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Micropropagation of an important medicinal plant *Catharanthus roseus* by using coconut water instead of synthetic plant growth regulators

Fizza Matloob[1], Zishan Gul[2]*, and Zafar Jamal[1]

1. Department of Botany, G. Post Graduate College, Abbottabad, Pakistan
2. Hazara Agriculture Research Station, Abbottabad, Pakistan

***Corresponding author**: Zishan Gul, Hazara Agriculture Research Station, Abbottabad, Pakistan.

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ABSTRACT

The present *in vitro* study was conducted for micropropagation of *Catharanthus roseus* by using coconut water. The coconut water was used in five different concentrations i.e T1 (3 %), T2 (6 %), T3 (9%), T4 (12 %) and T5 (15 %) v/v in 1L MS medium instead of synthetic plant growth regulators (PGRs). The results showed that in treatment (T4) 80% cultured explant segments showed shoot emergence and the mean number of leaves (4.80) nodes (2.60), average shoot length (3.15cm) and % rooting (60%) was also higher followed by the treatment (T3). At lower (T1, T2) and higher (T5) concentration of coconut water the growth of *Catharanthus roseus* plantlets was suppressed. It has been concluded that the treatment (T4) 12 % coconut water showed the best growth rate of *Catharanthus roseus* plantlets. The plant growth hormones present in coconut water affected the *in vitro* microprogation of *Catharanthus roseus* significantly, therefore, it can be used instead of synthetic plant growth regulators (PGRs) in MS media for micropropagation of *Catharanthus roseus.*

1. INTRODUCTION

Catharanthus roseus (L.) is a high value medicinal plant belongs to family Apocynaceae. It has two common cultivars on the basis of flower color i.e Rosea (pink flowers) and Alba (white flowers)[12]. *Catharanthus roseus* has anticancer activities due to the presence of more than 400 alkaloids among which actineo plastidemeric, Vinblastin, Vincrestine, Vindesine, Vindeline Tabersonine present in aerial parts while ajmalicine, vinceine, vineamine, raubasin, reserpine and catharanthine are present in basal stem and roots[13]. Methanolic crude extracts of *Catharanthus* have anticancer activity against numerous cell types [23]especially against the multidrug resistant tumor types[24]. Some alkaloids are used for the treatment of childhood leukemia, Hodgkin's disease, testicular cancer, diabetes, blood asthma, constipation and menstrual problem[6].

Nowadays, plant tissue culture is widely used technology for large scale plant multiplication[22]. Medicinal plants are the greatest source of valuable pharmaceutical drugs[8]. On commercial basis large scale production is required which can be achieved through in-vitro culture technique for mass multiplication of valuable medicinal plants[26,20]. The induction of multiple shoots in *Catharanthus roseus* from nodal segments was achieved on MS medium supplemented with 0.5 mg/l BAP ± 1mg/l NAA[16].

Attempts were made to find comparatively cheaper micro-propagation procedures by adopting low cost substituent in the culture medium. Many natural complex additives like coconut water, banana pulp, tomato juice, slap honey and beef extract were tested as growth factor substitute for the synthetic growth regulators. The use of coconut water as a plant growth regulator gives a better response on plant tissue culture[5].

Coconut water (CW) is the liquid endosperm of *Cocos nucifera* L used as a supplement in tissue culture media because it contained phytohormones especially cytokinins, indole-3-acetic acid (IAA)[27] , trans-zeatin [21] and gibberellins [9].

CW supplementation highly influenced the shoot bud initiation and multiplication in *in vitro* regeneration studies on *Cyamopsis tetragonolobust*[17]. It was reported that coconut water induced the callus growth and somatic embryogenesis of *Phoenix dactylifera* (Date palm)[4]. Supplementation of coconut water produces a high number of shoots in *in vitro* culture of orchid shoots (*Dendrobium Sp*)[10].

Due to the expensiveness of the pharmaceutically important alkaloids of *Catharanthus roseus* the interest in searching alternative ways of its production has been increased. Through tissue culture the medicinal plants can be produced rapidly throughout the year without depleting the natural resources. Plant tissue culture is an expensive technique for micropropagation of plants particularly due to use of expensive plant growth regulators. As Pakistan is an

underdeveloped country so the use of such expensive growth hormones for *in vitro* culture is not economical. Our study has been focused to evaluate the effect of various concentrations of coconut water on *in vitro* growth and micropropagation of *Catharanthus roseus* instead of using expensive synthetic plant growth hormones.

2. MATERIALS AND METHODS

The experiment was conducted in potato tissue culture laboratory at Hazara Agriculture Research Station (HARS), Abbottabad in 2017.

The coconut water was extracted aseptically from Coconut fruits by drilling holes through two of the micropyles and then heated at 60°C for 10 minutes with continuous stirring to precipitate out the undesirable proteins, fats and other materials and then filtered. MS medium[18] containing 1 mgl-1Ca-pentothenate, 100mgl-1 Myoinsitol , 30g/l sucrose and 6g/l agar was prepared and coconut water (CW) (v/v) was added as (T_1) 3% , (T_2) 6% , (T_3) 9% , (T_4) 12% and (T_5) 15%. The MS medium with no coconut water served as control (T_0) 0%. The pH of the media was adjusted to 5.8 with either 1 N NaOH or 1 N HCl solution prior to autoclaving. Each treatment was replicated 15 times.

The explants of *Catharanthus roseus* were collected from Department of Botany, Government Post Graduate College, Abbottabad and identified with the help of Flora of Pakistan[19]. After removing the leaves the shoots were washed under running tap water for 15 minutes, later washed with a mild detergent followed by rinsing thoroughly with distilled water for 5-6 times. After rinsing the explants were cut in to nodal segments and were surface sterilized in 0.1% (w/v) mercuric chloride for 3 minutes, washed with sterilized distilled water 3-4 times. Further the explants were treated with 0.1% (w/v) fungicide (Ridomil) for 1 min then washed thrice with sterilized distilled water. .

The nodal segments were trimmed at both ends after sterilization in order to expose fresh tissues and were inoculated aseptically on media in the test tubes under sterilized conditions in laminar flow cabinet. All cultured test tubes were incubated at 18-20ºC with 16 hours light and 8 hours dark in the growth chamber.

The Data were recorded after 30, 50 and 60 days of culturing for various growth parameters including root and shoot emergence, shoot length, number of nodes and number of leaves. The Data were analyzed by using computer software Statistix 8.1 and Least significance difference test (LSD) at 95% level of significance was performed to assess significant difference between various treatments. The Experiment was designed as Complete Randomized Design (CRD).

3. RESULTS AND DISCUSSION

The data revealed that different concentrations of coconut water showed great variation on the *in-vitro* growth of *Catharanthus roseus* as compared to control**.**

Shoot Emergence: Data regarding number of days to shoot emergence percentage (Table 1) showed that after 5 days of culturing the shoot emergence was observed in all the five treatments except control (T_0). The maximum shoot emergence was recorded in treatment T_4(60%) and T_3 (50%) in which CW was added @ 12% and 9% while after 15 days a significant increase ($P \leq 0.05$) in the shoot emergence percentage was recorded (Table 1) (Figure 1). Whereas in the control no emergence was observed even after 15 days of culturing.

Number of leaves: The data collected after 30 days showed that all the five treatments are significantly different ($P \leq 0.05$) regarding number of leaves (Table 2) (Figure 2). The maximum average number of leaves was observed in treatment T_4 (3.80) followed by T_3 (2.40) and T_2 (1.80). The minimum mean value for number of leaves was observed in treatment T_1 (1.60) and T_5 (1.10) in which 3% and 15% CW was added (Table 2). After 50 days of culturing a significant increase in average number of leaves between treatments was recorded (Table 3).

Table.1.Shoot emergence percentage in the cultured nodal segment of *Catharanthus roseus*

No of days	Shoot Emergence %					
	T1	T2	T3	T4	T5	T0
5Days	10 b	30 ab	50 a	60 a	10 b	0 b
15 Days	30 b	50 ab	70 a	80 a	30 bc	0 c

Means in a row followed by different letters are significantly different at P ≤ 0.05

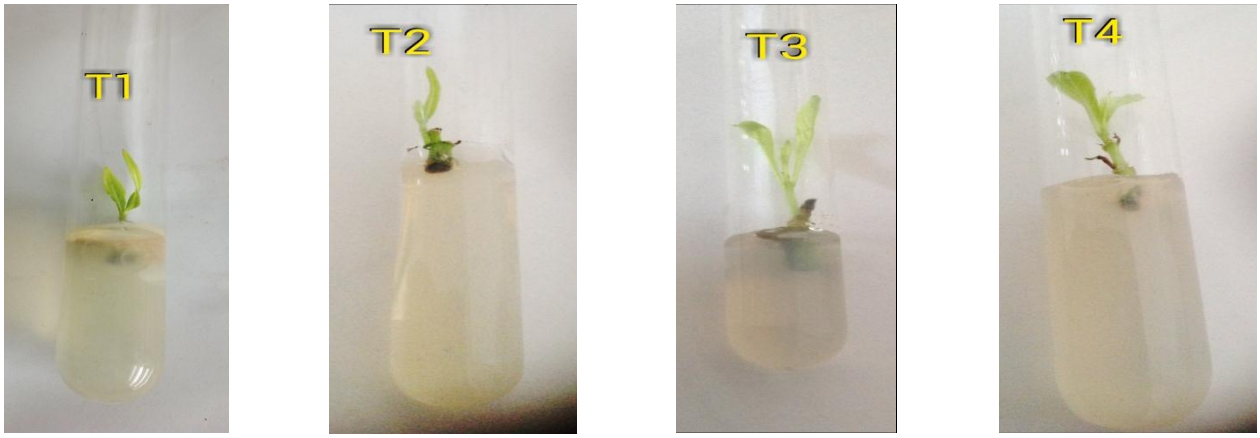

Figure.1.Treatment T1 , T2 T3 and T4 showing shoot emergence of *Catharenthus roseus* after 15 days of culturing

No of Nodes: Statistical analysis after 30 days of culturing revealed that the highest mean value (2.00) for number of nodes among the treatments was recorded in T_4 (Table 2) followed by treatment T_3(1.20) andT_2 (1.00). The minimum number of nodes was observed in treatment T_1(0.80) and T_5 (0.70) in which 3% and 15% CW was added. A similar trend was observed after 50 days (Table 3).

Figure.2.Treatment T3 and T4 showing number of leaves after 20 days of culturing

Table.2.Comparison of growth rate of *Catharanthus roseus* on MS media at different concentrations of coconut water after 30 days of culturing

Treatments	Shoot Length (cm)	Average no of leaves	Average no of nodes
T_1	1.06 bc	1.60 bc	0.80 b
T_2	1.24 bc	1.80 bc	1.00 b
T_3	1.80 b	2.40 b	1.20 b
T_4	2.70 a	3.80 a	2.00 a
T_5	0.68 cd	1.10 cd	0.70 b
T_0	0.00 d	0.00 d	0.00 c

Means in a column followed by different letters are significantly different at P ≤ 0.05

Shoot Length: The statistical analysis of the data after 30 days of culturing showed that the highest mean shoot length was observed in treatment T_4 (2.70cm) followed by treatment T_3 (1.80cm) and T_2 (1.24cm). The lowest mean value among the treatments for shoot length was observed in treatment T_5 (0.68). In control no shoot growth was observed. A similar trend in shoot growth was recorded even after 50 days (Table 3) (Figure 3).

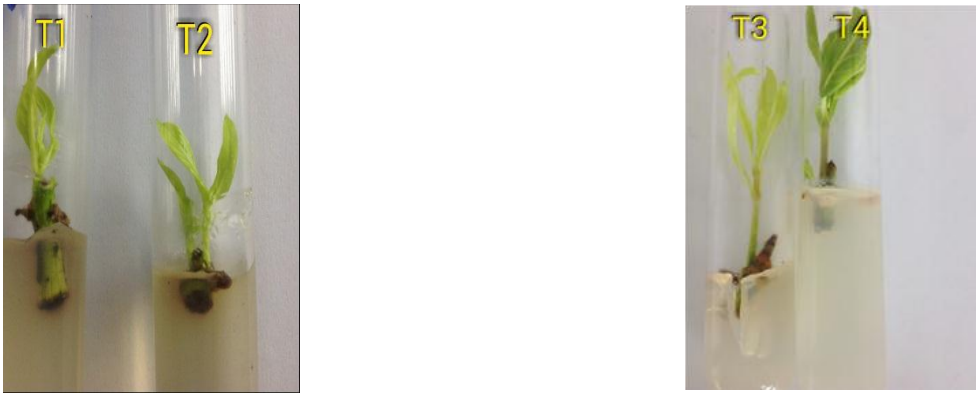

Figure.3.Treatment T1, T2 , T3 and T4 showing Shoot development of *Catharenthus roseus*

Table.3.Comparison of Growth rate of *Catharanthus roseus* on MS media at different concentration of coconut water after 50 days of culturing

Treatment	Shoot Length(cm)	Average no of leaves	Average no of nodes
T1	1.46 bc	2.70 bc	1.40 b
T2	1.59 bc	2.80 bc	1.50 b
T3	2.23 ab	3.60 ab	1.80 ab
T4	3.15 a	4.80 a	2.60 a
T5	0.90 cd	1.60 cd	0.90 c
T0	0.00 d	0.00 d	0.00 c

Means in a column followed by different letters are significantly different at P ≤ 0.05

Root emergence: After 60 days of culturing, the best rooting response (Figure 4) was observed in treatment T_4 (60%) (Figure 5a) followed by T_3 (30%) in which 12 % and 9 % coconut water (CW) was added while the rest of the treatments failed to induce rooting of the regenerated shoots. In vitro rooted plantlets were transplanted in the green house where their leaves expanded and plants grow quickly (Figure 5b).

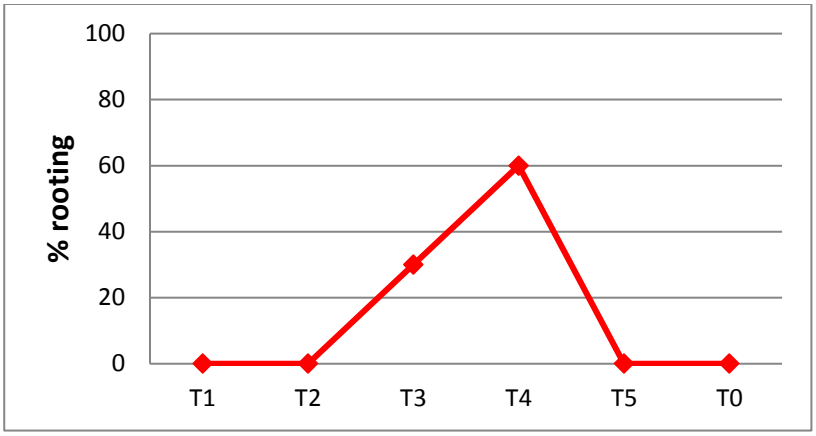

Figure.4.Treatments showing % root emergence in *Catharanthus roseus*

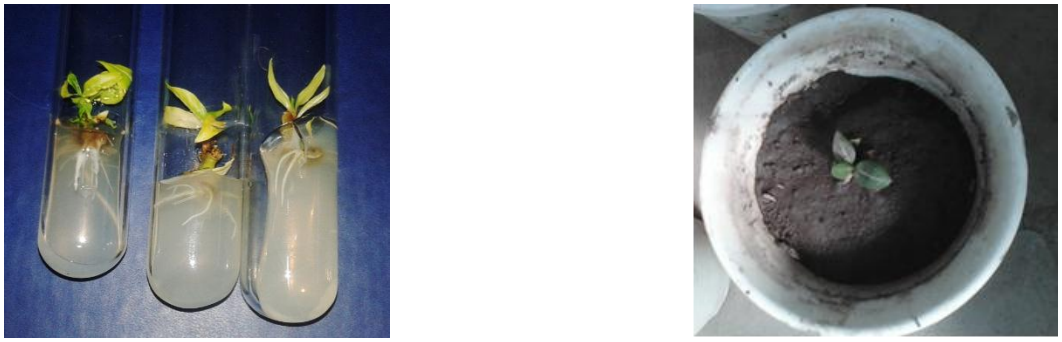

Figure.5.(A) Plantlets of treatment T4 showing root developments after 60 days of culturing (B) *Catharenthus roseus* plantlet transplanted in the green house

Discussion: In the present study coconut water was used instead of plant growth regulators for the micropropagation of *Catharenthus roseas*. Results showed that shoots were emerged after 5 days of culturing in every treatment except control. It might be due to presence of phytohormones cytokinin that stimulate *in vitro* growth of plants. Cytokinins are a major group of phytohormones that play significant role in plant growth and development e.g cell division, formation and activity of shoot meristem[14]. It was demonstrated that the addition of coconut water enhanced axillary shoots proliferation along with BA in *Faidherbia albida*[15].

Comparatively maximum shoot emergence was recorded in treatment T_4 (80%) in which 12 % coconut water was added while at higher and lower concentrations the shoot emergence and growth was suppressed. It was reported that *Dendrobium* needed medium doses (10-15%) of coconut water for improvement of shoot and leave number, and the higher concentration of coconut water causes the shoots to be stunted and eventually died[14]. It was also observed that the emerged shoots were stunted and eventually died in treatment T_5 in which CW at 15% concentration was used. The type and concentration of plant growth regulators (PGR) is a component of tissue culture medium that determines the success of tissue culture[14].

The maximum root emergence % was observed in treatment T_4 (60%) followed by treatment (T_3) in which 30%) rooting occurred. This may be due to presence of auxin in coconut water[3]. . Auxin plays a major role in formation of main root, lateral and adventitious roots[25], and possible reason for rapid root initiation in treatment (T_4) is may be that 12% coconut water concentration resulted in optimum auxin level in the medium that causes rooting of *Catharenthus roseus*. The physiological changes of rooting are correlated with changes in auxin concentration[11].

The data revealed that among the treatments the highest shoot length was observed in treatment T_4 (3.15cm). Shoot length can be affected by presence of Gibberellin in coconut water because it is also reported that *in vitro* addition of GA3 (0.5mg/L) combined with low cytokinin concentration was effective in shoot growth of potato and GA1 and GA3 were successfully detected and quantified in coconut water [7,14].

Regarding number of leaves the significant increase in the number of leaves (4.80) were found in treatment (T_4) in 50 days old plantlets of *Catharenthus roseas*. Higher concentration of coconut water (100 ml l^{-1}) decreased all the growth and morphological features, as well as induced abnormal plantlets growth in *Calanthe* hybrids[1]. The leaves arise from buds and it was reported that cytokinins are usually known to make promotion of buds formations in many *in vitro* cultured organs[2].

4. CONCLUSION AND RECOMMENDATIONS

It has been concluded from the present study that 12 % coconut water was most suitable for *in vitro* micropropagation of *Catharanthus roseus* which showed the maximum number of nodes, leaves, shoot length and highest percentage of root/shoot emergence.

Therefore, fresh coconut water extracted from coconut fruit can be used instead of synthetic plant growth regulators (PGR) in tissue culture of *Catharanthus roseus* and further research is needed to test its efficacy against micropropagation of other medicinal plants as a cost effective substitute for expensive synthetic plant growth hormones.

REFERENCES

1. Abdullahil MB, Shin YK, Elshmari T, Lee EJ and Paek KY, Effect of light quality, sucrose and coconut water concentration on the micro-propagation of *Calanthe hybrids* ('Bukduseong' × 'Hyesung' and 'Chunkwang' × 'Hyesung'). Australian Journal of Crop Sciences. 5, 2011, 1247-1254.

2. Afshin AH, Kaviani B, Tarang A and Zanjani SBI, Effect of different concentrations of kinetin on regeneration of ten weeks (*Matthiola incana*). Plant Omics Journal. 4, 2011, 236-238.

3. Agampodi VA and Jayawardena B, Identification and characterization of plant growth regulators present in coconut (*Cocos nucifera*) water using HPLC (High Performance Liquid Chromatography), Proceedings of the Annual Research Symposium 2007. Faculty of Graduate Studies, University of Kelaniya, 2007, 1- 118 P.

4. Al-khayri JM, Somatic embryogenesis of Date palm *(Phoenix dactylifera).* Biotechnology, 2010, 1-8.

5. Daud N, Taha RM, Noor NM and Alimon H, Provision of low cost media options for *in vitro* culture of *Celosia* sp. African Journal of Biotechnology, 10(80), 2011, 18349-18355.

6. Faheem M, Satyapal S, Babeet S, Moinuddin K and Anwar S, In-vitro Regeneration of multiplication shoots in Catharanthus roseus- An important medicinal plant, Pelagia Research Library, Advances in Applied Science Research, 2 (1), 2011, 208-213

7. Farhatullah, Abbas Z and Abbas SJ, In -vitro effects of gibberellic acid on morphogenesis of potato explant. International Journal of Agriculture & Biology, 1, 2007, 181–182.

8. Gajalakshmi S, Vijayalakshmi S and Rajeswari DV, Pharmacological Activities of *Catharanthus roseus*. A Perspective Review. Int J Pharm Bio Sci, 4(2), 2013, 431 – 439.

9. Ge L, Peh CYC, Yong JWH, Tan SN, Hua L and Ong ES, Analyses of gibberellins by capillary electrophoresis-mass spectrometry combined with solid-phase extraction. Journal of Chromatography A, 1159, 2007, 242–249.

10. Harahap F, The Growth of Orchids (*Dendrobium* sp) in in Vitro Giving With Coconut Water on Different Medium. First International Seminar on Trends in Science and Science Education, 2014, 46-53.

11. Heloir MC, Kevers C, Hausman JF and Gaspar T, Changes in the concentrations of auxin and polyamines during rooting of in-vitro-propagated walnut shoots. Tree Physiol, 16, 1996, 515-519.

12. Jaleel CA and Panneerselvam R, Variations inthe antioxidative and indole alkaloid status in differentparts of two varieties of *Catharanthus roseus*: An important folk herb. Chininese Journal of Pharmcology and Toxicology, 1(6), 2007, 487- 494.

13. Jaleel CA, Gopi R and Paneerselvam R, Alterations in non-enzymatic antioxidant components of *Catharanthus roseus* exposed to paclobutrazol, gibberellic acid and Pseudomonas fluorescens, Plant Omics J, 2, 2009, 30-40.

14. Jean WHL, Ng Y, Ge F and Tan SN, The chemical composition and Biological properties of coconut (*Cocos nucifera* L)Water. Molecules. 14, 2009, 5144-5164.

15. Kwapata MB, Kalengamaliro F, Bakuwa J and Manyela S, In-vitro rooting and axillary shoots proliferation of *faidherbia albida* (del.) A. Chev. Under varying levels of plant growth regulators. African Crop Science Journal, 7, 1999, 303-311.

16. Mehta J, Upadhyay D, Paras P, Ansari R, Rathore S and Tiwari S, Multiple Shoots Regeneration of (anti-cancer plant) *Catharanthus roseus* An important medicinal Plant. Am .J.PharmTech Res, 3(1), 2013, 2249-3387.

17. Mohammed S and Ali M, Effect of Coconut Water on Callus Growth on *Cyamopsis tetragonolobust*. Pharmacia, 1, 2010, 25-28.

18. Murashige T, and Skoog F, A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant physiology, 15, 1962, 473-497.

19. Nazimuddin, S and Qaiser, M, Flora of Pakistan, FI.Pak, 148, 1983, 1-141.

20. Pandey S, Bahadur AN, Kanungo VK and Tiwari, In-vitro propagation of a medicinal plant *Catharanthus roseus* L. (G.) Don. Indian J.L.Sci. 4 (1), 2014, 125-128.

21. Sridhar TM and Aswath CR, Influence of Additives on Enhanced in Vitro Shoot Multiplication of *Stevia rebaudiana* (Bert.)—An Important Anti Diabetic Medicinal Plant. American Journal of Plant Sciences, 5, 2014, 192-199.

22. Thorpe TA, Stasolla C, Yeung EC, de Klerk, GJ, Roberts A and George EF, The Components of Plant Tissue Culture Media II: Organic Additions, Osmotic and pH Effects, and Support Systems. In: George EF, Hall MA, De Klerk GJ (eds) Plant propagation by tissue culture, 3rd edn. Springer, Dordrecht, 2008, 115–173.

23. Ueda JY, Tezuka YA and Banskota H, Antiproliferative activity of *Vietnamese* medicinal plants. Biological Pharmaceutical Bulletin, 25(6), 2002, 753-60.

24. Wang S, Zheng Z and Weng Y, Angiogenesis and antiangiogenesis activity of Chinese medicinal herbal extracts. Life Science, 74(20), 2004, 2467-78.

25. Went FW and Thimann, KV, Phytohormones, New York, The Macnaillan Company, USA, 1937, 316.

26. Yadav, K., Singh, N and Verma, S. (2012): Tissue culture: a biotechnological tool for solving the problem of propagation of multipurpose endangered medicinal plants in India. Journal of Agricultural Technology. 8(1): 305-318.

27. Yong JWH, Ng L, Ge YF and Tan SN, The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water. Molecules, 14, 2009, 5144-5164.