EFFECT OF SOILLESS MEDIA ON GROWTH AND SOME PHYSIOLOGICAL TRAITS OF RUBBER (*Hevea brasiliensis*) SEEDLINGS

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ABSTRACT

This study evaluates the effect of newly prepared soilless media on rubber (Hevea brasiliensis) as alternative growth media to some of the poor soils in the tropics (including those used in rubber nursery in Malaysia). The materials used as media were selected based on their good physiochemical properties and local accessibility. Three different media and soil, which was designated the control treatment, were used. The soilless media significantly influenced the growth and biomass production of the rubber seedlings. The highest rate of seedling growth was recorded in the medium of 10% burned rice husk (BRH), 30% peat moss and 15% vermiculite (coded as M1). The effect was equally noticeable in root morphology, especially with regard to root length, surface area and the number of tips. The pH and EC were 6.5 and 2.3 dS m^{-1}, respectively. Higher concentrations of N and P were apparent in this medium while the Mg concentration was only significantly higher in soil (the control). Meanwhile, the urea-N used in the medium was lower than that used in the other media. However, the same amounts of rock phosphate (CIRP) were used in the respective media, and significant root growth was recorded. The least amount (5% N) used in the best medium (M1) could be maintained to reduce the use of fertilizer. These results show that the soilless mix that includes BRH with less urea-N as fortification would greatly increase plant growth. This is because it releases more essential plant nutrients due to the favorable pH when compared to the acidic soils used in many plantations in the tropics. The result shows that the soilless media used in this study could be adopted for rubber nursery seedlings.

Key words**:** Soilless, *Hevea brasiliens*, physiology, seedlings, rubber

Introduction

Soils used for rubber in the tropical Asia countries has been categorized as low organic C content and highly weathered as a result of overutilization in the last 100 years (Dharmakeerthi et al., 2012). This has been attributed to the poor growth of rubber (*Hevea brasiliensis*) especially at nursery stage which sometimes leads to plant death or long term irreparable root and shoot damage. Both the soil medium as well as the type of container (polybag) used may contribute to the poor performance of rubber seedlings. Many soils used for plantation crops in the tropics including Malaysia, are acidic and requires a lot of fertilizer for adequate support of plant growth (Shamshudeen, 2010). As a result, soilless growing system especially for young plants has been widely considered as an alternative growing medium to soil (Van Os and Postma, 2000) while root trainers are being considered as replacement for polybags.

Soilless may serve as an alternative planting medium because it reduces incidence associated with soil borne diseases and pests which leads to reduced use of soil fumigant. It improves water use efficiency and fertilizer use due to its high water-holding and cation exchange capacity (Cantliffe et al., 2007). Soilless medium or substrate with good physical and chemical properties gives farmers planting opportunity where soil conditions are certified to be detrimental to crop cultivation (Rodriguez et al., 2006). The natural rubber industry at present adopts a new approach such as the use of soilless media in nursery planting and the use of root trainers. Root trainers are conical cell plastic having 5 to 6 ridges inside and a small drainage hole on the bottom with various volumes ranging from 100, 150, 250, 300, and 600 cc each. It is scientifically designed to aid strong anchorage, excellent wind fastness, much faster growth; early maturity which may result in almost 100% survival upon transplanting.

Lateral roots of rubber seedlings grown in polybags grow in all possible directions and get themselves entangled into a mess (Josiah and Jones, 1992). However, growth and productivity of the plant is directly proportional to the growth of the lateral roots of *Hevea brasiliensis*. Thus, plant root damage is currently attributed to the heavy soil which leads to poor drainage and suppression of the plant root system which exposes plants to soil borne diseases. The effects which continue throughout plant's life span (Beattie and White, 1993). The use of plastic container (root trainers) has not received much attention in the tropics. This leads to poor shoot and root development of the plants (Miller and Jones, 1995). It also leads to poor performance of the plants when transplanted to the field.

Proper nursery management of plants at their early stage influences good establishment in the field (Baiyeri and Mbah, 2006). However, adoption of any container for planting seedlings requires the right soilless growing media. Organic and processed materials commonly adopted in the production of soilless for tree seedlings are rockwool, perlite, potting mix, vermiculite, expanded clay, pine bark, coconut coir, burned rice husk, EFB compost, peat moss and composted plant materials (Surrage et al., 2010). The materials must meet the optimum C/N ratio. In addition, nitrogen in the form of nitrogenous fertilizer such as urea and rock phosphate is also required (Mohanan and Sharma, 2005).

This is to ensure adequate supply of nutrients, maximum support and anchorage to the plants for plants healthy growth while serving as place of growth (Agbo and Omaliko, 2006). Soilless techniques in most greenhouses have been in practice for most plants including nursery trees. This is due to its superior physiochemical characteristics coupled with lower infestation rate of pathogenic pest at the initial stage. Plant fertilization and irrigation is equally easy to satisfy under this system (Raviv et al., 2002). Plant grown in commonly used polybags and soils, immediately strangled and distorted as a result of root coiling especially when the root reach the lower part of the polybag (Ginwal et al., 2001).

Considering the practical convenience and cost involved soilless and root trainer one- whorl plants had proved to be an ideal planting material for commercial planting of *Hevea* (George et al., 2012). Thus, the hypotheses for the study were that soilless media help; physical properties of soilless aids root penetration and increase quantity of lateral roots and shoot growth. This work relies on the previous study indicating acidic and low nutrient of many tropical soils and root damage caused by the type of containers used for nursery planting especially in the tropics.

Materials and Methods

Soilless Mix Combination
Soilless growing media were prepared using processed and locally sourced materials. Each soilless growing media contains proportions of the materials at different percentage. The media (M1) contains vermiculite, perlite, coconut husk, compost, peat moss, burnt rice husk, CIRP and urea. (M2) contains vermiculite, perlite, EFB, coconut husk, peat moss, sugarcane bagasse). Both media were fortified with Christmas Island Rock Phosphate (CIRP) and Urea. The materials except coconut husk, EFB compost, peat moss and burn rice husk were ground to pass through to 2-mm screen for easy decomposition. A commercial soilless medium as (M3) made for rubber seedlings by Accuplast Company in India was incorporated into the study for comparison with the newly produced soilless media (M1 and M2) while Oxisol soil, which had been categorized as the best soil for rubber plantation based on USDA soil (M4) as control evaluation.

Berkeley method was adopted as composting method for M1 and M2. This was based on suggested material composition and method for preparation of growing media for tree seedling nurseries by Miller and Jones (1995). The materials were spread in layers at the Universiti Putra Malaysia compost site and thoroughly mixed. Initial moisture content of the media was determined before adding more water. Moisture content at field capacity indicated 33.5% and 38% Media 1 and 2 respectively. Thereafter, additional water was added, thoroughly mixed and covered. The media were turned every week until ten weeks. Maturity of the media was determined when it was dark, crumbly, broken down with temperature at 30^{o}C and when the moisture content reached 50% respectively.

Trial Site and Planting Materials
The experiment was conducted under rain shelter at the field No. 2 Universiti Putra Malaysia. Rubber seeds from clone RRIM 2024 were germinated on seed beds size with 15 cm high, 90 cm width and 2 meter long under rain helter with 50% shading using 50% shade netting. The bed was filled with sawdust and 2 mm size river sand. Seeds were thoroughly washed, spread and arranged side by side touching one another in a single layer and gently pressed about 1cm deep. It was manually irrigated morning and evening. Germination commenced at day 6 and was completed at day 21. Germinated seeds were transplanted into root trainers size 26 cm length and 600 cc filled with 400 g of the three when radicle was noticed before emergence of leaves. Polybags 9 × 6 cm were filled with 5kg of Oxisol soil used as control. The design was RCBD with four treatments containing five replications. A week after transplanting, mixture of fertilizer solution and fungicides containing Bayfolan 5 mL, MZ – 45 2 g and Brightconil 75 WP 2g in 800ml of water were consistently sprayed as foliar spray two times weekly.

Data Collection
Following data were collected during and at the end of the experiments; Plant height and stem girth size was measured using standard measuring tape and digital Veneer Caliper respectively. Numbers of leaves were manually counted monthly. Plant fresh biomass was weighed (g) to constant weight of 0.01g. For the dry weight, plant tissues were oven-dried at 70°C and equally

weighed (g) to constant weight. Root/shoot ratio was also determined using the following equation (Hunt, 1978). Root to shoot ratio = (Total Root Dry Weight/Total Shoot Dry Weight).

Leaf area was measured at the end of the experiment using a LI-COR-3100 leaf area meter (LI-COR, Lincoln, NE, USA). Chlorophyll content was determined by first taking samples with a leaf punch. The 1.0 cm leaf disks were collected in scintillation bottles containing 15 ml aqueous 80% acetone and kept in dark for two weeks. Thereafter, the absorbance was determined at 664 and 647 nm using a UV-2550 visible spectrophotometer. Actual chlorophyll concentration was determined using described and published equation by Coombs et al. (1987).

In addition, leaf gas exchange was taken using portable photosynthesis system LI-6400XT device (LI-COR, US) on the third fully expanded leaves from top of each plant. Net photosynthetic rate (P_n) [µmol (CO_2) m^{-2} s^{-1}] and stomatal conductance (G_s) were recorded. Leaf temperature ranges between 27 and 28°C, relative humidity was 79% and light levels were 150 and 345µmol (CO_2) m^{-2} s^{-1} during the measurement period.

Foliar analysis was carried out according to rubber industry foliar sample techniques which specify four basal leaves from the first sub-terminal whorl for the leaf samplings. The leaves were oven dried at 60°C and ground. A 0.25 g of the weighed ground dried samples was put into digestion tube. Then 5 ml concentrated sulphuric acid (H_2SO_4) was added, shaken and left for about 2 hours to adsorb moisture. Thereafter, the mixture digestion tubes were placed in the digestion block at the temperature of 450°C in the Fume Chamber for approximately 45 minutes.

The digestion tubes were removed and allowed to cool, after which, 2 ml of hydrogen peroxide (H_2O_2) was added and the heating process was repeated in the Fume Chamber. After the stipulated heating period, the sample in the tube became colourless. The solution was left to cool and later diluted with distilled water to make up 100ml. The samples were analysed for N, P and K using Auto Analyzer, while Mg was analysed with Atomic Absorption Spectrophotometer (Perkin Elmer, Model AAS 3110) Lau *et al,* (1990). At the end of the experiment, after the last harvest, root morphological traits were examined. Roots were gently separated from the soilless media and containers, washed thoroughly with water to remove excess medium. Root samples were scanned using WinRHIZO pro software root analysis equipment. Parameters measured in all the treatments were total length, average diameter, surface area, total root volume and number of tips.

Statistical Analysis

Data obtained were subjected to analysis of variance at $p<0.05$ to determine the treatment effects. Means were seperated by the Fisher LSD test.

Results and Discussion

The result showed differences in both plant growth and biomass of rubber seedlings grown in various soilless media (Table 1). Plant growth and biomass production including plant height (cm), girth size (mm) number of leaves and fresh and dry weight (g), root/shoot ratio (RSR) were greatly influenced by the types of media used. Plant grown on M1 recorded highest plant height (25.40 cm) followed by M4 (23.0 cm). Plant grown on M2 had the lowest height. The influence of M1 on plant height may be related to (10%) burn rice husk in the media. Inclusion of rice husk in soilless media provides potassium and silicon elements and quality soilless media for greenhouse plant can be achieved (Kamenidou et al., 2008). In addition, Chaparro-Torres et al., (2006) reported that burned rice husk (BRH) when compared to other substrates, promotes plant growth.

Table 1: Growth and dry biomass of immature rubber as influenced by soilless media

Soilless/soil-based media	PH (cm)	GS (mm)	NoL	SFW (g/plant)	RFW (g/plant)	SDW (g/plant)	RDW (g/plant)	RSR
M1	25.40a	5.54a	33.80a	22.14a	10.35a	7.93a	1.89a	8.88a
M2	19.6b	3.82b	29.40a	15.67b	6.09b	4.55b	0.80b	3.74b
M3	21.4b	3.77b	18.80b	5.09c	2.92c	2.28c	0.70b	1.50b
M4	23.0ab	3.12b	16.20b	6.65c	3.06c	1.24c	1.26ab	2.32b
LSD	3.8424	0.8016	5.5961	2.6669	2.9975	1.7629	0.8932	4.5174
P >F	0.0371	0.0002	<.0001	<.0001	0.0005	<.0001	0.0488	0.0264

Mean values followed by the same letter within the same column are not significantly different at $p<0.05$, based on a least significant difference test (LSD).

PH; plant height, GS; girth size, NoL; the number of leaves, SFW; Shoot fresh weight, RFW; root fresh weight, SDW; shoot dry weight, RDW; root dry weight, RSR; root: shoot ratio.

The same scenario was equally recorded in plant stem diameter where M1 recorded highest values (5.54 mm) and significantly different at $p<0.0002$ while plant grown on other media were found to be the same with each other. Furthermore, plants in M1 and M2 were found to influence number of leaves, highly significant at $p<.0001$ and equal with each other (33.80) and (29.40) respectively. The performance of these media may be attributed to similar materials used in the two media. This is similar to the result by Gonbad et al., (2013) who stated that media containing peat moss, vermiculite, perlite increases number of leaves and similarly influence other growth traits. In addition, both fresh and dry biomasses were affected by M1 and significantly different when compared to other treatments. Addition of peat moss, vermiculite and perlite in soilless media may positively affect plant growth and could reflect in shoot dry weight because these materials especially perlite and peat moss increases air-filled and porosity in the container filled soilless media (Pill and Goldberger, 2009).

Similar responses were recorded on the physiological parameters of plants grown on the respective media (Table 2). For instance, net photosynthesis ranges between 5.9 and 9.9 µmol.m^{-2}s^{-1} with no significant difference among media. Similarly, there was no significant difference among media for stomata conductance. On the other hand, light intensity ranges between 143 and 194 µmol.m^{-2}s^{-1}, and M4 (Control) recorded the highest value. The amount of shade sometimes determines growth increment or reduction of plants (Terashima and Hikosaka, 1995). Where it favors plant, the effects would be quickly noticed. This is similar to the report by Valio, (2001) who noted that amount of shade influences girth circumference as previously observed in plant grown in M1.

Apart from other factors that may interfere in plant growth, Nugawela et al., (1995) reported a correlation between CO_2 assimilation rate and planting conditions. Plants experiences reduced dry biomass and this affects vegetative growth due to the reduction in CO_2 assimilation rate when planted under artificial shade such as green or shelter house. On the other hand, container and media interaction may affect fertility, pH, soluble salts, bulk density and root zone volume (Hockenberry and Cunliffe, 2004). These may greatly influence plant growth on soilless substrate. As a result, in choosing soilless growing media, environmental conditions and practices has always been a concern (Del and Gómez, 2009). Values of leaf area of seedlings grown on M1 were significantly higher compared to other treatments.

Table 2: Physiological responses of immature rubber grown in different soilless media

Soilless / soil-based media	Nett Photosynthesis (µmol.m^{-2}s^{-1})	Stomata Conductance (mol.m^{-2}.s^{-1})	Light Intensity (µmol m^{-2} s^{-1})	Chlorophyll (mV)	Leaf Area (cm^2/plant)
M1	9.722	0.23	145.83b	27.75a	720.66a
M2	8.596	0.23	143.64b	26.05a	231.21b
M3	5.934	0.23	155.27b	17.23ab	84.63b
M4	6.934	0.19	194.79a	14.09b	136.89b
LSD	3.2325	0.1022	15.506	11.037	168.29
P >F	0.1011	0.8146	<.0001	< 0.05	0.0003

Mean values followed by the same letter within the same column are not significantly different at p<0.05, based on a least significant difference test (LSD).

Leaf growth, leaf area and photosynthetic rate may be influenced by the level of N in the soilless media. This ensures control of photosynthetic elements and production of carbohydrates. Plant vegetative characteristics including sizes and number of plant organs may equally be greatly influenced (Enggels and Marschner, 1995) as previously noticed in seedlings grown in M1 and M2. In addition, chlorophyll content ranges between 14 and 27 mV and plants on M1 recorded highest value. Similar scenario was noticed in leaf area of plants grown on the same media. There may be probably a strong correlation and influence between chlorophyll and leaf area because the former indicate some level of N accumulation in leaves (Nageswara et al., 2001). More so nitrogen use efficiency is said to be attributed to leaf area and other growth traits such as plant height (Hirel et al., 2001). Generally leaf gas exchange may equally responsible for the seedlings positive response especially the seedlings grown on M1 especially in the dry weight. Similar to the report by Thornley and Johnson, (1990) who reported that increase in dry matter (DM) could be attributed to photosynthesis and nutrient concentration.

Foliar analyses showed how respective media significantly influenced the nutritional status of the plants and overall plant growth. M 3 recorded higher amount of P (0.33) (Table 3). M 2 on the other hand recorded higher N. This might be due to higher amount of Urea N and other materials used in the media. Increase in N and P could increase leaf growth and chlorophyll content while its decrease may also be detrimental to crops (Sinclair and Vadez, 2002). Furthermore, higher P and K were equally recorded in M3. There was a similar concentration of Ca and Mg in plants on all the media except M4 soil (Control). Though, concentration of these elements sometimes may be advantageous or detrimental to the plants (Salisu et al., 2013). Other factors which may equally stimulate plant growth and development are better gaseous exchange; improved drainage and uniform extension of root systems sometimes are more advantageous than other growth factors (Pinamonti et al., 1997).

Table 3: Nutrient concentration of soilless media in immature rubber

Soilless/soil-based media	N (mg.g^{-1})	P (mg.g^{-1})	K (mg.g^{-1})	Ca (mg.g^{-1})	Mg (mg.g^{-1})
M1	2.59b	0.23b	1.06b	0.80a	0.19a
M2	3.03a	0.26b	0.99bc	0.84a	0.19a
M3	2.78ab	0.33a	1.26a	0.77a	0.20a

M4	1.82c	0.13c	0.82c	0.68b	0.10b
LSD	0.4163	0.0344	0.1781	0.093	0.0447
P >F	0.0018	<.0001	0.006	0.022	0.0055

Mean values followed by the same letter within the same column are not significantly different at $p<0.05$, based on a least significant difference test (LSD).

All the media significantly influenced root morphology of plants. M1 produced significantly higher root length followed by M3 (Table 4) while M4 (Soil) which is the control recorded the lowest root length. Similar scenario was recorded in surface area. On the other hand M1 had a significantly higher number of tips than other media. The performance of M1 could be as a result of higher amount of vermiculite, perlite and peat moss in the media. Combination of these materials aids anchorage of young roots and promotes faster root growth (Hartman et al., 2007).

Table 4: Root morphological traits of immature rubber as influenced by soilless media

Soilless/soil-based media	Root length (cm)	Root Vol (cm^3)	AvgD (mm)	Surface Area (cm^2)	Number of Tips
M1	2158.9a	3.867	1.02	322.62a	9030a
M2	886.3ab	2.749	0.97	166.72ab	2655b
M3	290.1b	1.651	0.89	55.65b	1145b
M4	251.4b	2.022	1.14	79.38b	741b
LSD	1326	2.6757	0.3102	179.83	5110.7
P >F	0.0386	0.2865	0.3437	0.0384	0.0235

Mean values followed by the same letter within the same column are not significantly different at $p<0.05$, based on a least significant difference test (LSD).

Conclusion

In conclusion, the results clearly indicate that planting medium (M1) is the most suitable for growing rubber seedlings especially when seeds are considered for raising planting stocks. Based on nursery observation and as shown in the results, plants grown using this medium may experience little or no physiological stress. In addition, no leaf scorching and/or early nutrients deficiency was noticed during the study. The amount of Urea N and other soilless materials used in M1 significantly impact plant growth positively. As such the rates used could be maintained for preparation of this medium. At this stage of rubber, root morphology may not be negatively affected when the new media is adopted because no biological effect was noticed in the roots after final harvest and significant results such as optimal growth, vegetative development and quality and quantity of lateral roots of rubber seedlings would be achieved compared to soil (Control). Therefore, the media is recommended for use in nursery plantation especially in the tropics where some of the soils are certified to be acidic and negatively impact plant growth.

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