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POSSIBLE MECHANISM OF ENHANCEMENT OF CUCUMBER SEEDLING ESTABLISHMENT ACHIEVED BY COCO PEAT EXTRACT

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ABSTRACT

Coco peat is being considered as a new and viable ecologically friendly peat substitute. Experiments were conducted in order to investigate the effectiveness of coco peat as an alternative material to peat moss, especially in terms of its effect on cucumber cotyledon expansion. These were conducted in Tokyo University of Agriculture, Setagaya campus from 2022 to 2023. Enhanced seedling establishment of cucumber achieved by Sri Lankan coco peat extract was examined focusing on cytokinin activity, potassium content, ethylene production, respiration rate and root development in relation to the process of cotyledon expansion. Coco peat extract enhanced root development of cucumber seedlings producing higher amount of ethylene and CO_2 released from the enriched root system. The outstanding cotyledon expansion might be achieved by higher activity of cytokinin and increased potassium content. SEM observation illustrated that cotyledon expansion of cucumber seedlings grown in coco peat extract was derived mainly from the enlargement of cell size. Stomatal number was significantly increased in cotyledons grown in coco peat extract solution, which might be reflected in higher rate of respiration. "Super coco", a newly developed processed coco peat washed with 13mM $FeSO_4$ solution succeeded in developing bigger root system in cucumber seedlings but exerted lesser effect on cotyledon expansion in comparison with normal coco peat.

Key words: cotyledon expansion, cytokinin activity, ethylene production, potassium content, root development

INTRODUCTION

Potting mixes for growing horticultural crops provide adequate support for plants and serve as pool for water and nutrients, allowing exchange of gaseous oxygen between the roots and atmosphere (Awang et al. 2009). Peat moss is a primary component of transplant and potting media for various plants, because of high water-holding capacity and cation exchange capacity (Biernbaum 1992), but environmental and ecological concerns were expressed due to the detrimental effect of peat harvesting on wetland ecosystem (Barkham 1993; Barber 1993; Buckland 1993; Evans et al. 1996). The harvesting of peat may cause a potential degradation of fragile natural habitats of living organisms, since peat is very important in preserving groundwater and CO_2 sinks (Kitir et al. 2018). Huge peatlands are located mainly in Finland, northern Russia, Alaska and Canada (Sjörs 1980), the transportation makes it more costly. Therefore, for the purpose of usage in Southeastern Asian countries, the alternative growth media should be readily available, and coco peat is regarded as one of such promising organic substrates (Chulaka et al. 2003).

Coco peat is fiber from coconut husk (Abad et al. 2002) and the global coco peat market as of 2020 was reported over 2.4 billion US$ and estimated to expand at a compound annual growth rate (CAGR) of 4.4% between 2021 and 2031 to cross a valuation of 3.8 billion US$ towards the end of 2031 (Kilee et al. 2022). Since coco peat shows anti-bacterial and anti-fungal effects (Evans and Stamps 1996), and less acidity and higher EC in comparison with peat (Colla et al. 2007), it is regarded as a new and viable ecologically friendly peat substitute (Arenas et al. 2002; Ghehsareh et al. 2011; Meerow 1997; Noguera et al. 2000). In this article, the chemical properties of Sri Lankan coco peat were examined and the possible mechanism of enhancing effect of coco peat extract on cucumber seedling establishment was investigated, focusing on cytokinin activity, potassium content, ethylene production, respiration rate and root development in relation to cotyledon expansion. The characteristics of newly released "super coco" which is the product of Nihon Chiko Co. ltd. (Tokyo) made of normal Sri Lankan coco peat washed with 13mM $FeSO_4$ solution were also described.

MATERIALS AND METHODS

Chemical analysis of coco peat and "super coco". Coco peat made of Sri Lankan coconut coir and newly developed product "super coco" which was processed coco peat washed with 13mM $FeSO_4$ solution were provided by Nihon Chiko Co. Ltd. (Tokyo) (Fig. 1). "Super coco" was prepared based on the demand of farmers who dislike the colored drain water leached out from the irrigated coco peat.

Figure 1. Sri Lankan coco peat and "super coco" used in the experiments

The experiments were conducted at Tokyo University of Agriculture, Setagaya campus from April to October, 2022. The measurement of pH and EC were conducted as follows; 50 ml of deionized water was added to 10 ml of coco peat and "super coco", and after shaking for 1 hour, pH was measured with LAQUA-PH-SE (Horiba Co., Ltd., Kyoto, Japan) and EC was measured with LAQUAtwin (Horiba Co., Ltd., Kyoto, Japan).

Dried coco peat and "super coco" were used to determine total nitrogen, total carbon, and inorganic chemical composition. The materials were dried in a dryer (FO-60P, Tokyo Garasu Kikai Co., Ltd., Tokyo, Japan) at 60°C for 48 hours and ground into a fine powder. Automatic NC analyzer Sumigraph NCH-22F (Sumika Analysis Service, Osaka, Japan) was used to measure total nitrogen and total carbon.

To measure inorganic chemicals, coco peat and "Super coco" (0.5 g) were weighed using an analytical balance, placed in a polypropylene container (50 mL DigiTUBEs, GL Sciences, Inc., Tokyo, Japan), and after adding 5 ml of 61% nitric acid (Kanto Kagaku), these were left overnight. The container was placed in a heat block (DigiPREP LS, GL Sciences, Inc., Tokyo, Japan) at 100°C for 60 minutes. After cooling, the volume of the extract was adjusted to 50 ml with deionized water, filtered through filter paper No. 5B, and inorganic components were measured by ICP emission spectrometry (ICPE 9820, Shimadzu, Tokyo, Japan).

Quantification of total polyphenol content was carried out by UV-visible spectrophotometry (Samaniego et al. 2020; Colla et al. 2007). Coco peat and “Super coco” powder (1 ml) was placed in a 15 ml test tube with 6 ml of distilled water and 1 ml of Folin–Ciocalteau reagent and the mixture was allowed to equilibrate for 3 min. After that, 2 ml of 20% Na_2CO_3 (*w*/*v*) was added and heated to 40 °C for 2 min. The absorbance of the blue chromophore was measured at 760 nm using a UV-VIS spectrophotometer, Model 2600 (Shimadzu, Kyoto, Japan). Five extraction cycles were conducted to obtain total polyphenol content recovery. Quantification was carried out using gallic acid at 0–100 mg gallic acid/liter. Means of 5 replications were determined as mg of gallic acid equivalents per gram of dry sample (mg GAE g^{-1} DW).

Effect of water-soluble extract of coco peat and “Super coco” on cucumber seedling development. The experiments were conducted in Tokyo University of Agriculture, Setagaya campus from May to August 2023. A variety of extract solutions of coco peat and “Super coco” were prepared with different dilution ratio as follows. First, deionized water was added to coco peat and “super coco” at a volume ratio of 50:1 (deionized water: coco peat or “Super coco”), and after shaking for 1 hour, coco peat and “Super coco” were removed through the filtration with two layers of cotton gauze (10x extract solution). The 100 times diluted extract solution was prepared by diluting this extract solution with 9 times more deionized water (100x extract solution). In case of 1000 times diluted extract solution, it was prepared by adding 9 times volume of deionized water to the 100 times diluted extract solution (1000x extract solution). Deionized water was used for control.

Cucumber seeds (cv. Yoshinari, Sakata Seeds Co., Ltd., Kanagawa, Japan) were sown on mesh floats edged with styrofoam which was placed on 200 ml of each extract solution in 300 ml plastic cup. The cups were placed in the incubator (LH-220N Nippon Medical and Chemical Instrument. Co., Ltd., Osaka, Japan) at 25°C. After 6 days, shoot and root development were investigated.

Ethylene release and respiration rate of cucumber seedling roots grown in coco peat or “Super coco” extract solution for 10 days, were determined as follows: detached seedling roots were placed in 100 ml Erlenmeyer flask and incubated for 1 hour under dark at 25°C (LH-220N Nippon Medical and Chemical Instrument. Co., Ltd., Osaka, Japan) after sealing with silicon cup. To measure ethylene released, one ml headspace gas was analyzed by gas chromatography using a flame ionization detector (GC-14B, Shimadzu Japan), which was equipped with a Sumpack A column (Shinwa Kako, 2.1m×3.2mmφ, glass column filled with porous poly beads). The injector, column and FID temperatures were programmed at 180, 80 and 200°C, respectively. To measure respiration rate, one ml of headspace gas was analyzed for CO_2 by gas chromatography using a thermal conductivity detector (GC-14B, Shimadzu Japan), equipped with a glass column filled with porous poly beads (Shinwa Kako, 2.1 m×3.2 mmφ). The injector, column, and TCD temperatures were programmed at 150, 40, and 150°C, respectively. The identification of CO_2 was confirmed using standard CO_2 gas.

Effect of coco peat and “Super coco” extract solution on cotyledon development, cytokinin activity, ethylene production, respiration rate and potassium content of cucumber cotyledon. Cucumber seedlings were grown in coco peat and “Super coco” extract of 10 times dilution and cotyledon expansion was recorded with 12 replications from the 3rd to 6th day after sowing. Cell size and the number of stomata on the adaxial surface of cucumber cotyledon 4 days after sowing was checked with SEM observation by Hitachi TM-3000. The cotyledon weight was measured before SEM observation.

The analysis of ethylene and CO_2 was conducted using the same procedure as mentioned above, using gas chromatography (GC-14B, Shimadzu Japan).

The hormonal dynamics in cucumber cotyledons was monitored using cytokinin activity and ethylene release as well as potassium content.

The measurement of cytokinin activity was carried out according to the Fletcher's improved bioassay method (Fletcher et al. 1982). Ten pieces of cotyledons of the 5-day old cucumber seedlings (Tokiwa Shinjibai cv., Watanabe Noji Seed company, Chiba prefecture) were grown at 28℃ in an incubator (LH-220N Nippon Medical and Chemical Instrument. Co., Ltd., Osaka, Japan) under dark conditions in 200 ml of 10 times diluted extract solution of coco peat and "Super coco" in a 300 ml plastic cup. These were excised under dim green light and placed in a 5 cm Petri dish. All the cotyledons were then exposed to 2,000 lux florescent light for 1.5 hours, and extraction was conducted using 3 ml of acetone. Absorbance was recorded at 663 nm wavelength using UV-VIS spectrophotometer Model 2600 (Shimadzu, Kyoto, Japan). The standard curve of cytokinin activity was prepared using benzyladenine solution with 0, 0.001, 0.01, 0.1 and 1.0 ppm concentration (R^2=0.9606).

Potassium content was measured in the same way described in the section 1, using ICP emission spectrometry (ICPE 9820, Shimadzu, Tokyo, Japan).

RESULTS AND DISCUSSION

Chemical analysis of coco peat and "Super coco". The pH of coco peat was rather modest with high EC and high potassium content (Table 1a and 1b). Reduction in potassium content was remarkable for "Super coco", along with lesser total polyphenol, which might be leached away by washing with $FeSO_4$ solution. Fe content increased immensely and pH was reduced which could also be attributed to $FeSO_4$ treatment. "Super coco" can be applicable to alkaline soil to alleviate high pH and supplement Fe which might be difficult to be absorbed in alkaline conditions.

Table 1a. Chemical properties of coco peat and "Super coco"

	pH	EC (mS/cm)	C (%)	N (%)	C/N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
Coco peat	5.7	1.20	40.35	0.49	82.36	0.052	1.915	0.188	0.150	0.058
"Super coco"	4.5	0.12	47.08	0.44	107.46	0.045	0.381	0.258	0.123	0.056

Table 1b. Chemical properties of coco peat and "Super coco"

	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	B (ppm)	Total polyphenol (mg/g) (Gallic acid equivalent)
Coco peat	795.1	34.8	16.5	6.2	25.0.	0.24± 0.04
"Super coco"	4955.1	35.9	16.2	4.2	15.7	0.16± 0.02

Effect of water-soluble extract of coco peat and "super coco" on cucumber seedling development. The results were shown in Fig. 2 and Photo 1 for coco peat, and Fig. 3 and Photo 2 for "Super coco". The higher extract solution of coco peat resulted in more growth of shoot and roots, with almost double size in cotyledons in comparison with the control. The effect was milder in "Super coco", where only 10 times diluted extract significantly promoted the growth of shoot and roots. The root system growth and development rely on potassium ion, since protein synthesis and enzyme activity of root cells need adequate cytoplasmic potassium ion, and cell expansion zone requires turgor pressure, which builds up via osmotically active substances including potassium ion (Sustr et al. 2019). The higher amount of potassium in coco peat extract solution may well result in the enriched growth of cucumber seedlings due to high availability of potassium.

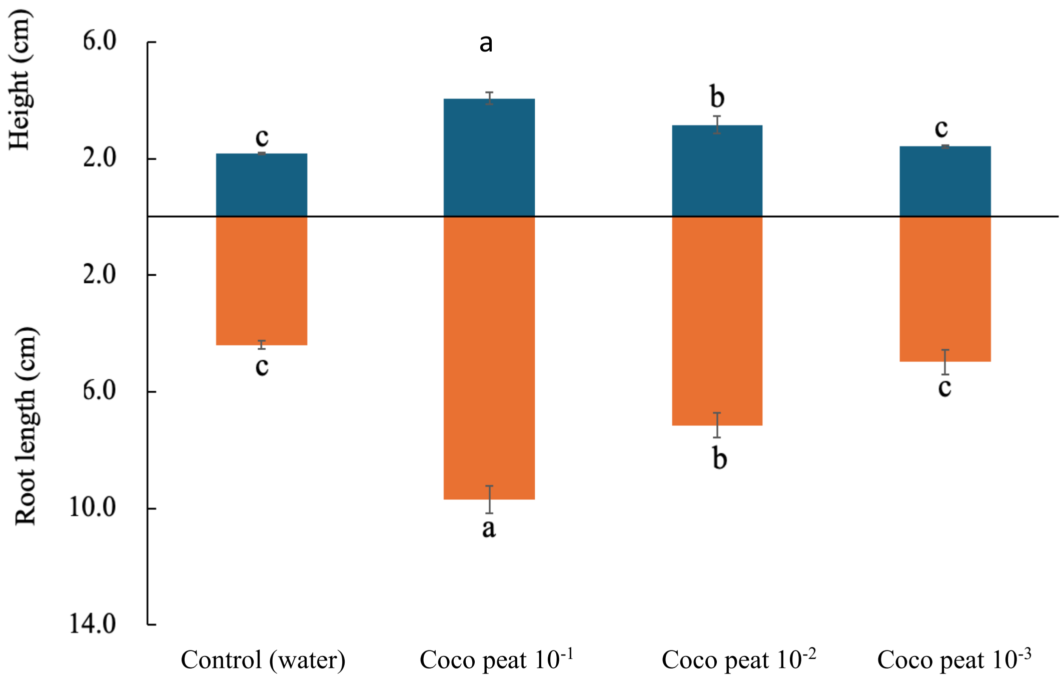

Figure 2. Effect of coco peat extract solution on growth of cucumber seedlings
Different letters indicate significant difference at ≤ 0.05 by Tukey's test.

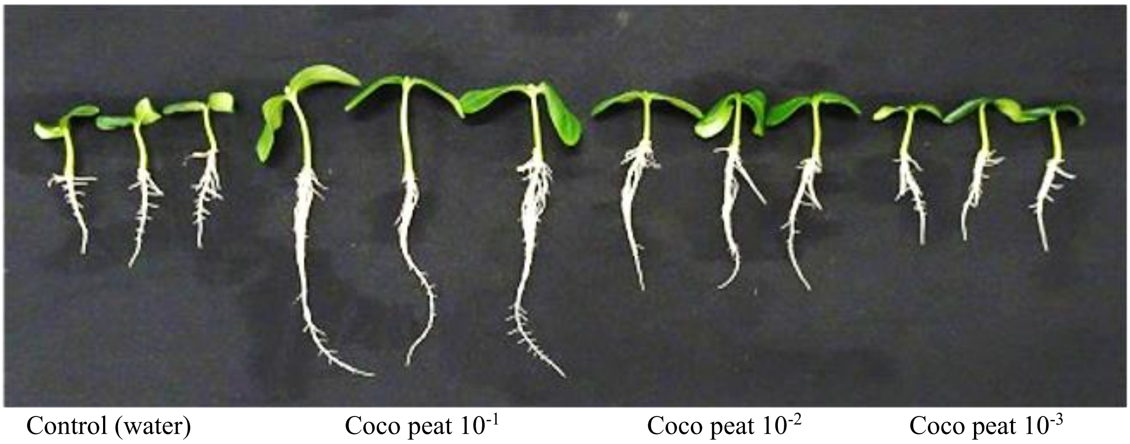

Photo 1. Effect of coco peat extract solution on growth of cucumber seedlings

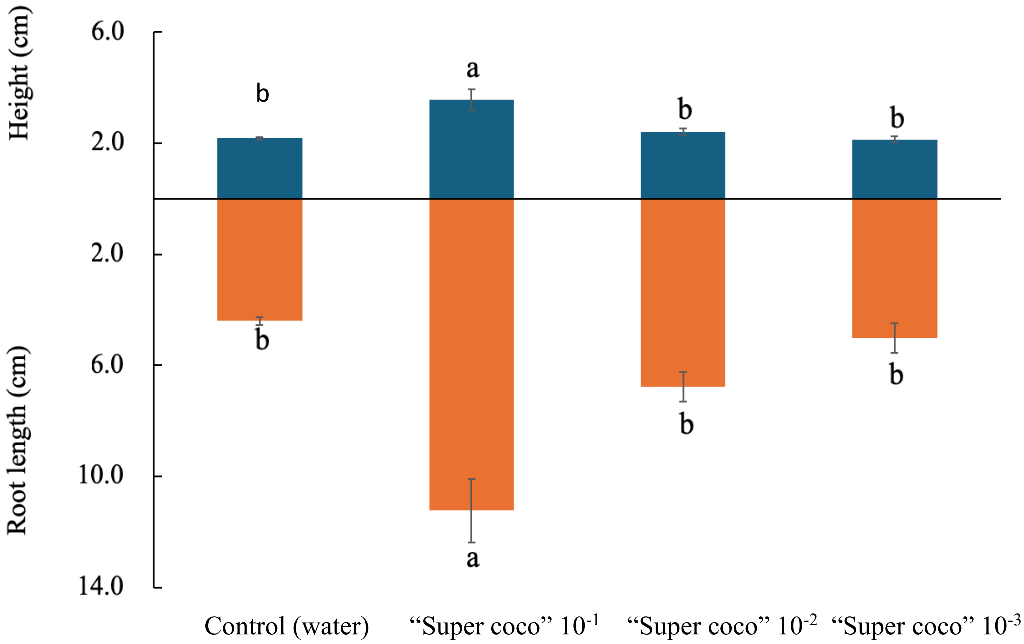

Figure 3. Effect of "Super coco" extract solution on growth of cucumber seedlings
Different letters indicate significant difference at ≤ 0.05 by Tukey′s test.

Control (water) "Super coco" 10^{-1} "Super coco" 10^{-2} "Super coco" 10^{-3}

Photo 2. Effect of "Super coco" extract solution on growth of cucumber seedlings

Effect of coco peat and "Super coco" on cotyledon development, cytokinin activity, ethylene production, respiration rate and potassium content of cucumber cotyledon grown in coco peat and "Super coco" extract solution. The effect of coco peat and "Super coco" extract solution on cucumber seedling growth was exceptional especially in cotyledon expansion both in length and width when grown in the 10 times extraction solution (Fig. 4 and 5), though slight growth retardation was observed in thickness (Fig. 6).

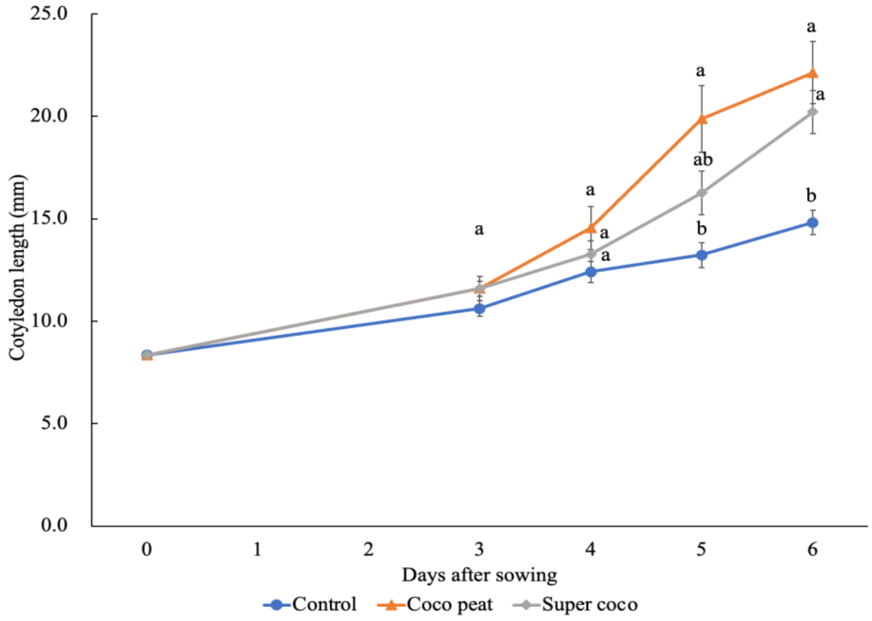

Figure 4. Effect of coco peat and “Super coco” extract solution on cotyledon expansion in apical-basal axis of cucumber seedlings
Data shown are means of 12 replications with SE, and different letters indicate significant difference at ≤ 0.05 by Tukey′s test.

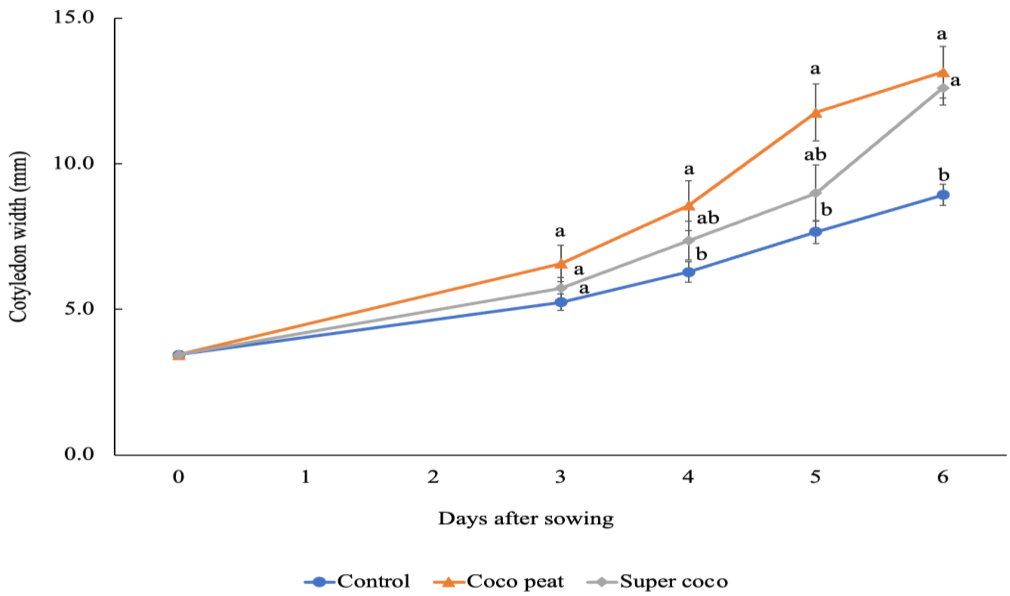

Figure 5. Effect of coco-peat and “Super coco” extract solution on cotyledon expansion in central-marginal axis of cucumber seedlings
Data shown are means of 12 replications with SE, and different letters indicate significant difference at ≤ 0.05 by Tukey′s test.

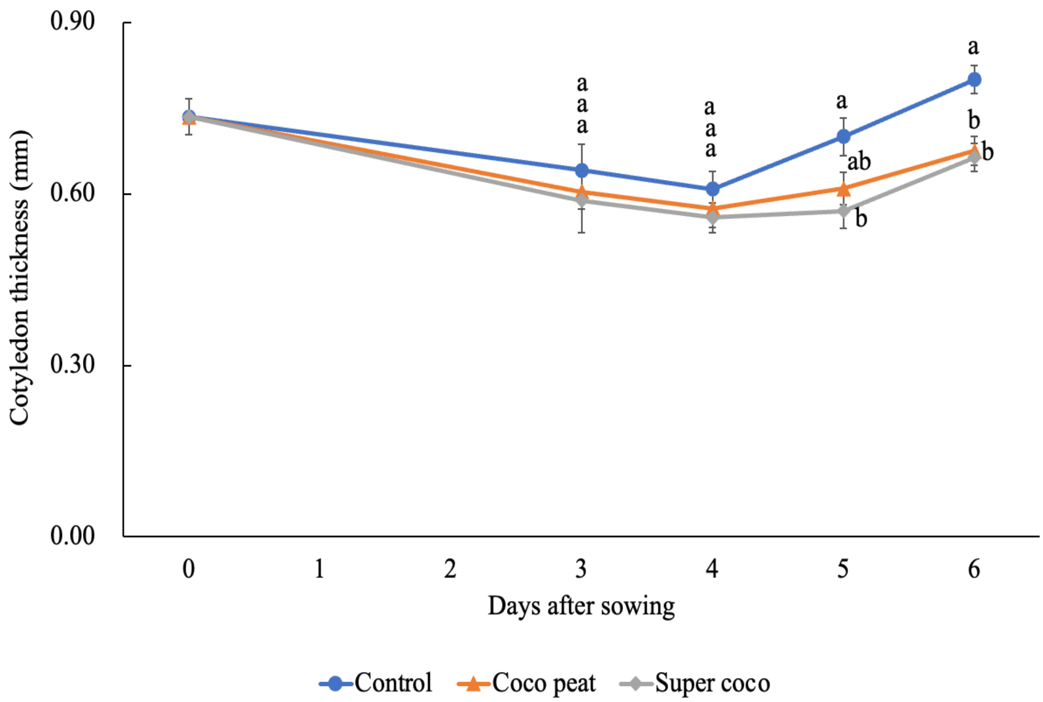

Figure 6. Effect of coco-peat and "Super coco" extract solution on cotyledon expansion in adaxial-abaxial axis of cucumber seedlings

Data shown are means of 12 replications with SE, and different letters indicate significant difference at ≤ 0.05 by Tukey′s test.

The length and width development of cucumber cotyledon reached around 3 times enlargement within 6 days, though almost no growth was observed in case of thickness. The direction of cotyledon expansion is controlled by at least two genetically independent pathways (Tsukaya et al. 1994), but in the present experiment, the rate of cotyledon expansion in length and width occurred almost parallel. Cell separation occurs as a result of traction of epidermal cells during the expansion of leaves and consequently the number of mesophyll cell layers decreases and the volume of intercellular spaces increase (Dale 1998), which might be well fitted with the study results that indicated rapid cotyledon expansion both in apical-basal axis and central-marginal axis was not be accompanied with the increase in the numbers of mesophyll cell layers.

SEM images of cucumber cotyledon 4 days after sowing were shown in Figure 7, illustrating the clear increase in cell size of cotyledon grown in coco peat and "Super coco" extract. The stomata can be seen as thick black lines.

The average cotyledon weight, cell size and number of stomata observed on the adaxial surface of cucumber cotyledon, and the respiration rate of cucumber cotyledon grown in coco peat and "Super coco" are listed in Table 2, and the effect of coco peat and "Super coco" extract solution on cytokinin activity, potassium content and ethylene production of cucumber cotyledon are shown in Table 3.

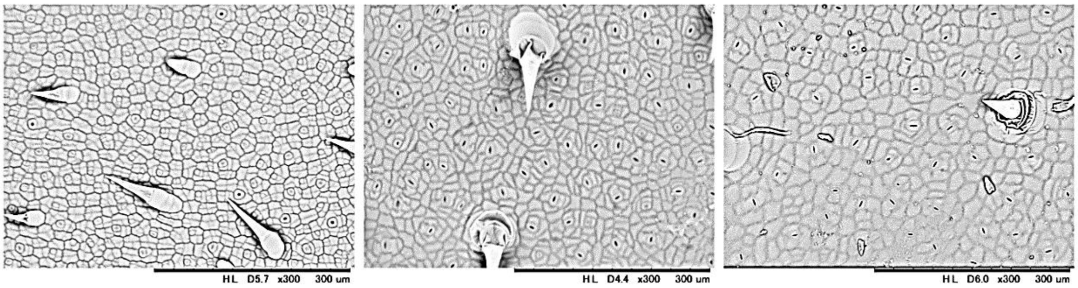

Figure 7. SEM images (x300) of cucumber cotyledons grown in water, coco peat and "Super coco" extract solution (50 times dilution)

Table 2. Effect of coco peat and "Super coco" extract solution on weight, cell surface area and the stomata number on the adaxial surface of cucumber cotyledon at 4 days after sowing

Treatment	Cotyledon (mg)	Cell surface area (μm^2/cell)	Stomata (number/mm^2)
Control	64.2± 2.5 c	136.2± 1.4 c	1.5± 0.5 c
Coco Peat	144.0± 5.8 a	320.0± 7.9 b	25.3± 1.3 a
"Super coco"	111.2± 5.3 b	582.4± 12.6 a	16.0± 0.4 b

Data shown are means ± standard error. Weight and respiration rate of cucumber cotyledon were measured with 12 replications and other parameters were observed with 4 replications. Different letters within the column indicate significant difference at ≤ 0.05 by Tukey′s test.

Table 3. Effect of coco peat and "Super coco" extract solution on cytokinin activity, potassium content, ethylene production and respiration rate of cucumber cotyledon at 7 days after sowing

Treatment	Cytokinin activity (BA equivalent) (ng/cotyledon)	Potassium (K) (mg/cotyledon)	Ethylene (C_2H_4) (pl/cotyledon/hr)	Respiration (CO_2) (µl/cotyledon/hr)
Control	0.30± 0.0 b	0.4±0.0 b	29.5± 2.6 b	29.2± 5.2 ab
Coco Peat	1.08± 0.3 a	3.1± 0.1 a	67.8±13.2 a	45.9± 6.2 a
"Super coco"	1.13± 0.2 a	2.9± 0.1 a	33.2± 5.3 b	26.4± 3.5 b

Data shown are means ± standard error. Cytokinin activity of cucumber cotyledon was measured with 7 replications and potassium content was analyzed with 4 replications. Ethylene production and respiration rate were measured with 6 replications. Different letters within the column indicate significant difference at ≤ 0.05 by Tukey′s test.

The outstanding cotyledon expansion was observed in coco peat and "Super coco" which might be derived from the cell size enlargement. Although cell size was significantly larger in the cotyledons grown in "Super coco" extract solution, the number of stomata and respiration rate were significantly greater in the cotyledons grown in coco peat extract solution, possibly enhanced by higher ethylene production (Serna and Fenoll 2001), which was promoted by high cytokinin level in combination with high potassium content in cucumber cotyledon (Green 1983;Green and Muir 1978). The role of cytokinin-induced cotyledon expansion has been studied by a number of groups from 1970's (Gordon and Letham 1975; Longo et al. 1978; Suresh et al. 1978), showing the crucial role of this phytohormone in hydrolysis of protein and lipids to transform them into amino acids or sugars to be distributed to growing organs in addition to the nucleic acid synthesis function (Tsui et al. 1980).

Inasmuch as the cotyledon is important in seedling development, either as an energy storage organ or photo assimilate providing organ (Lovell and Moore 1971; Penny et al. 1976), increased respiration rate in cucumber cotyledon grown in coco peat extract could result in quick mobilization or transference of storage materials to the developing organs (Bain and Mercer 1966; Penner and Ashton 1967). At the same time, increased number of stomata obtained in cotyledons grown in coco peat and "super coco" extract, might stimulate photosynthetic activity (Zelitch 1969) which promotes further growth in roots and shoots.

The promotion effect of seedling development induced by coco peat and "Super coco" extract solution derived from the promoted absorption of potassium encouraged to develop enriched root system, resulted in the enhancement of cytokinin production at roots and distribution to cotyledons which finally promoted cotyledon expansion, contributing to the early establishment of seedlings.

CONCLUSION

Sri Lankan coco peat showed great potential to be utilized as a horticultural or nursery planting medium to promote seedling development, with enhanced root development and cotyledon expansion in case of cucumber seedlings. It increased cytokinin activity, potassium content, ethylene production and respiration rate in cucumber cotyledon, leading to the early development of seedlings. The "super coco", produced from coco peat washed with 13mM $FeSO_4$ solution, exerted lower content of potassium and higher amount of iron in comparison with normal coco peat, and it might be adaptable in alkaline soil to adjust pH and promote Fe absorption. Sri Lankan coco peat and "Super coco", a processed coco peat washed with $FeSO_4$ solution, could therefore be a promising alternative to substitute peat moss.

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