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RESEARCH ARTICLE

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SUCCESSFUL INCORPORATION OF CRUSHED PINE (PINUS CARIBAEA) LEAVES INTO COCONUT COIR DUST NURSERY MEDIUM FOR VEGETABLE SEEDLING **PRODUCTION**

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ABSTRACT

Different soilless media are used in vegetable nurseries for high quality seedling production. In the current research, potential of incorporating crushed pine leaves (CPL) into coconut coir dust (CCD) as an economical and sustainable media alternative for vegetable nursery trays, was investigated. CPL was used as mixtures with CCD at the ratios of 4:0, 3:1, 2:2, 1:3 and 0:4 (Treatment 1-5). Suitability of each treatment as soilless medium was investigated by evaluating their physical and chemical properties as well as biodegradability. Growth performances of the seedlings grown in the treatments were evaluated using brinjal, capsicum, 'elabatu' (Solanuminsanum), knolkhol and tomato. The highest water holding capacity, pH and biodegradability (6.21ml/g; 7.38; 12.6%) were observed in CCD and lowest values were in CPL (1.30ml/g; 5.18; 8.6%). The highest air porosity and bulk density were in CPL (36.7%, 0.32g/cm³) and the lowest were in CCD $(2\%, 0.23g/cm^3)$. Mixing of these two materials brought those parameters to moderate values which were appropriate for vigorous plant growth. Capsicum, knolkhol and tomato showed higher seedling vigour index, growth rate and root lengths in T2 and T3 mixtures. Brinjal showed the highest values of growth rate and root length in T3 and T4, and seedling vigour index in T3. 'Elabatu' showed highest values of growth rate and root length in T4 and T5, and seedling vigour index only in T4. The result concludes that, CPL can be used in nursery medium without any harm to the plants but incorporation of CPL with CCD improves the properties of growth media and assures better seedling growth.

KEYWORDS

Bio-degradability, chemical and physical properties, crushed pine leaves, growth rate, media mixtures, seedling vigor.

1. Introduction

Production of vegetable seedlings in soilless nurseries is becoming popular rapidly as intensive nursery management provides several advantages over to soil-based nurseries (Raviv and Lieth, 2008). Soilless nurseries protect seedlings from adverse climatic conditions, pests and disease infections during seedling stage. Further, production of quality seedlings with appropriate plant characteristics is essential to get a healthy, uniform population of vegetables and optimum harvest at the end. Coconut coirdust (CCD) is a byproduct of coconut industry and it has long been a standard component of soil-less media due to its characteristic plant producing ability (Meerow, 1995; Abad, et al., 2002). However, the limited production of coconut-based products and higher trading in the export market made the Sri Lankan CCD production insufficient to cater the local demand. Farmers in main vegetable growing areas, where coconut plantations are not common are paying over prices for CCD in the seedling production. This raised the necessity of identification of alternative materials which are easily available and equal in performance as CCD in flower and vegetable nurseries. Researches on alternative growing media for soilless culture are in progress throughout the world and different substrates of biological origin have been proposed as soilless media (Mugnai et al., 2007; Kraska et al., 2018). The adaptability of proposed media has been evaluated based onthe plant growing ability, economic viability, environmental safety and availability.

Pinus species have been introduced to Sri Lanka as a forest tree during1960's for reforestation of catchment area of major rivers and reservoirs and *Pinus caribaea* is the most common species found in these plantations which were established with several economic expectations (Bandarathilake, 1989). However, the expected benefits from those plantations have not been accomplished. Several environmental problems such as poor regeneration of understory vegetation and lower soil fauna have been reported in pine forests (Nissanka et al., 2005). It suppresses under growth due to high allelopathy (Rice, 1984, Kimura, 2015). Accumulation of pine needles (Pine leaves) on forest floor leads to infertility of soil (Sharma et al., 2011). Dropped pine leaves are highly vulnerable to forest fires and poorly decomposing leaves act as hydrophobic cover in the ground thus minimizing water infiltration and

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increasing runoff. Removal of pine leaf layer would bring a remedy for this problem. Therefore, a series of studies was carried out to investigate the possibility of using pine leaves in agriculture.

Crushed pine leaves (CPL) were used as rooting medium of Draceana sanderiana 'White' and Cordyline fruticosa 'purple compacta' successfully (Karunananda and Wasala, 2010). Although this medium has several positive attributes such as good aeration, less compactness, good porosity and ideal pH and EC for plant growth, frequent watering is needed due to its poor water holding capacity. CCD is a material with higher water holding capacity, which will effectively improve water holding capacity of media mixtures (Fazlililahi and Ahmad, 2017). Improved CPL medium by overcoming minor weaknesses may offer good hope for vegetable farmers while minimizing the problems associated due to accumulation of pine leaves on the forest floor of main vegetable growing areas of Sri Lanka. Poor decomposition rate in tropics is a prominent feature of pine leaves due to its hard lignocellulosic outer cover (Dharmawan et al., 2016). Application of strong force helps to break lignocellulosic layers of pine leaves. Poor decomposition is a positive attribute of a good candidate in plant growth substrates or mixtures used in soilless media. In the current study, different combinations of CPL and CCD were evaluated to develop a suitable nursery medium for soilless vegetable seedling (plugs) production.

2. MATERIALS AND METHOD

The experiments were carried out in the Agronomy laboratory and plant houses of Horticulture Research and Development Institute (HORDI), Gannoruwa, Peradeniya, Sri Lanka and in the agriculture field of Jiffy Private Limited, Kobeigane, Sri Lanka using same experimental materials during 2018 and 2019.

2.1 Preparation of growth substrate using *Pinuscaribaea* leaves

Pinus careabia leaves were collected from Pine plantations of the Hanthana mountain range and transported to the experiment site at HORDI, Gannoruwa, Peradeniya. Leaves were sorted and crushed into small particles using a compost crushing machine (Model 8, Jinasena Private Limited, Sri Lanka). Small particles were collected separately and unbroken leaves were sent again through the crusher until average particle size becomes<1cm. Sieves with different-sized meshes were used to separate particles. Crushed and chopped pine leaf particles (CPL) of 1-3mm were isolated for this study. Coconut coir dust (CCD) obtained from a coir factory located in Kobeigane, was used to mix with CPL as the basic experimental material. Dried CCD was sieved and particles of 1-3mm were separated for the experiment. Both materials (CCD and CPL) were subjected to buffering process to remove tannin and excess Na+ and K+ions as done by commercial CCD substrate producers (Wittman, 2015). In the buffering process both media were thoroughly washed with clean running water followed by soaking with 50ppm Ca (NO₃)₂ solution for 2 days. The materials soaked in the Ca (NO₃)₂ solution was agitated slowly to speed up the cation replacement process. Both buffered substrates were heaped separately to drain out excess water.

2.2 Treatments

Five media mixtures were prepared as treatments from T1 to T5 by combining CCD: CPL at v/v ratios of 4:0, 3:1, 2:2, 1:3 and 0:4 respectively.

2.3 Evaluation of chemical and physical properties in the treatments

Following chemical and physical properties which may affect the plant growth in soilless media were determined in each treatment.

Chemical properties -pH and EC of each sample were measured with the help of pH and EC meters (model 86150AZ) using the method described (Barrett et al., 2016).

Physical properties – Total porosity (TP), air filled porosity (AFP), water holding capacity (WHC) and bulk density (BD) of each treatment were determined using the method described (Yahya, et al., 1997).

2.4 Evaluation of Biodegradability

Biodegradability of each treatment was evaluated by assessing decomposition rates of the treatments. This study was performed in

connection with the experiment of evaluation of tomato seedling growth conducted at Gannoruwa and same treatments were used here to collect the readings on media decomposition. Volume of a planting hole of a nursery tray (78.5 cm³) and dry weight of each media mixture (T1-T5) needed to fill a nursery hole (X1) were recorded at the beginning. Then the nursery trays filled with oven dried media mixtures were used for tomato seedling growth evaluation experiment. Seedlings were watered carefully without disturbing the medium. After 6thweek tomato seedlings were removed carefully leaving all medium particles in the nursery hole. Growth media mixture in each nursery holes were collected separately to metal core rings, oven dried at 105 °C for 24 hours and weighed (X2). Weight loss of used media mixtures was calculated after six weeks using following formula.

Weight loss % due to biodegradation = $\frac{(X_1 - X_2)}{X_1} 100$

2.5 Evaluation of seedling growth of each media combination

Evaluation of seedling growth under above treatments was done using tomato (Var. Padma hybrid) and capsicum (CA8) at Jiffy Private Limited, Kobeigane and repeated using tomato (Var. Padma), knolkhol, brinjal (Solanum melongena) and 'Elabatu' (Solanum insanum) at HORDI, during 2018 and 2019. In the trial at Kobeigane, nursery trays with square shape holes (2x2 cm) were filled with each treatment medium and 20 holes together were used as one experimental unit. In the trial at Gannoruwa. nursery trays with cylindrical holes (5cm diameter and 4cm height) filled with each treatment medium were used for tomato and 'elabatu' and 12 holes together used as one experimental unit. Evaluation of seedling growth of brinjal and knolkhol was done in the same site and nursery trays with square shape holes (2x2x2 cm) filled with each treatment medium were used. Twenty holes together were used as one experiment unit. In both locations, Completely Randomized Design was used with 4 replicates. Prepared nursery trays were placed in a protected house and watered well before seed sowing. Seeds were sown at the rate of 2 seeds per hole. Throughout the experiment, plants were sprayed with 20N: 20P: 20K +TE foliar fertilizer mixture. A seed with a radical longer than 2mm was considered as germinated and seedling growth data such as plant height and leaf number were recorded for 5 weeks along with seedling survival rate. At the end of the 5th week all plants were uprooted and root length of each plant was measured. Plant relative growth rate (RGR) of each treatment was calculated by using the following equation given (Hoffmann and Poorter, 2002).

$$RGR = \frac{(ln H2 - ln H1)}{(t2 - t1)}$$

t1 -time 1 (in days), t2 - time 2 (in days), H1- height at t1, H2 -height at t2

Seedling vigor index was calculated by the formula given by Adebisi *et al.* (2010).

Seedling Vigour Index =
$$\left(\frac{Seedling\ length*Germination\ percentage}{100}\right)$$

Statistical evaluations were performed using SAS statistical package (SAS portable 9.1 software). One-way variance analysis (ANOVA) was implemented by general linear model procedures to test the differences among different treatments and means were separated using LSD test at an alpha 0.05 level.

3. RESULTS AND DISCUSSION

3.1 Physical properties of the treatments

CCD and CPL showed dissimilarities in physical properties when use as growth media. Higher WHC was observed in pure CCD. In contrast to that higher BD, TP and AFP were observed in CPL (Table 1).

Table 1: Physical properties of the treatments								
Treatment (CCD:CPL)	Bulk density g/cm³	Total porosity %	Air filled porosity %	Water holding capacity ml/ cm ³				
T1 (4:0)	0.23	78	2.0	6.21				
T2 (3:1)	0.22	79	12.4	4.33				
T3 (2:2)	0.23	77	19.2	2.89				
T4 (1:3)	0.23	80	24.4	2.63				
T5 (0:4)	0.32	86	36.7	1.30				

N=5

Compactness or porosity is an important factor of a medium which facilitates to penetrate roots as well as to store and dispense water to plants. In this experiment, lower water retention ability was observed in pure CPL medium (T5), which demands for higher volume of water to fill its pores present among the medium particles. In contrast CCD (T1) needed comparatively low volume of water to saturate (78cm³ to 100cm³ of medium) (Table 1). However, the highest volume of water leached out from saturated medium was observed in T5 showing that it incurs higher % of AFP. CCD had the lowest AFP and only 2% water had leached out. This indicates CCD has very high WHC (6.21ml/cm³) compared to CPL and other mixtures.

Both macro and micro pores contribute to general pore space of a medium. The water in the large pores leaches out easily while water bound to the substrate particles remains comparatively long. The porosity and type of pores determine the water availability for plant growth (Bunt, 1988). Further, in some study have shown that 30-45% from water held in a root medium should be easily available water (EAW) for optimum plant growth (Verdonck et al., 1983; Gabriels and Verdonck, 1991). Therefore, it is important to have a balance between AFP and WHC in a medium as optimum WHC provides water to plant growth and air-filled spaces help to root penetration and provide aeration to root zone (Awang, 2009). However, excess water holding capacity makes the medium soggy and creates water logging conditions in the root zone. This is inversely related to the porosity of a medium and when AFP is high, it leaches out water easily making the water unavailable to the roots. In this experiment, WHC of the treatments gradually reduced when increasing the CPL ratio recording the lowest WHC (1.3ml/cm³) in the medium with only CPL (T5). This indicates that mixing of constituents with extreme levels of WHC and AFP, would bring these parameters to moderate levels in the mixtures.

Bulk density (BD) of a medium is important for root growth of plants. Potting media with very low BDs (0.1 to 0.2 g/cm³), such as all peat, or peat-perlite media, are unstable in windy conditions, especially if lightweight plastic pots or polythene bags are used as containers. However, soil-based media can have very high BDs (1-2 g/cm³), which also not suitable as it disturbs free root growth (Stirzaker et al., 1996). Generally, 0.2-0.5 g/cm³ bulk density is considered as suitable for containerized media (Bunt, 1988). Results of the experiment revealed that all the treatments used were in the acceptable range for soilless nurseries and containerized plant production.

3.2 Chemical properties of the treatments

Analysis of the chemical properties given in table 2, indicated that the pH and EC levels of all the treatments were in the acceptable range for plant growth. pH and EC of a medium determine the nutrient availability for the plants (Rahman et al., 2017). In general, plants can be grown in the pH range of 5.5-7.5 and most preferable level for tomato, capsicum and brinjal is slightly acidic (5.5-6.5) (Ikeda and Osava,1981; Pleasant, 2018). Plants prefer <2ms/cm EC in soilless media for better growth (Lodolini et al., 2016). pH of the CCD used in this study was 7.16. Results of this study

revealed that the mixing of CPL to CCD brings the pH to a range which is ideal for plant growth.

Table 2: Chemical properties of the treatments						
Treatment (CCD: CPL) pH EC ms/						
T1 (4:0)	7.16	0.19				
T2 (3:1)	6.56	0.208				
T3 (2:2)	6.14	0.24				
T4 (1:3)	5.96	0.25				
T5 (0:4)	5.18	0.46				

N=5

Table 3: Weight reduction due to biodegradation of treatments								
Treatment (CCD:	Initial	Final	Weight					
CPL)	weight	weight	reduction %					
T1 (4:0)	10.26	9.02	12.0a					
T2 (3:1)	11.93	10.11	11.08ab					
T3 (2:2)	13.44	12.0	10.7bc					
T4 (1:3)	15.60	14.1	9.6c					
T5 (0:4)	16.86	15.4	8.6d					
CV%			6.4					
LSD			1.03					

Means with the same letters are not significantly different at p=0.05

3.3 Biodegradability of the treatments

Table 3 shows the difference of weights of each medium before and after using in the nursery. Higher weight reduction was observed in CCD and weight reduction was gradually reduced with the increase of CPL component. However, CCD also remained less than 15% (W/W) weight reduction even after 6 weeks. The weight reduction of a growth medium is mainly taking place due to biodegradability. The materials used in this experiment had low weight reduction due to their bio stability. Three main essential biological properties are there to be considered in selecting soilless media, as free from pests, free from diseases and less prone to biodegradability (Barrett et al., 2016). This poor degradability (consistent texture) is one of the reasons for nominating CCD as a soilless medium (Meerow, 1995). However, pine leaves have become an environmental problem to Sri Lanka due to its low biodegradability, but this character befitted to use it as a plant growth substrate. Main components of Pine leaves are holocellulose, 57; lignin, 23; and extractives (alcohol, benzene, fibers, resins etc.), 20% (Sharma et al., 2011). Digestion of these materials does not take place easily, hence biodegradation is quite slow. Therefore, this character is highly useful in using CPL for many successive cultivation cycles.

3.4 Seedling production in different treatments

Table 4: Growth parameters of seedlings of tomato and capsicum tested in Kobeigane									
T		Tomato				Capsicum			
Treatment (CCD:CPL)	RGR*	SVI**	Root length	Loofno	RGR*	SVI**	Root length	Leaf no.	
(CCD.CFL)	mm/day	mm	cm	Leaf no.	mm/day	mm	cm		
T1 (4:0)	3.33a	87.1b	2.6d	3.8a	2.28b	46.32b	4.6c	3.41a	
T2 (3:1)	3.8a	134.3a	11.3a	4a	3.2ab	74.32a	7.4ab	3.33a	
T3 (2:2)	3.6a	124.5a	10.9a	4.1a	3.82a	81.55a	8.3a	3.54a	
T4 (1:3)	3.1ab	71.5bc	7.3b	4a	2.41b	32.45c	6.3bc	3.42a	
T5 (0:4)	2.7b	55.3c	3.4c	3.92a	1.81c	30.34c	4.3c	3.3a	
CV%	13.98	14.1	21.3	12.88	18.6	7.9	11.9	12.5	
LSD	1.05	18.74	2.4	0.618	1.02	11.15	1.35	0.43	

Means with the same letters are not significantly different at p=0.05

*RGR- Relative growth rate, **SVI- Seedling vigour index

	Table 5: Growth parameters of seedlings of tomato and 'elabatu' tested in Gannoruwa									
Treatment (CCD:CPL)	Tomato				'Elabatu' (S. insanum)					
	RGR* mm/day	SVI** mm	Root length cm	Leaf no.	RGR* mm/day	SVI** mm	Root length cm	Leaf no.		
T1 (4:0)	2.6b	78.1a	3.4d	3.8ab	1.3bc	22.2c	1.6c	2.26b		
T2 (3:1)	4.2a	155.2a	10.5a	4.1a	1.3bc	33.4b	3.8b	2.4b		
T3 (2:2)	4.4a	145.2a	9.8a	3.95a	1.41c	32.6b	3.56b	2.6ab		
T4 (1:3)	2.7b	66.45bc	7.6b	4.01a	1.98 ab	45.7a	4.26ab	2.88a		
T5 (0:4)	2.5b	45.3c	4.5c	3.84ab	2.4a	29.8b	5.7a	2.33b		
CV%	15.4	16.1	17.2	6.3	19.9	18.4	20.7	17.2		
LSD	1.1	21.77	1.3	0.44	0.86	11.6	1.1	1.0		

Means with the same letters are not significantly different at p=0.05

*RGR- Relative growth rate. **SVI- Seedling vigour index

	Table 6: Growth parameters of seedlings of brinjal and knolkhol tested in Gannoruwa									
Treatment (CCD:CPL)	Knolkhol				Brinjal(S. melongina)					
	RGR* mm/day	SVI** Mm	Root length cm	Leaf no.	RGR* mm/day	SVI** mm	Root lengthcm	Leaf no.		
T1 (4:0)	1.02c	23.5c	8.1b	4.2bc	1.54c	40.72c	9.23a	3.20b		
T2 (3:1)	1.41a	34.96a	9.07ab	4.2bc	1.43bc	48.5b	8.36a	4.21ab		
T3 (2:2)	1.29ab	34.5a	11.83a	4.87a	2.04a	55.81a	8.71a	4.46a		
T4 (1:3)	1.15bc	27.5b	12.25a	4.46ab	1.85ab	47.10b	8.13a	4.21ab		
T5 (0:4)	1.24b	15.6d	9.46ab	3.9c	1.75bc	42.25bc	9.36a	4.50a		
CV%	21.5	17.1	19.16	9.52	17.6	16.7	24.9	19.5		
LSD	0.15	0.38	3.5	0.41	0.024	4.9	2.59	0.41		

Means with the same letters are not significantly different at p=0.05

^{*}RGR- Relative growth rate, **SVI- Seedling vigour index





Figure 1: Elabatu (Solanum insanum) seedlings at the 6^{th} week (a) and Tomato (Solanum lycopersicum seedlings at the 4^{th} week (b) in treatments 1-5

Table 4, 5 and 6 show behavior of growth rate, vigour index, leaf number and root length of vegetable seedlings under different treatments in both experimental sites. The results revealed that all the treatments could produce seedlings and were not toxic to the plant growth. However, all growth parameters evaluated in the study had been significantly influenced by the treatments. Significantly highest seedling vigour index and growth rate of tomato (in both experimental sites), capsicum and knolkhol were observed in T2 and T3 but relative growth rate of both brinjal and 'elabatu' were higher in T4. Further, higher seedling vigour of brinjal was observed in T3, but 'elabatu' was in T4 (Table. 5, 6 and figure 1). There was no significant difference of leaf number in all the treatments of tomato, brinjal and capsicum trials but higher leaf number of elabatu was observed in T4 (2.88) while higher leaf number of knolkhol was observed in T3 and T4.

When consider root lengths all the treatments facilitated acceptable root growth for survival and growth of all 5 types seedlings. This proves that not only CCD but also CPL has no suppressive impact on the root growth. Root length of all the crops except 'elabatu' behaved in same manner and significantly better root lengths of tomato, capsicum and knolkhol were observed in T2, T3, and T4. Brinjal showed better root growth in all media mixtures. Higher WHC and low porosity of CCD reduce root growth in all crops. In 1995, Meerow has shown that root growth in pure CCD is lower than coir-based media mixtures. Mixing media increase porosity and reduce water to induce root growth. However, rapid water loss due to extreme porosity is also not desirable for plant growth. One reason for short root length in T5 might be due to lower availability of water in the medium. However, shortest route lengths were observed in CCD (T1) except brinjal and most possible reason is higher WHC of the medium, which limits the penetration of root due to easy availability of water in the root zone. Further, comparatively poor root growth of tomato, knolkhol and capsicum was observed in T5, but both brinjal and elabatu showed higher root length in T5, which may be due to perennial nature and tolerance to low water availability. Thus, in general mixing of both materials together performs better in nursery media than that of each material alone.

Although pine Leaves were reported to have allelopathic chemicals and hard wax coat around the leaves, we successfully used crushed pine leaves in media of vegetable seedling production process (Rice, 1984; Kimura, 2015; Dharmawan et al., 2016). Studies have shown that growth of plant in original form of pine leaves is unsuccessful and under growth of pine leaves accumulated grounds is zero or very low (Karunananda and Wasala, 2010; Nissanka et al., 2005). Two main inhibitory substances have been identified from pine leaves as 9α , 13β -epidioxyabeit-8(14)en-18-oic acid and abscisic acid- β -Dglucopyranosyl ester (Kimura et al., 2015). When pine leaves drop and remain in the forest floor, these chemicals may

deliver into the soil and leads to suppress under growth. However, release of toxic chemicals to ground may reduce the chemical content in leaves. Furthermore, crushing and washing of the material in the media processing, may have washed off the allelopathic chemicals as well as breaks the lignocellulosic coat. This improvement of chemical and physical properties of the CPL converted it to good plant growing substrate. Therefore, in agreement with CPL was used as plant growth substrate successfully in this experiment (Karunananda and Wasala, 2010). Moreover, more desirable growth media mixtures were resulted when CPL was incorporated into CCD.

The results revealed that mixtures of CCD and CPL show better performances compared to unmixed CCD and CPL. This might be due to improvement of chemical and physical properties due to mixing of the media. It can be presumed that high WHC of CCD as well as low WHC of CPL medium (Table 1) may negatively influence the plant growth. Nevertheless, mixing of CPL had brought WHC, pH of CCD closer to acceptable levels which help for proper seedling production (Verdonck et al., 1983; Lodolini et al., 2017). However, best levels of these properties of growth medium vary from crop to crop. 'elabatu' tolerates low water levels (Samarakoon et al., 2018). This may be the reason for showing higher seedling vigor index and growth rate by 'elabatu' in T4. Furthermore, when consider the environmental burden created by pine in central highlands of Sri Lanka, use of CPL as plant growth substrate discourage the leaf accumulation problem. Moreover, as pine plantations are available throughout the vegetable growing areas of central highland, partial replacement of CCD with CPL reduces the material and transport costs of nursery medium.

4. CONCLUSION

The potential of incorporating crushed pine leaves (CPL) into CCD as a plant growth medium in intensive vegetable nurseries was investigated. Mixing of CPL to CCD mainly improves WHC, air filled porosity, bulk density and pH towards better seedling production. The higher bio stability of CPL is an added advantage to use and reuse it as soilless media.CPL does not show any suppression of plant growth but media mixtures of CCD: CPL, 3:1 (T2), 2:2(T3) and 1:3(T4) have improved properties than pure forms, resulting in better plant growth rates, seedling vigour indexes in tomato, capsicum, Knolkhol, (in T2and T3), brinjal (in T3 and T4) and 'elabatu' (in T4). Therefore, it can be concluded that CPL can be used as an amendment to soilless medium for nursery trays in the vegetable seedling production and incorporation of it into CCD in different ratios depending on the crop, assures higher success of seedling production.

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AUTHORS CONTRIBUTIONS

Dayani Karunananda initiated, coordinated and guided the research and created the final manuscript. Harshani Chandrasiri conducted the experiment and recorded data of Peradeniya trials and Ajith Gunasena, conducted the experiment and recorded data of Kobeigane trials. Nishantha Kumara conducted all laboratory experiments and data analysis.

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