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Improving Bitter Gourd Seedling Characteristics by Some Alternative Compound Substrates

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Abstract

This research was carried out based on coconut husk as main material mixed with different proportions of sand, coconut charcoal, cow manure compost, and microbial fertilizer, to make a series of compound substrates. The rice husk, a commercial substrate, was selected as a control treatment. The physical and chemical properties of different compound substrates were analyzed, and the comparative tests for the development and physiological characteristics of bitter gourd seedlings growing on different substrates were performed. Additionally, the compound substrate formulas suitable for bitter gourd seedling were screened. The results indicated that among the researched formulas, the substrate formula with 706 dm^3/m^3 coconut husk + 59 dm^3/m^3 composting cow manure + 235 dm^3/m^3 sand + 4 g/L water-retaining agent + 2.5 kg/m^3 compound fertilizer, was most effective, compared to all others to promote the growth of bitter gourd seedlings.

Keywords: Bitter gourd; Seedling substrate; Coconut husk; Screening

Introduction

As a safety and health growth medium for crops, substrates can support fixed crop roots, provide necessary moisture through their water-retaining ability, supply nutrients and air for crop growth, and introduce a certain buffer capacity as well (Calkins *et al.*, 1997; Judd *et al.*, 2015). Substrates can improve management efficiency and provide more convenient conditions for crop growth and development. Seedling substrates provide necessary moisture, air, nutrients and other basic environmental conditions, and represent the key to cultivating healthy seedlings and shortening their breeding period (Abad *et al.*, 2005; Mazuela *et al.*, 2005; Eyheraguibel *et al.*, 2008). Thus, the selection of seedling substrates is an important part of industrialized seedling production. The physical and chemical properties of the substrate were closely related to the growth environment of the seedlings, and different types of crops have different requirements for substrates. Thus, each plant should be matched to its own corresponding seedling substrate, and producers should not use a universal substrate for different seedlings (Luo *et al.*, 2015; Zhao *et al.*, 2012). Generally, seedling substrates should be according to local cultivation conditions, using low-cost, simple materials from abundant local resources (Ghimire *et al.*, 2014; Montagne *et al.*, 2016).

As the main origin of coconut husks, Hainan currently designates a planting area of 3.3 hm^2 for coconut, with an annual output of 150 million coconuts. Furthermore, the planting area set aside for coconut continues to expand every year (SBHP and SONBSH, 2014). After processing, coconuts produce a large quantity of husks as a waste. Currently, the major disposal methods for husks consist of either burning them or accumulating them for natural degradation. Recently, international scholars have developed methods for using coconut husks as a main material in organic substrates. This process not only greatly reduces costs and increases revenues but also reduces environmental pollution related to coconut husks; moreover, it has launched a new coconut husk industry (Kowalska *et al.*, 2015). Coconut husks come in a variety of types and have many excellent properties, with good water-retaining ability and permeability, high concentrations of nutrients and freedom from pathogens. These qualities align coconut husks with the performance requirements for horticulture substrates in modern organic cultivation.

Bitter gourd is an important winter-cultivated vegetable in Hainan (SBHP and SONBSH, 2014). Long years of utilizing the single-planting model have led to increased soil borne diseases, for which the current treatment is to improve disease resistance in seedlings via grafting (Berg, 2009; Huang *et al.*, 2013). Because of the storage and transplanting operations for seedlings, light substrates are usually selected for use with seedlings. Rich coconut husk resources exist in Hainan, and coconut husk is reproducible and light, with good water- and fertilizer-retention abilities; therefore, suitable for seedling substrates. However, the existing coconut husk substrate formulas are

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coarse and inefficient, and no specific formula exists for use with bitter gourd seedlings. In this study, we used coconut husk as a main material and mixed it with different proportions of sand, coconut charcoal, cow manure compost, and microbial fertilizer to generate a series of compound substrates, using the rice husk substrate as a control. Firstly, physical and chemical analyses for the substrate formulas were performed; then the development and physiological characteristics of bitter gourd seedlings were experimentally compared and the most suitable compound substrate formulas were screened out.

Materials and Methods

The bitter gourd used for testing was "Reyan 3," glossy dark green type. It was obtained from Tropical Crop Genetics Resources Institute, Chinese Academy of Tropical Agricultural Sciences (CATAS). The seedling substrates tested included the rice husk substrate (Control, CK), 0.5mm-diameter sand, coconut husk and charcoal, microbial fertilizer, cow dung, compound fertilizer, and XM water-retaining agent.

Screening for Bitter Gourd Seedling Substrates

Twenty composite substrates made by mixing coconut husk, sand, and other tested substances with different volume ratios (Table 1) were dispensed into 8×8 cm seedling pots using a randomized complete block design. Each composite substrate treatment was repeated three times, with 20 seedling pots used for each repeat. Full bitter gourd seeds were germinated in a 30°C dark incubator after soaking for 12 h. Until the germs reached 0.5 cm, the consistently germinating seeds were selected and seeded in seedling pots with one seed per pot.

Determination of Indicators and Methods

The saturation extraction method was used to determine the bulk density (BD), total porosity (TP), and aeration porosity (AP) of each substrate (Lian, 1994). The dried substrate was added into a beaker (with empty weight W_1 and volume V), for a total weight of W_2. Then the substrate was soaked in water for 24 h, resulting in weight W_3. After the water in the beaker drained freely, the weight was W_4. From the following formulas we can calculate Bulk density (BD), Total porosity (TP), and Aeration porosity (AP) for each substrate.

Bulk density, BD (g/cm^3) = $(W_2 - W_1)/V$
Total porosity, TP (%) = $(W_3 - W_2)/V \times 100$
Aeration porosity, AP (%) = $(W_3 - W_4)/V \times 100$
Water retention porosity, WRP (%) = TP – AP
Air to water ratio = AP/WRP

Substrate's pH and electrical conductivity (EC, ms/cm) were determined by 5:1 extraction method. A sample of 5 g of dried substrate was transferred into a 250 mL flask, and small amounts of CO_2-free distilled water were used to moisten the sample. Then 25 mL of CO_2-free distilled water was added, and the mixture shaken for 40 min. The sample was immediately filtered, and kept in a 25 mL flask. Small amounts of CO_2-free distilled water were used to rinse the sample, and the filtration was transferred into the same flask. Finally, CO_2-free distilled water was added and mixed well to yield a volume of 25 mL. The EC and pH of the samples were measured by conductivity meter and pH meter, respectively.

Measurements of Agronomic Traits

The stem diameters and heights of bitter gourd seedlings were measured by vernier caliper and steel tape, respectively. The fresh weights of bitter gourd seedlings' shoot and root were measured after washing by deionized water, and the dry weights of shoot and root were measured after drying at 75°C for 72 h. The leaf area was calculated by equation:

Leaf area (cm^2) = x/y

Where x is the weight of the graph paper covered by the leaf outline (g) and y is the weight (g), of the cm^2 area of the graph paper. Outline of Bitter gourd seedlings leaves were drawn on the millimeter graph paper. The area of the graph paper covered by the outline was cut and weighed. A one cm^2 of the millimeter graph paper was cut and weighed.

Statistical Analysis

All statistical analyses were performed using SAS 9.0. Analysis of variance was used to assess significant differences between different parameters and the confidence interval for the Student *t*-test was calculated at α=0.05.

Results

Physical and Chemical Properties of Substrates

Most of the substrates fell within this range, but the bulk density values of samples A5 and A10 were less than 0.2 g/cm^3 (Table 2). This indicates that treatments A5 and A10 were too loose, with poor breathability and water absorption that made them inadequate for root stretching and made plant easy to lodging. In contrast, treatments A15, A16 and A17 all demonstrated higher BD values beyond the ideal range, indicating that they were too dense, with poor ability to retain water and nutrients.

Among the substrates, A1 and A10 had lowest and highest total porosity values of 50.4% and 91.2%, respectively. In addition, A1 had the smallest aeration porosity, with a value of 14.1%, while A10 had the largest AP, with a value of 47.1%. In terms of the air to water ratio (AP/WRP), the values for A5, A10, A15, A16 and A17 were relatively higher. The pH values for the different treatments were between 5.37 and 7.49. The EC value represents the total concentration of ions

Table 1: Composite Substrates for Bitter Gourd Seedlings

Treatment	CH	S	CC	CCM	MF	RHS	WRA	CF	Treatment	CH	S	CC	CCM	MF	RHS	WRA	CF
A1	750	250	0	0	0	0	0	2.5	A11	750	250	0	0	0	0	8	2.5
A2	706	235	59	0	0	0	0	2.5	A12	706	235	59	0	0	0	8	2.5
A3	706	235	0	59	0	0	0	2.5	A13	706	235	0	59	0	0	8	2.5
A4	706	235	0	0	59	0	0	2.5	A14	706	235	0	0	59	0	8	2.5
A5	1000	0	0	0	0	0	4	2.5	A15	0	800	200	0	0	0	8	2.5
A6	750	250	0	0	0	0	4	2.5	A16	0	800	0	200	0	0	8	2.5
A7	706	235	59	0	0	0	4	2.5	A17	0	800	0	0	200	0	8	2.5
A8	706	235	0	59	0	0	4	2.5	A18	0	250	0	0	0	750	0	0
A9	706	235	0	0	59	0	4	2.5	A19	0	250	0	0	0	750	8	0
A10	1000	0	0	0	0	0	8	2.5	CK1	0	0	0	0	0	1000	0	0

CH: Coconut Husk (dm^3/m^3), S: Sand (dm^3/m^3), CC: Coconut Charcoal (dm^3/m^3), CCM: Composting Cow Manure (dm^3/m^3), MF: Microbial Fertilizer (dm^3/m^3), RHS: Rice Husk Substrate (dm^3/m^3), WRA: Water-Retaining Agent (g/L), CF: Compound Fertilizer (kg/m^3)

Table 2: Physical and chemical properties of different composite substrates

Treatment	BD (g/cm^3)	TP (%)	AP (%)	WRP (%)	AP/WRP	pH	EC (ms/cm)
A1	0.56	50.4	14.1	36.4	0.39	5.37	1.02
A2	0.44	60.1	16.7	43.4	0.38	5.92	1.28
A3	0.56	63.2	17.9	45.3	0.39	6.48	1.73
A4	0.59	64.4	15.5	48.9	0.32	6.38	1.32
A5	0.13	89.8	45.2	44.6	1.01	5.75	1.65
A6	0.53	53.4	15.1	38.3	0.39	5.76	1.20
A7	0.41	64.1	17.5	46.6	0.38	5.92	1.63
A8	0.58	68.3	18.1	50.2	0.36	6.16	1.85
A9	0.42	66.6	18.1	48.5	0.37	6.52	1.46
A10	0.12	91.2	47.1	44.1	1.07	6.15	0.87
A11	0.42	58.3	17.2	41.1	0.42	5.91	1.36
A12	0.47	69.1	20.1	49.0	0.41	6.07	1.40
A13	0.52	74.1	21.3	52.8	0.40	6.33	2.12
A14	0.50	76.1	19.6	56.6	0.35	6.26	1.49
A15	1.30	40.1	37.1	3.0	12.37	6.78	1.34
A16	1.53	44.6	38.1	6.4	5.93	6.87	0.59
A17	1.44	43.6	37.6	6.0	6.32	7.49	1.33
A18	0.54	58.3	18.2	40.1	0.45	5.61	2.09
A19	0.66	67.1	20.5	46.7	0.44	5.67	2.25
CK	0.28	84.7	28.1	56.5	0.50	5.56	3.36

contained in the substrate solution, and the EC values of A10 and A16 were relative lower than other substrates.

Effects of Different Composite Substrate Formulas on the Agronomic Traits of Bitter Gourd Seedlings

Among the twenty composite substrates tested, the composite substrate A19 produced the largest leaf area, with a value of 52.57 cm^2, while the medium A1 exhibited the smallest leaf area, with a value of 25.85 cm^2 (Table 3). Seven treatments yielded leaf areas larger than the Rice husk substrate (CK treatment), which were A8, A9, A13, A14, A15, A17 and A19. The leaf areas of A8 and A19 were significantly higher than of CK indicating that these two substrates may produce better seedlings in terms of leaf area than the rice husk substrate. Inversely, the leaf area of the CK treatment was larger than of A1, A2, A3, A4, A5, A6, A10, A11, A16 and A18, and significantly larger than those of A1, A2, A3, A4, A12 and A16.

The stem heights of A8 and A19 were higher than 20 cm with the lowest 13.12 cm for A1 (Table 3). The stem heights associated with substrates A5, A6, A7, A8, A9, A10, A11, A12, A13, A14, A15, A17, A18 and A19 were higher than of CK. Among these, the stem heights of A8 and A19 were significantly higher than CK, indicating that substrates A8 and A19 may significantly enhance the stem height of the bitter gourd plant. In contrast, the stem heights of A1, A2, A3, A4 and A16 were lower than CK, and differences were significant for A1, A3, A4 and A16. These results led to treatments A1, A3, A4 and A16 being deemed not suitable substrates for the bitter gourd. The largest bitter gourd stem diameter resulted from treatment A15, with a value of 3.43 cm, while the smallest one resulted from A1, with a value of 2.79 cm. Although the stem diameters of A8, A13, A15, A17 and A19 were larger than CK, but differences were not significant. The stem diameter of CK was larger than A1, A2, A3, A4, A5, A6, A7, A9, A10, A11, A12, A14, A16 and A18, and it was significantly larger than A1, A2, A3, A4, A5, A7, A10, A11 A14, A16 and A18.

The seedling shoot fresh weight data indicated that the fresh weights of substrates A1, A2, A3, A4, A7, A10 and A16 were lower than CK, and of A1, A2, A3, A4 and A16 significantly lower (Table 4). The shoot fresh weights of substrates A5, A6, A8, A9, A11, A12, A13, A14, A15, A17, A18 and A19 were higher than CK, and those of substrates A5, A6, A8, A9, A15, A17 and A19 were significantly

Table 3: Effects of different composite substrates on the agronomic traits of the bitter gourd

Treatment	Leaf area	Stem		Treatment	Leaf area	Stem	
		height	diameter			height	diameter
	cm^2	cm	mm		cm^2	cm	mm
A1	25.85^{l}	13.12^{f}	2.79^{i}	A11	30.85^{hijk}	18.18^{bc}	3.08^{efg}
A2	28.53^{jkl}	14.37^{def}	2.86^{hi}	A12	32.43^{ghij}	18.65^{bc}	3.20^{bcde}
A3	28.10^{kl}	13.69^{ef}	2.94^{ghi}	A13	42.22^{bcde}	17.99^{c}	3.30^{abcd}
A4	30.85^{hijk}	13.70^{ef}	2.92^{ghi}	A14	43.11^{bc}	18.41^{bc}	2.98^{fgh}
A5	34.42^{efgh}	18.84^{bc}	2.95^{ghi}	A15	42.23^{bcde}	17.85^{c}	3.43^{a}
A6	36.81^{defg}	19.43^{abc}	3.20^{cd}	A16	32.60^{ghijk}	14.00^{ef}	2.99^{hgf}
A7	39.01^{cdef}	18.83^{bc}	3.17^{def}	A17	40.30^{bcdef}	17.84^{c}	3.30^{abcd}
A8	46.06^{b}	20.21^{a}	3.35^{abc}	A18	34.33^{efgh}	16.33^{cd}	3.02^{efg}
A9	42.51^{bcd}	19.24^{abc}	3.19^{cde}	A19	52.57^{a}	20.12^{ab}	3.37^{ab}
A10	32.58^{ghij}	18.14^{c}	2.98^{fgh}	CK	38.09^{bcdef}	16.12^{cd}	3.25^{abc}

Note: values with different superscripts in a column differ significantly ($P<0.05$)

Table 4: Effects of different compound substrates on the shoot and root weight of the bitter gourd seedlings

Treatment	Shoot weight		Root weight		Treatment	Shoot weight		Root weight	
	fresh	dry	fresh	dry		fresh	dry	fresh	dry
	g	g	g	g		g	g	g	g
A1	7.84^{gh}	0.91^{ef}	1.85^{bcd}	0.117^{ab}	A11	11.04^{ef}	0.99^{def}	1.83^{bcd}	0.106^{b}
A2	8.24^{gh}	1.29^{abcde}	1.86^{bcd}	0.107^{b}	A12	12.59^{cde}	1.19^{cdef}	1.77^{cd}	0.106^{b}
A3	6.87^{h}	0.84^{f}	1.46^{d}	0.094^{b}	A13	12.44^{de}	1.31^{abcde}	2.00^{abcd}	0.124^{ab}
A4	8.06^{gh}	1.33^{abcde}	2.01^{abcd}	0.124^{ab}	A14	12.81^{cde}	1.33^{abcde}	1.67^{cd}	0.100^{b}
A5	14.61^{a}	1.32^{abcde}	1.70^{cd}	0.099^{b}	A15	15.55^{b}	1.65^{ab}	1.79^{cd}	0.113^{b}
A6	14.17^{bcd}	1.24^{bcdef}	1.64^{d}	0.096^{b}	A16	8.14^{gh}	0.99^{def}	2.71^{a}	0.146^{ab}
A7	9.76^{fg}	1.06^{cdef}	2.18^{abcd}	0.125^{ab}	A17	14.12^{bcd}	1.38^{abcd}	1.81^{cd}	0.099^{b}
A8	15.60^{b}	1.44^{abc}	2.04^{abcd}	0.195^{a}	A18	12.33^{de}	1.28^{abcde}	1.93^{ab}	0.133^{ab}
A9	15.34^{b}	1.47^{abc}	1.56^{d}	0.099^{b}	A19	19.08^{a}	1.70^{a}	1.73^{cd}	0.102^{b}
A10	10.73^{ef}	1.11^{cdef}	1.85^{bcd}	0.123^{ab}	CK	11.02^{ef}	1.28^{abcde}	2.49^{abc}	0.157^{ab}

Note: values with different superscripts in a column differ significantly ($P<0.05$)

higher. The dry weights of seedling shoots showed different characters with fresh weights. The dry weights of substrates A4, A5, A8, A9, A13, A14, A15, A17 and A19 were higher than that of CK, although no substrate had a significant higher dry weight than CK. The dry weights of substrates A1, A3, A6, A7, A10, A11, A12 and A16 were lower than CK, and A3 had a significantly lower dry weight than CK. Only substrate A18 had the same shoot dry weight as CK.

The measured seedling root fresh weight data show that none of the nineteen substrates had a significantly higher root fresh weight than CK. All substrates except A16 were lighter than CK, and substrates A3, A6 and A9 have significantly lower fresh weights than CK. The measured seedling root dry weight data reveal no significant differences among the twenty substrates.

Discussion

Previous research indicated that the ideal bulk density range for a substrate is 0.2–0.8 g/cm^3, the total porosity range should fall in 60%–90% and the ideal range of air to water ratio is 0.25–0.67 (Li *et al*., 2002; Pan *et al*., 2003). Due to the coconut husk ratio in A5 and A10 was highest among all substrate formulas, the bulk density of A5 and A10 were less than 0.2 g/m^3, this indicated that these two substrates were too loose, and the total porosity values of substrates of A10 were out of the ideal total porosity range. The water retention porosity of substrates A15, A16 and A17 were lower than 6.4% and the aeration porosity of them were higher than 37.1%. It is because these three substrates had the highest ratio of sand.

The pH value is an important index for substrate, and the pH range requirements for common substrates were 5.5–7.5 (Li *et al.*, 2002; Qiu and Xie, 2014). The EC value can influence the mineral ions absorption of cops significantly, and the EC values should be within the range of 1.0–3.5 ms/cm, with an optimal value of 2.0 ms/cm (Calori *et al.*, 2014). The pH and EC values of most of the composite substrates promoted the normal growth and development of crops, with the exceptions of A1, A10 and A16. Due to the cation absorption ability of sand is relative lower, the EC of A16 was the lowest among all the substrates in this study. Since the ratio of coconut husk in substrate A1 was higher and did not added the water-retaining regent, which can buffer the pH decrease during the growth of bitter gourd seedlings, the pH of A1 was the lowest.

The contents of macro- and micronutrients in substrates can influence the stem diameter and leaf growth of plants (Osman and Rady, 2014; Rady and Rehman, 2016). Morphological indicators such as leaf area, stem diameter, and height are generally used to determine whether a plant is of “good” or “bad” quality. In this research, the first true leaf of each seedling was used to represent the plant’s leaf area. The comprehensive analysis of the agronomic traits of the bitter gourd showed that the leaf areas and stem heights for treatments A8 and A19 were obviously or significantly higher than control. On the other hand, one or more agronomic traits for substrates A1, A2, A3, A4, A5, A7, A10, A11, A12, A14, A16 and A18 were significantly lower than CK. The stem diameter and leaf growth of bitter gourd seedlings were mainly determined by the moisture contents, nutrients supply and capacity of substrates. Due to A1, A2, A3, A4 and A18 did not added the water-retaining regent, the moisture and nutrients supply capacity were relatively lower than CK, which lead the agronomic traits, which were stem diameter and leaf area, of bitter gourd seedlings for these five substrates were lower than CK. Since the substrates of A5, A10 were too loose and A16 too compact to growth of bitter gourd seedlings, the agronomic traits of A5, A10 and A16 were lower than CK. The leaf area and stem diameter of bitter gourd for A6, A11 and A12 were lower than CK mainly due to the nutrients contents of A6, A11 and A12, which compost mainly by coconut husk and sand, were relative lower than CK.

The fresh and dry weights of shoots and roots indicated that substrates A1, A2, A3, A4, A6, A7, A9 and A16 had one or more indicators that are significantly lower than CK. This mainly was due to the moisture and nutrients contents of these eight substrates lower than CK, which was rice husk substrate. Since some physical indicators of A5, A8, A15, A17 and A19 are significantly better than CK, one or more indicators of fresh and dry weights of shoots and roots were higher than CK. Thus medium A5, A8, A15, A17 and A19 showed an advantage over CK in terms of fresh or dry weights of shoots or roots, and the formulas A8, which was 706 dm^3/m^3 coconut husk + 59 dm^3/m^3 composting cow manure + 235 dm^3/m^3 sand + 4 g/L water-retaining agent + 2.5 kg/m^3 compound fertilizer, was most effective at promoting the growth of bitter gourd seedling.

Conclusion

In summary, substrates A1, A2, A3, A4, A5, A6, A7, A9, A10, A11, A12, A14, A16 and A18 had one or more indicators that is significantly lower than CK. Meanwhile, A8, A13, A15 and A19 were determined to be potential substitutes for rice husk substrate. Among them, substrates A8 and A19 have the greatest number of indicators significantly higher than CK. Substrate A19 included the rice husk substrate in the formula. Therefore, the substrate A8 (706 dm^3/m^3 coconut husk + 59 dm^3/m^3 composting cow manure + 235 dm^3/m^3 sand + 4 g/L water-retaining agent + 2.5 kg/m^3 compound fertilizer) can be applied in the future cultivation of bitter gourd seedlings.

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