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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(2): 392-395

www.thepharmajournal.com
Received: 22-12-2022
Accepted: 24-01-2023

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Impact of different hardening substrate on growth of *in vitro* raised plantlets of orchids (*Dendrobium nobile*)

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DOI: https://doi.org/10.22271/tpi.2023.v12.i3d.18956

Abstract
The present investigation was done on impact of different hardening substrates on growth of *in vitro* raised plantlets with objective to standardize hardening media for acclimatization of orchid plantlets. The research was laid out in Completely Randomized Design (CRD) in combination of 16 treatments containing charcoal, brick, cocopeat, bark, rice husk, groundnut shell, chopped coconut husk and chopped paddy straw in different combination all 16 treatments replicated thrice for analyzing the effect of media under poly house condition. Among the treatments, T_8 (chopped coconut husk + brick + charcoal + cocopeat) had superior result in almost all considerable aspects of study than all other treatments including control. The results revealed that combination of chopped coconut husk, brick, charcoal and cocopeat had been beneficial to significantly increase the vegetative growth and can be recommended for hardening media of orchids.

Keywords: Hardening, orchids, chopped coconut husk, cocopeat, charcoal, *in vitro*, rice husk, media

Introduction
Orchids are the most elegant and useful floricultural plants in nature's creation and also valued in cut flower production. Orchids belong to the family Orchidaceae. Orchids are the highly developed family among monocotyledons including 600-800 genera and 25000-35000 species. Orchid's prices on the international market are very high because of their longer lasting and fascinating beautiful flowers. Orchids show broad range of diversity in shape, size and colour of their flowers. Orchids are native to tropic regions of Asia such as the Himalayas and the Philippines. The eastern Himalayas and the north-eastern regions are the most prominent orchid regions in India. Chhattisgarh state blessed with incredible range of biological diversity in flora and fauna and widely varying climate is situated in central eastern part of India. This variability of climate couple with large forest cover makes the state very potential area for orchids. While most of the peasants in Chhattisgarh are still finding it extremely challenging and hard to move out of the paddy wheat cycle, a progressive farmer named Manoj Choudhary has growing orchid flowers in his farm sprawling over 11- acre land at village Tarra near Raipur and thereby earning a whooping profit of 15 lakh per acre each year.

Orchids are divided into two types on the basis of their growth habit these are sympodial and monopodial. *Dendrobiums* are the most favoured tropical orchid getting popularity as cut flower in the world. The propagation of Orchids is done asexually and sexually both. Sexually orchids can be propagated by seeds but seed propagation is difficult due to very small size of seeds and their symbiotic association with mycorrhizal fungi for germination. Asexual propagation of orchid is performed vegetatively through division, offshoots and cuttings but through the traditional asexual propagation results in rise of 2-4 plants per year which is very slow.

Micropropagation of orchids is the most commonly used appropriate technique in developed countries, for their exploitation as a major trade using different explants several orchid species have been effectively propagated under *in vitro* conditions. For the rapid multiplication of disease-free plants under *in vitro* conditions micropropagation has been widely used but mortality rate of plant is high during transferring of *in vitro* plantlets to *ex vitro* conditions, i.e., methods like pre-hardening and hardening makes orchid plantlets adaptable for growing environment of *ex vitro* condition as direct transfer of tissue culture raised plants to field is not possible because of high rate of mortality. Orchids in general regenerate in the culture having

environment of high humidity, variation of light and diversified temperature condition being guarded from the attack of microbial and other agents. Micro propagated plantlets are heterotrophs under laboratory condition and so they have to be progressively transformed into autotrophs for better survival and successful establishment, hardening is the method for making plantlets adaptable to growing environment. Thus, acclimatization of *in vitro* plants to greenhouse environment is an important stage to obtain quality plants of orchids for the commercial cultivation.

Materials and Methods

The experiment was done at the Poly house of Commercial Tissue Culture Laboratory, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh during the year 2019-2020. The study was designed under Completely Randomized Design (CRD) with three replications. The rooted plantlets of *Dendrobium* var. Sonia varying in height 2.0-3.0 cm were planted in perforated plastic pots filled with different Hardening media according to treatments specification. The media used for this study were charcoal, brick, cocopeat, bark, rice husk, groundnut shell, chopped coconut husk, chopped paddy straw in different combinations. All the media are sterilized before using to reduce the contamination at the time of making potting mixture. After planting, the plantlets were sprayed with fungicide solution and NPK fertilizer solution once in a week. The data were collected on shoot parameters like plant height, number of leaves plant $^{-1}$, leaf length, leaf width, leaf area (cm^2), leaf area index, shoot girth (mm) in response to different media combination. The results obtained were statistically evaluated using ANOVA for Completely Randomized Design (CRD).

Table 1: Details of different treatments

Treatments	Details
T_0	Charcoal + Brick + Cocopeat (1:1:1)
T_1	Bark + Brick + Charcoal (1:1:1)
T_2	Bark + Brick + Charcoal + Cocopeat (1:1:1:1)
T_3	Bark + Brick + Charcoal + Rice Husk (1:1:1:1)
T_4	Groundnut Shell + Brick + Charcoal (1:1:1)
T_5	Groundnut Shell + Brick + Charcoal + Cocopeat (1:1:1:1)
T_6	Groundnut Shell + Brick + Charcoal + Rice Husk (1:1:1:1)
T_7	Chopped Coconut Husk + Brick + Charcoal (1:1:1)
T_8	Chopped Coconut Husk + Brick + Charcoal + Cocopeat (1:1:1:1)
T_9	Chopped Coconut Husk + Brick + Charcoal +Rice Husk (1:1:1:1)
T_{10}	Chopped Paddy Straw + Brick + Charcoal (1:1:1)
T_{11}	Chopped Paddy Straw + Brick + Charcoal + Cocopeat (1:1:1:1)
T_{12}	Chopped Paddy Straw + Brick + Charcoal + Rice Husk (1:1:1:1)
T_{13}	Rice Husk + Brick + Charcoal (1:1:1)
T_{14}	Rice Husk + Brick + Charcoal + Cocopeat (1:1:1:1)
T_{15}	Charcoal + Brick + Cocopeat + Rice Husk + Bark + Groundnut Shell + Coconut Husk +Paddy Straw (1:1:1:1:1:1:1:1)

Results and Discussion

In the present study, the collected observational data was statistically analysed using the method of analysis of variance at 5% level of significance. ANOVA revealed that the different treatments showed significant influence on growth at 120 DAT.

1. Plant Height

It is observed from the table that significant difference in plant height was observed among different hardening media treatment at 120 DAP the plant height range was 10.76 to 21.08 cm. The maximum plant height 21.08 cm was observed with application of treatment T_8 (Chopped coconut husk + Brick + Charcoal + Cocopeat) at 120 DAP respectively which showed considerable variation from other treatments at 120 DAP. The Minimum plant height 10.76 cm was noted in treatment T_{10} (Chopped paddy straw + Brick + Charcoal) at 120 DAP.

The maximum value obtained in T_8 (Chopped coconut husk + Brick + Charcoal + Cocopeat) may be attributed to the enhanced moisture and nutrient holding capacity of the substrates due to coconut husk, charcoal and cocopeat at the initial stages of plant growth further good mechanical support provided by brick pieces and charcoal may increases height of growing plants. This has been found to be in consonance with earlier work done in Orchid by Muna *et al.*, (2016) [4] and Lakshanthi and Seran (2019) [3].

2. Number of leaves plant^{-1}

Significant difference in number of leaves plant^{-1} was observed among different hardening media treatment at 120 DAP. The maximum number of leaves per plant^{-1} was noted 6.43 on application of treatment T_8 (Chopped coconut husk + Brick + Charcoal + Cocopeat) at 120 DAP which was noted at par with T_2 (Bark + Brick + Charcoal + Cocopeat) and T5 (Groundnut shell + Brick + Charcoal + Cocopeat) at 120 DAP. The minimum number of leaves plant^{-1} 2.00 was observed with application of treatment T_{10} (Chopped paddy straw + Brick + Charcoal) at 120 DAP respectively.

Enhancement in number of leaves per plant might be credited to combined effect of T_8 (Chopped Coconut Husk + Brick + Charcoal + Cocopeat) (1:1:1:1). Nutritional supplements to the plant N is a chief constituent of protoplasm providing metabolic energy to the cell division and cell enlargement. This substrate mixture may have provided nutrient directly to the plant causing stimulation of new leaves. The results are in accordance with the reports of Indhumati *et al.*, (2003) [2] and Muna *et al.*, (2016) [4] in orchids.

3. Leaf length (cm)

Significant difference in leaf length has been noted among different treatment at 120 DAP. The maximum leaf length 10.26 cm was noted on application of Treatment T_8 (Chopped coconut husk + Brick + Charcoal + Cocopeat) at 120 DAP which was noticed *at par* with treatment T_2 (Bark + Brick +

Charcoal + Cocopeat) and T_5 (Groundnut Shell + Brick + Charcoal + Cocopeat). However, the minimum leaf length 3.80 cm was noted with the application of treatment T_{10} (Chopped paddy straw + Brick + Charcoal) at 120 DAP.

Maximum leaf length in T_8 (Chopped coconut husk + Brick + Charcoal + Cocopeat) may be due to better growth and development of plants because of the proper supply of essential nutrient to the plants through the rich organic content in coconut husk and cocopeat. The media combination might be stimulating metabolic activity with stimulating effect on cell wall loosing, increase cell elongation along with cell enlargement and cell differentiation resulting in increased photosynthesis and translocation of food material which might be enhanced the leaves length. Similar outcome has been reported by Lakshanthi and Seran (2019) [3] and Sabreeswaran *et al*., (2019) [7] in orchids.

4. Leaf width (cm)

The maximum leaf width 3.48 cm noted on application of treatment T_8 (Chopped coconut husk + Brick + Charcoal + Cocopeat) at 120 DAP which was noted at par with T_5 (Groundnut Shell + Brick + Charcoal + Cocopeat) and T_2 (Bark + Brick + Charcoal + Cocopeat). However, the minimum leaf width 1.68 cm was observed with application of treatment T_{10} (Chopped paddy straw + Brick + Charcoal) at 120 DAP.

The increased width of leaves in T_8 (Chopped coconut husk + Brick + Charcoal + Cocopeat) may be due to coconut husk with cocopeat, charcoal and brick gave maximum width in account of favorable physio-chemical properties of media and nutritional value. It is due to the fact that more N, P, and K in substrate lead to increase in cell number and cell size. Identical result has been also reported by Muna *et al*., (2016) [4] and Sabreeswaran *et al*., (2019) [7].

5. Leaf area (cm^2)

Significant difference in leaf area was observed among different treatment at 120 DAP the range was 6.44 cm^2 to 35.66 cm^2. The maximum leaf area 35.66 cm^2 was observed on application of treatment T_8 (Chopped coconut husk + Brick + Charcoal + Cocopeat) 120 DAP which were noticed at par with treatment T_5 (Groundnut Shell + Brick + Charcoal + Cocopeat) at 120 DAP. The Minimum leaf area 6.44 cm^2 was noted in treatment T_{10} (Chopped Paddy straw + Brick + Charcoal) at 120 DAP.

The increased leaf area may be attributed by higher carbohydrate accumulation in leaves facilitated by favorable nutrients has led to higher photosynthetic activities resulting in an increased leaf area. Mirani *et al*., (2016) [8] and Muna *et al*., (2016) [4] in *Dendrobium* reported similar outcome.

Table 2: Effect of hardening media on shoot parameters of orchid plants at 120 DAP

Treatments	Plant height (cm)	Number of leaves plant^{-1}	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)	Leaf area index (cm^2)
	120 DAT	120 DAT	120 DAT	120 DAT	120 DAT	120 DAT
T_0	18.34	5.23	8.23	2.73	22.59	0.22
T_1	13.87	3.00	7.00	2.03	14.26	0.14
T_2	19.48	6.13	9.67	3.07	29.72	0.29
T_3	16.08	4.33	8.10	2.57	20.88	0.20
T_4	13.75	2.90	6.86	2.00	13.72	0.13
T_5	19.02	5.83	9.54	3.27	31.10	0.30
T_6	16.03	4.19	7.70	2.45	18.89	0.18
T_7	14.00	3.16	7.28	2.31	16.82	0.16
T_8	21.08	6.43	10.26	3.48	35.66	0.35
T_9	18.23	4.61	8.22	2.46	20.24	0.20
T_{10}	10.76	2.00	3.80	1.68	6.44	0.06
T_{11}	12.85	2.33	5.00	1.84	9.21	0.09
T_{12}	15.73	4.21	7.87	2.43	19.02	0.19
T_{13}	13.43	2.45	6.38	1.98	12.80	0.12
T_{14}	18.86	5.63	8.27	2.86	23.64	0.23
T_{15}	15.48	3.28	7.40	2.34	17.27	0.17
$SE_{m\pm}$	0.25	0.21	0.29	0.20	1.67	0.02
CD	0.71	0.62	0.84	0.58	4.82	0.05

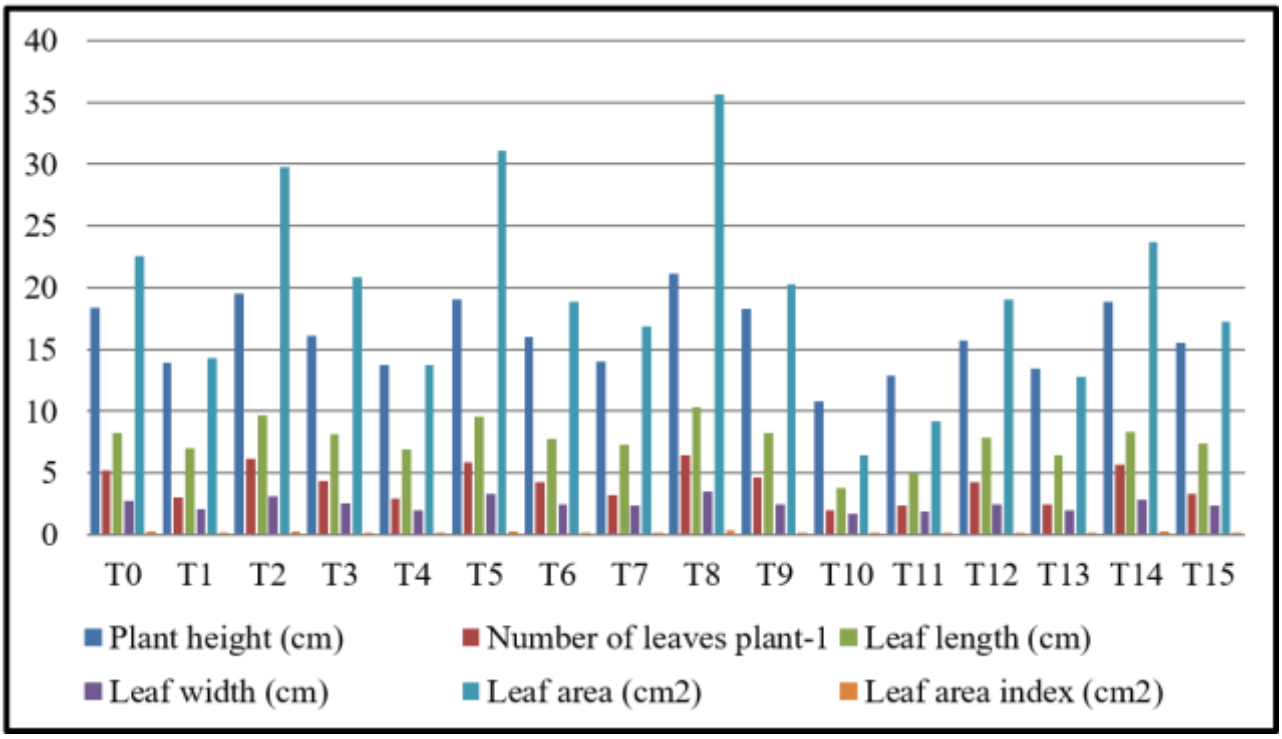

Fig1: Graphical representation of effect of different hardening substrates on Growth parameters of Orchids plants

6. Leaf area index (cm^2)

Significant difference in leaf area index was observed among different treatment at 120 DAP the range was 0.06 to 0.35. The maximum leaf area index 0.35 was observed with treatment T_8 (Chopped coconut husk + Brick + Charcoal + Cocopeat) at 120 DAP which was noticed at par with treatment T_5 (Groundnut Shell + Brick + Charcoal + Cocopeat) at 120 DAP. The Minimum leaf area index 0.06 was noted in treatment T_{10} (Chopped paddy straw + Brick + Charcoal) at 120 DAP. The results have been in contradiction to the findings of Mirani *et al*., (2016) [8].

Conclusion

Perusal of the data from the research result concluded that the hardening media combination T_8 (chopped coconut husk + brick + charcoal + cocopeat) had a superior result in all considerable aspects of study.

The results revealed that hardening medium with moderate aeration and high water holding capacity would be the best media for acclimatization of *in vitro* propagated orchids. The treatment T_8 achieved better results as it contained coconut husk, brick pieces, charcoal and cocopeat. Where, coconut husk is best suited for improving the water holding ability of the substrate and nutrient content at initial phase, brick pieces help in providing good aeration and mechanical support to plants, charcoal is also helpful in providing aeration and retaining fertilizer when pouring fertilizer solution and it steadily transfer nutrients to developing plants on subsequent watering, cocopeat carries eight times of water of its own weight it improves water holding capacity of media and also releases nutrients over long periods. While treatment T_{10} gives minimum result in almost all parameters might be due to the reason that medium containing chopped paddy straw, brick and charcoal could not supply enough support and nutrients required for the growth. Therefore the media combination T_8 (chopped coconut husk + brick + charcoal + cocopeat) may be recommended for hardening media of orchids.

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