

Photosynthetic Response and Antioxidant Content of Hydroponic Bitter Gourd as Influenced by Organic Substrates and Nutrient Solution

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Additional index words. stomatal conductance, photosynthesis, chlorophyll content, growth and yield, antioxidant content, protein, bitter gourd

Abstract. Crop-specific nutrient solution and suitable growing media mixtures are necessary in hydroponic system to improve the yield of vegetables. Therefore, an experiment was conducted to specify the required amount of nutrient solution and ecofriendly mixtures of growing substrates of a hydroponic bitter gourd. Treatments consisted two factors, viz., four different types of growing substrate ($M_1 = 60\%$ rice husk + 30% coconut coir + 10% vermicompost, $M_2 = 60\%$ coconut coir + 30% khoa + 10% vermicompost, $M_3 = 60\%$ sawdust + 30% khoa + 10% vermicompost, and $M_4 = 60\%$ ash + 30% khoa + 10% vermicompost) and three different composition of nutrient solutions [$N_1 =$ full strength Rahman and Inden (2012), $N_2 =$ full strength Hoagland and Arnon (1940), and $N_3 = \frac{1}{2}$ strength Rahman and Inden (2012)]. Photosynthetic responses and its related parameters, namely, stomatal conductance (g_s), transpiration (E), and photosynthetic rate (P_N) were significantly affected by nutrient solution composition and growing substrate mixtures. Results revealed that the leaf gas exchange parameters, yield contributing characters, and biochemical parameters showed that full strength of Rahman and Inden (2012) nutrient solution application was better in a growing mixture of 60% rice husk + 30% coconut coir + 10% vermicompost in soilless culture for obtaining high yield and high quality of bitter gourd.

Use of suitable growing substrate is essential for production of high-value horticultural crops. It directly affects the development and later maintenance of the functional rooting system. A suitable growing medium provides a sufficient support to the plant, serves as reservoir for nutrients and water, allows oxygen diffusion to the plant roots, and permits gaseous exchange between the roots and atmosphere (Abad et al., 2002). Many organic materials are widely available in the tropics, e.g., rice hull, coco peat, carbonized rice husk, and sawdust. These materials are mainly agricultural byproducts obtained after the extraction of fiber from the coconut husk,

paddy, and saw mills and may be used as horticultural growing substrates. As a growing medium, coco peat can be used to produce several crop species with acceptable quality (Yahya et al., 1997). Coco peat is considered as a good growing substrate component with acceptable pH, electrical conductivity (EC), and other chemical attributes (Abad et al., 2002). However, coco peat has been recognized to have high water-holding capacity which causes poor air–water relationship, leading to low aeration within the medium, and it affects the oxygen diffusion to the roots. Carbonized rice husk may be used as a horticultural growing substrate, although it has the problem of air–water relationship. Rice hull is often incinerated to form fine charcoal-like dust. When it is used as a component for growing substrate, it might behave like fine sand. However, it is lighter and sterile and may contain some nutritional elements. Carbonized

rice husk induced faster cell division and differentiation for root formation (Moe, 1988) and it was the best growing substrate for growing horticultural crops (Budiarta et al., 2006) and sweet pepper production. Sawdust is used as growing substrate and is available in almost all over the world and it could be renewable. Wood residues (i.e., sawdust and bark) have been used in containers for growing ornamentals (Klett et al., 1972). But, microorganisms involved in decomposition of raw wood residues are more efficient than higher plants in nitrogen absorption and assimilation (Alexander, 1961). Large amount of nitrogen must, therefore, be added to wood residues used as media to grow plants. This problem can be solved, however, by composting residues before using them for growing substrates (Still et al., 1974). Furthermore, decomposition of old sawdust is not necessary for using as growing substrates. However, desirable physical and chemical properties of these substrate components are highly dependent on their processing technique and handling. It is desirable to improve physical and chemical properties of them before using as growing substrates. Incorporation of coarser materials into the substrate components could improve the aeration and drainage status of the substrate mixtures (Sambo et al., 2008). Perlite (Islam, 2008; Sambo et al., 2008), bora (volcanic soil), but rice hull may be used as the alternative of perlite, brick broken, gravel, etc. may be the possible coarser materials. These materials can be used to improve the air–water relationship of the substrate components. Furthermore, a suitable combination of different growing substrate components influences the growth and yield of horticultural crop production like bitter gourd as a test crop.

The nutrient solution is directly involved in producing higher yield and quality of horticultural crops (Rahman and Inden, 2012a). Nutrient solution formulation and its nutritional constituents may have the effect on quality and antioxidant components of horticultural crop production. Nowak (1980) stated that all nutrient combinations had the most beneficial effect on hydroponically grown plants. Monforte-González et al. (2010) stated that nitrate accumulation in placenta depended on its availability in the nutrient solution and the pod presenting placenta with low nitrate content accumulated, which may be related to limited nitrogen availability in this tissue of crops, such as pepper. Thus, improving the quality and yield contributing characteristics in bitter gourd are important factors in soilless culture technique. These may be improved by managing external proper nutrient availability in the growing substrates. Proper nutrient combinations in the solution may improve the yield and yield contributing characters in the crop. Consequently, the present experiment was aimed to find out a suitable and sustainable growing media mixture and a nutrient strength for producing high yield and quality of soilless bitter gourd producing in greenhouse in the tropics.

Received for publication 21 May 2018. Accepted for publication 17 June 2018.

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Materials and Methods

Experimental site. Two trials were conducted in the greenhouse at Horticulture Farm of the Sher-e-Bangla Agricultural University, Dhaka, from February to September of 2016 and 2017.

Growing environment. Both the trials were conducted in 180 × 25 × 25 cm wooden channels, and six plants were transplanted into each channel/box. Seedlings were transplanted into the boxes on 11 Feb. for both the years. Bitter gourd (Marutane Seed Co., Kyoto, Japan) was used in these experiments. Six 10-cm, fourth-leaf stage, 6-week-old seedlings were transplanted into each box containing growing media mixtures as treatments. Three replications were tested for each treatment. Nutrient solutions were applied to the plants continuously by a drip irrigation system according to the treatments. The pH and EC of the nutrient solutions were controlled during application. Average minimum and maximum temperatures and relative humidity were 24 ± 2 °C and 32 ± 2 °C and 60% ± 10% and 75% ± 10%, respectively, during the experiment.

Experimental design and treatments. A randomized complete block design with 3 × 3 factorial treatments and three replications were used for both the trials. Three plants

served as an experimental unit. A two-factor experiment was conducted with four different types of growing media mixtures (M_1 = 60% rice husk + 30% coconut coir + 10% vermicompost, M_2 = 60% coconut coir + 30% khoa + 10% vermicompost, M_3 = 60% sawdust + 30% khoa + 10% vermicompost, and M_4 = 60% ash + 30% khoa + 10% vermicompost) and three different composition of nutrient solutions [N_1 = full strength Rahman and Inden (2012), N_2 = full strength Hoagland and Arnon (1940), and N_3 = ½ strength Rahman and Inden (2012)] An equal volume of nutrient solution was applied for all the treatments and it was on an average 0.85 L/plant/d. The nutrient solution treatments were applied to the plants for all the treatments until 2 weeks after transplanting until harvest.

Data collection. Data were collected on the growth parameters and yield contributing characters (vine length, number of branches per plant, leaf area, number of fruits, fruits length, fruit girth, fruit weight, seed number, and seed weight); leaf gas exchange parameters (E , g_s , P_N); fruit characters (fruit color, flesh thickness, flesh color, and placenta color). The biochemical parameters, viz., chlorophyll content (Chl a and Chl b), ascorbic acids, total carotenoids, protein, total soluble solids (TSS), and reducing sugar were measured.

Data recording procedure. The leaf chlorophyll content was measured by following Dey et al. (2016) procedure. The P_N , E , and g_s were measured using the LC-pro portable photosynthetic system (ADC BioScientific Ltd., UK) as described by Quamruzzaman et al. (2017). Ascorbic acid was determined by titrating a known weight of sample with 2,6-dichlorophenol-indophenol dye using metaphosphoric acid as a stabilizing agent (Albrecht, 1993). Total carotenoids were determined by a modified method of one described by Ranganna (1986). The protein content was evaluated by the multiplication of total nitrogen with 6.25, which was determined by following the micro-Kjeldahl method (Kjeldahl, 1883). The TSS of the fruit juice was determined by hand held refractometer (manufactured by Global Water, Xylem Inc., USA), values corrected to 20 °C and expressed as °Brix. Acidity (as citric acid) was determined by using standard N/10 NaOH solution in the presence of phenolphthalein as an indicator, AOAC (1984). Reducing sugar was also analyzed by following the standard methodologies (AOAC, 1984).

Statistical analysis. Data from the two trials were combined and analyzed by two-way analysis of variance using SPSS (version 20.0) (SPSS Inc., Chicago, IL) and differences among the treatment means were determined by Tukey's test (Tukey, 1977) at $P \leq 0.05$.

Table 1. Influence of growing media mixture and nutrient solution on vegetative growth and chlorophyll content of bitter gourd.

Treatments	Vine length (cm)	No. branches per plant	Leaf area (cm ²)	Chlorophyll content	
				Chl a	Chl b
Growing media mixture (<i>M</i>)					
<i>M</i> ₁	291.14 c ^z	5.12 c	41.46 c	1.28 c	0.44 c
<i>M</i> ₂	345.24 a	12.11 a	65.39 a	1.40 a	0.52 a
<i>M</i> ₃	314.92 b	9.33 b	50.35 b	1.36 b	0.48 b
<i>M</i> ₄	255.73 d	4.11 d	31.44 d	1.23 d	0.42 d
Nutrient solution (<i>N</i>)					
<i>N</i> ₁	305.14 a	8.60 a	50.60 a	1.32 a	0.47 a
<i>N</i> ₂	299.02 c	6.67 c	43.50 c	1.31 b	0.49 b
<i>N</i> ₃	301.11 b	7.74 b	47.38 b	1.32 a	0.47 a
Level of significance (<i>P</i>)					
<i>M</i>	<0.001	<0.001	<0.001	<0.001	<0.001
<i>N</i>	<0.001	<0.001	<0.001	<0.001	<0.001
<i>M</i> × <i>N</i>	<0.001	0.939	<0.001	0.105	0.322

^zMeans with different letters are significantly different by Tukey's test at $P \leq 0.05$. *P* represents the level of significance of two-way analysis of variance. M_1 = 60% rice husk + 30% coconut coir + 10% vermicompost, M_2 = 60% coconut coir + 30% khoa + 10% vermicompost, M_3 = 60% sawdust + 30% khoa + 10% vermicompost, and M_4 = 60% ash + 30% khoa + 10% vermicompost; and three different compositions of nutrient solutions, viz., N_1 = full strength Rahman and Inden (2012), N_2 = full strength Hoagland and Arnon (1940), and N_3 = ½ strength Rahman and Inden (2012).

Table 2. Influence of growing media mixtures and nutrient solution on leaf gas exchange of bitter gourd.

Treatments	Stomatal conductance (mol·m·s ⁻¹)	Transpiration (mmol·m ⁻² ·s ⁻¹)	Photosynthetic rate (μmol·m ⁻² ·s ⁻¹)
Growing media mixture (<i>M</i>)			
<i>M</i> ₁	0.10 c ^z	2.07 c	5.23 c
<i>M</i> ₂	0.17 a	2.59 a	12.02 a
<i>M</i> ₃	0.13 b	2.13 b	8.27 b
<i>M</i> ₄	0.07 d	1.72 d	2.12 d
Nutrient solution (<i>N</i>)			
<i>N</i> ₁	0.13 a	2.35 a	8.08 a
<i>N</i> ₂	0.11 b	1.88 c	5.66 c
<i>N</i> ₃	0.11 b	2.15 b	6.99 b
Level of significance (<i>P</i>)			
<i>M</i>	<0.001	<0.001	<0.001
<i>N</i>	<0.001	<0.001	<0.001
<i>M</i> × <i>N</i>	<0.001	<0.001	<0.001

^zMeans with different letters are significantly different by Tukey's test at $P \leq 0.05$. *P* represents the level of significance of two-way analysis of variance. M_1 = 60% rice husk + 30% coconut coir + 10% vermicompost, M_2 = 60% coconut coir + 30% khoa + 10% vermicompost, M_3 = 60% sawdust + 30% khoa + 10% vermicompost, and M_4 = 60% ash + 30% khoa + 10% vermicompost; and three different compositions of nutrient solutions, viz., N_1 = full strength Rahman and Inden (2012), N_2 = full strength Hoagland and Arnon (1940), and N_3 = ½ strength Rahman and Inden (2012).

Table 3. Influence of growth media and nutrient solution on number of fruits, fruit length, fruit girth, and fruit weight of bitter gourd.

Treatments	No. fruits	Fruit length (cm)	Fruit girth (cm)	Fruit wt (g)	Seed no. per fruit	Seed wt per fruit (g)
Growing media mixture (<i>M</i>)						
<i>M</i> ₁	40.10 c ^z	17.93 c	16.48 c	165.94 c	24.31 c	4.53 c
<i>M</i> ₂	75.41 a	24.18 a	21.42 a	214.25 a	35.29 a	5.12 a
<i>M</i> ₃	55.20 b	21.87 b	17.74 b	185.06 b	29.33 b	4.81 b
<i>M</i> ₄	24.38 d	15.52 d	11.39 d	103.93 d	19.27 a	3.08 d
Nutrient solution (<i>N</i>)						
<i>N</i> ₁	54.11 a	21.20 a	17.99 a	170.79 a	27.96 a	4.44 a
<i>N</i> ₂	43.41 c	18.31 c	15.87 c	164.17 b	26.06 c	4.31 b
<i>N</i> ₃	48.80 b	20.12 b	16.42 b	166.93 c	27.13 b	4.39 c
Level of significance (<i>P</i>)						
<i>M</i>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<i>N</i>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<i>M</i> × <i>N</i>	0.001	0.139	<0.001	<0.001	0.980	<0.001

^zMeans with different letters are significantly different by Tukey's test at $P \leq 0.05$. *P* represents the level of significance of two-way analysis of variance. *M*₁ = 60% rice husk + 30% coconut coir + 10% vermicompost, *M*₂ = 60% coconut coir + 30% khoa + 10% vermicompost, *M*₃ = 60% sawdust + 30% khoa + 10% vermicompost, and *M*₄ = 60% ash + 30% khoa + 10% vermicompost; and three different compositions of nutrient solutions, viz., *N*₁ = full strength Rahman and Inden (2012), *N*₂ = full strength Hoagland and Arnon (1940), and *N*₃ = ½ strength Rahman and Inden (2012).

Table 4. Influence of growing media mixtures and nutrient solution on fruit color, flesh thickness, flesh color, and placenta color of bitter gourd.

Treatments	Fruit color ^z	Flesh thickness (mm)	Flesh color ^z	Placenta color ^z
Growing media mixtures (<i>M</i>)				
<i>M</i> ₁	2.30 b ^y	1.91 c	2.30 b	2.91 a
<i>M</i> ₂	1.11 c	2.88 a	1.11 c	1.12 c
<i>M</i> ₃	2.20 b	2.61 b	2.20 b	2.10 b
<i>M</i> ₄	2.83 a	1.61 d	2.83 a	3.10 a
Nutrient solution (<i>N</i>)				
<i>N</i> ₁	2.23	2.30 a	2.23	2.33
<i>N</i> ₂	2.05	2.22 b	2.05	2.32
<i>N</i> ₃	2.05	2.23 b	2.05	2.28
Level of significance (<i>P</i>)				
<i>M</i>	<0.001	<0.001	<0.001	<0.001
<i>N</i>	0.381	<0.001	0.381	0.659
<i>M</i> × <i>N</i>	0.908	<0.001	0.908	0.693

^zFruit color, flesh color: 1-green, 2-light green, and 3-greenish white; placenta color: 1-white, 2-cream, 3-light pink, and 4-pink.

^yMeans with different letters are significantly different by Tukey's test at $P \leq 0.05$. *P* represents the level of significance of two-way analysis of variance. *M*₁ = 60% rice husk + 30% coconut coir + 10% vermicompost, *M*₂ = 60% coconut coir + 30% khoa + 10% vermicompost, *M*₃ = 60% sawdust + 30% khoa + 10% vermicompost, and *M*₄ = 60% ash + 30% khoa + 10% vermicompost; and three different compositions of nutrient solutions, viz., *N*₁ = full strength Rahman and Inden (2012), *N*₂ = full strength Hoagland and Arnon (1940), and *N*₃ = ½ strength Rahman and Inden (2012).

Table 5. Influence of growth media and nutrient solution on ascorbic acid, total carotenoids, protein, total soluble solids (TSS), and reducing sugar in bitter gourd.

Treatments	Ascorbic acids (mg/100 g)	Total carotenoids (mg/100 g)	Protein (mg/100 g)	TSS (%)	Reducing sugar (%)
Growing media mixtures (<i>M</i>)					
<i>M</i> ₁	95.60 c ^z	2.61 c	2.15 c	5.25 c	5.78 b
<i>M</i> ₂	119.63 a	3.21 a	2.56 a	5.55 a	6.62 a
<i>M</i> ₃	105.59 b	2.93 b	2.32 b	5.41 b	6.32 a
<i>M</i> ₄	85.70 d	2.32 d	1.10 d	4.52 d	4.94 c
Nutrient solution (<i>N</i>)					
<i>N</i> ₁	105.53 a	2.88 a	2.07 a	5.24 a	5.96 a
<i>N</i> ₂	98.05 c	2.67 c	1.99 c	5.14 c	5.91 a
<i>N</i> ₃	101.30 b	2.76 b	2.04 b	5.17 b	5.86 a
Level of significance (<i>P</i>)					
<i>M</i>	<0.001	<0.001	<0.001	<0.001	<0.001
<i>N</i>	<0.001	<0.001	<0.001	<0.001	0.760
<i>M</i> × <i>N</i>	<0.001	0.107	0.006	<0.001	0.398

^zMeans with different letters are significantly different by Tukey's test at $P \leq 0.05$. *P* represents the level of significance of two-way analysis of variance. *M*₁ = 60% rice husk + 30% coconut coir + 10% vermicompost, *M*₂ = 60% coconut coir + 30% khoa + 10% vermicompost, *M*₃ = 60% sawdust + 30% khoa + 10% vermicompost, and *M*₄ = 60% ash + 30% khoa + 10% vermicompost; and three different composition of nutrient solutions, viz., *N*₁ = full strength Rahman and Inden (2012), *N*₂ = full strength Hoagland and Arnon (1940), and *N*₃ = ½ strength Rahman and Inden (2012).

Results and Discussion

Vegetative growth and chlorophyll content.

Growing media mixtures affect the vegetative growth and chlorophyll content of the bitter gourd. The significant differences were observed in the vine length, number of branches, leaf area, and chlorophyll content (Table 1). The result revealed that *M*₂ indicated the highest values of vine length, number of branches per plant, leaf area, and chlorophyll content (Chl a and Chl b),

whereas treatment *M*₁ denoted lower values of these traits. This might be due to proper aeration, water-holding capacity, lower bulk density, and biostability of *M*₂ treatment as compared with other treatments. Lemaire (1995) reported that lack of biostability may cause severe volume loss resulting in compaction, reduction in air volume, readily available water, and porosity due to mineralization and also changes in gaseous phase composition due to carbon dioxide production. These changes may

finally reduce the plant growth (Lemaire, 1995). Thus, the lower plant growth was observed in *M*₁ that strongly supported by Rahman et al. (2017).

The nutrient solution had significant effect on the vegetative growth and chlorophyll content of bitter gourd (Table 1). The treatment *N*₁ resulted in the highest vine length, number of branches, leaf area, and chlorophyll content. In the case of chlorophyll content (Chl a and Chl b) it showed the same trend where *N*₁ and *N*₃ resulted in statistically

similar values. The plants required optimum nutrient combination for proper growth and development. The possible reason behind the finding is that N_1 provided proper nutrient elements compared with other treatments. The present finding is consisted with the finding of Quamruzzaman et al. (2018).

The combined effect of growing media mixtures and nutrient solutions showed significant effect on vine length and leaf area, whereas number of branches and chlorophyll content was nonsignificant (Table 1) (data not shown).

Leaf gas exchange. Leaf gas exchange parameters significantly influenced the different growing media mixtures and nutrient solution compositions. The result revealed that both growing media and nutrient solutions enhanced the g_s , P_N , and E (Table 2). All the parameters were observed highest in M_2 , whereas the lowest in M_4 . This might be due to proper aeration, water-holding capacity, lower bulk density, and biostability of M_2 treatment. Furthermore, leaf gas exchange parameters performed better in N_1 nutrient solution. It might be due to adequate supply of nutrient of N_1 treatment as compared with the others. Rahman et al. (2017) stated that growing media mixtures play an important role in plant growth and development. On the other hand, full strength of Rahman and Inden (2012) solution (N_1 treatment) provided optimum and proper nutrient combination for high-value crops. Quamruzzaman et al. (2017) suggested that maximum stomatal pores in the epidermal layers of leaves were opened because of proper supply of nutrient solution. The result suggesting that the increased E was associated with the increased P_N and g_s agreed with the finding of Lu et al. (2014). Higher g_s in plants increases in the diffusion of CO_2 in the leaves and, thus, increases in the rate of photosynthesis. The P_N is progressively increased because of increasing g_s . Rahman et al. (2012a) also reported the almost similar result.

The combined effect of growing media mixtures and nutrient solutions also showed a significant impact on leaf gas exchange parameters (Table 2) (data not shown).

Yield and yield contributing characters. Yield and yield contributing characters, viz., number of fruits, fruits length, fruit girth, fruit weight, seed number, and seed weight were influenced by different growing media mixtures and nutrient solutions (Table 3). Significantly higher number of fruits, fruit length, fruit girth, fruit weight, seed number, and seed weight were observed in M_2 , whereas the lowest in M_4 . Better root growth of plants grown on M_2 could be attributed to the greater water availability and favorable aeration following the incorporation of 60% coconut coir + 30% khoa + 10% vermicompost. Under such a condition, the plants were provided with sufficient water and oxygen. Furthermore, better vegetative growth and physiological changes attributed to higher yield (Rahman et al. 2017). The yield contributing char-

acters performed better in N_1 nutrient solution. This might be due to an adequate supply of nutrient combination provided by treatment N_1 , resulting in the maximum reproductive growth (Quamruzzaman et al., 2017) because the plants required optimum nutrient combination for proper growth and better yield. When all the plant nutrients with the growth promoters and the regulators were supplied, the metabolic function of the plants would progress in the right direction and rate, whereas the yield and yield contributing characters increased gradually (Rahman and Inden, 2012b).

The combined effect of growing media mixtures and nutrient solutions also showed significant impact on yield and yield contributing characters where fruit length and seed number showed nonsignificant effect (Table 3) (data not shown).

Fruit characters. For the different growing media mixtures, the fruit color, flesh thickness (mm), flesh color, and placenta color ranges from 1.11 to 2.83, 1.61 to 2.88 mm, 1.11 to 2.83, and 1.12 to 3.10, respectively (Table 4). For the application of different nutrient solution, the fruit color, flesh thickness (mm), flesh color, and placenta color ranges from 2.05 to 2.23, 2.22 to 2.30 mm, 2.05 to 2.23, and 2.28 to 2.33, respectively. All of the fruit characters were determined by eye observation. Here, 1 represents green, 2 represents light green, and 3 represents greenish-white color for fruit and flesh of bitter gourd. Similarly, for the placenta color, 1 represents white, 2 represents cream, 3 represents light pink, and 4 represents pink.

Biochemical properties. The biochemical properties of bitter gourd showed significant effect for different growing media mixtures and nutrient solution (Table 5). The highest values of the biochemical properties, viz. ascorbic acids, total carotenoids, protein, TSS, and reducing sugar were found in the bitter gourd fruits grown in the media mixture of M_2 and the lowest in M_4 . The physical properties of substrate are important factor in determining the plant development in soilless substrate that helped produce higher biochemical activities (Rahman et al., 2017). As for why, the biochemical components in fruits recorded in this study were higher in M_2 and were linked with the differences in the chemical and physical properties of the substrate mixtures. On the contrast, the higher biochemical compounds were found in N_1 solution. It might be due to the appropriate supply of nutrient solution of N_1 that contained the required nutrient elements to produce the higher biochemical components. Quamruzzaman et al. (2017, 2018) and Rahman et al. (2012b) reported that adequate supply of nutrient helped get higher mineral elements.

The combined effect of growing media mixtures and nutrient solutions also showed significant impact on biochemical properties except total carotenoids and reducing sugar (Table 5) (data not shown).

Conclusion

In conclusion, the values obtained for the growth parameters, leaf gas exchange, yield contributing characters, and biochemical compound of bitter gourd for treatment M_2 and N_1 were higher than those obtained for the other treatments. Thus, M_2 and N_1 can be used to culture bitter gourd in hydroponic system as it provides higher vegetative growth and yield with the highest biochemical components.

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