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SELECTION OF SUITABLE GROWING MEDIA AND NUTRITIONAL ASSESSMENT OF MICROGREENS

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Now-a-days malnutrition and chronic diseases have become serious issues due to low intake of vegetables in both developed and developing countries (Miller and Welch, 2013). Vegetables are the noble source of vitamins, minerals and bioactive compounds which are documented to have defensive benefits against malnutrition and oxidative stress related chronic diseases (Martinez-Tomas *et al.,* 2012; Alarcon-Flores *et al.,* 2014). Rice-Evans and Miller (1995) reported that diets rich in vegetables provide an abundance of bioactive compounds, vitamins and minerals which confer protective benefits against different chronic disease and malnutrition. Alissa and Ferns (2017) demonstrated that vegetables consumption is associated with a reduction in the development of different chronic diseases such as cancer, Type 2 diabetes and cardiovascular diseases. So, World Health Organization has been stressing upon increasing the consumption of vegetables (WHO 2005).

In Bangladesh, vegetables consumption per capita is very poor. Besides, unauthorized chemicals and frequent pesticide application during growing period and post-harvest level make the marketable fruits and vegetables more dangerous for keeping good health. That is why, attention on fresh, functional and nutraceutical food (Ebert 2013). Accordingly, it is in the premium interest of specialty researchers to tap forthcoming trends and opportunities for niche products i.e. microgreens.

Microgreens are leafy vegetables distinguished by a central stem, cotyledonary leaves and very young true leaves that are harvested between 7-14 days in growth and are consumed fresh (Xiao *et al.,* 2015). Although small in size, microgreens have higher concentrations of bioactive compounds, antioxidants, vitamins and up to 40 times more vital nutrients, than their mature greens or seeds in the absence of genetic engineering and bio-fortification (Treadwell *et al.,* 2010; Xiao *et al.,* 2012). So, the production of fast-growing, nutrient-dense and health promoting traits containing microgreens may be a significant solution for pesticides free vegetables consumption which could assist in reduction of chronic diseases and malnutrition in Bangladesh. To the best of our knowledge, no scientific investigation was undertaken in Bangladesh on microgreens. Therefore, the current research work is designed to develop microgreens production technologies and determine the nutritional and health promoting traits.

Through sensory attribute test (Xiao *et al.,* 2015), we selected four (mustard, leaf mustard, radish and cabbage) out of fifteen microgreens for our main experiments. The seeds of selected microgreens were surface sterilized with 2.5% sodium hypochlorite solution. Followed by washing, seeds were sown in pre drawn pots under different treatments. The treatments combinations were tissue media (tissue paper with water), foam media (foam with water), soil media and soil plus cow dung (1:1) media. All treatments were replicated for three times under Completely Randomized Design. The seedlings were grown for 14 days and meanwhile different morphological assessments were performed and after harvesting, the nutritional parameters were assayed.

Germination percentage and vigor index was calculated by the following formulae,

$$\text{Per cent germination} = \frac{\text{Number of germinated seed}}{\text{Number of seed tested}} \times 100$$

$$\text{Vigor index} = \frac{\text{Per cent germination}}{\text{Average seedling length in cm}} \times 100$$

Seedling height was assessed by measuring the seedling from root tip to the leaf tip with a meter scale. 50 seedlings from every treatment were weighed as fresh weight and dry weight was determined after drying the sample in an oven at 70±2°C for three days till it attained a constant weight. Proximate composition of shoots was determined by adopting procedures described by Helrich (1990). Dried microgreens were manually ground into a fine powder and digested in concentrated di-acid mixture to determine minerals according to Hassan and Umar (2004).

For determining bioactive contents, leaves of plants (1g) were extracted in 10 ml of chilled acetone solution in dark. After centrifugation at 4000 rpm for 10 minutes the absorbance of supernatants was taken at 453, 505, 645 and 663 nm wave length. Contents were calculated according to the equation as described by Barros *et al. (*2010).

Vitamin C content was determined by the procedure described by Xaio *et al.* (2012) with 2.6-dichloroindophenl and measuring the content by titrimetric method. The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay was carried out with some modifications described by Sanja *et al.* (2009). Radical scavenging activity was calculated using the following formula:

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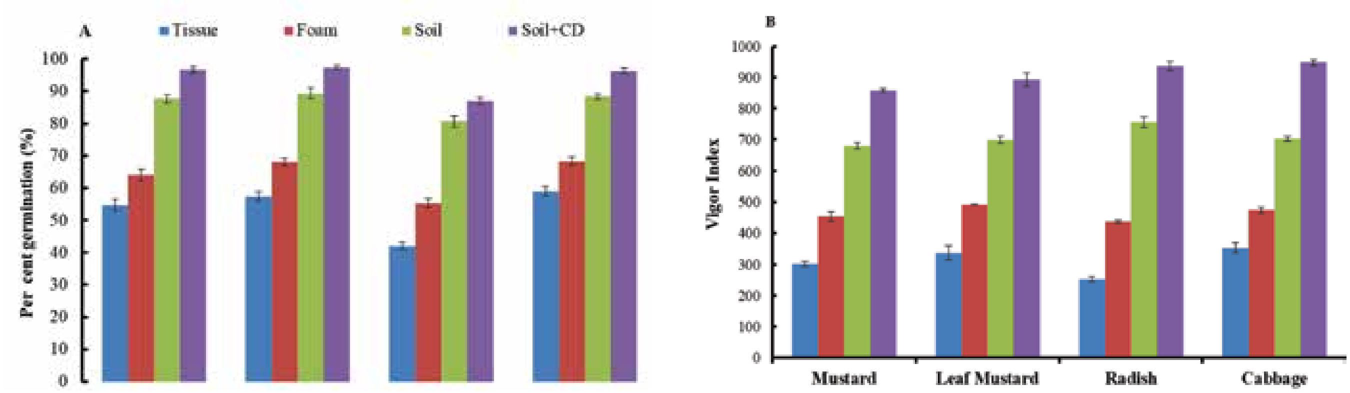

Fig. 1. Effect of media on (A) per cent germination and (B) vigor index of four tested microgreens. The vertical bars represent the mean ± SE (n=3). CD= Cow dung.

$$\% \text{ radical scavenging activity} = \frac{\text{OD of control} - \text{OD of sample}}{\text{OD of control}} \times 100$$

Where OD is Optical density

The collected data were statistically analyzed by using Minitab 17. Fisher,s LSD test was applied to compare the treatment means at 0.05 level of confidence.

The per cent germination of mustard, leaf mustard, radish and cabbage were 96.67, 97.33, 87.00 and 96.33% respectively, in soil plus cow dung medium (1:1), which showed reduction for the same microgreens by 9.31, 8.22, 7.28 and 8.30%, respectively in soil medium; 33.80, 30.13, 38.70 and 29.06%, respectively in foam medium and 43.45, 41.09, 51.72 and 38.75%, respectively in tissue medium paralleled to soil plus cow dung medium (Fig. 1A). The possible reason could be soil plus cow dung medium possessed optimal water holding capacity and aeration that favors the entire germination processes. Abad *et al.* (2001) and Bewley (1997) reported that growing media should have optimal water holding capacity 55 to 70% v/v and aeration 20 to 30% v/v for imbibition, hydration of protoplasm and restoration of enzymatic activity that leads to germination. Besides soil with high organic matter shows low bulk density (1 g cm^{-3}) that facilitates abundant movement of air and water (Soane, 2017) that are essential for higher germination.

Soil plus cow dung medium showed the maximum vigor index than the rest of media. Soil medium showed reduction at 20.65, 21.64, 19.34 and 25.77% in mustard, leaf mustard, radish and cabbage, respectively while it was 47.02, 44.84, 53.31 and 49.95% in foam and 64.67, 62.22, 73.73 and 62.82% in tissue medium for the same microgreens as compared to soil plus cow dung medium (Fig. 1B).

Soil plus cow dung medium exhibited the maximum seedling height than rest of the media. In soil, mustard, leaf mustard, radish and cabbage showed reduction in seedling length at 12.40, 14.61, 12.99 and 18.92%, respectively; in tissue medium at 19.95, 21.16, 26.65 and 29.50%, respectively; and the foam medium showed reduction at 37.66, 38.16, 44.01 and 39.26%, respectively compared to soil plus cow dung medium. Mustard, leaf mustard, radish and cabbage showed 22.50, 25.23, 30.17, 28.93g fresh weight and 1.43, 1.58, 2.10, 2.00 g dry weight, respectively, in soil plus cow dung medium, which exhibited reduction for the same microgreens at 20.31, 17.44, 17.03 and 22.33% (fresh weight) and 16.08, 19.62, 17.62 and 23% (dry weight) respectively in soil; 45.2, 49.15, 47.53 and 51.95% (fresh weight) and 47.55, 51.89, 47.62 and 52% (dry weight) respectively in tissue medium and 46.67, 50.73, 50.85 and

Table 1. Effect of media on seedling height (cm), fresh and dry weight (g) of four tested microgreens at harvest

	Seedling height (cm)				Fresh weight (g)				Dry weight (g)			
	M	LM	R	C	M	LM	R	C	M	LM	R	C
F	5.53± .09d	5.67± .07d	6.03± .03d	5.97± .12d	12.00± .17c	12.43± .12c	14.83± .12d	13.07± .12c	0.72± .01b	0.74± .01c	1.04± .04c	0.91± .01c
T	7.10± .06c	7.23± .09c	7.90± .12c	6.93± .03c	12.33± .9c	12.83± .09c	15.83± .09c	13.90± .10c	0.75± .01b	0.76± .01c	1.10± .04c	0.96± .01c
S	7.77± .12b	7.83± .12b	9.37± .03b	7.97± .07b	17.93± .12b	20.83± .09b	25.03± .09b	22.47± .12b	1.20± .12a	1.27± .09b	1.73± .07b	1.54± .12b
S+CD	8.87± .03a	9.17± .18a	10.77± .03a	9.83± .09a	22.50± .15a	25.23± .07a	30.17± .12a	28.93± .09a	1.43± .15a	1.58± .02a	2.10± .06a	2.00± .06a

Values marked with the same letter within the columns do not differ significantly @ 5% level of confidence. M= Mustard; LM= Leaf Mustard; R= Radish; C= Cabbage; CD= Cow dung, T= Tissue medium, F= Foam medium, S= Soil medium and S+CD= Soil plus Cow dung medium.

Table 2. Proximate composition of four tested microgreens at harvest (g 100g^{-1} on dry weight basis)

Microgreens	Moisture	Ash	Fiber	Carbohydrates	Proteins	Fat
Mustard	88.0±.46b	2.0±.17a	0.4±.04b	5.0±.15a	4.0±.12b	0.6±.03b
Leaf Mustard	89.5±.29b	1.1±.21b	0.4±.03b	3.8±.06b	4.8±.17a	0.4±.04c
Radish	92.5±.45a	0.6±.05b	0.6±.03a	5.5±.10a	0.7±.12c	.08±.01d
Cabbage	92.0±.72a	0.6±.05b	0.6±.04a	5.5±.15a	0.8±.06c	0.8±.06a

Values marked with the same letter within the columns do not differ significantly @ 5% level of confidence.

54.82% (fresh weight) and 49.65, 53.16, 50.47 and 54.5% (dry weight) respectively in foam medium compared to soil plus cow dung medium (Table 1). Sinha (2005) demonstrated that vigor, seedling height, fresh and dry weight are directly proportional to the nutrient supply, that is why being deficit of nutrients the tissue, foam and soil media showed less vigor, lower seedling height, minimal fresh and dry weight as well. On the other hand, soil plus cow dung (1:1) medium contains NPK at a ratio 3:2:1 (Raj *et al*., 2014) that might supply nutrients and consequently the soil plus cow dung showed the highest vigor, seedling height, fresh and dry weight.

Moisture, ash, fiber, carbohydrates, protein and fat content of mustard microgreen was 88, 2, 0.4, 5, 4 and 0.6 g, respectively whereas these were 89.5, 1.1, 0.4, 3.8, 4.8 and 0.4 g respectively in leaf mustard microgreen. Radish microgreen showed moisture, ash, fiber, carbohydrates, protein and fat content at 92.5, 0.6, 0.6, 5.5, 0.7 and 0.08 g, respectively while cabbage microgreen contained 92, 0.6, 0.6, 5.5, 0.8 and 0.8 g moisture, ash, fiber, carbohydrates, protein and fat, respectively (Table 2). Microgreens contain higher amount of moisture in contrast of low amount of carbohydrates and fat, this could protect human body from weight gain and type 2 diabetes(Seidelmann *et al*., 2018). Diet rich in protein not only maintains and helps lose weight, it also stabilizes the blood sugar, boost energy level and supports the absorption of important nutrients (Westerterp-Plantenga *et al.,* 2009). Fiber plays a crucial role in digestive, heart and skin health and it can also help in lowering blood cholesterol and glucose levels (Anderson *et al.,* 2009).

Total chlorophyll in mustard, leaf mustard, radish and cabbage microgreens was 0.81, 0.92, 0.67 and 0.86 mg 100g^{-1} fresh weight, respectively. Mustard, leaf mustard, radish and cabbage microgreens contained β-carotene at 0.24, 0.18, 0.23 and 0.21 mg 100g^{-1} fresh weight, respectively while they contained lycopene at 0.44, 0.5, 0.43 and 0.42 mg 100g^{-1} fresh weight, respectively (Table 3). Chlorophyll is good source of nutrients with anti-inflammatory, wound-healing (Lanfer-Marquz *et al.,* 2005) and antioxidant properties that reduces the binding of carcinogens to DNA (Ferruzzi and Blakeslee, 2007). β-carotene and lycopene protect our body from free radicals and help to maintain the health of our skin, eyes and immune system (Burri, 1997; Kang *et al.,* 2003).

Microgreens contain different mineral nutrients. Mustard microgreen contained 0.18, 0.41, 0.51, 9.50, 0.80 and 0.38 g Na, K, S, P, Ca and Mg, respectively. Na, K, S, P, Ca and Mg contents were 0.23, 0.52, 0.52, 9.20, 1.80 and 0.78 g, respectively in leaf mustard microgreen; 0.12, 0.28, 0.57, 10.40, 1.12 and 0.38 g, respectively in radish microgren; and 0.13, 0.18, 0.52, 10.53, 1.24 and 0.47 g, respectively in cabbage microgreen as well. Microgreens also contained some trace mineral nutrients i.e. Fe and Zn. Fe and Zn contents were 293.67 and 653.33 mg, respectively in mustard microgreen whereas 319.67 and 748.67 mg, respectively in leaf mustard microgreen. Radish and cabbage microgreens showed 215 and 299.67 mg, Fe respectively while they showed 261.67 and 243.33 mg, Zn respectively (Table 4). In general, microgreen cotyledon leaves had considerably higher nutritional densities than their mature counterparts (Xiao *et al.,* 2012 Pinto *et al.,* 2015; Xiao *et al.,* 2016). Potassium (K) helps in maintaining normal physiological function, water balance and pH balance of the body (Tazoe *et al.,* 2007). Sodium (Na) helps in regulating blood pressure and in maintaining proper function of muscle and nerves (Hassan and Umar 2004). Deficiencies of calcium (Ca) leads to osteoporosis and it assists in bone formation and prevents heart diseases (Hassan and Umar, 2006). Magnesium (Mg) is involved in calcium metabolism in bones and helps to prevent heart diseases (Hassan and Umar, 2006). Sulfur (S) seems to have antibacterial effects against the bacteria that cause acne (Gupta and Nicol, 2004). The main function of phosphorus (P) is the formation of bones and teeth (Anderson, 1996). It also assists in growth, maintenance and repair of cells as well as tissues (Bernhardt and Kasko, 2008). Iron (Fe) is not only essential in the formation of haemoglobin and oxidation of

Table 3. Bioactive compounds (mg 100g^{-1} fresh weight) of four tested microgreens at harvest

Microgreens	Chl a	Chl b	Total Chl	β-carotene	Lycopene
Mustard	0.57±.01a	0.24±.16c	0.81±.06b	0.24±.01a	0.44±.01a
Leaf Mustard	0.63±.14a	0.29±.16b	0.92±.06a	0.18±.01b	0.50±.02a
Radish	0.41±.11c	0.26b±.16c	0.67±.08c	0.23±.01a	0.43±.02a
Cabbage	0.47±.05b	0.39±.15a	0.86±.08ab	0.21±.01ab	0.42±.02a

Values marked with the same letter within the columns do not differ significantly @ 5% level of confidence.

Table 4. Mineral composition of four tested microgreens at harvest (dry weight basis)

Vegetables	Na (g100g-1)	K (g100g-1)	S (g100g-1 -)	P (g100g-1)	Ca (g100g-1)	Mg (g100g-)	Fe (mg 100g-1)	Zn (mg 100g-1)
M	0.18± .01ab	0.41± .02b	0.51± .01b	9.50± .36ab	0.80± .02c	0.38± .01b	293.67± 2.60b	653.33± 4.41b
LM	0.23± .01a	0.52± .01a	0.52± .00b	9.20± .21b	1.80± .17a	0.78± .02a	319.67± 2.60a	748.67± 6.96a
R	0.12± .01b	0.28± .01c	0.57± .01a	10.40± .21a	1.12± .07bc	0.38± .01d	215± 2.89d	299.67± 6.06c
C	0.13± .02b	0.18± .00c	0.52± .00b	10.53± .24a	1.24± .02b	0.47± .01c	261.67± 6.01c	243.33± 4.81d

Values marked with the same letter within the columns do not differ significantly @ 5% level of confidence. M= Mustard; LM= Leaf Mustard; R= Radish and C= Cabbage.

carbohydrates, protein and fats (Adeyeye and Otokiti, 1999) but also helps in preventing of anemia (National Research council, 1989). Zinc (Zn) involves in protein and DNA synthesis, wound healing and in normal cell division (Prasad, 1995).

All microgreens contained abundant vitamin C. Mustard, leaf mustard, radish and cabbage microgreens contained vitamin C at 12.3, 16, 8.53 and 8.53 mg 100g^{-1} fresh weight, respectively. We found the maximum vitamin C content in leaf mustard microgreen (16 mg 100g^{-1} fresh weight) followed by mustard microgreen (12.30 mg 100g^{-1} fresh weight). Radish and cabbage microgreens showed the lowest (8 mg 100g^{-1} fresh weight) vitamin C (Fig. 2). Generally, young edible seedlings are dynamic source of vitamin C, an antioxidant that helps protect our body from the harmful carcenogenic free radicals (Stratton and Godwin, 2011). It is also useful against common cold and skin infection (Hodges *et al.,* 1969; Heimer *et al.,* 2009**)**. The DPPH radical scavenging activity with an IC_{50} value of mustard, leaf mustard, radish and cabbage microgreens was 1.20, 0.75, 2.90 and 3,57 µg, respectively. Here, the lowest IC_{50} value reveals the highest anti-oxidant capacity (Fig 2). DPPH itself is a radical that binds with other free radicals and leads removal of free radical from body thus ensures healthy and long life (Sharma and Bhat, 2009).

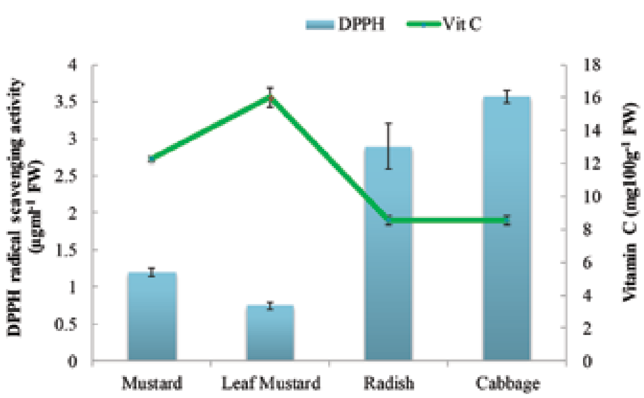

Fig. 2. DPPH radical scavenging activity and Vitamin C content in four tested microgreens. The vertical bars represent the mean ± SE (n=3). (FW= Fresh weight)

In conclusion we could say that soil plus cow dung medium showed the peak performance over the remaining media. It might be due to optimum water holding capacity, low bulk density and nutrients composition of soil plus cow dung medium which gives proper germination, easy root penetration and anchorage and adequately supplements nutrients. With regard to nutritional aspects, all microgreens contained abundant bioactive compounds, vitamin C, mineral nutrients, and DPPH radical scavenging activity that protect from malnutrition and chronic diseases.

Authors' contribution

Conceptualization of research work and designing of experiments (MAH, MASP); Execution of field/lab experiments and data collection (MASP, SS); Analysis of data and interpretation (MASP); Preparation of manuscript (MAH, MASP, MAS)

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