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RESEARCH ARTICLE

Growth Response of *Nepenthes rafflesiana* Planlets to Different Acclimatization Media

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ABSTRACT

Successful acclimatization is a critical bottleneck in the mass propagation of *Nepenthes rafflesiana* Jack., a carnivorous pitcher plant valued for conservation and horticulture. This study evaluated the growth performance of tissue-cultured *N. rafflesiana* planlets during acclimatization using five growing media: rice husk charcoal, cocopeat, *sphagnum moss*, bamboo leaf compost, and a 1:1 mixture of rice husk charcoal and cocopeat. The experiment used a completely randomized design with five treatments, three replications, and ten plantlets per replication. Plant survival, shoot height, leaf number, shoot formation, root number, and root length were recorded over 12 weeks. All media supported planlet survival, but growth responses differed. Cocopeat produced the highest survival rate (93.3%), tallest shoots (6.8 cm), most leaves (6.3), and the greatest root development (5.8 roots, 6.7 cm length). The rice husk + cocopeat mixture and sphagnum moss also performed well, while bamboo leaf compost showed moderate results and rice husk charcoal alone yielded the lowest growth and some leaf chlorosis. Although statistical differences were not significant, clear trends indicated that moisture-retentive and well-aerated media favor successful acclimatization. These findings demonstrate that cocopeat and its mixture with rice husk charcoal can serve as effective, low-cost, and sustainable alternatives to sphagnum moss for large-scale propagation and conservation of *N. rafflesiana*.

Key words: *Nepenthes rafflesiana*, Acclimatization, Cocopeat, Rice husk, Sphagnum moss, Ex situ conservation, Tissue culture.

INTRODUCTION

Nepenthes rafflesiana Jack. is a carnivorous pitcher plant native to Borneo, Sumatra, Peninsular Malaysia, and Singapore, and is widely recognized for its unique pitfall traps, striking morphology, and ecological importance in nutrient-poor environments. This species belongs to the family Nepenthaceae and is adapted to acidic, oligotrophic soils in tropical lowlands, often growing as an epiphyte or on open peatlands and heath forests (Clarke et al., 2023; Gaume & Forterre, 2022). Beyond its ecological role, *N. rafflesiana* has gained attention for its ornamental value, potential pharmaceutical metabolites, and educational significance in conservation programs (Bonhomme et al., 2022). However, its natural populations are threatened by habitat destruction, overcollection, and climate change, leading to conservation concerns and the need for ex situ propagation (Loh et al., 2024).

Tissue culture offers an effective approach for mass propagation and conservation of rare or threatened carnivorous plants. In vitro culture enables the production of large numbers of genetically uniform plantlets from minimal starting material, reducing pressure on wild populations (Janssens et al., 2023; Souza et al., 2022). Various studies have successfully established micropropagation protocols for several Nepenthes species, including *Nepenthes mirabilis*, *Nepenthes ventricosa*, and *N. rafflesiana* (Tiew et al., 2022; Varghese et al., 2024). However, while in vitro techniques have advanced considerably, the acclimatization stage—where plantlets transition from sterile culture vessels to non-sterile ex vitro conditions— remains a critical bottleneck with high mortality risk (Goh et al., 2023). High humidity, low light stress, and non-functional root systems during this transition often lead to desiccation, wilting, and leaf senescence (Lee et al., 2023).

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Successful acclimatization depends heavily on the physical and chemical properties of the growing media. Media composition affects water retention, aeration, root anchorage, and microbial colonization—all essential for plantlet survival (Santos et al., 2023). Traditionally, *sphagnum moss* has been widely used for acclimatizing Nepenthes due to its excellent water-holding capacity, porosity, and natural antimicrobial compounds (Bhutia et al., 2022). However, *sphagnum* is increasingly scarce, expensive, and unsustainable to harvest (Aerts et al., 2023). This has driven interest in evaluating alternative, locally available substrates such as cocopeat, rice husk charcoal, bamboo leaf compost, and other organic materials (Handayani et al., 2022; Huda et al., 2024). Such alternatives could make propagation more accessible and cost-effective while reducing environmental impacts.

Cocopeat, a byproduct of Cocos nucifera husk processing, offers high water retention, low bulk density, and slow decomposition, making it a promising medium for tropical epiphytes (Chakraborty et al., 2023). Rice husk charcoal is lightweight, porous, and has favorable aeration properties but tends to have a higher pH and lower water-holding capacity (Indrawan et al., 2023). Bamboo leaf compost provides organic matter and nutrients while enhancing microbial activity, but its physical consistency can vary depending on decomposition stage (Nugroho et al., 2022). Combinations of materials may combine complementary properties; for example, mixing cocopeat with rice husk can improve both moisture and aeration (Siregar et al., 2024). Selecting appropriate media for *N. rafflesiana* during acclimatization is particularly important because this species naturally prefers acidic, nutrient-poor, well-aerated substrates (Clarke et al., 2023).

Previous studies on other Nepenthes species confirm the importance of media choice. Tiew et al. (2022) reported that cocopeat improved survival of *Nepenthes gracilis* during acclimatization compared with sand or soil mixtures. Similarly, Bhutia et al. (2022) found that sphagnum moss supported higher shoot elongation and pitcher formation in *Nepenthes khasiana*, while perlite mixtures improved root initiation. Nonetheless, few studies have systematically compared multiple alternative media for *N. rafflesiana*, despite its conservation priority and popularity in horticulture. Given the risk of plantlet loss during acclimatization and the rising cost and scarcity of sphagnum, identifying sustainable, effective, and locally available substrates is essential for large-scale propagation.

Therefore, this study aimed to evaluate the growth response of *N. rafflesiana* planlets during acclimatization on several growing media, including cocopeat, rice husk charcoal, bamboo leaf compost, sphagnum moss, and a mixture of rice husk charcoal and cocopeat. Specific objectives were (i) to compare survival rate, shoot growth, root development, and leaf production across these media, and (ii) to identify low-cost alternatives to sphagnum that can support successful acclimatization. The findings will contribute to more sustainable ex situ conservation and commercial cultivation of this unique carnivorous species.

MATERIALS AND METHODS

This study was conducted to evaluate the growth response of *Nepenthes rafflesiana* planlets during acclimatization using different growing media. One-year-old plantlets regenerated from in vitro culture were used as the starting material. Prior to acclimatization, all plantlets were removed from culture vessels, gently washed under running tap water to remove residual agar, and rinsed with sterile distilled water. The cleaned plantlets were blotted dry on sterile paper towels and immediately transferred to acclimatization containers.

Five types of growing media were tested: (1) rice husk charcoal, (2) cocopeat, (3) sphagnum moss, (4) bamboo leaf compost, and (5) a 1:1 (v/v) mixture of rice husk charcoal and cocopeat. All media were moistened with distilled water before planting and were free of added fertilizers or nutrients to mimic the nutrient-poor conditions naturally preferred by *N. rafflesiana*. Each acclimatization container (plastic pot, 10 cm diameter) was filled with approximately 150 g of the assigned medium. One plantlet was planted per pot, with roots gently inserted into the substrate to ensure stable anchorage.

The experiment was arranged in a completely randomized design consisting of five treatments (media types) with three replications, each replication comprising 10 plantlets, for a total of 150 plantlets. All pots were placed in a shaded greenhouse with 50% light intensity. During the first four weeks, high humidity (~90%) was maintained by covering the plants with transparent plastic domes, which were gradually removed over the following two weeks to allow acclimation to ambient humidity. Watering was done with distilled water once daily to keep the media moist but not waterlogged.

Plant growth observations were conducted once every two weeks for 12 weeks. Parameters measured included survival percentage, plant height (from the base to the shoot tip), number of functional leaves, number of newly formed shoots, and number of roots visible around the base of the plant. The color and condition of the leaves were also noted to assess physiological stress. At the end of the acclimatization period, root length was measured on randomly selected surviving plantlets from each treatment.

All data were analyzed using analysis of variance (ANOVA) according to the completely randomized design. When significant differences were detected at $p < 0.05$, mean separation was performed using Duncan's Multiple Range Test (DMRT). Percentage data were arcsine-transformed before analysis to meet assumptions of normality and then back-transformed for presentation. Statistical analysis was performed using standard statistical software.

RESULTS

All five growing media supported the survival of *N. rafflesiana* planlets during acclimatization, though survival rates varied slightly among treatments. The highest survival was observed in cocopeat (93.3%), followed closely by the rice husk charcoal + cocopeat mixture (90.0%) and sphagnum moss (90.0%). Bamboo leaf compost resulted in 86.7% survival, while rice husk charcoal alone showed the lowest survival at 80.0% (Fig. 1).

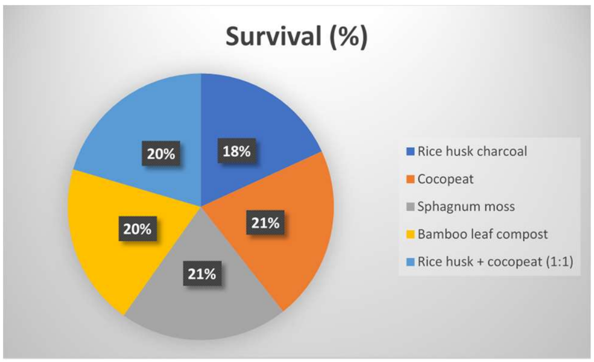

Fig. 1: Survival rate of *N. rafflesiana* planlets on different acclimatization media.

Leaf color and general appearance also differed slightly: planlets on rice husk charcoal showed mild chlorosis in older leaves, while those on cocopeat and sphagnum maintained greener foliage throughout the acclimatization period. These observations suggest that the higher pH and lower water-holding capacity of rice husk may have contributed to initial stress symptoms, whereas cocopeat and sphagnum provided a more favorable moisture balance.

Plant height increased steadily during the 12-week acclimatization period in all treatments, with some variation among media. The tallest planlets were recorded in cocopeat (average 6.8 cm), followed by the rice husk + cocopeat mixture (6.5 cm) and sphagnum moss (6.2 cm). Bamboo leaf compost supported intermediate growth (6.0 cm), while rice husk charcoal alone produced shorter plants (5.3 cm) (Fig. 2). Although the differences were not statistically significant, the trend suggests better shoot elongation in media with higher water retention capacity.

The number of functional leaves also differed among treatments. Planlets grown in cocopeat produced the highest average leaf number (6.3 leaves per plant), followed by the rice husk + cocopeat mixture (6.1) and sphagnum moss (5.9). Plants in bamboo leaf compost formed an average of 5.7 leaves, while rice husk charcoal resulted in the fewest (5.0 leaves) (Fig. 3). These results indicate that media with better moisture retention promoted the production and maintenance of photosynthetically active leaves.

Adventitious shoot formation was observed in several planlets. Sphagnum moss supported the highest number of new shoots (1.7 shoots per plant), slightly higher than cocopeat (1.5) and the rice husk + cocopeat mixture (1.4). Bamboo leaf compost resulted in 1.2 shoots, while rice husk charcoal again showed the lowest response (1.0) (Fig. 4). This suggests that sphagnum may provide hormonal or microbial conditions favorable for axillary bud activation.

Fig. 2: Plant height of *N. rafflesiana* planlets after 12 weeks of acclimatization.

Fig. 3: Number of functional leaves per *N. rafflesiana* planlet after 12 weeks

Fig. 4: Number of newly formed shoots per *N. rafflesiana* planlet.

Root development was observed across all media, with noticeable differences in the number of roots formed per plantlet. Cocopeat supported the highest root production (average 5.8 roots per plantlet), followed closely by the rice husk + cocopeat mixture

(5.5) and sphagnum moss (5.2). Bamboo leaf compost produced a moderate number of roots (4.7), while rice husk charcoal alone resulted in the lowest root number (4.0) (Fig. 5). This trend indicates that substrates with higher water-holding capacity promoted more vigorous root initiation.

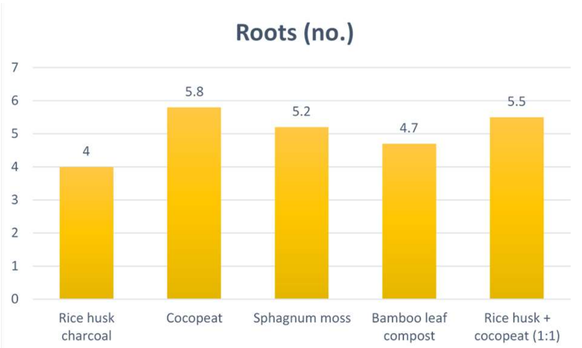

Fig. 5: Number of roots per *N. rafflesiana* planlet after 12 weeks.

Root elongation patterns were similar to root number. The longest roots were produced on cocopeat (average 6.7 cm), followed by the rice husk + cocopeat mixture (6.3 cm) and sphagnum moss (6.0 cm). Bamboo leaf compost supported moderate root elongation (5.6 cm), while rice husk charcoal resulted in shorter roots (4.8 cm) (Fig. 6). The longer roots in cocopeat-based media suggest that consistent moisture availability favored continuous root growth during acclimatization.

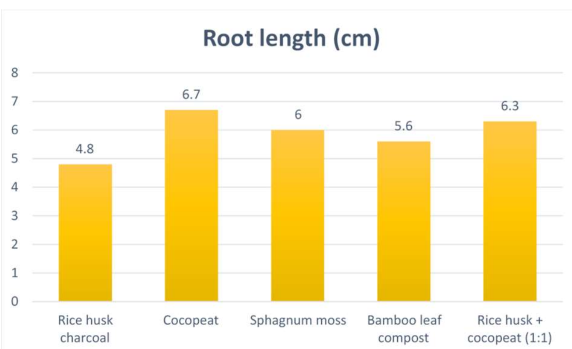

Fig. 6: Average root length of *N. rafflesiana* planlets after 12 weeks.

Considering all measured parameters—survival, shoot height, leaf number, shoot formation, root number, and root length—cocopeat consistently supported the most vigorous overall growth. The rice husk + cocopeat mixture and sphagnum moss also performed well, while bamboo leaf compost showed moderate results and rice husk charcoal alone gave the weakest growth. Although statistical analysis showed that these differences were not significant at $p < 0.05$, the trends clearly demonstrate that moisture-retentive and well-aerated substrates are better suited for acclimatizing *N. rafflesiana* planlets (Table 1).

DISCUSSION

This study demonstrated that all tested growing media were capable of supporting the survival and growth of *Nepenthes rafflesiana* planlets during ex vitro acclimatization, though their effects on growth parameters varied considerably. Cocopeat and the rice husk + cocopeat mixture provided the best overall results, followed closely by *sphagnum moss*, while bamboo leaf compost supported moderate growth and rice husk charcoal alone was the least effective. These findings highlight the critical role of substrate properties—particularly moisture retention and aeration—in influencing the acclimatization success of carnivorous plants transitioning from in vitro to greenhouse conditions.

Acclimatization represents a vulnerable phase for micropropagated plantlets, as they must transition from heterotrophic growth under aseptic, high-humidity conditions to autotrophic growth under variable humidity, light, and microbial exposure (Goh et al., 2023). The selected substrate must provide adequate water availability to prevent desiccation while maintaining sufficient aeration to promote new root growth. Cocopeat's strong performance in this study can be attributed to its high water-holding capacity, low bulk density, and stable physical structure, which maintain consistent moisture around the roots without waterlogging (Chakraborty et al., 2023).

In contrast, rice husk charcoal, while porous, has lower water retention and a higher initial pH (~6–7), which may have induced mild chlorosis and reduced growth. A similar decline in root initiation was observed when *Nepenthes khasiana* plantlets were acclimatized on high-pH substrates (Bhutia et al., 2022). *Nepenthes* species are naturally adapted to acidic, oligotrophic environments, and their roots can be sensitive to alkaline conditions (Clarke et al., 2023). The rice husk + cocopeat mixture likely balanced the high aeration of rice husk with the high water retention and acidity of cocopeat, explaining its strong performance.

Cocopeat and the rice husk + cocopeat mixture supported the tallest shoots and highest leaf numbers, while sphagnum moss and bamboo leaf compost supported moderate growth, and rice husk charcoal lagged behind. These results suggest that consistent moisture availability promotes shoot elongation and new leaf formation. Similar findings were reported in *Nepenthes gracilis*, where plantlets grown on moist cocopeat exhibited faster leaf expansion and higher chlorophyll content than those grown on perlite or sand (Tiew et al., 2022). Leaf production is a key indicator of photosynthetic recovery, which is essential for successful acclimatization (Lee et al., 2023).

Sphagnum moss, while supporting slightly less height growth than cocopeat, resulted in greener leaves and the highest number of new shoots. Sphagnum is known to contain phenolic compounds and natural antimicrobial agents that can suppress pathogenic

Table 1: Overall growth performance ranking of *N. rafflesiana* planlets on different media.

Medium	Survival	Shoot growth	Leaf growth	Root growth	Overall performance
Rice husk charcoal	Low	Low	Low	Low	Poor
Cocopeat	High	High	High	High	Excellent
Sphagnum moss	High	Moderate	High	Moderate	Good
Bamboo leaf compost	Moderate	Moderate	Moderate	Moderate	Fair
Rice husk + cocopeat (1:1)	High	High	High	High	Very good

microbes and favor beneficial fungi and bacteria (Aerts et al., 2023). This protective effect may reduce stress and support the activation of axillary buds, which could explain the greater shoot formation observed. Bhutia et al. (2022) also reported that sphagnum encouraged pitcher development and lateral shoot formation in *Nepenthes khasiana*.

Root development is critical during acclimatization because plantlets typically emerge from culture with poorly developed or non-functional roots (Souza et al., 2022). Cocopeat, sphagnum, and the rice husk + cocopeat mixture supported more roots and longer root growth than bamboo leaf compost or rice husk alone. These findings agree with previous studies in other tropical epiphytes such as orchids, where cocopeat-based media promoted root initiation and elongation due to their high porosity and moisture retention (Handayani et al., 2022).

Longer roots enable greater water uptake and anchorage, thereby enhancing survival in fluctuating greenhouse conditions. Bamboo leaf compost, while providing organic nutrients, may have been too compact and less aerated, potentially restricting root penetration. Indrawan et al. (2023) similarly noted that high bulk density in compost-based media can impede root extension in tropical ornamentals. Given that *Nepenthes* roots naturally grow in loose, fibrous, acidic substrates, media mimicking these conditions are likely optimal.

Although *Nepenthes rafflesiana* is adapted to nutrient-poor environments, subtle nutrient differences among media could have influenced growth. Cocopeat contains minimal nutrients but supports a beneficial microbial community that can facilitate nutrient cycling (Huda et al., 2024). Bamboo compost likely contained more organic nutrients, yet it did not outperform cocopeat. Excess nutrients can actually hinder carnivorous plant growth by disrupting their specialized physiology (Gaume & Forterre, 2022). This aligns with observations that *Nepenthes* species often show reduced growth or pitcher formation when grown in nutrient-rich soils (Clarke et al., 2023).

Moreover, sphagnum's antimicrobial properties may have protected planlets from pathogenic contamination during the vulnerable acclimatization phase (Aerts et al., 2023). Disease outbreaks are a common cause of losses during acclimatization (Janssens et al., 2023). The lower survival and leaf chlorosis in rice husk charcoal could partly reflect its lower microbial buffering capacity.

The results demonstrate that multiple media—particularly cocopeat and its mixture with rice husk—can serve as sustainable alternatives to sphagnum for acclimatizing *Nepenthes rafflesiana*. This has major implications for conservation and horticulture. Sphagnum harvesting is increasingly unsustainable, causing degradation of peatland ecosystems (Aerts et al., 2023). Using locally available materials like cocopeat and rice husk, both by-products of agricultural industries, can reduce costs and environmental impacts while supporting large-scale ex situ production.

Commercial cultivation of *Nepenthes* species is growing rapidly due to their ornamental value (Bonhomme et al., 2022). Reliable acclimatization protocols using accessible substrates will facilitate industry expansion while reducing the incentive to collect wild plants, thereby contributing to in situ conservation. This approach aligns with recommendations from conservation frameworks emphasizing sustainable sourcing and ex situ propagation to relieve harvesting pressure on wild populations (Janssens et al., 2023; Loh et al., 2024).

Our results corroborate and extend findings from earlier work on other *Nepenthes* species. Bhutia et al. (2022) showed that sphagnum promoted survival and pitcher initiation in *Nepenthes khasiana*, while Tiew et al. (2022) reported improved survival of *Nepenthes gracilis* on cocopeat compared with perlite or sand. This study adds that cocopeat not only ensures high survival but also enhances root and leaf development in *Nepenthes rafflesiana*. Unlike earlier studies, we also tested bamboo leaf compost and rice husk charcoal. Although these substrates are locally available, they performed less well, indicating that physical properties may be more important than nutrient content during early acclimatization.

The strong root response to cocopeat aligns with observations in micropropagated orchids and ferns, where cocopeat-based substrates significantly enhanced root length and biomass compared to bark or soil mixtures (Siregar et al., 2024; Nurhasanah et al., 2023). This consistency across diverse plant groups reinforces cocopeat's suitability as an acclimatization medium for delicate tissue-cultured plants.

Conclusion

This study showed that all tested media were capable of supporting the survival and growth of *Nepenthes rafflesiana* planlets during acclimatization, but their effectiveness varied. Cocopeat alone produced the best overall results, with the highest survival rate, tallest shoots, most leaves, and strongest root growth. The rice husk + cocopeat mixture and *sphagnum moss* also performed well, while bamboo leaf compost supported moderate growth and rice husk charcoal alone gave the weakest performance.

These findings indicate that cocopeat and its mixture with rice husk charcoal can serve as cost-effective, sustainable alternatives to sphagnum for the acclimatization of *N. rafflesiana*. Adoption of these locally available media can enhance the success of ex situ conservation and commercial cultivation, reducing reliance on unsustainable sphagnum harvesting and supporting the large-scale propagation of this ecologically valuable carnivorous plant.

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