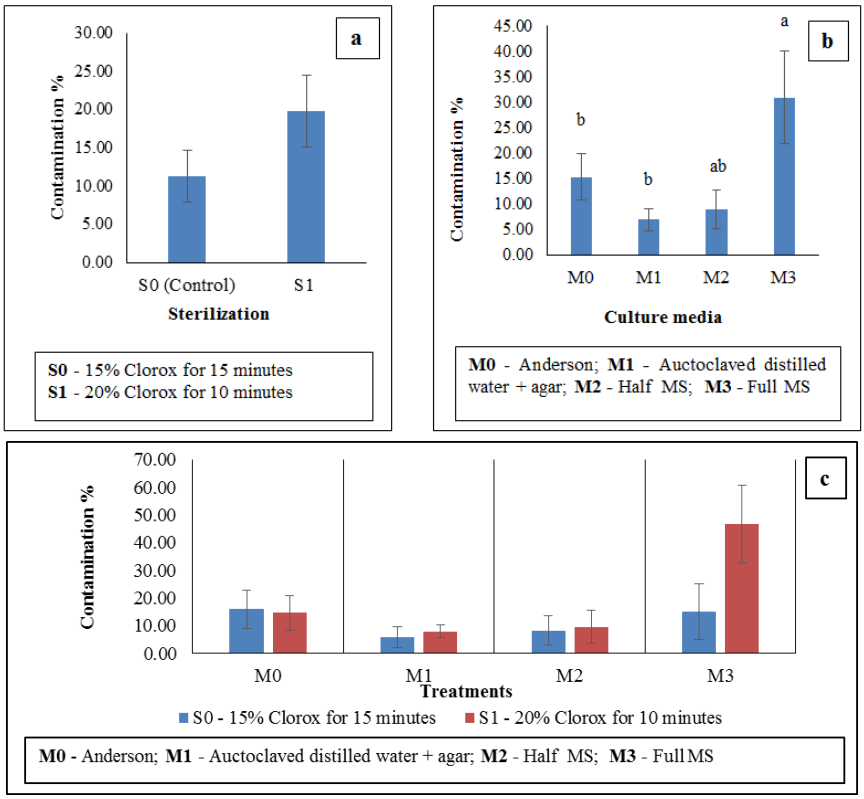


Fig-7: The effect of sterilization protocol and culture media on the contamination percentage (a) The effect of the sterilization protocol; (b) The effect of the culture medium; (c) The interaction between culture media and sterilization protocol.
Note: The results are represented as average ± SD of ten replicates, n = 30 (n = total number of seeds per replicate) and different letters indicate significant differences (p value <0.05).

CONCLUSION

The research results revealed that the *Hakgala* is better than *Peradeniya* for *in vivo* seed germination of *Rhododendron arboreum subsp. zeylanicum*. It can be disclosed that coir dust: sand (1:1) can be used as a successful medium for *in vivo* seed germination. The results demonstrated the autoclaved distilled water + agar medium has potential as a culture medium for *in vitro* seed germination of *Rhododendron arboreum subsp. zeylanicum* and also full-strength MS medium cannot be used for *in vitro* seed germination studies of this species. 20% Clorox with few drops of Teepol shake for 10 minutes is better than 15% Clorox with few drops of Teepol shake for 15 minutes for seed sterilization for *in vitro* cultures. Research results will be useful for both *in situ* and *ex situ* conservation programs of *Rhododendron arboreum subsp. zeylanicum*, the vulnerable and endemic plant species in Sri Lanka.

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