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Effects of growing substrates and seed density on yield and quality of radish (*Raphanus sativus*) microgreens

VU THI THUONG[1] AND HOANG GIA MINH[2,*]

[1]*Hanoi Pedagogical University 2*
Nguyen Van Linh Str., Xuan Hoa, Phuc Yen, Vinh Phuc, Vietnam
**(e-mail : hoangminhpra@gmail.com)*

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ABSTRACT

Microgreens are a new class of edible fresh vegetable salads gaining more popularity as a new culinary ingredient which are harvested when cotyledons fully expanded before the true leaves completely emerge (7 to 21 days after germination). Being new to the market proper substrate and seed density are yet to be determined in order to increase the productivity. This present study was conducted during 2019 at the high-tech greenhouse, VinEco Company, Vinh Phuc province, Vietnam to evaluate effects of growing substrates and seed density on yield and quality of radish microgreens in greenhouse conditions. The studied growth parameters were germination rate, fresh weight, dried weigh and quality of radish microgreens grown on different substrates of white sphagnum peat and coco-coir dust. Results showed that white sphagnum peat and coco coir dust had no effects on germination rate, dried matter yield and shoot height of radish microgreens. The use of higher fertilizer concentration as coco coir substrate did not make fresh shoot yields higher than white sphagnum peat substrate with lower fertilizer concentration. Seed density significantly affected fresh shoot yield of radish microgreens grown on white sphagnum peat and coco coir substrate. Radish microgreen cultivation obtained highest fresh yield when sown seeds with density of 8 seeds/cell (approximate 109 g seeds). Nitrate level and microbiological contamination (*E. coli* and *Salmonella spp.*) of radish microgreens grown on these substrates are within legal and in safe limit and therefore can be considered as a safe and good fresh vegetable salad in the human diet.

Key words : Growth media, microgreen production, radish, *Raphanus sativus*, seed density

INTRODUCTION

Microgreens (*Raphanus sativus*) are a fresh agricultural product cultivated and sold as a salad, similar to sprouts and baby leaves. Microgreens are generally harvested after 7 to 21 days of growth from seed. Stems and cotyledons are harvested and the root is left behind in the growth medium. The first true leaves may or may not be present depending on growth rate and preference. Unlike sprouts, the roots of microgreens growing in substrates are not edible, making them a safer vegetable in general (Xiao *et al.*, 2015; Naserzadeh et al., 2018; Reed *et al.*, 2018; Bayat *et al.*, 2019). Increasingly used by chefs as garnish, toppings and in salads, microgreens are gaining popularity as a functional food by high content of vitamins, bioactive compounds and antioxidants. More importantly, microgreens contain more nutrients than mature crops of the same type (Xiao *et al.*, 2012; Weber, 2016).

With the development of the urban farming industry, there is an increasing interest in high-value commercial production. Microgreens may be grown in greenhouses or indoors with artificial light sources (Choi *et al.*, 2015) in the soil or most commonly in soil-less systems using organic or in-organic growing substrates or hydroponic medium such as vermiculite, perlite and peat moss (Murphy *et al.*, 2010; Murphy and Pill, 2010; Di Gioia *et al.*, 2015; Mir *et al.*, 2017). Gutierrez (2018) claims that the microgreen production industry is split evenly between conventional and hydroponic growing methods. There is currently not enough evidence available to growers to make informed decisions on which production methods would

[2]Institute of Ecology and Biological Resources (IEBR), Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Str., Cau Giay Dist., Hanoi city, Vietnam.

work best for their situation. For instance, very few articles are available which compare seed density for all the varieties. No comparisons were found for the seed density differences of each variety among growing mediums. Currently, the detailed comparison is available only for broccoli microgreens, where the results suggested no difference for seed density in soil and hydroponics (Di Gioia *et al.*, 2017).

Every year, Vietnam imports a large quantity of growing substrates for nursery and horticultural production including microgreens. In spite of big advantages of locally available raw coco-peat materials for substrate production, no standardized growing substrates for microgreen are available for commercial production at large scale. There are very limited scientific investigations and studies on growing substrates for microgreen production in greenhouse. Especially, growers still do not know suitable seed density to maximize microgreen yield. Based on all these constraints, this study was conducted to study the effects of different growing substrates and seed density on the yield and quality of radish microgreens grown in greenhouse.

MATERIALS AND METHODS

Experimental Site, Plant Material and Growing Substrates

The experiment was conducted during October 2019 at the high-tech greenhouse, VinEco Company located in Gia Khang town, Binh Xuyen district, Vinh Phuc province, Vietnam (21°20' N ;105°38' E). The average air temperature was monitored daily during the experiment by the internal sensors Galcon in the greenhouse. Air humidity level was controlled at 85% by humidifier. Greenhouse screen was opened automatically during the day-time and closed before darkness (about 1 hour before darkness). During the experimental period, the screens open when temperature in the greenhouse exceed 34°C, or radiation level exceeds 600 watts/SM.

Red radish (*Raphanus sativus*) cultivar "Sango" was used throughout the experiment. The seeds were untreated and imported directly from Suba Seed Company, Italy with high quality and up to 93% germination at a constant temperature of 20°C with relative humidity of 85%.

Irrigation and Fertilization for Microgreen

The irrigation system was done using moving spray system "boom" to provide water and fertilizer solution for microgreens. In each experiment during first three days in the greenhouse, microgreens were irrigated with drinking water by using the auto irrigation system. After 4 days of germination, the seedlings were fertigated daily using a nutrient solution containing all the essential macro and micro nutrients with the concentrations expressed in ppm: N 100, P 18, K 109, Ca 45, Mg 30, S 13, Zn 0.04, Fe 0.26, Cu 0.03, Mn 0.13, B 0.06, Mo 0.02. The fertilizer solution (EC = 0.8 mS/cm, pH = 5.5-6.5) was sprayed daily using auto irrigation system at spraying time of 8:00 and 16:00. There was no irrigation on the harvesting day to avoid water on the surface of microgreen leaves before cutting.

Preparation of Growing Substrates

The specifications of the tested growing substrates are presented in Table 1.

Experiment 1. Effects of Different Growing Substrates on Growth Attributes

Thirty plastic trays (14.5 x 9.5cm, height 5cm) for each growing substrate with drainage holes were prepared and filled with each substrate to a height of 4cm in the case of peat. Seeds were sown manually on the surface of the growing substrate in each tray at the rate of 150 seeds/tray. After sowing, the trays were irrigated manually using a water-nozzle and then placed into a dark germination room under full climate control, maintaining 24 hours and 7 days temperature of 25°C and 85-90% relative humidity. After 24 hours in the germination room, the trays started to germinate were taken out and be placed on the aluminum benches in the greenhouse. All treatments were replicated for three times under Completely Randomized Design. Each experimental unit contained 10 trays. After 8 days of sowing, germination rate was calculated by the following formula:

$$\text{Germination rate (\%)} = \frac{\text{No. of germinated seeds}}{\text{No. of seeds tested}}$$

Table 1. Chemical characterization of the tested growing substrates used in the production of red radish microgreens

Substrates	T_1	T_2	T_3
Origin	Import	Import	Vietnam
Materials	75% white sphagnum peat +25% vermiculite (size 4-6mm)	75% white sphagnum peat +25% vermiculite (size 4-6mm)	75% coco coir dust+25% vermiculite (size 4-6mm)
pH (H_2O)	5.7	6	6.2
EC (mS/cm)	0.21	0.45	1.2
Nutrient content:			
N water soluble (mg/L)	110	115	120
NO_3^- - N (mg/L)	60	75	80
NH_4^- - N (mg/L)	50	40	40
P_2O_5 soluble (mg/L)	55	50	40
K_2O soluble (mg/L)	150	140	125
Ca (mg/L)	-	-	2.5
Mg (mg/L)	6 mg/L	7 mg/L	-
Soluble humic acid	-	-	0.41%
Screening	Fine (0-10 mm)	Fine (0-5 mm)	Fine (1-7 mm)
Moisture content (%)	35-40%	25-30%	40-45%

T_1 : White sphagnum peat substrate I, T_2 : White sphagnum peat substrate II, T_3 : Coco coir dust.

Microgreens of each tray were harvested by cutting the seedling just above the surface of the growing substrate with a sterilized knife. Productivity variables evaluated were shoot fresh weight (FW) per unit area, shoot dried matter yield and shoot height at harvest. The shoot height was measured with a ruler from the stem base to the top of the seedling in five points of the tray. Harvested biomass from each treatment was placed into pre-weighed foil cups and weighed. The foil cups were placed into a drying oven at 80°C for 48 hours prior to weighing again to determine the dried matter yield (DMY, g/m^2).

Experiment 2. Effect of Seed Density and Growing Substrates on Microgreens Yield

The styrofoam trays (70 × 46cm, height 5cm, 442 cells "2 × 2cm") were used in this experiment. All trays were washed and sterilized by chlorine solution 200 ppm for 3 minutes, and then dried naturally during 24 hours in greenhouse. Seeds were sown automatically by the seeding machine (Model ALFA65 Urbinati Italia) filled with the tested white sphagnum peat substrates (T_1 and T_2), and coco coir dust T_3. Quantity of seeds per tray was calculated considering 1,000 seed weight (approximate 10g/1000 seeds). There were 4 treatments of the seed density programmed on the machine including: 6 seeds/cell (82.36 g/m^2), 8 seeds/cell (109.81 g/m^2), 10 seeds/cell (137.27 g/m^2) and 12 seeds/cell (164.72 g/m^2).

After completing the sowing of seeds with the help of seeding machine, the trays of the experiment were placed into a dark germination room as described in the Experiment 1. Five trays were designed for each treatment. All treatments were replicated for three times under Completely Randomized Design.

Eight days old microgreens were harvested by cuts at the base of the seedling near the substrates, using the cutting machine. Harvested microgreens from each treatment were placed into pre-weighed foil cups and weighted to determine the fresh weight (FW, kg/m^2). Based on shoot FW of microgreens in each treatment of seed density, substrate costs were calculated in USD per kg of harvested microgreens.

Experiment 3. Quality Assessment of Harvested Radish Microgreens

Harvested microgreens from greenhouse were sent to the laboratory of Vietnam National Institute of Food Control (Cau Giay district, Hanoi city, Vietnam) for analyses of nitrate concentration and micobiological evaluation (*E. coli* and *Salmonella spp.*). Standard limit of nitrate residue and microbiological contamination in each sample were adopted from the standard regulated by World Health Organization (WHO).

From each tested substrate, 20 g microgreens harvested from each tray were mixed and washed with distilled water and placed in specific packets, then samples were placed in an oven at 70°C temperature for 48

hours. These samples were powdered after parching and extracted by 0.2% acid citric solution. One gram powder was taken from each sample and 100 ml of 0.2% acid citric solution was added to samples and placed in shaker device for 20 to 30 minutes to combine samples and solutions. Then extraction was done by filter paper and juice kept in a specific bottle. Then, nitrate reading was done by spectrophotometer and Palin test (photometer 7100) to compare nitrate values in different devices and nitrate of some samples was recorded randomly.

Microbiological analyses were performed to evaluate the microbiological contamination of the three growing media and microbial growth levels on microgreens grown on each substrate. For detection and enumeration of *Escherichia coli* and *Salmonella spp.* contamination, the following analysis standards were used :

For *E. coli :* TCVN 7924-2:2008, ISO 16649-2:2001;

For *Salmonella spp.*: TCVN 10780-3:2016, ISO/TR 6579-3:2014.

The collected data from treatment were statistically analyzed by using Minitab 16. The values represent the mean of germination rate, fresh weight, dried matter yield and shoot height ± SE (standard errors), p-values from Fisher's LSD test at P=0.05 level of confidence.

RESULTS AND DISCUSSION

Effects of Different Growing Substrates on Growth of Radish Microgreens

The local substrate coco coir presented the highest electrical conductivity (EC = 1.2 mS/cm), the EC of white sphagnum peat substrates T_1 and T_2 were 0.21 and 0.45 mS/cm, respectively. The pH and EC of the growing substrates effects significantly the performance of crops, including germination and rooting stage. The optimal pH and EC for seedlings and salt sensitive crops are neutral (5.4-6.2) and (1.0-2.6), respectively (Diane *et al.*, 2009). The tested substrates had pH values very close to the recommended range. In this study, the material of local substrate coco coir was treated to reach EC ≤ 0.5 mS/cm before used for producing substrate and then mixed with nutrients to make a growing substrate for testing, with EC =1.2 mS/cm higher than the substrates of white sphagnum peat imported from overseas. Additionally, coco coir substrate had a small amount of humic acid mixed with an aim of promoting germination and root growth.

The germination rate of radish microgreen seeds sown in the substrates white sphagnum peat (T_1, T_2) and coco coir (T_3) was 91.11, 88.80 and 88.27%, respectively (Table 2). The substrates had no significant effect on germination rate (P = 0.178), dried matter yield (P = 0.169) and mean shoot height (P = 0.184). However, the growing substrates had significant effect on fresh weight of radish microgreens. Radish microgreens grown in white sphagnum peat T_1 and coco coir T_3 had higher FW than the substrate of white sphagnum peat T_2 (P = 0.001 < 0.05). All of the microgreen trays had good performance on three tested growing media and no disease infection during growing days. According to Bewley (1997), growing substrates with good aeration (20-30%) and optimal water holding capacity (50-70%) promoted higher seed germination. In this study, the tested growing substrates had good quality and were suitable for germination. In Vietnam, there are several local biomaterials that could be substituted for imported vermiculite and white sphagnum peat

Table 2. Effects of different growing substrates on germination rate, shoot fresh weight (FW), shoot dried matter yield (DMY) and shoot height of radish microgreen at harvest in greenhouse (values are means ± standard errors)

Substrates	Germination rate (%)	FW (g/m^2)	DMY (g/m^2)	Mean shoot height (cm)
T_1	91.11 ± 1.21[a]	2567.2 ± 102[a]	103.97 ± 4.12[a]	8.11 ± 0.08[a]
T_2	88.80 ± 1.10[a]	2109.7 ± 77.5[b]	95.07 ± 3.41[a]	7.89 ± 0.08[a]
T_3	88.27 ± 1.10[a]	2455.2 ± 87[a]	103.61 ± 3.67[a]	7.99 ± 0.08[a]
df	2	2	2	2
F	1.76	7.13	1.81	1.72
P	0.178	0.001	0.169	0.184

T_1 : White sphagnum peat substrate I, T_2 : White sphagnum peat substrate II, T_3 : Coco coir dust.
Figures in a column followed by the same letter are not differed significantly.

substrates resulting in more affordable growth media. Coconut coir dust could be a good alternative substrate for microgreens production.

The microgreens were harvested by cuts at the base of the seedling at lower hypocotyl level near the substrates (Fig. 1). The fresh shoot yield of radish microgreens grown in the substrate white sphagnum peat T_1 and coco coir T_3 was 2567.2 and 2455.2 g/m^2, respectively. These values were higher than those reported by Johnny (2017). In this study, vermiculite (size 4-6mm) was used to mix with the tested substrate to increase aeration for the root system. The substrate experiment conducted by Muchjajib *et al.* (2015) showed that using sugarcane filter cake mixed with coconut coir produced higher microgreen yield of radish microgreens (3900 g/m^2). In this study, coco coir substrate had EC higher (EC = 1.2) than other tested substrates but FW of radish microgreens had not significantly different from white sphagnum peat T_1 (EC = 0.21). It could be found that the use of other sources of fertilizers as growing media may not be much useful as seed itself had enough food storage, which could provide nutrients during germination. The storage food was enough to achieve the cotyledon stage for most of microgreens. The true-leaf stage was achieved within 2-3 days after the development of cotyledons and this is the time period when platelets start photosynthesis and uptake of nutrients from growing substrate (Treadwell *et al.*, 2010).

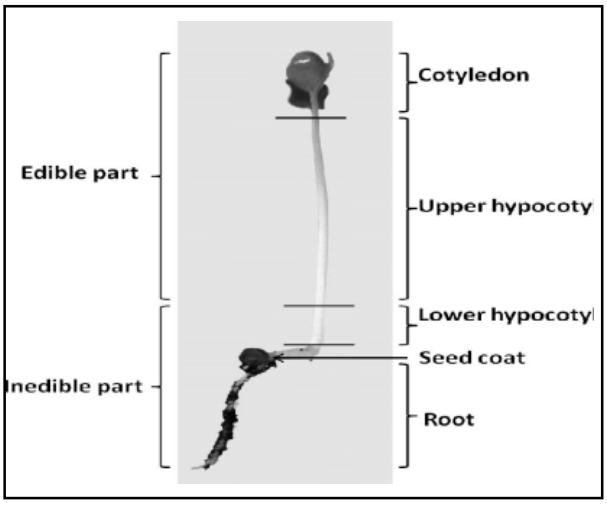

Fig. 1. Diagram of 7 days old radish microgreen. The upper hypotcotyl and cotyledon sections are the edible parts (Xiao *et al.,* 2015).

Effect of Seed Density and Growing Substrates on Microgreens Yield

All tested substrates had a moisture content ranging from 25 to 45% that was suitable for the automatic seeding machine. The seeding line includes the following automatic steps:

- Mixing of tested substrates with vermiculite (size 4-6mm)
- Filling of each substrate in the 442-hole trays
- Sowing of seeds
- Spreading of light layer of vermiculite (size 2-3mm) over the seeds
- Light irrigation in seeded trays using drinking water

Seed density had significant effects on FW of radish microgreens grown in different growing substrates (P <0.001) (Table 3). This study showed that seed density of radish ranging from 8 to 10 seeds per cell (approximate 110 to 140g/m^2) produced highest FW when grown in each substrate. At seed densities higher 10 seed/cell (approximate 140 g/m^2), the FW yield of radish microgreens tends to decrease in all tested substrates.

Table 3. Effect of seed density on shoot fresh weight (FW) yield grown on different growing substrates (values are means ± standard errors)

Substrates	Seed density (seeds/cell)	Approximated seed density (g/m^2)	FW (g/m^2)
T_1	6	82.36	839.86 ± 37.7^c
	8	109.81	1318.01 ± 28.8^a
	10	137.27	1135.92 ± 50.4^b
	12	164.72	910.14 ± 39.3^c
T_2	6	82.36	638.92 ± 25^c
	8	109.81	1102.38 ± 48.2^a
	10	137.27	1018.22 ± 38.3^{ab}
	12	164.72	888.82 ± 35.5^b
T_3	6	82.36	708.59 ± 37.7^c
	8	109.81	1271.84 ± 35.7^a
	10	137.27	1164.70 ± 43.4^a
	12	164.72	869.88 ± 38.3^b

T_1 : White sphagnum peat substrate I, T_2 : White sphagnum peat substrate II, T_3 : Coco coir dust.
Figures in a column followed by the same letter are not differed significantly.

Seed density is one of the most important factors influencing on microgreen yield and production efficiency (Storey, 2017). Bulgari *et al.* (2017) reported that microgreen production with substrate vermiculite in cell

trays used densities of 45 to 242 g/m^2. According to experimental results of Nolan (2019), with higher densities the FW yield could be bigger but the cost of seeds would be increasing. However, there was no or little correlation between seed density and microgreen yield. Additionally, sowing seeds with higher densities can cause fungal disease infections that reduce the quality and productivity of microgreens. In the present study, the root rot disease occurred in some trays when sowing was done with higher densities of 12 seeds/cell. Several other workers also reported that higher seed densities results in development of fungal diseases that reduce quality and productivity of microgreens (Treadwell *et al.*, 2010; Storey, 2017; Johnny, 2017). The seed density may be an overlooked factor to crop success when producing microgreens in greenhouse. Besides impact of seed density, microgreens yield is also influenced by other factors such as environmental conditions in greenhouse, quality of irrigation water (Kaiser and Ernst, 2018), quality of growing media (Muchjajib *et al.*, 2015) and type of microgreen cultivation technology (Di Gioia *et al.*, 2017; Nolan, 2019).

White sphagnum peat substrates are imported directly from Europe and quite costly. In fact, the price of coconut coir dust substrate has lower price than other tested substrates. The seed density of 8 seeds/cell had lowest cost of growing substrates for radish microgreens production (Table 4). At 8 seeds/cell density, the radish microgreens had highest sheet fresh yield as presented in Table 3, resulting in reducing production cost. At lower seed density of 6 seeds/cell obtained lowest shoot fresh yield, and therefore, cost of growing media was highest in comparison with higher seed densities. This study showed that coco coir dust had potential to substitute imported white sphagnum peat substrates for microgreens production costs. However, further studies should be conducted using other local raw materials to mix with coco coir substrate to have more alternative substrates for microgreens production in particular and for nursery use in general.

Quality Assessment of Harvested Radish Microgreens

Table 4 presented the nitrate residue and bacterial contamination (*E. coli* and *Salmonella spp.*) of radish microgreen shoots grown on different growing substrates. The limits detected for nitrates and bacteria in microgreen samples of this study were within the legal limits recommended by European Union (2011) and WHO (2011) standard, therefore, from the point of view of nitrates and microbiological contamination, microgreens grown on these tested substrates are safe.

Nitrates are compounds considered as harmful for human health and the most part of the daily intake of nitrates by foods is related to fresh vegetable consumption (Santamaria, 2006; Iammarino *et al.*, 2014; Majeed, 2020; Nguyen and Tran, 2020). The contaminant level for nitrate in radish microgreens found on coco coir substrate was 135 mg/kg FW whereas, on white sphagnum peat T_2 was 91 mg/kg FW and on white sphagnum peat substrate T_1 was 59 mg/kg FW (Table 5). The coco coir substrate had highest nitrate concentration but still lower than legal levels. From a case of coco coir substrate (EC = 1.2), it seems that nitrate levels were significantly affected by N fertilizer concentration in growing substrates. Similar results were found by Pussemier *et al.* (2006), who reported significant differences in average nitrate contents from types of organic and inorganic production. Moreover, these result

Table 4. Cost of each growing substrate in radish microgreen production at different seed density : A case of using the automatic seedling machine

Substrates	Mean quantity of substrate (g/m^2)	Price of substrate (USD/kg)	Cost of growing substrate at different seed density (USD/kg havested microgreen)			
			6 seeds/cell	8 seeds/cell	10 seeds/cell	12 seeds/cell
T_1	3663	0.62	2.7	1.72	2	2.5
T_2	3163.5	0.54	2.67	1.55	1.68	1.92
T_3	4329	0.32	1.95	1.09	1.19	1.59

T_1 : White sphagnum peat substrate I, T_2 : White sphagnum peat substrate II, T_3 : Coco coir dust.
Figures in a column followed by the same letter are not differed significantly.

Table 5. Nitrate residue and bacterial contamination (*E. coli* and *Salmonella spp.*) of radish microgreen shoots grown on different growing substrates

Parameters \ Substrates	Unit	T_1	T_2	T_3	Residue/contamination limit	
					Minimum	Maximum
NO_3^-	Mg/kg FW	59	91	135		1000
E. coli	CFU/g	0	0	2	0	10
Salmonella spp.	CFU/25g	0	0	0	0	0

T_1 : White sphagnum peat substrate I, T_2 : White sphagnum peat substrate II, T_3 : Coco coir dust.
Figures in a column followed by the same letter are not differed significantly.

showed that nitrate level in radish microgreens was much lower than that in mature radish recommended by WHO (2011).

Concerning microbiological contamination of radish microgreen in greenhouse, *E. coli* was observed only on microgreens grown on coco coir dust but was within acceptable limit. *Salmonella spp.* was not detected on microgreens grown on all tested substrates. These results showed that microgreens grown in greenhouse with using clean water for irrigation had lower microbiological contamination than those grown in soil as reported by Balkhair (2016). Similarly, Johnny (2017) also found that the microgreens grown in a protected environment as greenhouse, the risk of bacteria and fungus disease contamination on microgreens was lower than unprotected plants grown in soil or in field conditions. The previous studies conducted by Xiao *et al.* (2015) and Xiao *et al.* (2014) indicated that initial inoculation levels of radish seeds could proliferate *E. coli* strains significantly during microgreen growth. Also, Di Gioia *et al.* (2017) revealed that before sowing, seeds treated with NaOCl was effective in eliminating or reducing microorganisms from the seed surface. However, in this study, source of microbial contamination may occur on source of coco coir dust before sowing. The initial raw material disinfection treatments of growing substrates must be considered to ensure quality of substrate for microgreen production.

CONCLUSIONS

The results of the present study demonstrate that radish microgreens yield was highly influenced by quality of growing substrates and seed densities. The coco coir dust could be applied at low-cost and renewable alternative substrates for microgreens production. However, the raw materials of coco coir dust growing substrates represent a potential source of microbial contamination for microgreens. Therefore, selection of the growing substrates with great attention on the microbiological characteristics is required to ensure better quality and yield of radish microgreens production.

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