Tropical Agricultural Research & Extension 27 (3): 2024

RESEARCH ARTICLE

EFFECT OF CUTTING TYPE, POTTING MEDIA, AND IRRIGATION FREQUENCY ON THE SUCCESS OF VEGETATIVE PROPAGATION OF IRIWERIYA (*Plectranthus zeylanicus* FORSSK)

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Received: 24 January 2024, Accepted: 29 August 2024, Published: 30 September 2024

Abstract

***Plectranthus zeylanicus* is an indigenous, multi-functional herb used in traditional medicine. Due to over-exploitation from the natural habitats, the species is on the verge of extinction. Therefore, commercial cultivation of the species is needed to satisfy the rising demand while protecting its genetic base. The current study was designed with three trials to identify the most suitable cutting type, potting media, and irrigation schedule for *P. zeylanicus*. Four distinct cutting types (tip cutting, triple nodal cutting, double nodal cutting and single nodal cutting), five different potting mixtures (topsoil: sand: compost-1:1:1, topsoil: sand: compost-1:2:1, topsoil: sand: compost-1:1:2, topsoil: compost -1:1, topsoil: compost-1:2) and four different irrigation schedules (daily watering to field capacity level, daily watering to half of the field capacity level, every other day to the field capacity level, every other day to half of the field capacity level) were used in Completely Randomized Design with ten replicates. Survival percentage (%), number of leaves, the height of the plant (cm), average length of roots (cm), average leaf area (mm^2), and chlorophyll content (SPAD Unit) were measured as growth parameters. Data were analyzed using SAS software with a 5% significance levels. Highest survival percentage (95%), highest average root length (26.8 cm), average leaf area (80.5 mm^2) and a higher chlorophyll content (31.1 SPAD Unit) were recorded from tip cuttings of *P. zeylanicus.* Furthermore, the topsoil: sand: compost-1:1:1 combination showed a higher survival percentage (90%), number of leaves (3), highest average plant height (43.9cm) and average root length (20.2 cm) with significantly ($P<0.05$) higher average leaf area (52.3 mm^2) and chlorophyll content (31.07 SPAD Unit). Daily watering to the field capacity showed a higher survival percentage (90%), significantly higher ($P<0.05$) number of leaves (3.7), plant height (51.6 cm), higher root length (25.5 cm), the highest average leaf area (80.0 mm^2) and chlorophyll content (32.7 SPAD Unit). Therefore, tip-cutting planted in topsoil: sand: compost-1:1:1 media can be recommended for successful propagation of *P. zeylanicus*.**

Keywords: Growth parameters, Irrigation schedule, *Plectranthus zeylanicus,* Potting media, Stem cutting

INTRODUCTION

With the global resurgence in conventional and alternative healthcare systems, the market for herbal medicines has expanded dramatically, and as a result, medicinal plants are significant economically (Sen *et al.,* 2011). Among other medicinal plants, *Plectranthus zeylanicus*, is a widely used medicinal plant in Sri Lanka (Soyza *et al*., 2017). It is a member of the family Lamiaceae, which includes about 200 genera and 3,200 species. The family has a history of use in both food and medicine (Punet *et al*., 2020). Lamiaceae is one of the most significant families that contains volatile oils. As an antiemetic, anti-inflammatory, anti-spasmodic, carminative, choleretic, diaphoretic, emmenagogue, and antibacterial substances, they are employed in traditional medicine (Hamed *et al*., 2020).

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P. zeylanicus plant adds to its enormous therapeutic value due to its unique medicinal characteristics. The active ingredients of *P. zeylanicus* include hexatriacontane, lupeol, tannins, and volatile, antioxidant oleananes (Mirihagalla & Fernando, 2021). Plectranthus species contain highly fragrant essential oils such as geraniol and geranyl acetate (Muthukumarana and Dharmadasa, 2014). Napagoda *et al*. (2022) found *P. zeylanicus* to be a highly effective antibacterial and disinfecting agent, confirming its long-standing use in Sri Lanka for the treatment of microbial infections and inflammatory disorders.

Throughout the ages, a wide variety of wild and semi-wild plant species, medicinal, and fragrant plants have been utilized for human welfare, health promotion, and as ingredients in medications and fragrances (Rahman and Fakir, 2015). Therefore, the natural populations of plants are under a lot of pressure due to the rising demand for both domestic and international markets. The future of several species is seriously threatened by the unsustainable exploitation that collectors of medicinal plants are now undertaking (Mahindapala, 2004). Due to the importance of aromatic compounds and essential oils in the fragrance industry, flavoring industry, pharmaceuticals, and several other industries, production of *P. zeylanicus* must rise while taking place with less effort and expense (Fonseka & Katepearachchi, 2020). *P. zeylanicus* is an indigenous plant in Sri Lanka. Thus, it's important to avoid excessive harvesting to meet its increasing requirements. Therefore, it is crucial to build commercial farming using cost-effective agricultural technologies to ensure the production and steady supply of high-quality raw materials of *P. zeylanicus.*

Vegetative cuttings are the usual method of propagating *P. zeylanicus*. Cuttings are the most popular propagation method since they are both successful and inexpensive (Leakey, 2004). However, using the improper type of cutting results in a laborious process with relatively few successful propagules. Specific cutting types are required depending on the species to be propagated and its maturity. Depending on the species, certain species can produce roots quickly in cuttings; hardwood, semi-hardwood, or in softwood (Hartmann and Kester, 1959). Aside from cutting type, growth media is important for the growth and development of plants. At the same time, having a suitable water supply is crucial for the successful development of plants. Therefore, this study was designed to determine the optimal cutting type as the most basic vegetative propagation technique. Following that, to choose the best potting medium and irrigation schedule for *P. zeylanicus* propagules to produce high-quality planting materials.

MATERIALS AND METHODS

The present study was conducted in a protected house, Faculty of Agriculture, University of Ruhuna, Sri Lanka. Three separate sub-experiments were conducted to select the best cutting type and the most suitable potting media to identify the most promising irrigation schedule for the best growth performances of *P. zeylanicus*.

Experiment 1: Selection of the best cutting type of *P. zeylanicus* for its vegetative propagation

Four different cutting types; tip cutting/ softwood cutting (C_1), triple nodal cutting (semi-hardwood) (C_2), single nodal cutting (Semi-hardwood) (C_3), and double nodal cutting (semi-hardwood) (C_4) at the same height (8 cm) taken from uniform, quality, healthy mother plants were established in polybags (gauge 300 and 25 x 40 cm size) filled with a common growing media: topsoil: sand: compost-1:1:1. Potting media were drenched using fungicide. Established cuttings were kept in a propagator after saturating the media with water. A Completely Randomized Design (CRD) was used with ten replicates from each cutting type. Data on survival percentage (%), average root length (cm), average leaf area (mm^2), and chlorophyll content (SPAD Unit) were measured at six weeks after establishment.

The survival percentage was calculated by counting the successful plants and calculating the percentages for each treatment, as shown in the equation below.

Survival percentage (%) =

$$\left(\frac{\text{Number of cuttings succeed}}{\text{Number of cuttings established}}\right)\text{x100}$$

...Equation 1

The average root length was calculated using the following equation after measuring the length of each root from the carefully uprooted plants. Leaf area was measured using the grid method. The same positioning leaf of each plant was taken to calculate the leaf area and chlorophyll content was measured using a SPAD meter.

Average Root Length=

$$\frac{\text{Sum of all root lengths}}{\text{Number of roots}}$$

...Equation 2

Experiment 2: Selection of the best potting media for *P. zeylanicus* for better growth performances

Similar size (25 x 40 cm) black polythene (300 gauge) polybags were used, and water-draining holes were made at the bottom of each bag. Five different potting mixtures such as topsoil: sand: compost-1:1:1(M_1), topsoil: sand: compost-1:2:1 (M_2), topsoil: sand: compost-1:1:2(M_3), topsoil: compost -1:1 (M_4), topsoil: compost-1:2 (M_5) were used to fill the pots. Media-filled polybags were laid out in a Completely Randomized Design (CRD) inside the protected house with ten replicates from each potting mixture. The cutting type that exhibited the most robust growth performance in experiment 1 (tip cuttings) was established, and the same management practices were followed. Four weeks after establishment, survival percentage (%), number of leaves, average plant height (cm), average root length (cm), average leaf area (mm^2), and chlorophyll content (SPAD Unit) were measured as in experiment 1.

Physio-chemical parameters of five different growing media were examined for pH, Electrical Conductivity (EC) (mS/cm), Ammonium nitrogen (mg/kg), Nitrate nitrogen (mg/kg), available Nitrogen content (%), available phosphorus content (mg/kg), potassium (mg/kg), and organic matter content (%). Data on pH and EC were determined using the pH and EC meter (HANNA HI 83099). Ammonium nitrogen, nitrate nitrogen, and available phosphorous were analyzed using a UV visible spectrophotometer (Shimadzu UV160). Available potassium was measured using the flame photometer (Sherwood model 360).

Experiment 3: Identifying the most promising irrigation schedule for better growth performances of *P. zeylanicus cuttings*

Similar size *P. zeylanicus* tip cuttings were established in polybags (gauge 300 and 25 x 40 cm size) filled with growing media (topsoil: sand: compost 1:1:1), which exhibited the most favorable growth performance in experiment 2. After four weeks of establishment of cuttings, irrigation was practiced according to the designed treatments (daily watering to field capacity level (I_1), daily watering to half of field capacity level (I_2), every other day to field capacity level (I_3), every other day to half of field capacity level (I_4)). A Completely Randomized Design (CRD) was used with ten replicates from each irrigation schedule. Irrigation was accomplished to the field capacity level, which was determined using the gravimetric method. The water requirement to reach field capacity was determined using the equation below.

Water needs for the plants to reach field capacity =

$$\left[\left(\begin{array}{c}\text{Moisture amount}\\\text{in field capacity level}\end{array}\right)-\left(\begin{array}{c}\text{Initial moisture}\\\text{in the soil}\end{array}\right)\right]$$

...Equation 3

Data on survival percentage (%), number of leaves per plant, average plant height (cm), average root length (cm), average leaf area (mm^2), and chlorophyll content (SPAD Unit) were measured at twelve weeks after establishment.

The data of all experiments were analyzed using SAS statistical software at 5% Significance level. Duncan's multiple range test was employed to compare treatment means.

RESULTS AND DISCUSSION

Experiment 1: Selection of the best cutting type of *P. zeylanicus* for its vegetative propagation for better growth performance

Based on the results of the study, softwood cuttings of *P. zeylanicus* (C_1) exhibited the maximum survival percentage of 95% (Figure 1). Further, it was found that softwood cuttings of *P. zeylanicus* performed significantly ($P<0.05$) the highest root length (26.8cm) and average leaf area (80.5 mm^2) (Table 1). Further, the same cutting type (C_1) recorded a higher chlorophyll content (31.1 SPAD unit) (Table 1). Therefore, it seems that tip/softwood cuttings of *P. zeylanicus* has performed well in all the parameters compared to other cutting types used in this experiment.

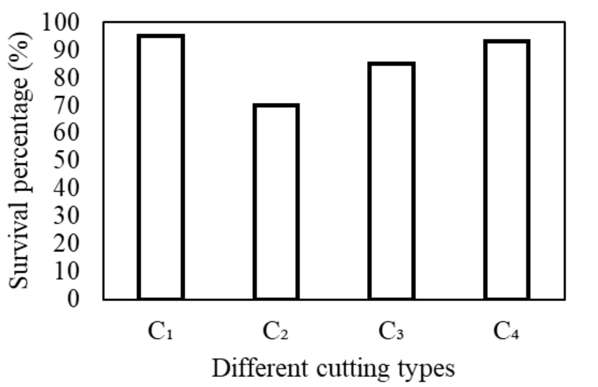

Figure 1: Effect of different cutting types on survival percentage of *P. zeylanicus* at six weeks after establishment

C_1: tip/top cutting, C_2: triple nodal cutting, C_3: single nodal cuttings, C_4: double nodal cuttings

Although softwood cuttings need specific care and a unique rooting environment, they typically root more easily than other cutting forms (Dumroese *et al*., 2009). Many species, including mints (Mentha spp.), Brahmi (*Bacopa monnieri*), and other herbs, are commonly propagated via softwood and herbaceous cuttings (Waman *et.al*., 2019). As stated by Kassahun and Mekonnen (2011), using the upper cutting portion of *Stevia rebaudiana* Bertoni; a perennial herb is recommended in its vegetative propagation. It's interesting to note that softwood cutting has been successful not only with non-woody herbs but also with some woody plants like cherry (Gulen *et al*., 2004), soursop (Santos *et al*., 2010*)* and cashew (Saranga and Cameron, 2007). On the contrary, an experiment by Patel (2016) indicates that *Plectranthus amboinicus (Louris*): a valuable medicinal plant has greater potential to regenerate effectively with mature stem cuttings. Similarly, hardwood stem cuttings of *Gongronema latifolia* generated a notably greater quantity of roots in comparison to semi-hardwood and softwood cuttings (Agbo and Obi, 2006). However, all these variations may be based on several possible explanations including physiological state of the cuttings, carbohydrate content related to the preservation of leaves on cuttings, nutrient richness of the substrate as well as the temperature (Danthu *et al*., 2008). According to the physiological age of the wood, stem cuttings are divided into three categories: softwood, semi-hardwood, and hardwood (Agbo and Obi, 2008). As the experiment's results indicate, softwood cuttings of *P. zeylanicus* perform better than semi-hardwood cuttings. This lowered success capacity of

Table 1: Effect of different cutting types on average root length (cm), average leaf area (mm^2), and chlorophyll content (SPAD unit) of *P. zeylanicus*

Different cutting types	Average root length (cm)	Average leaf area (mm^2)	Chlorophyll content (SPAD unit)
Tip cutting (C_1)	26.8^a	80.5^a	31.1^a
Triple nodal cutting (C_2)	21.8^b	43.5^b	33.4^a
Single nodal cutting (C_3)	15.3^c	22.0^c	29.7^a
Double nodal cutting (C_4)	11.3^d	15.8^d	31.1^a

Note: Means with similar letters in a column are not significantly different at $p<0.05$

C_1: tip/top cutting, C_2: triple nodal cutting, C_3: single nodal cuttings, C_4: double nodal cuttings semi-hardwood cuttings could be related to the natural aging process of mature cuttings. Furthermore, since the rooting hormone plays a key role in the success of cuttings (Ingle and Venugopal, 2009), we hypothesized that the success of softwood cuttings may be due to the favorable concentration of natural hormones in softwood cuttings of *P. zeylanicus*. Therefore, further thorough research is suggested to identify the variation of hormone and carbohydrate concentrations in different cutting positions of *P. zeylanicus*.

Selection of the best potting media type for *P. zeylanicus* cuttings for its better growth performances

As revealed by the experimental results, it is evident that the highest survival percentage (97%) was shown by M_3 potting media, followed by M_1 and M_4 (90%) (Figure 2). As noted by Singh and Verma (2015), growth media exhibit profound influence on the survival rate of cuttings. Further, they discovered the highest survival rate for *Stevia rebaudiana* Bertoni (Sweet herb) using a mix of vermicompost, soil, and farmyard manure in balanced proportion. For the vegetative propagation of *Salacia reticulata* (Kothalahimbatu) semi-hardwood stem cuttings planted in topsoil: compost-1:1 resulted the highest rate of survival (Nayana *et al*., 2015). Comparably, an experiment conducted by Jayawardene *et al*. (2021) detailed how three distinct herb seedlings (*Andrographis paniculata, Barleria prionitis and Rhinacanthus polonnaruwensis*) from the same family (Aacanthaceae) responded differently to various growing media in terms of their seedling survival rates. Overall, these findings show that different herbs can respond uniquely to different growing media on their survival rate. Therefore, it is important to identify species-specific media for the successful propagation of herbs. In the present study top soil: sand: compost- 1:1:2 mixture's had ideal physical, chemical and biological properties (Table 2) which may be associated with the favourable impact of this media on the highest survival rate of *P. zeylanicus* (Figure 2).

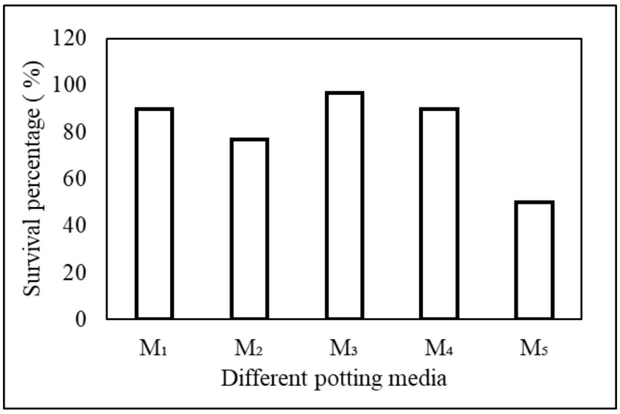

Figure 2: Effect of different potting media on survival percentage of *P. zeylanicus* at four weeks after establishment

M_1: topsoil: sand: compost-1:1:1, M_2: topsoil: sand: compost-1:2:1, M_3: topsoil: sand: compost-1:1:2, M_4: topsoil: compost: 1:1, M_5: topsoil: compost-1:2

Table 2: Composition analysis of different media types used to cultivate *P. zeylanicus* softwood cuttings

Soil Properties	M_1	M_2	M_3	M_4	M_5
pH	7.00 ± 0	7.39 ± 0.021	7.59 ± 0.028	7.33 ± 0.049	6.75 ± 0.007
EC (mS/cm)	0.26 ± 0	0.27 ± 0.002	0.28 ± 0.074	0.21 ± 0.005	0.31 ± 0.019
Ammonium nitrogen (mg/kg)	0.13 ± 0.009	0.12 ± 0.008	0.10 ± 0.003	0.12 ± 0.023	0.17 ± 0.005
Nitrate nitrogen (mg/kg)	0.50 ± 0.006	0.51 ± 0.077	0.44 ± 0.005	0.43 ± 0.001	0.48 ± 0.007
Nitrogen %	0.14 ± 0.012	0.13 ± 0.029	0.18 ± 0.039	0.23 ± 0.065	0.41 ± 0.026
Phosphorous (mg/kg)	0.04 ± 0.019	0.01 ± 0.056	0.31 ± 0.028	0.27 ± 0.028	0.63 ± 0.033
Potassium (mg/kg)	65.7 ± 0.707	73.7 ± 0.707	75.7 ± 0.707	62.7 ± 0.707	95.7 ± 0.707
Organic matter %	5 ± 0.070	3.23 ± 0.162	4.83 ± 0.056	7.41 ± 0.042	13 ± 0.021

Note: M_1: topsoil: sand: compost-1:1:1, M_2: topsoil: sand: compost-1:2:1, M_3: topsoil: sand: compost-1:1:2, M_4: topsoil: compost -1:1, M_5: topsoil: compost-1:2

Values represent means ± standard errors

The highest number of leaves (3.2) was observed in M_4 followed by M_1 (Figure 3A). A crop with a high leaf count will develop and grow more quickly, and it also has a good relationship with crop production (Kumaresan *et al*., 2023). An experiment by Nawarathna *et al*. (2020) reported that, *Momordica dioica* cuttings on coir dust that were planted in the field had noticeably more leaves per vine compared other two soil combinations. Media composition of topsoil, river sand, and cow dung showed the noticeably higher leaf count of *Annona muricata* L. seedlings (Ibeh *et al*., 2024). The highest average plant height (43.9 cm) was given by M_1, followed by M_4 (42.9 cm) (Figure 3B). However, *Punica granatum* L. displayed the highest growth in cocopeat soil, with a noticeable rise in plant height (Choudhary *et al*., 2024). An another study reported the maximum plant height of *Dendranthema grandiflora* L. in the potting media combination of Soil: Sand: FYM: Vermicompost - 2:1:0.5:0.5 (Kala *et al*., 2020). Results also showed that the highest average root length of 20.2cm was recorded in M_1 closely followed by M_3 while that of the lowest was reported in M_5 (Figure 3C). Root length is directly affected by the physico-chemical properties of the growth media. The cuttings of *Buchholzia coriacea* require a medium that is not overly porous but still permits proper drainage and enough room for the growth of cuttings (Akinyele, 2010). The physical and chemical properties of the substances used to prepare potting media directly effect on the growth performances of the plant. Further, physiochemical characteristics of growing medium greatly influence on quality of plant materials (Saleh *et al*., 2022).The quality of the root system that forms depends significantly on the structure, texture, porosity, chemical

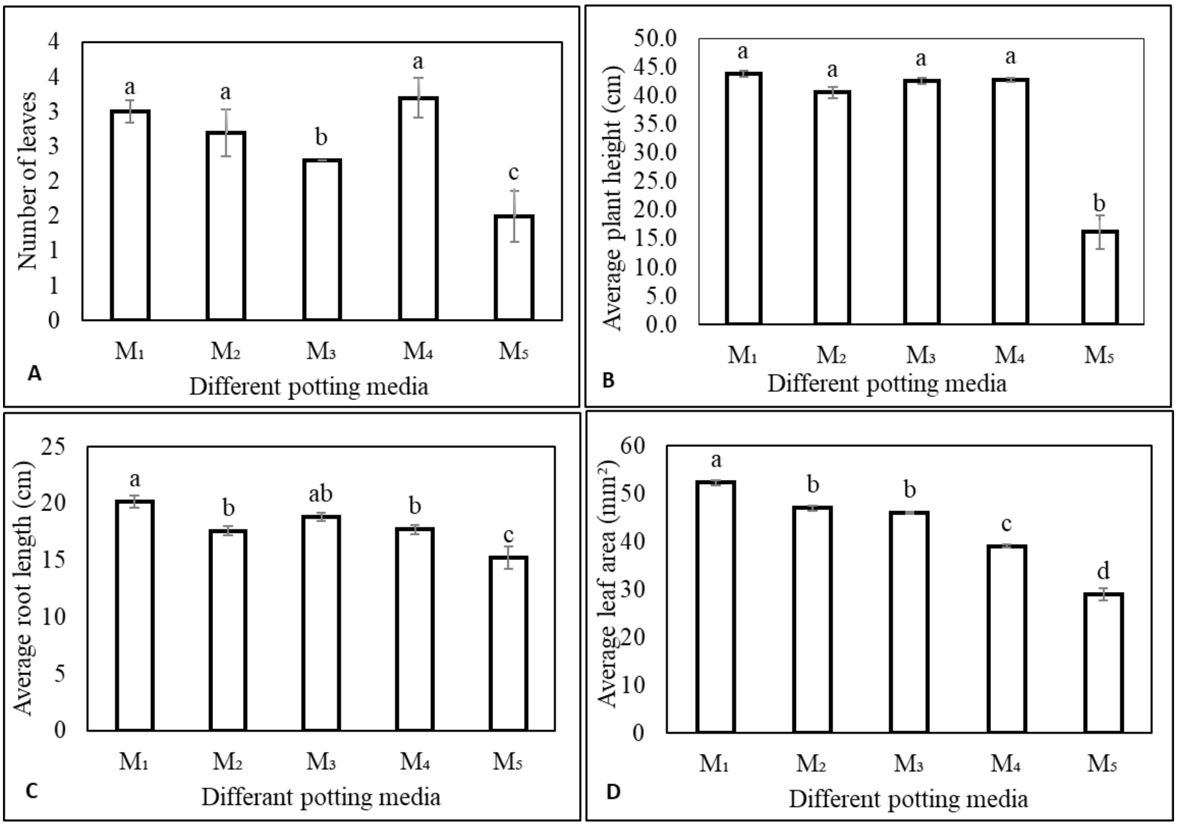

Figure 3: Effect of different potting media on the number of leaves (A), the height of plant (cm) (B), root length (cm) (C), Average leaf area (mm^2) (D) of *P. zeylanicus*

M_1: topsoil: sand: compost-1:1:1, M_2: topsoil: sand: compost-1:2:1, M_3: topsoil: sand: compost-1:1:2, M_4: topsoil: compost -1:1, M_5: topsoil: compost-1:2

composition, and water-holding capacity of the rooting medium (Waman *et al*., 2019). As demonstrated by the experiment's results, significantly (P<0.05) the highest leaf area (52.3 mm^2) (Figure 3D) and the significantly (P<0.05) highest chlorophyll content (31.07 SPAD Unit) (Figure 4) were recorded in M_1. Five different growth media were subjected to a composition analysis for further explanations of how these different growing media influence on growth and development of *P. zeylanicus* (Table 2). Based on the results, M_1 media comprise a comparatively higher amount of Nitrate nitrogen (0.50 ± 0.006 mg/kg) (Table 2).

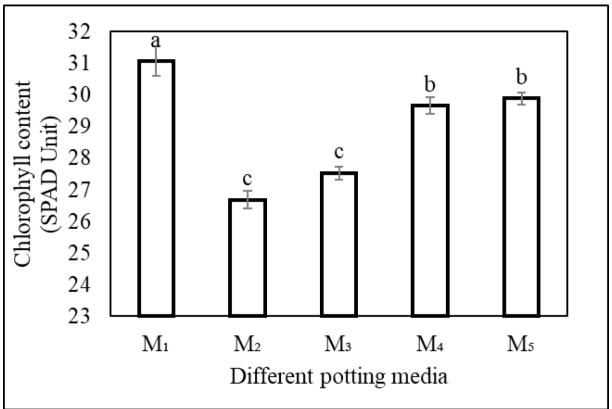

Figure 4: Effect of different potting media on Chlorophyll content (SPAD Unit) of *P. zeylanicus*

M_1: topsoil: sand: compost-1:1:1, M_2: topsoil: sand: compost-1:2:1, M_3: topsoil: sand: compost-1:1:2, M_4: topsoil: compost -1:1, M_5: topsoil: compost-1:2

Similarly, an experiment by Kumaresan *et al*. (2023) revealed that a medium with a greater rate of accessible N had the largest chlorophyll content of two medicinal plants, such as *Coleus forskohlii* and *Centratherum punctatum*. In the present study, topsoil: compost: sand-1:1:1 media (M_1) performed well in most of the growth parameters of *P. zeylanicus*. Further, pH, electrical conductivity (mS/cm), Ammonium nitrogen (mg/kg), Nitrate nitrogen (mg/kg), available Nitrogen %, available phosphorus (mg/kg), available potassium (mg/kg) and organic matter content (%) can be observed between five different growing media (Table 2). The results for the chemical properties indicated that the M_5 medium had the highest values for all measured parameters except for pH value and Nitrate Nitrogen. Further, M_5 had a lower value for most of the parameters considered in the experiment than other media. The reason could be the high proportion of compost in M_5 media. Compost phytotoxicity is a crucial factor to consider because applying immature compost in greater levels will result in free ammonia, organic acids, and other water-soluble chemicals that can stunt plant growth and root development (Council, 2002). Available Nitrogen and Potassium content has a significant effect on growth parameters. According to Nakandalage *et al.* (2022), selecting a growing medium with relatively low amounts of nitrate, nitrogen, and potassium is imperative for achieving greater development of *P. longum*. However, M_1 media has relatively higher Nitrate nitrogen (0.50 ± 0.006 mg/kg), and lower Potassium (65.7 ± 0.707 mg/kg) content compared to five different media and that may be associated with the high growth rate of *P. zeylanicus* under current experimental conditions. The greatest growth and yield observed in *P. zeylanicus* softwood cuttings may be associated with ideal moisture availability and aeration provided by the M_1 medium.

Identifying the most promising irrigation schedule for better growth performances of *P. zeylanicus* tip cuttings

Based on the results of the study, I_2 resulted in the highest rate of survival (96%) followed by I_4 (95%) (Figure 5). The presented results appear unrelated to water stress, as there is no discernible pattern corresponding to different levels of water stress. Similarly, Guo *et.al.* (2009) report that relationships between plants and water at the cellular and overall plant levels produce both targeted and non-targeted reactions. Plant response to water stress is a complex procedure that often involves the synthesis of polyamines and a group of unique proteins whose roles are still mostly unknown (Khan *et.al*., 2020). A species may possess a complement of survival mechanisms that allow it to survive in minimal and fluctuating precipitation (Liu *et. al*., 2013).

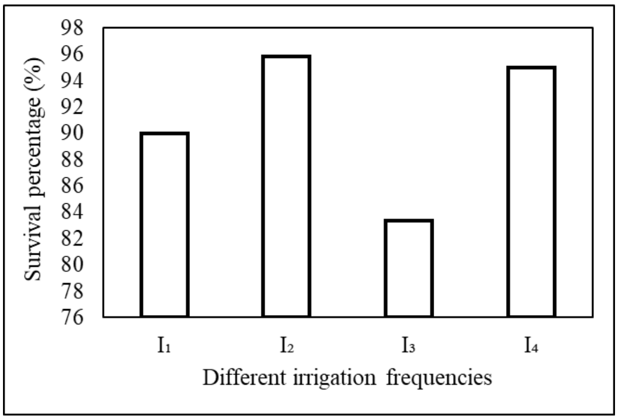

Figure 5: Effect of Different Irrigation Schedules on Survival Percentage of *P. zeylanicus at* twelve weeks after establishment

I_1 : daily watering to field capacity level, I_2 ;daily watering to half of field capacity level, I_3 : every other day to field capacity level, I_4 : every other day to half of field capacity level

The results indicated that the I_1 irrigation schedule produced significantly (P<0.05) the highest number of leaves (3.7) (Figure 6A). Remarkably, the same irrigation schedule (I_1) has recorded significantly (P<0.05) the highest plant height (51.6 cm) (Figure 6B) and significantly (P<0.05) a higher root length (25.5 cm) as shown in Figure 6C. Similarly, numerous studies conducted by researchers have confirmed that providing a sufficient moisture content is necessary to achieve the highest possible growth characteristics. For example, Ping *et al.* (2014) have described that the two medicinal plants' growth indices at 75% of field water holding capacity were higher than those at 30% and 50%. On average, drought is characterized by stunted growth, decreased stomatal conductance, and reduced plant biomass, which results in less efficient photosynthesis of carbon dioxide (Amalero *et al.*, 2003).

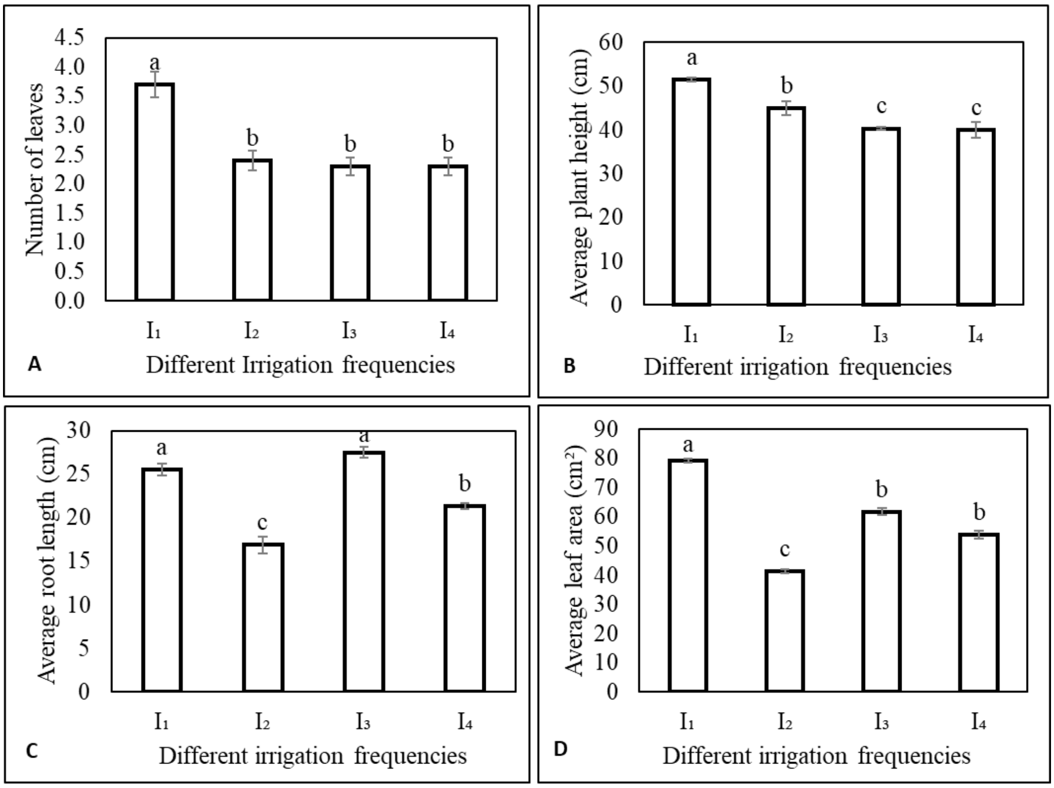

Figure 6: Effect of different irrigation frequencies on the number of leaves (A), the average height of plant (cm) (B), average root length(cm) (C) and Average leaf area (mm^2) (D) of *P. zeylanicus*

I_1 : daily watering to field capacity level, I_2 : daily watering to half of field capacity level, I_3 : every other day to field capacity level, I_4 : every other day to half of field capacity level

Moreover, our experiment found that water stress has a more severe impact on plant height and leaf count than on average root length. Consistent with these results, a study by Foday *et al.* (2012) also observed that shoot growth is more severely restricted than root growth under low irrigation frequency. However, Nakandalage *et al.* (2022) reported that root parameters had an interaction effect with growing media and irrigation frequency. Therefore, it is very important to select the best-growing media with the promizing irrigation schedule to obtain higher growth and yield especially for potted plants. Plants are only able to tolerate the range of easily accessible water in the growing medium, up to the point of refilling the container (Wang, 2013).

As for the above parameters, the highest leaf area (80.0 mm^2) and the highest chlorophyll content (32.7 SPAD Unit) were also recorded from I_1 as presented in Figure 6D and Figure 7 respectively. Different water regimes directly affect plant physiology. As a result of the physiology of the plant may act differently, changes in photosynthetic pigments, together with other parameters, are of utmost significance for drought resistance (Jaleel *et al.*, 2009). Significant photosynthetic impairment and notable chlorophyll degradation are expected by drought or flooding (Abbey *et al.*, 2023). In summary, the results of this experiment indicate that daily irrigation to field capacity is the most effective practice for achieving higher yields in *P. zeylanicus.* Likewise, Rao *et al*. (2010) discovered that *Piper longum* responded considerably better in terms of growth, yield, and quality when given an irrigation schedule of 0.8 cumulative pan evaporation. They also highlighted the fact that *P. longum* needed relatively high watering. Additionally, as the production of secondary metabolites in medicinal plants is highly dependent on water availability (Albergaria *et al*., 2020). Most importantly, present study direct future research to discover how secondary metabolites of *P. zeylanicus* vary depending on differing water levels.

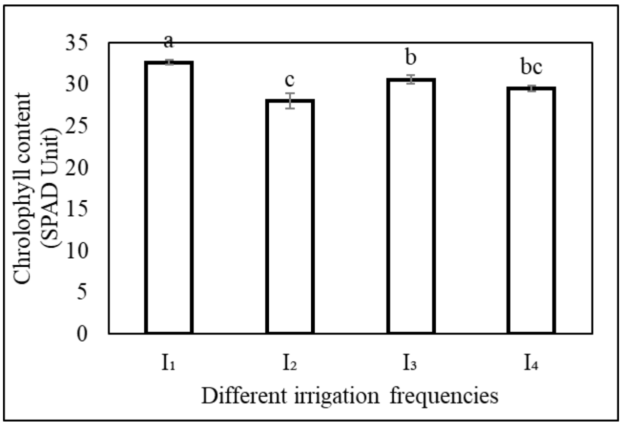

Figure 7: Effect of different irrigation frequencies on chlorophyll content (SPAD Unit) of *P. zeylanicus*

I_1: daily watering to field capacity level, I_2: daily watering to half of field capacity level, I_3: every other day to field capacity level, I_4: every other day to half of field capacity level

CONCLUSION

The presented study attempted to find out the best cutting type, growing media and irrigation schedule for the successful propagation of *P. zeylanicus* under three separate subsequent experiments.

Based on the results of experiment 1, softwood/ tip cuttings of *P. zeylanicus* resulted in higher survival %, average root length, average leaf area and chlorophyll content. In the second experiment tip cuttings established in topsoil: sand: compost-1:1:1 media performed well in most of the growth parameters such as: survival %, number of leaves, plant height, average root length, leaf area and chlorophyll content considered in the experiment. Moreover, daily irrigation to the field capacity level of *P. zeylanicus* tip cuttings established in topsoil: sand: compost-1:1:1 media resulted in all most all the highest growth parameters in experiment 3.

Therefore, softwood/tip cuttings of *P. zeylanicus* established in topsoil: sand: compost-1:1:1 media, irrigated with daily irrigation to the field capacity level is recommended for the optimal success of vegetative propagation of *P. zeylanicus.* These findings may provide valuable insights for enhancing the propagation and cultivation practices of *P. zeylanicus*, thereby contributing to its sustainable cultivation and utilization.

AUTHOR CONTRIBUTION

NN conceptualized and designed the experiment. RMAP performed the experiment. NN and WRS contributed in drafting the manuscript and critically revised the manuscript.

REFERENCES

Abbey, L., Ofoe, R., Wang, Z. and Chada, S., 2023. How Central Carbon Metabolites of Mexican Mint (Plectranthus amboinicus) Plants Are Impacted under Different Watering Regimes. *Metabolites*, *13*(4), p.539. https://doi.org/10.3390/metabo13040539

Agbo, C.U. and Obi, I.U., 2006. Macro-propagation technique for different physiological ages of Gongronema latifolia Benth cuttings. *African journal of biotechnology*, *5*(15).

Agbo, C.U. and Obi, I.U., 2008. Patterns of vegetative propagation of stem cuttings of three physiological ages of Gongronema latifolia Benth over two seasons in Nsukka. *Agro-Science*, *7* (3).

Akinyele, A.O., 2010. Effects of growth hormones, rooting media and leaf size on juvenile stem cuttings of Buchholzia coriacea Engler. *Annals of Forest Research*, *53*(2), pp.127-133. https://doi.org/10.15287/afr.2010.105

Albergaria, E.T., Oliveira, A.F.M. and Albuquerque, U.P., 2020. The effect of water deficit stress on the composition of phenolic compounds in medicinal plants. *South African Journal of Botany*, *131*, pp.12-17.

Amalero, E.G., Ingua, G.L., Erta, G.B. and Emanceau, P.L., 2003. Review article Methods for studying root colonization by introduced. *Agronomie*, *23*(2007), pp.407-418.

Choudhary, R., Gurjar, P.K.S., Tripathia, M.K., Kantwa, R.C., Astha, A., Sharma, A., Khandoliya, S. and Ram, G., 2024. Effect of different Potting Mixtures on Acclimatization of In vitro Developed Plants on Survival Percentage under Glass House Condition. *International Journal of Environment and Climate Change*, *14* (3), pp.445-449.

Council, U.C., 2002. Test methods for the examination of composting and compost. *Rokonkoma, NY*.

Danthu, P., Ramaroson, N. and Rambeloarisoa, G., 2008. Seasonal dependence of rooting success in cuttings from natural forest trees in Madagascar. *Agroforestry systems*, *73* (1), pp.47-53.https://link.springer.com/article/10.1007/s10457-008-9116-7

De Soyza, S.G., Wijayaratne, W.M.D.G.B., Napagoda, M. and Witharana, S., 2017. Antimicrobial potential in biogenic silver nanoparticles synthesized from Plectranthus zeylanicus. *Journal of Molecular Nanotechnology and Nanomedicine*, *1*, p.105.

De Soyza, S.G., Wijayaratne, W.M.D.G.B., Napagoda, M. and Witharana, S., 2017. Antimicrobial potential in biogenic silver nanoparticles synthesized from Plectranthus zeylanicus. *Journal of Molecular Nanotechnology and Nanomedicine*, *1*, p.105.

Dumroese, R.K., Luna, T. and Landis, T.D. eds., 2009. *Nursery manual for native plants: a guide for tribal nurseries* (No. 730). US Department of Agriculture, Forest Service.https://books.google.lk/books?hl=en&lr=&id=7sfmOiy-1IkC&oi=fnd&pg=PP6&dq=Luna,+T.+(2009).+Vegetative+propagation.+Nursery+manual+for+native+plants:+A+guide+for+tribal+nurseries,+1,+153-175.&ots=cQcjnh4c_g&sig=7jlTfuBvRrXhtK2Ogak2skIganU&redir_esc=y#v=onepage&q&f=false

Foday, T.I., Xing, W., Shao, G. and Hua, C., 2012. Effect of water use efficiency on growth and yield of hot pepper under partial root-zone drip irrigation condition. *International Journal of*

Scientific & Engineering Research, *3* (1), pp.1-14.

Fonseka, D.K. and Katepearachchi, K.G.,2020, Mass Production of Plectranthus amboinicus: A valuable medicinal and aromatic plant with a future value. http://www.botanyjournals.com/

Gulen, H., Erbil, Y. and Eris, A., 2004. Improved rooting of Gisela-5 softwood cuttings following banding and IBA application. *HortScience*, *39* (6), pp.1403-1405.

Guo, P., Baum, M., Grando, S., Ceccarelli, S., Bai, G., Li, R., Von Korff, M., Varshney, R.K., Graner, A. and Valkoun, J., 2009. Differentially expressed genes between drought-tolerant and drought-sensitive barley genotypes in response to drought stress during the reproductive stage. *Journal of experimental botany*, *60*(12), pp.3531-3544.

Hamed, A., Ahmed, N., Attia, E. and Desoukey, S., 2020. Phytochemical investigation of saponifiable matter & volatile oils and antibacterial activity of Moluccella laevis L., family Lamiaceae (Labiatae). *Journal of Advanced Biomedical and Pharmaceutical Sciences*, *3*(4), pp.213 -220. DOI: 10.21608/ JABPS.2020.38933.1096 https://jabps.journals.ekb.eg/article_115271.html

Hartmann, H.T. and Kester, D.E., 1959. Plant propagation: principles and practices. *Plant propagation: principles and practices.https://www.cabidigitallibrary.org/doi/full/10.5555/19590303074*

Ibeh, K.G., Anozie, E.L., Osigwe, C.C. and Umeh, P.C., 2024. Early Growth Response of Annona muricata (L.) Seedlings to Different Potting Media. *Asian Journal of Research in Agriculture and Forestry*, *10*(1), pp.135-144. https://doi.org/10.9734/ajraf/2024/v10i1277

Ingle, M.R. and Venugopal, C.K., 2009. Effect of different growth regulators on rooting of stevia (Stevia rebaudiana Bertoni) cuttings.

Jaleel, C.A., Manivannan, P.A.R.A.M.A.S.I.V.A.M., Wahid, A., Farooq, M., Al-Juburi, H.J., Somasundaram, R.A.M.A.M.U.R.T.H.Y. and Panneerselvam, R., 2009. Drought stress in plants: a review on morphological characteristics and pigments composition. *Int. J. Agric. Biol*, *11*(1), pp.100-105. http://www.fspublishers.org/

Jayawardhane, J., Damunupola, J.W. and Jayasuriya, K.M.G.G., 2021. Propagation of three important medicinal plants in Aacanthaceae; Andrographis paniculata, Barleria prionitis and Rhinacanthus polonnaruwensis. *Ceylon Journal of Science*, *50*(2), pp.129-137. http://doi.org/10.4038/cjs.v50i2.7874

Kala, D., Mahawer, L.N. and Bairwa, H.L., 2020. Response of potting media composition for pot mum chrysanthemum production (Dendranthema grandiflora L.). *International journal of chemical studies*, *8*(2), pp.1246-1251. https://doi.org/10.22271/chemi.2020.v8.i2s.8936

Kassahun, B.M. and Mekonnen, S.A., 2011. Effect of cutting position and rooting hormone on propagation ability of stevia (Stevia rebaudiana Bertoni). *J. Plant. Biochem. Biotechnol*, *6*(1), pp.5 -8.

Khan, N., Ali, S., Tariq, H., Latif, S., Yasmin, H., Mehmood, A. and Shahid, M.A., 2020. Water conservation and plant survival strategies of rhizobacteria under drought stress. *Agronomy*, *10* (11), p.1683.

Kumaresan, M., Nadhiya Devi, K. and Rajaselvam, M., 2023. Effect of organic media on growth and rooting performance of medicinal plants. *Research Journal of Agricultural Sciences*, *14*(6), pp.1855-1858. http://rjas.org/Article/Article/5697

Leakey, R.R., 2004. Physiology of vegetative reproduction. Academic Press.

DOI: 10.1016/B0-12-145160-7/00108-3

Liu, F., Xing, S., Ma, H., Du, Z. and Ma, B., 2013. Cytokinin-producing, plant growth-promoting rhizobacteria that confer resistance to drought stress in Platycladus orientalis container seedlings. *Applied microbiology and biotechnology*, *97*, pp.9155-9164.

Mahindapala, R., 2004. Medicinal plants: conservation and sustainable use in Sri Lanka. DOI: 10.11588/xarep.00003857

Mirihagalla, M.K.P.N. and Fernando, K.M.C., 2021. Medicinal plants use for home remedies in Sri Lanka: a review. http://ir.lib.ruh.ac.lk/xmlui/handle/iruor/8809

Muthukumarana, R. and Dharmadasa, R.M., 2014. Pharmacognostical investigation of Plectranthus hadiensis (Forssk.) Schweinf. ex Sprenger. and Plectranthus amboinicus (Lour.) Spreng. *World Journal of Agricultural Research*, *2*(5), pp.240-246. DOI:10.12691/wjar-2-5-7

Nakandalage, N., Athukorala, W.A.A.D.P.K., Sandamali, B.N., Ekanayake, E.M.U.I., Amarasinghe, S.R. and Hettiarachchi, C.S., 2022. Effect of growing media and irrigation frequencies on early vegetative growth of Piper longum L. *Journal of Dry Zone Agriculture*, *8*(2). DOI: http://doi.org/10.4038/jdza.v8i2.59

Napagoda, M., Gerstmeier, J., Butschek, H., Lorenz, S., De Soyza, S., Qader, M., Nagahawatte, A., Wijayaratne, G.B., Schneider, B., Svatoš, A. and Jayasinghe, L., 2022. Plectranthus zeylanicus: a rich source of secondary metabolites with antimicrobial, disinfectant and anti-inflammatory activities. *Pharmaceuticals*, *15*(4), p.436. https://doi.org/10.3390/ph15040436

Nawarathna, S.L., Subasinghe, S., Vidanapathirana, N.P. and Kumarasinghe, K.K.S., 2020. Impact of potting medium and cutting types on rooting and survival performance of male plants of Momordica dioica. Roxb (Thumba Karawila).

Nayana, E.K.E., Subasinghe, S., Amarasinghe, M.K.T.K., Arunakumara, K.K.I.U. and Kumarasinghe, H.K.M.S., 2015. Effect of maturity and potting media on vegetative propagation of Salacia reticulata (Kothalahimbatu) through stem cuttings.

Patel DK., 2016, Vegetative propagation strategy of *plectranthus amboinicus* (Lour) spreng in herbal garden. Adv Plants Agric Res.;5 (4):603-609 DOI: 10.15406/apar.2016.05.00190

Ping, C.H.E.N., Ping, M.E.N.G., Jin-song, Z.H.A.N.G., Chun-xia, H.E., Chang-rong, J.I.A. and Jian-zhong, L.I., 2014. Effects of drought stress on growth and water use efficiency of two medicinal plants. *Yingyong Shengtai Xuebao*, *25*(5).

Punet Kumar, S. and Kumar, N., 2020. Plectranthus amboinicus: A review on its pharmacological and pharmacognostical studies. *Am. J. Physiol. Biochem Pharmacol*, *10*, pp.55-62. DOI 10.5455/ajpbp.20190928091007

Rahman, M.M. and Fakir, M.S.A., 2015. Biodiversity of medicinal plants in Bangladesh: prospects and problems of conservation and utilization.

Rao, G.G.E., Vasundhara, M., Nuthan, D. and Reddy, K.M., 2010. Influence of irrigation regimes and nitrogen levels on growth, yield and quality of long pepper (Piper longum L.).

Saleh, R., Gunupuru, L.R., Lada, R., Nams, V., Thomas, R.H. and Abbey, L., 2022. Growth and biochemical composition of microgreens grown in different formulated soilless media. *Plants*, *11*(24), p.3546. https://doi.org/10.3390/plants11243546

Santos, M.Q.C., Lemos, E.E.P., Salvador, T.L., Rezende, L.P., Lima Salvador, T., Silva, J.W., Barros, P.G. and Campos, R.S., 2010, August. Rooting soft cuttings of soursop (Annona muricata)'GIANT OF ALAGOAS'. In *XXVIII International Horticultural*

Congress on Science and Horticulture for People (IHC2010): International Symposium on Micro 923 (pp. 241-245). DOI-10.17660/ActaHortic.2011.923.36

Saranga, J. and Cameron, R., 2007. Adventitious root formation in Anacardium occidentale L. in response to phytohormones and removal of roots. *Scientia horticulturae*, *111*(2), pp.164-172.

Sen, S., Chakraborty, R. and De, B., 2011. Challenges and opportunities in the advancement of herbal medicine: India's position and role in a global context. *Journal of Herbal medicine*, *1* (3-4), pp.67-75. https://doi.org/10.1016/j.hermed.2011.11.001

Singh, A. and Verma, P.P.S., 2015. Survival and growth performance of stevia cutting under different growing media. *Journal of Medicinal Plants Studies*, *3*(2), pp.111-113.

Waman, A.A., Smitha, G.R. and Bohra, P., 2019. A Review on Clonal Propagation of Medicinal and Aromatic Plants through Stem Cuttings for Promoting their Cultivation and Conservation. *Current agriculture Research Journal*, *7*(2). Doi: http://dx.doi.org/10.12944/CARJ.7.2.01

Waman, A.A., Smitha, G.R. and Bohra, P., 2019. Review on clonal propagation of medicinal and aromatic plants through stem cuttings for promoting their cultivation and conservation. .DOI- http://dx.doi.org/10.12944/CARJ.7.2.01

Wang, T., 2013. *Chemical, physical, and moisture release characteristics of compost based potting media* (Master's thesis, Clemson University).