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In-Vivo Propagation Protocols for Kapparawalliya (*Plectranthus amboinicus* Lour)

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ABSTRACT

***Plectranthus amboinicus*, a member of the family Lamiaceae, is an herb commonly employed in traditional medicinal systems owing to its distinct therapeutic qualities. The demand for this rare herb is rising rapidly, suggesting that wild harvesting may be insufficient and could even result in the extinction of this precious herb. Thus, initiating commercial cultivation of the species is imperative to meet the growing demand for the herb while preserving its genetic foundation. The current study comprised three independent experiments that evaluated the most suitable cutting type, potting media, and irrigation schedule for *in-vivo* propagation of *P. amboinicus* under protected house conditions. Four distinct cutting types, five different potting mixtures and four different irrigation schedules were tested in Completely Randomized Design. Data on plant height (cm), number of new buds and number of newly emerged leaves were collected at weekly intervals. Data on chlorophyll content (SPAD value) and leaf area (mm^2) were recorded six weeks after cutting establishment. Data were analyzed using analysis of variance (ANOVA), and treatment means were compared at the 5% level of significance. The results showed that softwood cuttings of *P. amboinicus* exhibited significantly higher growth and yield ($p \leq 0.05$), while the potting medium was composed of topsoil: sand: compost (1:2:1). The optimum irrigation schedule, out of the four examined, was daily watering to field capacity. In conclusion, softwood cuttings, topsoil: sand: compost with a 1:2:1 ratio with daily watering to field capacity could be used as the most promising agronomic practice for the successful *in-vivo* propagation of *P. amboinicus*.**

Keywords: Growing media, *In-vivo* propagation, Irrigation frequency, *Plectranthus amboinicus*, Softwood cuttings

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INTRODUCTION

Plectranthus amboinicus is a well-known member of the family Lamiaceae, or "mint" family comprises roughly 200 genera and species out of 3200, and it has a history of being used as food and as medicine (Arumugam *et al*., 2016). It is also a member of the Nepetoideae subfamily, which includes over 300 species of herbs and subshrubs, many of which are succulents that can be either annual or perennial (Wagner & Lorence, 2016). Due to its unique pharmaceutical properties, *P. amboinicus* is widely used in traditional medicinal systems (Arumugam *et al*., 2016). According to a phytochemical analysis by Janakiraman & Somasundaram (2014), it contains flavonoids such as luteolin, salvigenin, and apigenin. Essential oil of *P. amboinicus* had notable antibacterial activity, which could be attributed to the presence of two important monoterpenoid compounds: camphor and carvacrol (Erny Sabrina *et al*., 2014). Also, this herb is useful for healing wounds with minimal side effects (Punet & Kumar, 2020). According to Archana (2013), the alcoholic leaf extract of *P. amboinicus* is an effective anxiolytic agent. Similarly, the ethanol extract of *P. amboinicus* leaves exhibits hypo-glycaemic and antihyperlipidemic properties that are mediated by the re-establishment of pancreatic tissue functions and an insulinotropic impact (Viswanathaswamy *et al*., 2011). The leaves of *P. amboinicus* have a variety of biological activities that support their historic usage as a remedy for ulcers, infections, and colds (Gupta *et al*., 2013). Moreover, the potential of this plant to strengthen immunity by inhibiting the growth of bacteria and other diseases has been reported by Sreedharan *et al*. (2010). According to Chang *et al*. (2010), *P. amboinicus* may be useful in treating rheumatoid arthritis, and this herb may be produced as an anti-rheumatic medication that modifies illness. Interestingly, *P. amboinicus* is a rich source of bioactive compounds with nutritional value that improve food flavour and extend their shelf life (Chang *et al*., 2010).

Therefore, the demand for this herb increases rapidly, given the growing global desire for natural products, indicating that wild collection alone will not be enough and will ultimately result in its extinction (Fonseka & Katepearachchi, 2020). The use of medicinal plants without conservation efforts results in the extinction of species, particularly endangered species (Shahidullah *et al*., 2015). Therefore, developing a well-organized commercial cultivation of *P. amboinicus* is urgently needed. In contrast to wild-gathered raw materials, the herbal business increasingly favours cultivated medicinal and aromatic plant material since it is easier to predict plant production, quality, and drug composition (Dajic-Stevanovic & Pljevljakusic, 2015). Producers of medicinal plants should adapt to the agro-environmental factors, involve selecting the appropriate cutting type, potting medium, and irrigation schedule, all of which have an impact on the growth and quality of the final product on a commercial scale. Although these factors are directly linked with the growth performance and the yield characteristics of *P. amboinicus,* limited research has addressed the success of its vegetative propagation under commercial cultivation. In this regard, the current experiment was planned to find the best cutting type, potting media and most promising irrigation schedule on the success of vegetative propagation of *P. amboinicus* under protected house conditions.

MATERIALS AND METHODS

This experiment was carried out at a protected house of the Department of Crop Science, Faculty of Agriculture, University of Ruhuna. Three independent subsequent experiments were carried out to determine the best cutting type, potting media and irrigation schedule on *in-vivo* propagation of Kapparawalliya (*Plectranthus amboinicus* Lour.*).* During the study period, average monthly temperature, relative humidity and the light intensity of the protected plant house were 33.0 ± 1.2 ℃, 70 ± 4.5% and 25000 ± 2300 lux, respectively.

Experiment 1: Selection of the Best Cutting Type of P. amboinicus for In-Vivo Propagation

Four different cutting types, such as: C_1-tip/softwood cutting, C_2-single nodal semi-hardwood cuttings, C_3-double nodal semi-hardwood cuttings and C_4-triple nodal semi-hardwood cuttings, were taken from healthy and quality mother plants. Cuttings were planted in poly bags (300 gauge; 25x15x40 cm size) filled with common potting media (topsoil: sand: compost; 1:1:1) and placed randomly inside a protected house. Irrigation and other management practices were applied uniformly. The experiment was laid out in a Completely Randomized Design (CRD) with 04 treatments, each replicated 20 times, resulting in 80 experimental units. Data on plant height (cm), number of new buds and number of newly emerged leaves were collected at weekly intervals. Data on chlorophyll content (SPAD value) and leaf area (mm^2) were recorded six weeks after cutting establishment. Plant height was assessed by measuring the distance from the upper level of the potting medium to the tip of the fully opened leaves. The Grid method was used to measure the leaf area of three fully opened, randomly selected leaves (Pandey & Singh, 2011).

Experiment 2: Selection of the Best Potting Media for Successful Propagation of P. amboinicus

The best cutting type from experiment 1 (C_1) was tested with five different potting media such as 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 and M_5- topsoil: compost-1:2. Softwood cuttings were established in poly bags (300 gauge; 25x15x40 cm size) filled with five different potting media and placed randomly inside a protected house. Irrigation and other management practices were applied uniformly. The experiment was laid out in a Completely Randomized Design (CRD) with five treatments, each replicated 20 times, resulting in 100 experimental units. Data on plant height (cm), number of new buds and number of newly emerged leaves were collected at weekly intervals. Data on chlorophyll content (SPAD value) and leaf area (mm^2) were recorded six weeks after cutting establishment.

Measuring Physiochemical Parameters of Five Different Potting Media

Physiochemical parameters of five different growing media, including pH, Electrical Conductivity (EC), organic matter content, available phosphorus content (mg/kg), ammonium nitrogen (mg/kg) and nitrate nitrogen (mg/kg) were analyzed. Data on pH and EC were determined using the pH and EC meter (HANNA HI 83099). Ammonium nitrogen and nitrate nitrogen were analyzed colourimetrically using the UV-visible spectrophotometer (Shimadzu UV160) according to Keeney and Nelson (1982). Available phosphorus was analyzed using the Olsen sodium bicarbonate extraction method (Olsen et al., 1954). Available potassium was measured using the flame photometer (Sherwood model 360) following Black (1965)

Experiment 3: Identifying the Most Promising Irrigation Schedule for P. amboinicus

Softwood cuttings of *P. amboinicus* established in poly bags (300 gauge; 25x15x40 cm size) filled with the best potting media from the sub-experiment 2 (M2- topsoil: sand: compost -1:2:1) was tested with four different irrigation schedules such as I_1-daily watering to field capacity, I_2- daily watering to 50% of field capacity, I_3-watering at one-day intervals to field capacity, I_4- watering at one-day intervals to 50% of field capacity. All plants were irrigated regularly for four weeks for better establishment. After four weeks, irrigation treatments were adopted (I_1, I_2, I_3 and I_4). At each irrigation event, pots were irrigated to reach the specified field capacity determined by the gravimetric method. A Completely Randomized Design (CRD) was used in the experiment with four treatments, each replicated 20 times, resulting in 80 experimental units. Data on plant height (cm), number of new buds and number of newly emerged leaves were collected at weekly intervals. Data on chlorophyll content (SPAD value) and leaf area (mm^2) were recorded six weeks after the establishment of stem cuttings.

DATA ANALYSIS

Data from all three independent experiments were subjected to analysis of variance (ANOVA) separately by Statistical Analysis System (SAS statistical software 9.1 version). Duncan's multiple range test was employed to compare treatment means. The relationship between the physical properties of potting media and plant growth parameters were assessed using Pearson correlation analysis in SPSS Statistics version 29.0. Correlation coefficients were evaluated for significance at $p \leq 0.05$ and $p \leq 0.01$.

RESULTS AND DISCUSSION

Experiment 1: Selection of the Best Cutting Type of P. amboinicus for In-Vivo Propagation

Based on the results of the study, softwood cuttings of *P. amboinicus* exhibited significantly ($p \leq 0.05$) the highest plant height (38.5 ± 0.9 cm) (Figure 1a), the highest total number of leaves (19 ± 1.6) (Figure 1b), total number of buds (12.7 ± 1.3) (Figure 1c), average root length (19 ± 2.5 cm) (Figure 1d), average leaf area (83 ± 3.4 mm²) (Figure 1e) and average chlorophyll content (33.78 ± 2.9 SPAD Unit) (Figure 1f). The experiment's findings indicated that the softwood cuttings of *P. amboinicus* performed significantly better than other cutting types and exhibited higher success

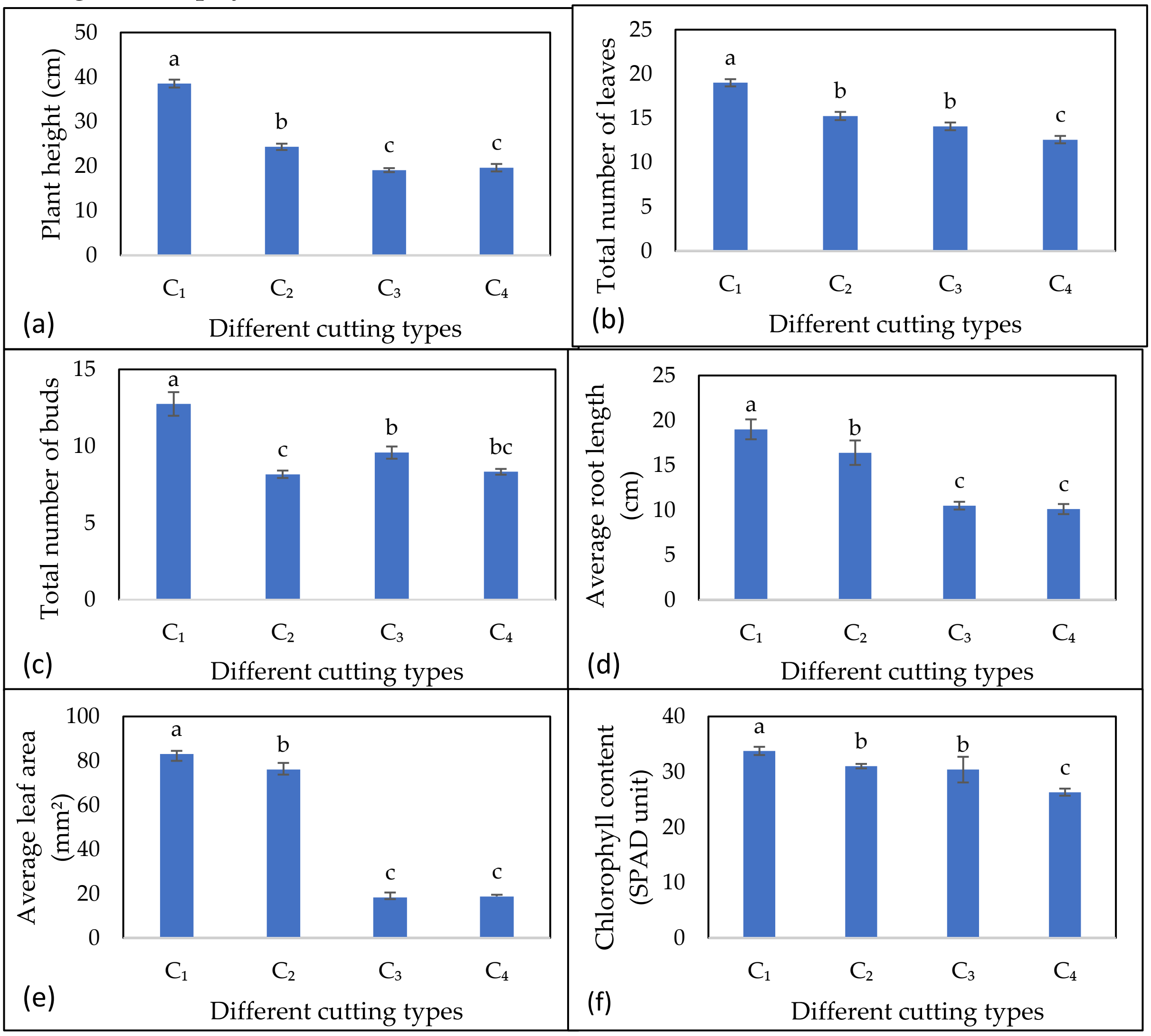


Figure 1: Effect of different cutting types on: (a) plant height, (b) total number of leaves, (c) total number of buds, (d)average root length (cm), (e) average leaf area (mm²), (F) chlorophyll content (SPAD unit) of *P. amboinicus* across the evaluated parameters, highlighting that the maturity of the stem cutting has a direct and unique impact on the success of vegetative propagation of *P. amboinicus.* Similar observations were reported by Yogish Kumar *et al*. (2018), whose study found that softwood cuttings exhibited a higher number of leaves, longer roots, higher number of roots, the highest rooting percentage, and earlier sprouting and rooting ability than semi-hardwood and hardwood cuttings. Similarly, Zargar and Kumar (2018) have discovered that the average number of leaves and sprouts per cutting varied significantly depending on the maturity stage of the donor plant. Also, the same experiment has found that juvenile cuttings of *Diploknema butyracea*, a medicinal plant, had the highest average number of leaves and sprouts per cutting. According to the findings of an experiment conducted by Baul *et al*. (2010), *H. pubescens* has also regenerated by juvenile single-node leafy stem cuttings. When it comes to vegetative propagation of the beneficial herb, *Embelia ribes*, semi-hardwood cuttings perform better than hardwood cuttings, which have a lower maturity (Saumya *et al*., 2014). According to Nakandalage *et al*. (2024), softwood cuttings of *Plectranthus zeylanicus* outperformed semi-hardwood cuttings due to the natural ageing process of mature cuttings and the higher concentration of natural endogenous hormones present in softwood cuttings.

The findings show that the maturity degree of the cuttings had a significant ($p<0.05$) impact on the root length (Figure 1d). This effect may be attributed to the internal physiology of the planting material. For instance, the observed difference in root performance could be due to the heterogeneous distribution of natural root-promoting and inhibiting substances related to cutting age. The proportional amounts of endogenous auxins and inhibitors produced by the branches are considered to reflect the degree of rooting (Nandi *et al*., 2002). To better understand the maturation process, a more thorough examination of auxin metabolism during root induction and root initiation of juvenile and mature material would be beneficial (Osterc & Stampar, 2011). Therefore, we recommend carrying out additional comprehensive studies to examine how these endogenous hormone levels or concentrations can change depending on the maturity stages of cuttings and their impact on the growth parameters of *P. amboinicus*

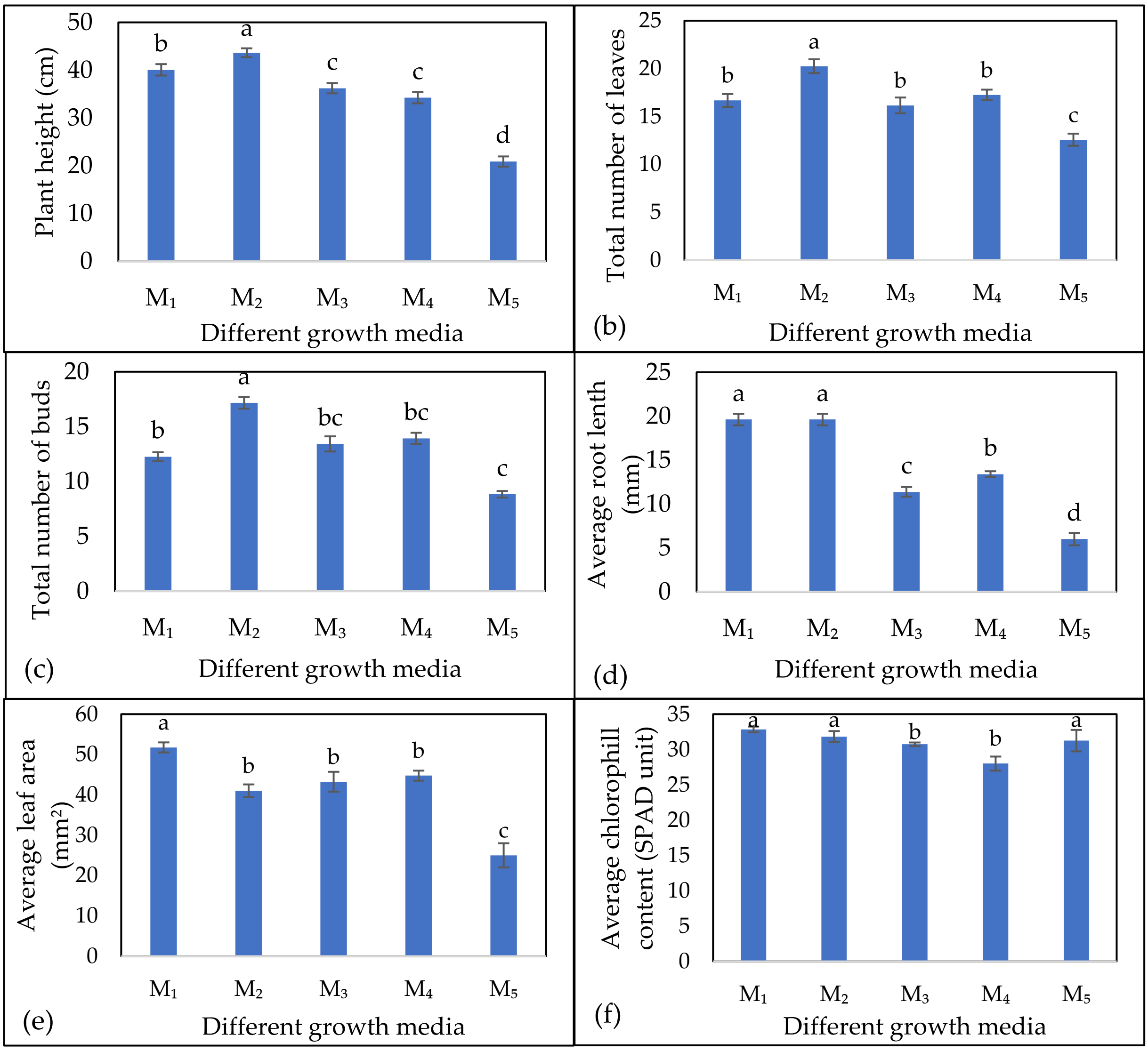


Figure 2: Effect of different growth media on: (a) plant height, (b) total number of leaves, (c) total number of buds, (d) average root length (cm), (e) average leaf area (cm2), (f) average chlorophyll content (SPAD unit) *P. amboinicus*.

Experiment 2: Selection of the Best Potting Media for Successful Propagation of P. amboinicus

As revealed by the experimental results, significantly ($p<0.05$), the highest plant height (43.64 ± 3.3 cm) (Figure 2a), the total number of leaves (20.25 ± 3.8) (Figure 2b) and the total number of buds (17.17 ± 2.3) (Figure 2c) were shown by M_2 growth media. Regarding the parameter, average root length, both M_1 and M_2 exhibit a significant difference ($p<0.05$) from other treatments (Figure 2d). M_1 recorded the highest chlorophyll content (32.83 ± 0.3 SPAD Unit), but it did not differ significantly ($p>0.05$) from M_2 and M_5. Numerous investigations on the optimal growth media for medicinal plants have produced varying findings, such as for *Stevia rebaudiana*: vermicompost + soil + FYM (Singh & Verma, 2015) and for *Ipomea pes-carpe*: soil: compost media (1:1) (Shinde & Chavan, 2018), highlighting the need to choose the finest and unique potting media for optimum growth (Figure 3). Also, these results convey the idea that each plant uniquely produces vegetative cuttings over a range of media and that determining the ideal medium is crucial to producing high-quality finished products on a commercial scale. Regarding the current experiment, the M2 media appears to perform well for most of the parameters evaluated of *P. amboinicus* under the given experimental conditions. This may be attributed to the higher sand content in the medium (50% of the total composition), and M_2 possesses certain distinct physical properties that aren't present in other examined media. Due to the high porosity and drainage properties of sand, it exhibits a faster water infiltration rate compared to substrates composed of 100% organic matter, which typically has a lower water infiltration rate (Bilderback & Jones, 2001). Additionally, the enhanced aeration provided by sand may positively influence the M_2 media performance, as poor aeration is a major issue that causes cuttings to deteriorate before root initiation can occur (Schmitz *et al*., 2013).

Beyond its physical characteristics, the M_2 medium's composition seems to have produced a favourable physiochemical environment that supported the vegetative propagation of *P. amboinicus*. According to the composition analysis of growth media, the unique pH (7.39 ± 0.021) and EC (0.27 ± 0.002 dS m^{-1}) values of M_2 may affect the solubility and availability of essential nutrients, thereby affecting

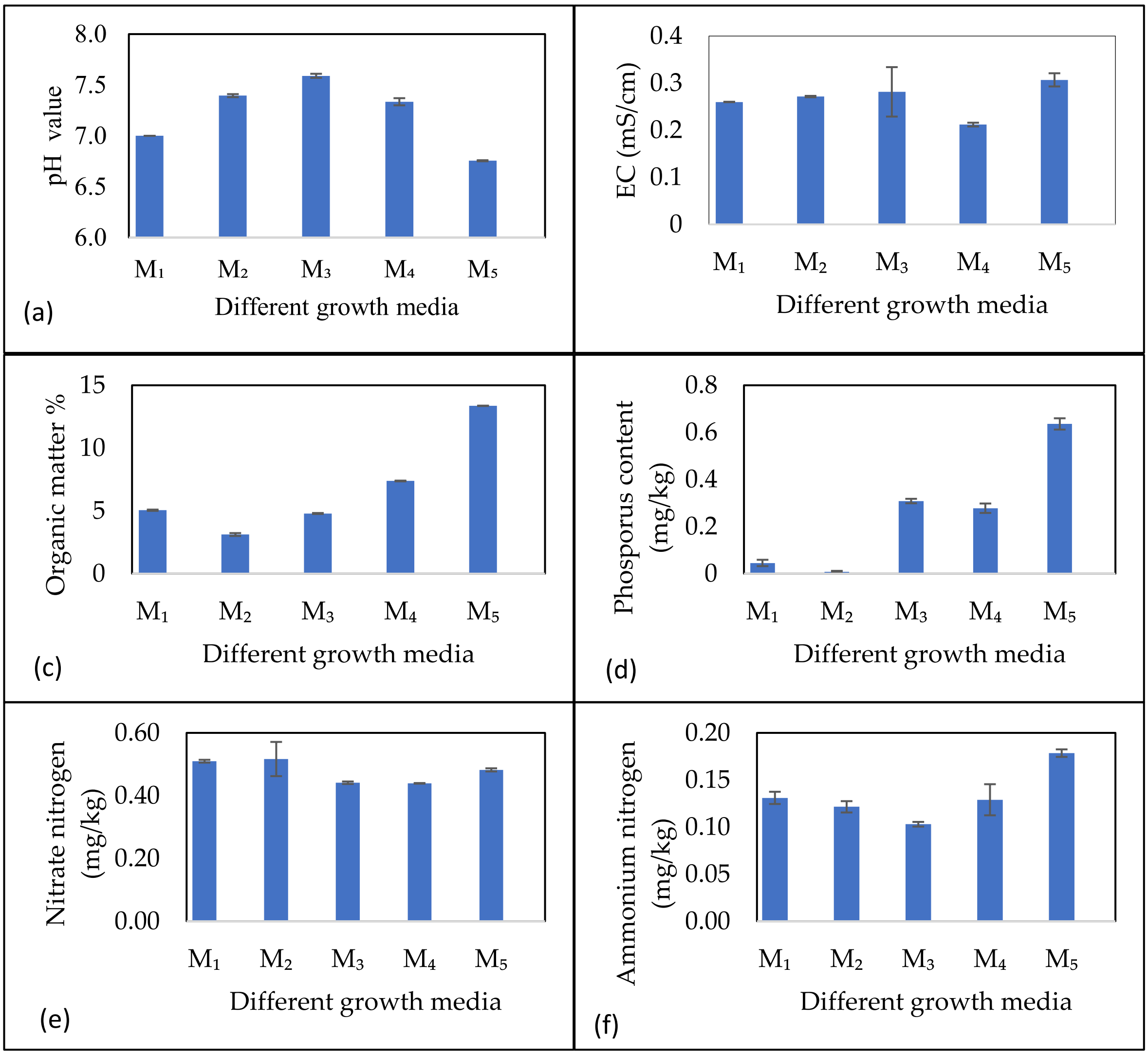


Figure 3: Composition analysis of different media types used to cultivate *P. amboinicus* softwood cuttings; (a) pH value, (b) EC value, (c) Organic matter content, (d) Phosphorus content (mg/kg), (e) Nitrate nitrogen (mg/kg), (f) Ammonium nitrogen (mg/kg)).

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 their bioavailability and mobility, which leads to optimal growth of *P. amboinicus* softwood cuttings. Similarly, M_2 was reported to have the lowest organic matter percentage (3.23 ± 0.162%) out of five different growth media. Althougadding compost enhanced various chemical characteristics by improving nutrientutilization and promoting acquisition is a fundamental process for all living organisms, where N is typically, a limiting component that affects agricultural productivity (Fernandez & Galvan, 2008). M_2 demonstrated superior performance in nitrogen (N) acquisition compared to the other media, thereby supporting improved growth of *P. amboinicus*. According to the relationship between the growth parameters of *P. amboinicus* and the physiochemical characteristics of different growth media present in Table 1, correlation with plant height ($p \leq 0.01$, r = -0.96) and total number of leaves ($p \leq 0.05$, r = -0.88). Phosphorus content (Figure 3) was also significantly Correlation analysis revealed several growth (Shamshuddin *et al*., 2004), the lower levels of organic matter have positively impacted the growth of *P. amboinicus* softwood cuttings under the current experimental conditions. As Figure 3 presents, M_2 contains the highest nitrate nitrogen (0.51 ± 0.077mg/Kg) with an average amount of ammonium nitrogen (0.12 ± 0.008 mg/kg). Nitrogen

significant negative relationships between soil properties (Figure 3) and plant growth traits. Organic matter content (Figure 3showed a strong and significant negative negatively correlated with plant height ($p \leq 0.01$, r = -0.98), total number of leaves ($p \leq 0.05$, r = -0.89) and average root length ($p \leq 0.01$, r = -0.99). These high correlation coefficients (r) indicate that organic matter and phosphorus have a strong negative relationship with these growth parameters, giving explanations for better growth performance in M2 medium which has more sandy soil. No other correlations were statistically significant ($p \geq 0.05$), and therefore, they were not interpreted further.

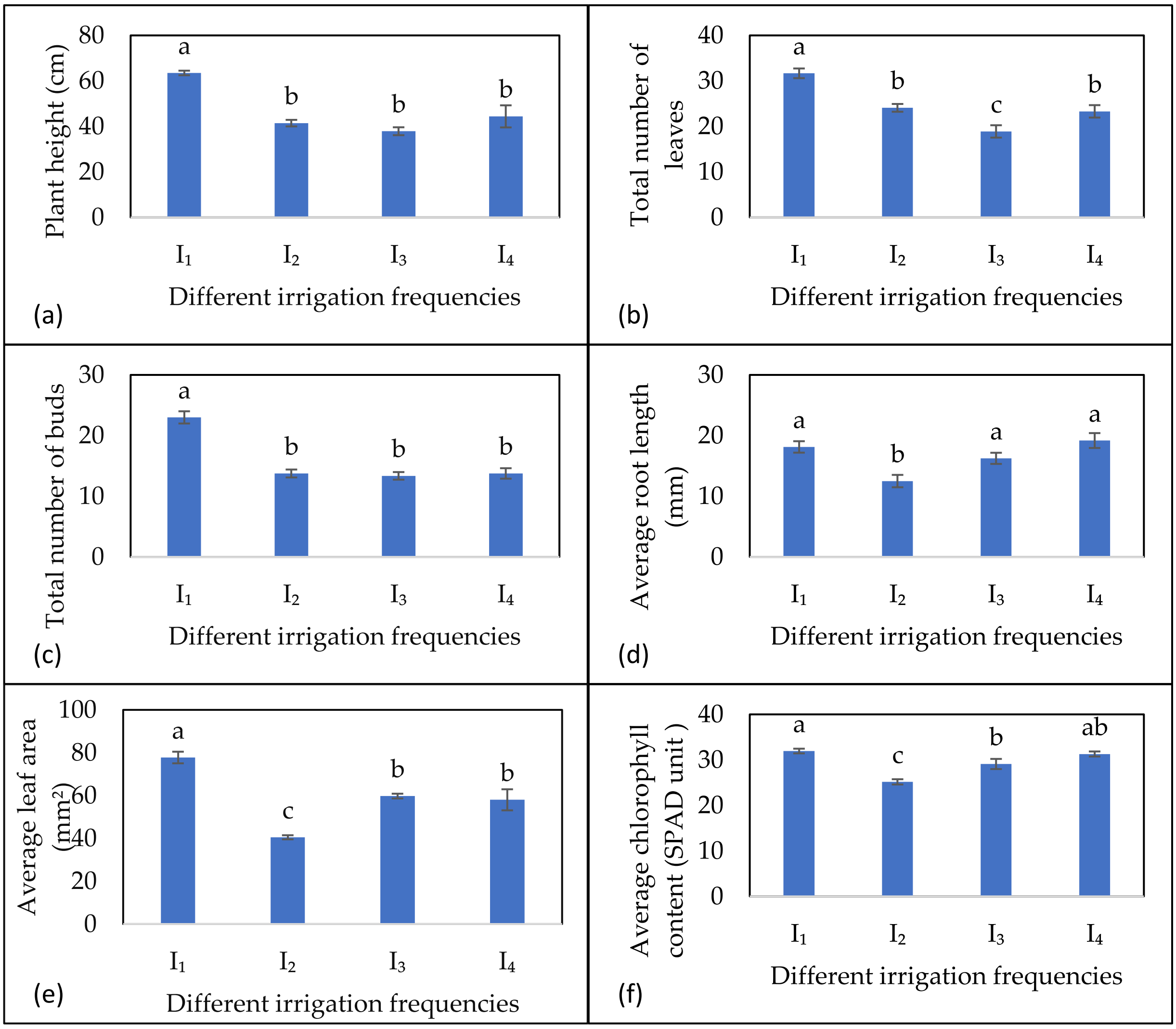
Table 1: Relationship between growth parameters of *P. amboinicus* and physiochemical characteristics of different growth media

Trait	pH value	EC (mS/cm)	Organic matter (%)	Phosphorus (mg/kg)	Nitrate N (mg/kg)	Ammonium N (mg/kg)
Plant height (cm)	0.63	-0.41	-0.98**	-0.98**	0.33	-0.81
Total number of buds	0.77	-0.44	-0.87	-0.79	0.16	-0.75
Total number of leaves	0.66	-0.49	-0.88*	-0.89*	0.29	-0.7
Average root length (cm)	0.31	-0.42	-0.84	-0.99**	0.57	-0.55
Average leaf area (mm^2)	0.48	-0.64	-0.79	-0.82	0.03	-0.75
Average chlorophyll content (SPAD)	-0.34	0.64	-0.21	-0.32	0.82	0.09

Experiment 3: Identifying the Nost Promising Irrigation Schedule for P. amboinicus

The results indicate that, significantly ($p \leq 0.05$) the highest plant height (63.5±7.2 cm) (Figure 4a), the total number of leaves (31.67±2.5) (Figure 4b), the total number of buds (23±1.4) (Figure 4c), average leaf area (77.75±1.5 mm^2) (Figure 4e) and average chlorophyll content (31.95±1.4 SPAD unit) (Figure 4f) were recorded by I_1 daily watering to field capacity irrigation schedule. Although I_4 showed the highest average root length (19.18±2.6 cm), it did not differ significantly ($p > 0.05$) from I1 (18.13±1 cm) and I_3 (16.25±1 cm) (Figure 4d). Accordingly, among the four irrigation schedules tested for softwood cuttings of *P. amboinicus* in topsoil: sand: compost (1:2:1) medium, daily watering to field capacity was identified as the optimum schedule.

As the results indicate, irrigation frequency has made a significant ($p \leq 0.05$) impact on the growth of *P. amboinicus*. Similarly, an experiment for the same herb by Sabra *et al*. (2018) also reveals that irrigation interval and harvest timing significantly influence biomass, essential oil content, and yield of *P. amboinicus*. Irrigation can benefit the overall growth and production of plants by promoting the growth and development of the root systems of *H. officinalis* and *P. incarnate* (Bilalis *et al*., 2014). An experiment conducted on *Gynura procumbens* grown hydroponically revealed that shorter irrigation cycles enhanced plant height, yield and leaf marketability. In contrast, longer irrigation intervals resulted in the highest total polyphenol and Vitamin C content (Lee *et al*., 2020).



** Significant level at $p \leq 0.01$ and * Significant level at $p \leq 0.05$ growing media

Figure 4: Effect of different irrigation frequencies on: (a) plant height, (b) total number of leaves, (c) total number of buds, (d) average root length (cm), (e) average leaf area (cm^2), (f) average chlorophyll content (SPAD unit) of *P. amboinicus*

An important result of this experiment is that, while increased irrigation frequency did not markedly reduce growth, significantly lower values were observed for most parameters at lower irrigation frequencies. The longer irrigation intervals recorded the decreased fresh weight and essential oil yield in *P. amboinicus* (Sabra *et al*., 2018). Some *Curcuma* spp. plants' (*Curcuma aromatica* and *Curcuma domestica*) growth characteristics and chemical composition were considerably lowered by the extended irrigation intervals (Mohamed *et al*., 2014). Extension of the 7–day to 14-day irrigation intervals minimizes all characteristics, such as grain yield, the number of fruits per plant, the number of umbels per plant (fertile or sterile), the thousand-grain weight, and the total biomass, except the percentage of essential oil of *Carum copticum*: traditional medicinal plant (Vahidipour *et al*., 2013). The growth of the medicinal herb *T. edulis* was significantly suppressed by prolonged drought treatment compared to well-watered conditions (Miao *et al*., 2015). Anyway, examining the results closely reveals that increased irrigation frequencies typically had a favourable impact on the growth and yield parameters of *P. amboinicus.*

CONCLUSION

Three independent experiments were carried out to find the most suitable cutting type, growth media and irrigation schedule for *P. amboinicus* for its effective vegetative propagation. Based on the results of experiment 1, softwood cuttings of *P. amboinicus* exhibit significantly ($p \leq 0.05$) the highest values for all the selected parameters, including plant height, the total number of leaves, the total number of buds, average root length, average leaf area and chlorophyll content. In experiment 2, growing media of topsoil: sand: compost (1:2:1) performed well for almost all the selected parameters, except average leaf area. Further, daily irrigation to field capacity showed the highest results for all selected parameters.

Therefore, it can be concluded that softwood cuttings of *P. amboinicus*, established in topsoil: sand: compost (1:2:1) growth media with daily irrigation to field capacity, are recommended for the successful vegetative propagation of *P. amboinicus* for its commercial production.

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