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Exploring the potential use of *Moringa olifera* Lam and *Azadirachta indica* additives in Orchid tissue culture

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***Abstract*—This study was conducted in the Floriculture Research and Development Unit at the Royal Botanical Gardens, Peradeniya to explore the potential of *Moringa olifera* Lam. (Moringa) and Azadiracta indica (Neem) organic supplements for promoting Orchid invitro culture of *Dendrobium* spp . The Knudson C (KnC) basal media was prepared by additionally supplemented with either 5g/l, 10g/l and 15g/l of moringa powder, moringa leaf extract and neem leaf extract separately. The experiment was laid out in completely randomized design with 10 treatments having 10 replicates. One month after explant incubation, the number of leaves, shoots and roots per plantlets and shoot length were recorded. According to the results, different treatments had significantly affected the tested parameters ($p<0.05$). Both moringa and neem leaf extracts gave improved responses compared to moringa powder. ¼ KnC+15g/l of moringa leaf extract performed well in terms of producing increased number of leaves and roots per plantlet, whereas full KnC+5g/l of moringa leaf extract resulted the highest number of shoots per plantlet (3.5). On the contrary, neem leaf extracts (5g/l, 10g/l and 15g/l) increased the shoot length of orchid explants and produced significantly higher number of roots per plantlet. Hence, moringa and neem leaf extracts can be used to develop a simple and cost effective culture media for promoting orchid tissue culture.**

***Keywords*—**Azadiracta indica, *Dendrobium*, *Moringa olifera* Lam., organic supplements

I. Introduction

Orchids belong to the family Orchidaceae which is widely cultivated in tropical and temperate regions owing to their attractive, long-lasting flowers. The crop is dominant as the most needed decorative plants with thousands of species and hybrids (Nambiar *et al*., 2012). Micropropagation technique has been frequently used for the mass production of this plants (Rittirat *et al*., 2012). The availability of growth regulators and the nutritional elements provided by the culture media determine the plantlet growth (Gnasekaran *et al*., 2010). In general, the medium being used for orchid tissue culture contains water, vitamins, and mineral salts (Murdad *et al*., 2010). Supplementing the culture medium with organic additives is simple, practical, beneficial and convenient way to promote the in vitro growth of orchid. (Arditti and Ernst, 1993). Generally coconut water, tomato juice, peptone, extracts from potato, banana and beef are used as organic additives to orchid culture medium (Murdad *et al*., 2010). A number of recent studies have provided strong evidences that media additives facilitate germination, micropropagation and growth of different orchid varieties (Tawaro *et al*., 2008). Moreover, Ichihashi and Islam, (1999) found out that taro extract (50 – 300 ml/l) was the best additive tested for orchid explant growth compared to extract derived from apple and potato extract, coconut water, banana homogenate. According to Aktar *et al*., (2008), Knudson C (KnC) medium with banana pulp produced the longest shoots for *Dendrobium* orchid explants, whereas the KnC and ½ strength of MS media with banana pulp produced the longest leaves.

Moringa, originally from Asia is a tree with high biotechnology potential (Moreno *et al*., 2018). As a bio-stimulant, moringa leaf extract is consisted of macro and micronutrients, amino acid, ascorbic acid, minerals, and growth-promoting properties (Makkar *et al*., 2007). Additionally, the extract of Moringa leaves could be utilized to hasten plant growth because it includes minerals like Ca, K, and Fe as well as zeatin, cytokinin, ascorbate, and phenolics that can promote the growth of plant (Sari *et al*. 2020). Neem is mainly used in medicine and pesticide production. Main nutrient components (N, P, K, Ca an Mg) and antifungal chemicals in neem are considered to facilitate the growth and development of plants (Yi *et al*., 2021). Hence, the aims of present study were to determine the effectiveness of Moringa (*Moringa olifera*) and Neem (*Azadirachta indica*) as a nutrient media

Table I: Different treatments used in this study

Treatment no	Treatments
T1	Full KnC + 5 g/l of moringa leaf powder
T2	½ KnC + 10 g/l of moringa leaf powder
T3	¼ KnC + 15 g/l of moringa leaf powder
T4	Full KnC + 5 ml/l of moringa leaf extract
T5	½ KnC + 10 ml/l of moringa leaf extract
T6	¼ KnC + 15 ml/l of moringa leaf extract
T7	Full KnC + 5 ml/l of neem leaf extract
T8	½ KnC + 10 ml/l of neem leaf extract
T9	¼ KnC + 15 ml/l of neem leaf extract
T10 (Control)	Full KnC media only

additive for *Dendrobium* orchid tissue culture and to develop a cost effective culture media

II. Materials and Method

A. Experimental location

The experiment was carried out in the tissue culture laboratory facilities of Floriculture Research and Development Unit at the Royal Botanical Gardens, Peradeniya (7° 16' N, 80° 35' E), which is located in the WM_{3a} agro ecological zone of Sri Lanka.

B. Preparation of planting materials

Young plantlets of *Dendrobium* orchid species were taken from the existing collection of explants from Royal Botanical Gardens. The plantlets (1 cm height) were immersed in a 10 % Sodium Hypochlorite solution for 5-10 minutes and washed thrice using autoclaved distilled water for surface sterilization before incubation (Pradhan *et al*., 2013). The moringa and neem leaves collected from young plants were washed thoroughly 2-3 times and spread on a sterilized kitchen napkin to absorb extra moisture. Leaves were ground using a blender and filtered to obtain the leaf extracts separately. To get the powder form, leaves were dried under shade for 2-3 days and ground into powder after they became crispy. Finally powder was stored in an airtight container.

C. Media preparation and culture conditions

Three different strengths of KnC (Knudson C, 1946) basal media were prepared (full strength, ½ strength and ¼ strength) in which 5 g/l, 10 g/l and 15 g/l of moringa powder, moringa leaf extract and neem leaf extract were added separately as shown in Table I. After that 4 % (W/V) of sugar and 1.27 % (W/V) of agar were added and the pH of the media was adjusted to 5.60 - 5.63 (Gansau *et al*., 2016). The media were then autoclaved at 120 °C for 15 minutes and 40 ml of each medium was poured into sterilized culture bottles (100 ml). Then the surface sterilized orchid explants were established into culture bottles inside a laminar flow with one culture vial held four small plantlets. Cellophane layers were used to seal the culture bottles, which were then kept in a growth room at 25 °C and 16 hours of photoperiod under fluorescent lighting (40 μ mol photons $m^{-2}s^{-1}$).

Figure 1: Measuring the shoot length of orchid explant using a graph paper

D. Data Collection and analysis

The following data were recorded after one month of explant inoculation. The number of leaves, shoots and roots per plantlet were counted. Shoot length was measured using 1 mm graph paper (Figure 1). The treatments were arranged in a CRD (Completely Randomized block Design) method having 10 replicates where each replicates consisted 4 plantlets. Then the obtained data were evaluated using the SPSS software and analysis of variance (ANOVA) was performed to check if there are any treatments that differ significantly at the Tukey's 5 % level of probability.

III. Results and Discussion

A. Impacts of different organic supplements on number of leaves produced per plantlet in orchidt

Number of leaves per plantlet was significantly impacted by different treatment combinations ($p<0.05$). The highest number of leaves was observed in T6 (¼ KnC+15 ml/l of moringa leaf extract) (5.2) followed by T7 (Full KnC+5 ml/l of neem leaf extract) (4.8) and T5 (½ KnC+10 ml/l of moringa leaf extract) (4.3) which were higher than that of the control (4.1). T2 (½ KnC+10 g/l moringa powder had the lowest number of leaves per plantlet (3.6) (Figure 2). This corresponds with previous work by Aktar *et al*. (2008) obtained the highest number of leaves per plant in *Dendrobium* orchid (2.52) when supplementing the ½MS with banana pulp at 40 and 60 days after inoculation. Further, they obtained 2.11, 1.20 and 1.20 leaves per plant in KnC media when added with banana extract, charcoal and coconut water respectively.

B. Impacts of different organic supplements on number of roots produced per plantlet in orchid

Different treatments significantly affected the number of roots produced from orchid explants ($p<0.05$). Out of all

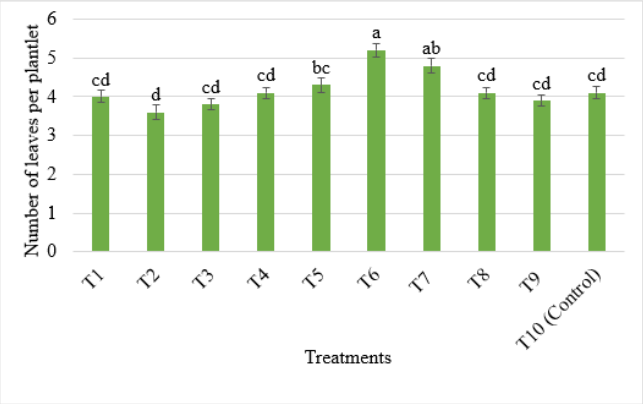

Figure 2: Effects of different organic additives on number of leaves per plantlet in orchid explant. Bars with similar letters are not significantly different.

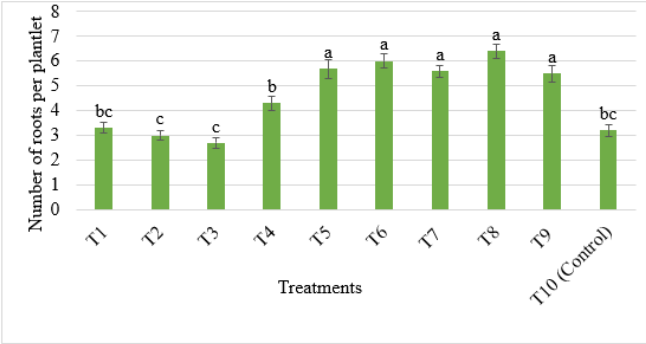

Figure 3: Effects of different organic additives on number of roots per plantlet in orchid explant. Bars with similar letters are not significantly different.

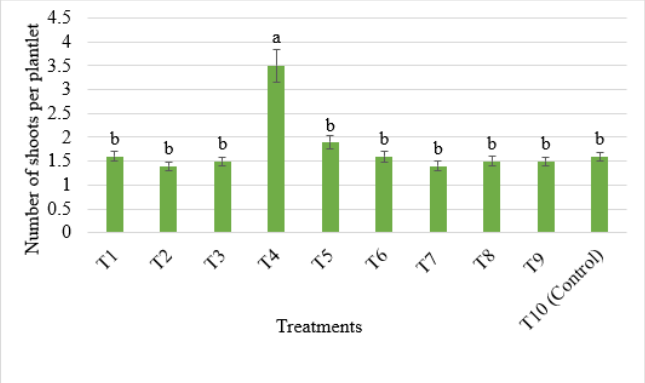

Figure 4: Effect of different organic additives on number of shoots per plantlet in orchid explant. Bars with similar letters are not significantly different.

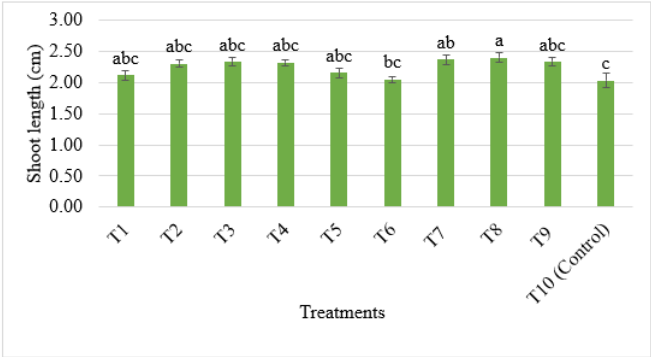

Figure 5: Effects of different organic additives on shoot length of orchid explant. Bars with similar letters are not significantly different.

treatments, the number of roots per plantlet were significantly higher in T8 (½ KnC + 10 ml/l of neem leaf extract) (6.4), T6 (¼ KnC + 15 ml/l of moringa leaf extract) (6), T5 (½ KnC + 10ml/l of moringa leaf extract) (5.7), T7 (Full KnC + 5 ml/l of neem leaf extract) (5.6) and T9 (¼ KnC + 15 ml/l of neem leaf extract) (5.5) compared that of control (3.2), while the lowest was observed in T3 (¼ KnC + 15 g/l of moringa leaf powder) (2.7) (Figure 3). This results in line with previous work reported by Islam *et al*. (2015), obtained 7 roots per plant for *Dendrobium* Orchids when adding 25 ml/l of banana extract in ½ MS media.

C. Impacts of different organic supplements on number of shoots per plantlet in orchid explant

Different treatments had significantly affected the number of shoots per plantlet produced from orchid explant ($p<0.05$). T4 (Full KnC + 5 ml/l of moringa leaf extract) resulted significantly higher shoots per plantlet (3.5) compared with that of other treatments (Figure 4). In a previous study, KnC media supplemented with charcoal and coconut water resulted 6.52 and 5.50 shoots per plant respectively in *Dendrobium* orchids at 40 days after explant inoculation (Akter *et al*., 2008).

D. Impacts of different organic supplements on shoot length of orchid explant

Shoot length of orchid cultures were significantly affected by different treatments ($p<0.05$). All the treatments had significantly higher shoot length than that of the control. The highest shoot length was observed in T8 (½ KnC + 10 ml/l of neem leaf extract) (2.40 cm) followed by T7 (Full KnC + 5 ml/l of neem leaf extract) (2.37 cm) while the lowest was recorded in control (2.03) (Figure 5). Islam *et al*. (2015) obtained shoot length of 2.8 cm for *Dendrobium* spp. Orchid when adding 25 ml/l of banana extract in ½ MS media.

Of the identified responses of treatment, the ¼ KnC + 15 ml/l of moringa leaf extract promoted number of leaves and roots per plantlet whereas, full KnC + 5 ml/l of moringa leaf extract resulted the production of highest number of shoots per plantlet. On the other hand, all three concentration (5 ml/l, 10 ml/l and 15 ml/l) of neem leaf extracts increased the shoot length of orchid explants and produced higher number

of roots per plantlet than that of other treatments. Conversely, moringa leaf powder did not give improved results in the tested parameters. As opposed to nutritional values, moringa leaves which is believed to have higher amount of zeatin and can act as natural sources of cytokinin (Basra *et al*., 2011). This could be the reason for its best performances in producing increased number of leaves, shoots and roots of *Dendrobium* orchids explants. Moreover, moringa leaf is abundant in ascorbate, carotenoid, phenols, potassium and calcium, which promote the plant growth (Foidl *et al*., 2001). Neem extracts also have reported to supply certain level of growth promoting abilities that are similar to gibberellin and cytokinin (Micheli *et al*., 2018). As opposed to plant tissue culture studies, under natural soil and plant systems, a number of recent studies have provided strong evidences that, neem leaf extract had increased the growth of root and shoot in stem cuttings of Rosa spp. (Sharief *et al*., 2020). In terms of economic aspects, moringa and neem leaf extracts are also affordable, eco-friendly, and practical. Hence, these organic additives could be utilized in developing simple tissue culture media.

IV. Conclusions

Simplification of a medium is one of the major goals of commercial growers and the utilization of organic supplements to facilitate rapid in vitro growth of plants. Organic additives contain a various amount of nutrient compounds and PGR which promote the growth and development of plantlets. According to this study, moringa leaf extract and neem leaf extract performed better than that of powdered forms in the in vitro growth of *Dendrobium* orchids. Since moringa and neem leaves are easily available in tropical Asian countries, using them may contribute to developing a simple and cost-effective tissue culture medium. Future researches are need to focus on the chemical composition analysis of studied leaf extract in orchid cultures.

References

Aktar, S., Nasiruddin, K.M. Hossain, K. (2008). 'Effects of different media and organic additives interaction on in vitro regeneration of *Dendrobium* orchid'. *Journal of Agriculture Rural Development, 6*(1), 69-74. Arditti, J. Ernst, R. Micropropagation of orchids. New York: John Wiley Son; 1993, 682.

Basra, S.M.A., Iftikhar, M.N. Afzal, I. (2011). 'Potential of Moringa (Moringa oleifera) leaf extract as priming agent for hybrid maize seeds'. *International Journal of Agriculture Biology. 13*, 1006-1010.

Foidle, N., Makkar, H.P.S. Becker, K. (2001). 'The potential of Moringa oleifera for agricultural and industrial uses'. p. 45-76. In L.J. Fuglie (ed.) The miracle tree: The multipurpose attributes of Moringa. CTA Publications, Wageningen, The Netherlands.

Gansau, J.A., Indan, H., Abdullah, S.N., David, D., Marbawi, H. Jawan, R. (2016). Effects of organic additives and plant growth regulators on protocorm development of *Dendrobium* lowii. *Transactions on Science and Technology, 3*(3), 462-468.

Gnasekaran, P., Rathinam, X., Sinniah, U.R. Subramaniam. S. (2010). A study on the use of organic additives on the protocorm-like bodies (PLBs) growth of Phalaenopsis violaceae orchid. Journal of Phytology, 2, 29–33. Ichihashi, S. Islam, M.O. (1999). 'Effects of complex organic additives on callus growth in three orchid genera, Phalaenopsis, Doritaenopsis, and Neofinetia'. *Journal of the Japanese Society for Horticultural Science, 68*(2), 269-274.

Islam, M.O., Islam, M.S. Saleh, M.A. (2015). 'Effect of banana extract on growth and development of protocorm like bodies in *Dendrobium* sp. Orchid'. *The Agriculturists, 13*(1), 101-108.

Knudson, C. (1946). 'A new nutrient solution for germination of orchid seed', Amer Orchid Soc Bull, 15, 214-217.

Makkar, H.P., Siddhuraju, P. Becker, K. (2007). Plant secondary metabolites (Vol. 393, pp. 1-122). Totowa, NJ, USA:: Humana Press.

Micheli, M., da Silva, D.F., Farinelli, D., Agate, G., Pio, R. Famiani, F. (2018). 'Neem oil used as a "complex mixture" to improve in vitro shoot proliferation in olive',HortScience, 53(4), 531-534.

Moreno, A., Bernal, Á., Ugarte, F., Lima, K., Coig, M., Sánchez, C., Aldrey, A. Vidal, N. (2018). 'Use of liquid medium and biofortificants for improving micropropagation and acclimation of Musa AAA cv. Williams'. Clonal Trees in the Bioeconomy Age: Opportunities and Challenges.

Murdad, R., Latip, M.A., Aziz, Z.A. Ripin, R. (2010). 'Effects of carbon source and potato homogenates on in vitro growth and development of Sabah's endangered orchid: Phalaenopsis gigantea'. *Asia-Pacific Journal Molecular Biology and Biotechnology. 18*, 199–202.

Nambiar, N., Siang, C.T. Mahmood, M. (2012). 'Effect of 6-benzylaminopurine on flowering of a *Dendrobium* orchid'. *Australian Journal of Crop Science, 6*, 225–231.

Pradhan, S., Paudel, Y.P. Pant, B. (2013). 'Efficient regeneration of plants from shoot tip explants of *Dendrobium* densiflorum Lindl., a medicinal orchid'. *African Journal of Biotechnology, 12*(12). 1378-1383.

Rittirat, S, Thammsiri, K. Te-Chato, S. (2012). 'Effect of media and sucrose concentrations with or without activated charcoal on the plantlet growth of P. cornucervi (Breda) Blume Rchb. f. J Agric Sci Tech, 8, 2077–2087.

Sari, P.N., Auliya, M., Farihah, U. Nasution, N.E.A. (2020). 'The effect of applying fertilizer of moringa leaf (Moringa oliefera) extract and rice washing water to the

growth of pakcoy plant (Brassica rapa L. spp. Chinensis (L.)). *In Journal of Physics: Conference Series 563*(1), p. 012021, IOP Publishing.

Sharief, T.M., Eisa, E.M. AbdElseid, K.B. (2020). 'Effects of Osher, Neem and Argel as natural product growth hormone'. *Journal of Chemical Research Advances, 1*(1). 18-21.

Tawaro, S., Suraninpong, P. Chanprame, S. (2008). 'Germination and regeneration of Cymbidium findlay sonianum Lindl. on a medium supplemented with some organic sources'. *Walailak Journal of Science and Technology. 5*, 125–135.

Yi, U., Zaharah, S.S., Ismail, S.I. Musa, M.H. (2021). 'Effect of Aqueous Neem Leaf Extracts in Controlling Fusarium Wilt, Soil Physicochemical Properties and Growth Performance of Banana (Musa spp.)'. *Sustainability, 13*(22), p.12335.