RESEARCH ARTICLE

Yield optimization, microbial load analysis, and sensory evaluation of mungbean (Vigna radiata L.), lentil (Lens culinaris subsp. culinaris), and Indian mustard (Brassica juncea L.) microgreens grown under greenhouse conditions

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Abstract

Microgreens have been used for raw consumption and are generally viewed as healthy food. This study aimed to optimize the yield parameters, shelf life, sensory evaluation and characterization of total aerobic bacteria (TAB), yeast and mold (Y&M), Escherichia coli, Salmonella spp., and Listeria spp. incidence in mungbean (Vigna radiata L.) | Wilczek), lentil (Lens culinaris Medikus subsp. culinaris), and Indian mustard (Brassica juncea (L.) Czern & Coss.) microgreens. In mungbean and lentil, seeding-density of three seed/cm², while in Indian mustard, eight seed/cm² were recorded as optimum. The optimal time to harvest mungbean, Indian mustard, and lentil microgreens were found as 7th, 8th, and 9th day after sowing, respectively. Interestingly, seed size was found highly correlated with the overall yield in both mungbeans (r² = .73) and lentils (r² = .78), whereas no such relationship has been recorded for Indian mustard microgreens. The target pathogenic bacteria such as Salmonella spp. and Listeria spp. were not detected; while TAB, Y&M, Shigella spp., and E. coli were recorded well within the limit to cause any human illness in the studied microgreens. Washing with double distilled water for two minutes has shown some reduction in the overall microbial load of these microgreens. The results provided evidence that microgreens if grown and stored properly, are generally safe for human consumption. This is the first study from India on the safety of mungbean, lentils, and Indian mustard microgreens.
Introduction

Microgreens are nutritionally superior food that can be produced from several crops including vegetables, herbs, grains, and some wild species and offer a good option for addressing the problems arising due to rapid urbanization [1]. Microgreens are generally seven to twenty-one days old tender immature greens of 5–10 cm height having three major parts: cotyledonary leaf, stem, and a pair of true leaves [2, 3]. The use of microgreens was reported for the first time during the late 1980s by the chefs working in some restaurants located in San Francisco, California, United States of America (USA) for culinary purposes [4]. The global microgreens market has four broad segments, (i) Green types (Brassicaceae, Asteraceae, Fabaceae or Leguminosae, etc.); (ii) Farm types (outdoor farming, greenhouse farming, vertical farming); (iii) End-uses (food & beverages, cosmetics, etc); and (iv) Region-based (North America, Latin America, Europe, Asia Pacific, Middle East, and Africa) [5].

The USA is a major contributor to the microgreens global market, followed by Canada and Mexico. By geography, North America is leading the microgreens market with a share of nearly 50% in terms of dollar sales in 2019 [6]. The large-scale microgreens farming in the USA and consumption (mostly in the restaurants) are supporting the market in this region [6]. During 2020–2025, the microgreens market at the global level is anticipated to grow at a compound annual growth rate (CAGR) of 7.5–8.0% [7]; while the microgreens market in the USA is projected to register a CAGR of 10.1% [5]. Microgreens are generally produced as an organic product; however, now trend is shifting towards biofortification of microgreens for various minerals including Selenium [8], Fe, and Zn [9]. Microgreens can be comfortably grown irrespective of season in a variety of growing media, depending on the scale of production [3, 10]. Optimization of seeding density and day of harvesting will help in minimizing the microgreens production cost.

Microgreens are usually consumed without heat treatment or decontamination [11] and when compared to sprouts, microbial contamination is not so rampant in microgreens [11, 12]. However, many recalls have happened in the USA and Canada due to Salmonella [13, 14], and Listeria contamination [15–18]. Salmonella spp., Escherichia coli, and Listeria spp. are the most common bacterial pathogens associated with fresh produce and sprouts [19–23]. The microflora of microgreens is reportedly influenced by the type and composition of growing-medium (soil, peat, vermiculite, or hydroponics) [12, 24, 25], seed-contamination, care taken during harvesting and storage of the microgreens [18].

Being fresh-cut product, microgreens have a relatively very short shelf life, which does vary depending upon the species [26, 27]. The quick post-harvest quality deterioration is also due to their high surface area to volume ratio, delicate leaves, and high respiration rate [28–30]. Immediately after harvest, microgreens can be marketed or should be washed, packed, and stored under cool conditions (1–5 °C) [24, 26]. Thus, various post-harvest treatments such as washing, packaging, and storage conditions become very crucial for extending their shelf life including the sensory qualities of freshly cut microgreens [29–31]. Several sanitizers, including washing of microgreens with tap water, chlorinated water, citric acid, ascorbic acid, and their subsequent storage at 5°C for up to 9 days was reported on Chinese cabbage microgreens [29]. Amongst all the variables, storage temperature is considered as the key factor affecting overall quality including microgreens shelf life [26, 31]. This study was aimed to optimize various yield parameters including seeding density and day of harvesting of microgreens of mungbean, lentil, and Indian mustard. These microgreens were also washed, packed, and stored for a variable duration at refrigeration temperature for shelf life and evaluation of microbial load (TAB, Y&M, E. coli, Salmonella spp., and Listeria spp.).
Material and methods

Genotypes used and growing conditions

To optimize the seeding density, harvesting stage, and marketable yield, seeds of 20 mungbean and lentil genotypes each and two Indian mustard genotypes (Table 1; Fig 1) were sown in three replications with different seeding-density: two-seed/cm², three-seeds/cm², and four-seeds/cm² for mungbean and lentil, while six, eight, and ten seeds/cm² for Indian mustard.

The selected mungbean and lentil genotypes were very diverse for several parameters including antioxidant activities and the mineral profiles [3]. In addition, PDZM31 (Pusa Double Zero Mustard-31) is the first double zero (erucic acid <2% and glucosinolates <30ppm) mustard variety of India [32], while PM28 is a short duration variety. Microgreens are commercially produced under partially controlled conditions on different growing-medium like cocopeat or a combination of cocopeat, vermiculite, and sand. In addition, to avoid any variations (in quality of the produce) due to the growing conditions (temperature, photoperiod, etc.), the genotypes were grown under partially controlled conditions in the National Phytotron Facility, IARI, New Delhi which is located at the latitude, longitude, and altitude of 28.6412˚ N, 77.1627˚ E, and 228.61 m AMSL, respectively. The desired temperature was maintained for mungbean (28/26˚C), lentils (21/18˚C), and Indian mustard (21/18˚C) along with a 10:14 h of day and night cycles.

Freshly harvested seeds (Table 1) were obtained from the Division of Genetics, IARI, New Delhi having more than 90% germination. The seeds were surface-sterilized at room temperature.

Table 1. Genotypes, seed-weight, and moisture content in the studied lentil, mungbean, and Indian mustard microgreens.

| S. No. | (a) Mungbean microgreens | (b) Lentil microgreens | (c) Indian mustard microgreens (100 seed weight, g) |
|--------|--------------------------|------------------------|-----------------------------------------------|
|        | Genotype                 | 10 seed weight (g)     | Moisture (%)                                  | Genotype   | 10 seed Weight (g) | Moisture (%) |
| 1      | Pusa Baisakhi            | .37±.02                | 92.76±1.03                                   | L4076      | .21±.006           | 83.59±1.64   |
| 2      | Pusa Ratna               | .41±.01                | 91.14±.69                                    | L4147      | .25±.015           | 85.53±1.73   |
| 3      | Pusa Vishal              | .54±.015               | 90.01±1.44                                   | L4594      | .35±.01            | 83.99±2.27   |
| 4      | Pusa105                 | .43±.021               | 90.46±.82                                    | L7903      | .35±.02            | 83.59±2.24   |
| 5      | Pusa0672                | .42±.02                | 92.59±2.00                                   | HM1        | .37±.06            | 85.70±1.52   |
| 6      | Pusa9072                | .40±.031               | 90.13±.93                                    | BM4        | .22±.03            | 84.21±2.35   |
| 7      | Pusa9531                | .33±.015               | 91.77±1.32                                   | JL1        | .27±.015           | 84.55±1.37   |
| 8      | MH96-1                  | .40±.021               | 92.00±1.41                                   | Sehore74-3 | .24±.02           | 84.32±1.81   |
| 9      | MH318                   | .48±.01                | 92.08±1.35                                   | NDL1       | .22±.06            | 85.21±1.13   |
| 10     | MH421                   | .40±.017               | 92.46±1.66                                   | IPL81      | .21±.06            | 85.62±1.11   |
| 11     | MH521                   | .38±.06                | 92.22±.86                                    | IPL321     | .34±.01            | 83.97±1.90   |
| 12     | MH810                   | .33±.06                | 92.41±2.30                                   | K75        | .28±.015           | 82.69±2.48   |
| 13     | ML512                   | .31±.015               | 92.78±.92                                    | KLS218     | .21±.006           | 85.19±1.28   |
| 14     | ML818                   | .37±.08                | 92.92±1.43                                   | DPL58      | .32±.015           | 84.61±2.45   |
| 15     | PS16                    | .30±.01                | 91.70±1.01                                   | DPL62      | .33±.006           | 84.37±1.76   |
| 16     | TM96-2                  | .36±.02                | 91.76±1.33                                   | PL1        | .38±.015           | 83.45±1.28   |
| 17     | IPM02-3                 | .41±.012               | 93.06±1.07                                   | PL2        | .30±.03            | 83.65±1.96   |
| 18     | IPM02-14                | .41±.08                | 91.95±1.34                                   | PL6        | .28±.015           | 84.95±1.39   |
| 19     | IPM409-4                | .32±.06                | 91.54±1.78                                   | L830       | .20±.006           | 85.95±1.01   |
| 20     | PMR-1                   | .35±.012               | 91.80±1.02                                   | L4602      | .39±.01            | 82.93±1.29   |

(c) Indian mustard microgreens (100 seed weight, g)

1. PM-28    | .44±.03       | 90.5±1.3   | 2. PDZM-31 | .33±.02       | 89.83±1.9   |

Where values are expressed as mean±SD (n = 3).
temperature (24˚C) for one minute in 1% sodium hypochlorite (NaOCl) solution and rinsed twice with sterile water [33] and then sown in plastic trays (38×28×6 cm) in three replicates. The autoclaved (120˚C, 120 Pa, 90 min) growing-medium consisted of coco peat: vermiculite: sand (2:1:1) was used for growing these microgreens. Based on the growth rate, harvesting was performed at different durations for mungbean (after 5th, 7th, and 9th day), lentil (after 7th, 9th, and 11th day), and Indian mustard (after 6th, 8th and 10th day) microgreens.

Microgreens were harvested using ethanol-cleaned scissors by cutting the stem approximately 1.0 cm above the growing medium and were immediately weighed using analytical balance to determine the total fresh weight (FW). Afterward, these were dried in hot air GenLab vertical oven (40˚C for 72h), then weighted and kept in an airtight container for further biochemical analysis. The moisture content was calculated as per the equation:

\[
\text{Moisture} \% = \left( \frac{\text{Initial Weight} \ (g) - \text{Final weight} \ (g)}{\text{Initial Weight} \ (g)} \right) \times 100
\]

Where, Initial weight (g) = Weight of fresh microgreens after harvesting; Final weight (g) = Weight of microgreens after 72h of drying at 40˚C

**Microgreens microbial load analysis**

The incidence of total aerobic bacteria (TAB), yeast & mold (Y&M), *Salmonella* spp., *Shigella* spp., *Listeria* spp., and *E. coli* were assessed for both unwashed (freshly harvested) and washed samples of mungbean (genotypes MH-810, MH-318, PS-16), lentil (genotypes K75, L4594, L830), and Indian mustard (genotypes PM28, PDZM31) microgreens as obtained from the partially controlled conditions. The washing was performed using double distilled water for two minutes and then samples were air-dried in the laminar airflow (Svision, India). The mungbean, Indian mustard, and lentil microgreens were harvested on 7th, 8th, and 9th day of...
sowing, respectively, and were stored in zip lock bags at 4˚C and 1.0 g tissue was used to study the microbial load at 1st, 2nd, 4th, 8th, and 12th day (day of harvest was considered 1st day).

Microbial growth was assayed following the standard protocols [34, 35]. A 1.0 g sample was incubated in 10.0 mL sterile phosphate-buffered saline (PBS, 10x solution from Sigma Aldrich, USA) and vortexed (15 min). Plating of serially diluted samples (1.0 mL) was done on different agar plates. TAB population was identified by plating samples on nutrient agar (NA) supplemented with Amphotericin b (5.0mg/mL; an anti-fungal agent) and incubated at 37˚C for 24–48h. Y&M enumeration was performed by plating samples on potato dextrose agar (PDA, Merck, Germany) supplemented with 50.0 mg/mL Chloramphenicol and incubated at 25˚C for 48 to 72h.

Salmonella and Shigella were recorded (based on their colony morphology) by plating the samples on Xylose Lysine Deoxycholate agar (XLD, Merck, Germany) supplemented with Amphotericin b (5.0mg/mL) and incubated in dark at 35±2˚C for 24 h. Listeria spp. was identified by plating the samples on Chromed Listeria Agar (Merck, Germany) supplemented with Nalidixic acid (13.0mg/mL), Ceftazidime (10.0mg/mL), and Amphotericin b (5.0mg/mL) and incubated at 37˚C for 24 h. E. coli O157:H7 population was identified by plating the samples on Sorbitol MacConkey agar (Merck, Germany) and incubated at 37˚C for 24 h. Each microbial count was determined as the mean of three measurements and the result was expressed as log CFU per g of tissue.

Microgreens shelf life and sensory evaluation

For shelf life and sensory evaluation, two genotypes each of mungbean (MH810 and MH318), lentil (L830 and K75), and Indian mustard (PM28 and PDZM31) microgreens were harvested at the optimum stage and stored in food-grade linear low-density polyethylene (LLDPE) bags to avoid cross-contamination [30]. For mungbean and lentils, the genotypes having largest and smallest seed sizes were selected. The LLDPE bags are of 16x12 cm size (8.0 g per bag) and 51 µ thickness. The samples were then stored in three replications at 4˚C (in dark) for different durations. A panel of seven semi-trained judges (aged 24–45 years) from the IARI, New Delhi (India) performed the sensory evaluation [36]. Sensory evaluation (color & appearance, aroma, taste, and overall acceptability) was performed after 2nd, 4th, and 6th day of storage using a 10-point hedonic scale (10 = like ultimate, 9 = like extremely, 8 = like strongly, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly; 3 = dislike moderately, 2 = dislike strongly, and 1 = dislike extremely [34]). A score of 6 was considered as the limit of salability [37]. A sample size of 2.0 g of each microgreen was used for evaluation.

Electrolyte leakage analysis

The electrolyte leakage of freshly harvested and stored microgreens was measured to find the possible tissue deterioration during storage. For this, 20.0g microgreens sample (from each replicate) was dipped in 400 mL deionized double distilled water (at 20˚C) and gently shaken for 30.0 min. The solution conductivity (µS/cm) was then measured using a conductivity meter (Orion 4-star portable pH/conductivity meter; Thermo Electron Corporation, U.S.A.) by dipping the probe in the sample solution [29, 38].

Statistical analysis

The experiments were conducted thrice and the results were presented as mean±SD. One-way analysis of variance (ANOVA) was performed using SPSS11.5 to compare the groups, and Pearson’s correlation test was used to assess the correlation between means. The mean comparison was performed using Tukey’s test and a P ≤ 0.05 was regarded as significant.
Results and discussion

There has been a growing interest in promoting a healthy lifestyle including consumption of nutritious and quality foods, and microgreens offer a very good option [27, 38–40]. However, growing conditions of microgreens affect their overall yield, while their storage for some time may facilitate the growth of certain harmful microorganisms having food safety risks. Thus, this study was aimed to find the optimum growth parameters, sensory details, and existing microbial load during storage of mungbean, lentil, and Indian mustard microgreens.

Optimization of microgreens yield

The growing medium is very crucial for proper germination, growth of microgreens and its physical properties including porosity and water holding capacity [41]. Three combinations of growing medium consisting of cocopeat: vermiculite: sand in the ratio of 1:1:1, 2:1:1, and 2:2:1 was studied and 2:1:1 medium was found best in terms of water holding capacity. For this growing-medium combination, we generally need only 1–2 light irrigation when microgreens were grown in plastic trays (without holes) under greenhouse conditions [42]. The trays were placed on a leveled surface on benches [24] and two most common factors affecting the total yield include seeding density and plant growth. Also, harvesting microgreens at the right stage is the key production strategy, since the time from sowing to harvesting varies greatly from crop to crop [3, 42].

Optimum seeding density is very specific to the crop species and is generally based on mean seed weight and germination (%) [42]. The yield of microgreens in this study showed an increasing trend with increasing seeding-density (Table 2a and 2b, Fig 2a). But, once it crossed the optimum seeding density, the marketable quality of microgreens got deteriorated. In

| S. No. | Genotype (a) Mungbean | Yield (g/m²) | Genotype (b) Lentil | Yield (g/m²) |
|-------|------------------------|-------------|---------------------|--------------|
|       | 2-Seed/cm² | 3-Seed/cm² | 4-Seed/cm² | 2-Seed/cm² | 3-Seed/cm² | 4-Seed/cm² |
| 1     | Pusa Baisakhi  | 1854.38±19.69e | 1952.94±36.63g | 2125.46±18.68f | L4067 | 948.60±36.28defg | 1059.79±64.44de | 1192.44±40.53c |
| 2     | Pusa Rattna   | 2080.59±49.80abc | 2258.32±54.82bcd | 2359.00±54.75bcd | L1477 | 976.54±47.54defg | 1083.73±86.59cde | 1186.88±81.20c |
| 3     | Pusa Vishal   | 2168.47±55.70a | 2450.11±46.45a | 2572.04±46.75a | L4594 | 1068.92±77.35abcd | 1160.95±78.12abcd | 1308.94±66.62abc |
| 4     | Pusa105      | 2137.43±60.88ab | 2438.23±73.03abc | 2469.06±54.31abc | L7903 | 1073.90±29.65abcde | 1165.06±62.68abcde | 1293.06±87.81abc |
| 5     | Pusa0672     | 2010.75±21.22abcde | 2170.39±51.06def | 2306.78±64.19cde | HM1 | 1093.66±75.70abcde | 1146.97±84.08abcde | 1270.76±85.09abcde |
| 6     | Pusa0972     | 2143.69±50.95a | 2396.13±50.25ab | 2498.30±61.15ab | BM4 | 962.85±36.99defg | 1071.18±77.97cde | 1219.92±48.80abc |
| 7     | Pusa9531     | 1922.23±59.60de | 2021.69±66.35g | 2215.75±70.09def | JLI | 900.63±27.88g | 1053.69±98.26de | 1213.72±94.52bc |
| 8     | MH96-1       | 1886.18±94.93e | 1974.84±48.69g | 2159.79±71.78f | Sehore74-3 | 941.36±45.25efg | 1024.47±28.27de | 1129.1±29.31c |
| 9     | MH318        | 2078.95±41.11abcd | 2359.50±81.37abc | 2461.43±69.90abc | NDLE-1 | 899.45±8.41g | 1028.14±58.64de | 1148.75±79.44c |
| 10    | MH421        | 1946.48±66.58cde | 2069.36±83.16def | 2213.84±47.42def | IPL81 | 934.37±66.02g | 1061.88±69.52de | 1215.28±58.52bc |
| 11    | MH521        | 1953.81±25.14cde | 2063.26±33.97efg | 2200.01±40.77ef | IPL321 | 1147.37±55.78abc | 1281.78±29.61ab | 1393.13±84.96ab |
| 12    | MH108       | 1961.55±51.24cde | 2128.19±54.61def | 2222.92±66.69def | K75 | 1009.39±19.00cdefg | 1108.51±80.61cdef | 1241.32±56.44abc |
| 13    | ML512       | 1983.22±16.43cde | 2116.78±68.51def | 2237.82±56.56def | KL5218 | 1060.88±67.23abcdef | 1127.87±92.47abcde | 1292.17±37.23abc |
| 14    | ML818       | 1950.20±52.34cde | 2088.94±85.37defg | 2193.02±82.51f | DPLS8 | 1105.28±41.91abcde | 1190.85±60.31abcde | 1300.61±13.18abc |
| 15    | PS16        | 1906.26±12.39e | 1986.40±49.02ef | 2123.35±17.74f | DPL62 | 1222.56±60.79a | 1283.51±36.74ab | 1417.95±56.23a |
| 16    | TM96-2      | 1918.21±22.95e | 2045.65±83.68efg | 2241.09±55.77def | PL1 | 1176.23±41.11ab | 1272.97±67.25abc | 1404.65±71.61ab |
| 17    | IPM02-3     | 1980.96±43.88cde | 2173.12±81.03cde | 2307.45±38.02cde | PL2 | 1078.94±84.25abcde | 1201.25±48.27abcd | 1322.46±47.53abc |
| 18    | IPM02-14    | 1978.93±37.96cde | 2213.03±67.78bcde | 2348.99±46.91bcde | PL6 | 977.05±6.30defg | 1044.06±48.41de | 1171.65±49.79bc |
| 19    | IPM409-4    | 1939.30±93.27cde | 2110.64±66.59def | 2219.83±50.50def | L830 | 900.89±28.73g | 977.14±52.80e | 1138.75±57.31c |
| 20    | PMR-1       | 1891.20±42.48e | 1966.22±61.63f | 2161.29±70.50f | L4602 | 1171.95±65.19ab | 1289.38±31.36a | 1392.77±36.13ab |

Where mungbean at 07th day, while lentil was harvested on 09th day after sowing. Values are expressed as mean±SD (n = 3) and different letters indicate a significant difference (P≤.05). Values in bold represent maximum and minimum values.

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mungbean, the microgreens yield at 2-seed/cm² was recorded from 1854.38±19.69 to 2168.47±55.70 g/m², while at 4-seed/cm² this was 2123.35±17.74 to 2572.00±47.76 g/m². Similarly, in lentils, the microgreens yield recorded at 2-seed/cm² was 899.45±8.41 to 1222.56±60.79 g/m² while at 4-seed/cm² this was 1129.10±29.31 to 1417.90±56.23 g/m². The yield of Indian mustard microgreens at 6-seed/cm² ranged from 1091.56±41.09 to 1101.70±21.37 g/m², while at 10 seed/cm² this was from 1333.88±31.32 to 1355.78±28.04 g/m². The yield details are presented in Table 2a and 2b and Fig 2a. For mungbean, and lentils 3-seed/cm² was found optimum, while for Indian mustard it was 8-seed/cm². Any increase in the seeding density beyond
optimum resulted in poor marketable-quality produce. Excessive plant stand resulted in undesirably elongated shoots (due to more congestion and competition). Higher seeding density also hampered air circulation, favorable for fungal growth [43]. In addition, increase in seeding density result in higher seed cost.

Day of harvesting is also equally important for reaping the best marketable-quality yield. On 5\textsuperscript{th} to 9\textsuperscript{th} day, the mungbean microgreens yield ranged from 1773.8±30.32 to 2645.06 ±52.60 g/m\textsuperscript{2}; while on 7\textsuperscript{th} to 11\textsuperscript{th} day, the lentil microgreens yield ranged from 840.14±45.87 to 1411.29±77.66 g/m\textsuperscript{2} (Table 2) and the Indian mustard microgreens yield on 6\textsuperscript{th} to 10\textsuperscript{th} day ranged from 1070.6±35.13 to 1346.87±27.27 g/m\textsuperscript{2} (Fig 2b).

Different microgreens species have different harvesting stages to achieve their marketable hypocotyl length and leaf area to reap maximum economic benefit. The mungbean, Indian mustard, and lentil microgreens have different growth rates and under studied conditions; 7\textsuperscript{th}, 8\textsuperscript{th}, and 9\textsuperscript{th} day, respectively were found optimum for harvesting (Table 3; Fig 2b). Even though the overall yield recorded was higher during the later stages of harvesting, the quality of microgreens got deteriorated (S1 Fig). Additionally, significant genotypic differences for yield were observed in the studied microgreens. Similarly, the microgreens yield was recorded as 659 g/m\textsuperscript{2} in Brassica oleracea L. and 1548 g/m\textsuperscript{2} in Cichorium intybus L. [27]. Also, the

| Table 3. Microgreens yield (g/m\textsuperscript{2}) of twenty lentil and mungbean genotypes at different days of harvesting. |
|---|---|---|---|---|
| S. No. | Genotype (Mungbean) | 7\textsuperscript{th} Day Yield (g/m\textsuperscript{2}) | 9\textsuperscript{th} Day Yield (g/m\textsuperscript{2}) | 11\textsuperscript{th} Day Yield (g/m\textsuperscript{2}) |
| 1 | Pusa Baisakhi | 1813.20±69.61 | 1982.5±36.63 | 2100.09±68.40 |
| 2 | Pusa Ratna | 2102.33±80.50abc | 2258.32±54.82bcd | 2368.95±47.67bcde |
| 3 | Pusa Vishal | 2160.8±51.31a | 2450.11±46.45a | 2557.94±67.71ab |
| 4 | Pusa105 | 2057.28±53.13abcd | 2348.23±73.03abc | 2645.06±52.60a |
| 5 | Pusa0672 | 1908.95±51.06def | 2170.39±51.06cdef | 2372.25±55.92cde |
| 6 | Pusa9072 | 2111.14±59.04ab | 2396.13±50.25ab | 2478.54±90.27abcd |
| 7 | Pusa9531 | 1887.31±60.65def | 2021.69±66.35 | 2133.67±51.02g |
| 8 | MH96-1 | 1871.41±49.61f | 1974.81±48.69g | 2154.17±46.56abcd |
| 9 | M318 | 2053.67±77.74abcd | 2359.50±81.37abc | 2543.63±59.45abc |
| 10 | MH421 | 1864.41±66.45ef | 2069.36±38.13def | 2143.01±59.81g |
| 11 | MH521 | 1973.14±81.49bcde | 2063.26±33.97efg | 2243.03±62.12efgh |
| 12 | MH110 | 1894.74±34.21def | 2128.19±54.61def | 2152.58±56.69g |
| 13 | ML512 | 1930.35±50.62cdef | 2116.78±68.51def | 2283.53±67.24efgh |
| 14 | ML818 | 1901.11±46.00def | 2088.94±45.37def | 2304.72±36.49efgh |
| 15 | PS16 | 1778.65±28.04f | 1986.40±9.02g | 2176.42±51.58gh |
| 16 | TM96-2 | 1849.26±52.26ef | 2045.65±63.86eg | 2136.16±39.63gh |
| 17 | IPM02-3 | 1932.44±49.40cdef | 2173.12±81.03cdef | 2275.52±88.07efgh |
| 18 | IPM02-14 | 1955.46±48.08bcde | 2213.03±67.78bcde | 2323.77±38.86def |
| 19 | IPM409-4 | 1869.92±55.11ef | 2110.64±66.59g | 2279.31±41.62efgh |
| 20 | PMR-1 | 1773.87±30.32f | 1996.22±21.63fg | 2120.55±32.77h |

Where values are expressed as mean±SD (n = 3) and different letters indicate a significant difference (P<0.05). Values in bold represent maximum and minimum values. 

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optimum days to harvest for radish microgreens were 7th day, arugula–9th day, and red cabbage–11th day under specific growing conditions [28].

A very high correlation was recorded between mean seed weight and yield in both mungbean ($r^2 = .73$) and lentil ($r^2 = .78$) genotypes. As we used only two Indian mustard samples with nearly the same seed weight, the correlation analysis could not be performed.

**Microbial counts**

In many countries, various microbial outbreaks have been reported mainly due to the consumption of contaminated sprouts [19–21]. Thus, it becomes imperative to monitor and evaluate the microbial load in the microgreens too. Among different studied microbes, we have recorded the growth of Y&M, TAB, Shigella, and E. coli (O157:H7) in the studied microgreens. The target pathogenic bacteria Salmonella spp. and Listeria spp. were not detected in any of the tested samples. On contrary, Bergspica et al. [11] have detected the presence of Listeria innocua in the radish and sunflower microgreens, and Salmonella spp. in sunflower microgreens. In general, Listeria innocua is considered to be a non-pathogenic Listeria species [44].

Washing of microgreens was done using double distilled water for 2-minutes and results of both washed and unwashed samples for the growth of various microbes in mungbean was found comparable to lentil and mustard microgreens (Table 4). On contrary, Chandra et al. [29] reported a very high value for the TAB count (7.8 logCFU/g) of unwashed cabbage microgreens; which after washing get reduced to 7.2 logCFU/g. Washing has shown a significant ($P \leq 0.05$) reduction in the TAB (2.6 to 3.4 logCFU/g) and Y&M (1.1 to 2.2 logCFU/g) over fresh-cut beetroot samples [45]. Survival of E. coli O157:H7 was reported on radish [46], arugula, kale, lettuce, and mizuna microgreens [47]; while Di Gioia et al. [48] reported microbial growth on brassica microgreens. Inoculation of seed and irrigation water with Shiga toxin-producing E. coli (STEC) has resulted in the growth of bacteria on eight microgreens species [49].

No significant difference was recorded in the overall microbial load between the microgreens of different studied genotypes (Table 4a–4c). In general, an increasing trend was recorded for various microbial counts when samples were stored at 4°C from 1st day to 12th day (S2 Fig). Similarly, Chandra et al. [29] also recorded an increasing trend in the microbial population during storage of Chinese cabbage and beetroot samples [45].

In mungbean microgreens, washing significantly decreased the load of Shigella spp., whereas, amicrobial count (TAB, Y&M, and E. coli) did not show any significant decreasing effect (Table 4a). However, in lentil microgreens, washing significantly reduced the overall load of Shigella spp. and E. coli; but TAB and Y&M count did not show much reduction (Table 4b). This means that the survival and growth need of Shigella spp. and E. coli are different from that of aerobic bacteria and Y&M in the microgreens, as also recorded by Chandra et al. [45]. In mustard microgreens, although washing reduced overall microbial load, it was not very significant (Table 4c).

Washing of harvested microgreens has been practiced to remove the attached soil particles, to reduce the initial microbial load, and also for clean packaging. However, washing reportedly creates humid environmental conditions suitable for microbial growth, thus necessitating careful removal of excess moisture without causing any damage to the greens [18]. Relatively faster loss of shelf life was reported for the washed radish [31] and buckwheat microgreens over unwashed microgreens; which could be due to the damage caused during washing and dewatering, and also the presence of excess moisture in washed microgreens packages [30].

Microgreens are prone to bacterial internalization as the bacteria present in the seeds can become part of endophytic microflora [50]. Also, during germination, the bacteria present in
Table 4. Microbial load in three genotypes of lentil (K75, L4594 & L830) and mungbean (MH810, MH318 & PS16) and two genotypes of Indian mustard (PM28, PDZM-31) microgreens after 1\textsuperscript{st}, 2\textsuperscript{nd}, 4\textsuperscript{th}, 8\textsuperscript{th}, and 12\textsuperscript{th} day of storage at 4°C under washed and unwashed conditions.

| Microbes | Genotype | Day-1 | Day-2 | Day-4 | Day-8 | Day-12 |
|----------|----------|-------|-------|-------|-------|-------|
|          |          | Washed | Washed | Washed | Washed | Washed |
| TAB      | MH810    | 3.32±0.13bc | 2.81±0.19bc | 3.59±0.12bc | 3.21±0.13bc | 3.63±0.14bc |
|          | MH318    | 3.98±0.39ab | 3.65±0.76ab | 4.06±0.28ab | 3.82±0.41ab | 4.19±0.32ab |
|          | PS16     | 3.65±0.50ab | 3.32±0.43ab | 3.83±0.14ab | 3.43±0.29ab | 4.04±0.45ab |
|          | Y&M      | 1.56±0.29cd | 1.64±0.38ef | 1.98±0.18ef | 1.88±0.43de | 2.14±0.12de |
|          | MH318    | 2.05±0.12ab | 1.34±0.42bc | 2.14±0.29de | 2.10±0.44cd | 2.23±0.49cd |
|          | PS16     | 1.95±0.12ab | 1.57±0.26bc | 2.06±0.54de | 2.23±0.16de | 2.11±0.16de |
| E. coli  | MH810    | 2.18±0.62cd | 2.14±0.25de | 2.52±0.14cd | 2.35±0.104cd | 2.69±0.64cd |
|          | MH318    | 3.48±0.44ab | 3.06±0.17ab | 4.39±0.29a | 3.41±0.32ab | 4.03±0.56ab |
|          | PS16     | 3.45±0.40ab | 2.42±0.51bc | 3.61±0.29bc | 2.93±0.35bc | 3.97±0.15ab |
| Shigella | MH810    | 3.07±0.40bc | 1.02±0.40cd | 3.14±0.56bc | 1.23±0.23df | 3.40±0.25bc |
|          | MH318    | 3.97±0.61ab | 1.04±0.28bc | 4.06±0.19ab | 1.15±0.41bc | 4.35±0.39ab |
|          | PS16     | 4.12±0.65ab | 1.19±0.17bc | 4.37±0.24bc | 1.38±0.32bc | 4.54±0.25bc |
| (b) Lentil |          | Unwashed | Washed | Unwashed | Washed | Unwashed | Washed | Unwashed | Washed | Unwashed | Washed | Unwashed | Washed |
| TAB      | K75      | 4.59±0.15a | 4.49±0.30a | 4.96±0.38a | 4.79±0.01a | 4.99±0.02a |
|          | L4594    | 4.80±0.12a | 4.60±0.04a | 4.97±0.02a | 4.77±0.38a | 5.09±0.06a |
|          | L830     | 4.72±0.10a | 4.49±0.04a | 4.97±0.03a | 4.84±0.14a | 5.21±0.17a |
|          | L830     | 4.20±0.12a | 3.78±0.05ab | 4.16±0.15bc | 3.83±0.01bc | 4.33±0.07bc |
| E. coli  | K75      | 3.16±0.16bc | 1.89±0.05cd | 3.23±0.32cd | 2.00±0.02cd | 3.67±0.24cd |
|          | L4594    | 3.03±0.15c | 1.90±0.07cd | 3.23±0.35cd | 2.08±0.18cd | 3.37±0.41c |
|          | L830     | 3.17±0.21c | 1.89±0.02cd | 3.27±0.60cd | 1.99±0.01cd | 3.45±0.07c |
| Shigella | K75      | 2.81±0.17cd | 1.90±0.13cd | 3.02±0.03cd | 1.99±0.02cd | 3.23±0.05c |
|          | L4594    | 2.98±0.05cd | 1.76±0.23cd | 3.14±0.05cd | 1.96±0.04cd | 3.22±0.08c |
|          | L830     | 3.07±0.15c | 1.92±0.30bc | 3.18±0.02cd | 1.96±0.03cd | 3.45±0.14c |
| (c) Indian mustard |          | Unwashed | Washed | Unwashed | Washed | Unwashed | Washed | Unwashed | Washed | Unwashed | Washed |
| TAB      | PM28     | 4.39±0.13a | 3.67±0.76ab | 4.71±0.44a | 4.59±0.26a | 4.82±0.29a |
|          | PDZM31   | 4.41±0.39a | 3.84±0.57ab | 4.68±0.51a | 4.60±0.42a | 4.84±0.33a |
| Y&M      | PM28     | 2.49±0.17c | 1.71±0.54c | 2.20±0.17c | 2.61±0.18c | 2.32±0.39c |
|          | PDZM31   | 1.37±0.11 | 1.59±0.33c | 2.10±0.14c | 2.17±0.15c | 2.37±0.10d |
| E. coli  | PM28     | 4.57±0.24a | 4.00±0.36ab | 4.90±0.65a | 4.53±0.28a | 4.49±0.29a |
|          | PDZM31   | 4.54±0.51a | 4.42±0.45a | 4.70±0.17a | 4.52±0.12a | 4.88±0.23a |
| Shigella | PM28     | 3.40±0.54a | 3.20±0.23b | 3.60±0.25b | 3.90±0.16c | 3.90±0.46b |
|          | PDZM31   | 4.30±0.14a | 3.90±0.29ab | 4.70±0.55a | 3.60±0.43b | 4.30±0.19bc |

Where Y&M: Yeast & mold, TAB: total aerobic bacteria. All the microbial counts are expressed in logCFU/g of microgreens. Values are expressed as mean±SD (n = 3) and different letters indicate a significant difference (P ≤ 0.05). Values in bold represent maximum and minimum values.

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the rhizosphere are attracted by the seed exudates and may enter through the germinating rad-
icals or secondary roots [50]. Therefore, once contaminated, it is nearly impossible to elimi-
nate the microbes from the living plant system. Thus, sanitization or washing of harvested
microgreens may not be a very effective control strategy. In addition, microgreens being very
delicate are quite prone to the damage caused by any such treatments [18]. A much lower
value of microbial load in the present experiments could be due to the good agronomic prac-
tices used during the growth of the microgreens including the use of freshly harvested seeds,
seed treatment, autoclaving of growing media, and use of alcohol cleaned scissors while har-
vesting the microgreens.

Microgreens shelf life and sensory evaluation
Since microgreens are very tender, they are extremely vulnerable to dehydration and quality
deterioration. Therefore, to maintain the quality and shelf life of microgreens, proper refrigera-
tion and packaging become extremely crucial [18]. At the time of harvesting, microgreens
have a very high respiration rate [29] and can be stored comfortably for nearly a week time
at <5˚C [30, 31]. Immediately after the harvest, microgreens should be washed and cooled
(1–5˚C) [26] or this can be marketed in trays with growing-medium [24]. Thus, two genotypes
each of mungbean (MH810 & MH318), lentil (L830 and K75), and Indian mustard (PM28 &
PDZM31) were used for the shelf life and sensory evaluation. These were stored at 4˚C for 6
days and analyzed at 1st, 2nd, 4th and 6th day of storage. The visual appearance of microgreens
declined gradually as the storage time increased under cool (4˚C) conditions (S1 Fig). Mung-
bean and Indian mustard microgreens showed nearly 4-day shelf life, while lentil microgreens
could be used till 6th day of their storage in 51μ thick LLDPE zip-lock bags (16×12 cm) at 4˚C
conditions. On contrary, based on visual parameters, the shelf life of arugula, radish, and red
cabbage was recorded as 14, 21, and 14 days, respectively at 4˚C; whereas, at 10˚C this was 7,
14, and 7 days, respectively [28].

Till 4th day of the storage at 4˚C, all the studied sensory parameters such as color and
appearance, aroma, taste, and overall acceptability of the studied microgreens showed the
hedonic score of >6, which was considered as the limit of salability [37]. However, on the 6th
day of storage, a drastic reduction in all the sensory parameters of mungbean and mustard
microgreens was recorded. Interestingly, lentil microgreens showed >6 hedonic scores for all
the studied sensory parameters, even on 6th day of its storage (Table 5a–5c). This could be due
to relatively less moisture content in the lentil microgreens over mungbean or Indian mustard
microgreens. In general, the moisture content in mungbean, lentil, and Indian mustard micro-
greens ranged from 90.01±1.44 to 93.06±1.07, 82.69±2.48 to 85.95±1.01, and 89.83% to 90.5%,
respectively. An inverse relationship was found between the moisture content and the shelf-
life (and sensory qualities) in the studied microgreens. Many reports underlined the impor-
tance of temperature in prolonging the overall post-harvest shelf life of various fresh-cut prod-
ucts including microgreens [51–53]. A slower respiration rate at low temperature can be
directly correlated with the lower rate of cellular metabolism and cause a direct effect on visual
microgreens quality and hence increased self-life [28].

Electrical conductivity (EC) of washed and unwashed microgreens
EC can be associated with the overall quality and shelf life of microgreens and is used as an
indirect measure of the same [37]. With increasing storage duration, EC showed an increasing
trend in both washed (with sterile double distilled water for 2.0 min) and unwashed micro-
greens over fresh samples (Fig 3). EC was recorded more for the mungbean microgreens, espe-
cially at 4th day (6.97 μs/cm) and 6th day (15.63 μs/cm) of storage over lentil (3.37 & 6.57 μs/
cm) or Indian mustard (6.4 & 14.0 μs/cm) microgreens, respectively. Relatively less EC was recorded for 4th and 6th -day samples under 4°C storage; while it was a bit more for washed samples for 2nd day. Cell surface damage caused during washing treatment might have got repaired by 4th day of storage. Interestingly, 6th day of storage showed a sudden rise in EC for all the microgreens (Fig 3). Conductivity values showed a positive association with the storage duration of the studied microgreens.

On a similar note, the EC values showed a 7-fold increase (over initial value) for the tap water-washed Chinese cabbage microgreens, until the end of storage (9th day), when packed in polypropylene (PP) film [29]. Similar observations were also recorded for fresh-cut cilantro [54]. An increase in EC values under storage may be due to the irreversible membrane damage and accumulation of CO₂ from respiration [29]. On contrary, a decreasing trend in the electrolyte leakage was recorded for broccoli microgreens for various washing treatments and O3 washing (180s) under 09-day storage conditions [38].

**Conclusions**

Rapid growth cycle, limited space requirement, rich flavor, diverse color, and highly economic produce makes microgreens a nutrient alternative that may contribute to the nutritional security of a large population. To the best of our knowledge, no study about the yield optimization and microbial aspects of mungbean, lentil, and Indian mustard microgreens has been reported so far from India. The use of good agricultural practice is the key to manage the microbial

| Genotypes | Storage (days) | Color & appearance | Aroma | Taste | Overall acceptability |
|-----------|---------------|-------------------|-------|-------|-----------------------|
| (a) Mungbean | | | | | |
| MH810 | D1 | 9.80±.076a | 9.50±.177a | 9.34±.261a | 9.47±.255a |
| | D4 | 7.64±.261b | 7.79±.290b | 7.19±.422b | 7.30±.282b |
| | D6 | 4.93±.492c | 3.73±.301c | 4.19±.666c | 3.91±.488c |
| MH318 | D1 | 9.66±.090a | 9.30±.200a | 9.50±.283a | 9.23±.225a |
| | D4 | 7.93±.183b | 7.57±.353b | 7.53±.353b | 7.30±.203b |
| | D6 | 4.67±.480c | 3.56±.424c | 4.41±.615c | 4.16±.450c |
| (b) Lentil | | | | | |
| K75 | D1 | 9.36±.226ab | 8.99±.188b | 9.06±.184b | 9.06±.159b |
| | D4 | 9.24±.184b | 8.59±.259c | 8.56±.261c | 8.60±.278c |
| | D6 | 7.34±.447c | 7.20±.374d | 7.16±.430d | 7.14±.358d |
| L830 | D1 | 9.66±.325a | 9.56±.282a | 9.57±.291a | 9.53±.446a |
| | D4 | 9.47±.361ab | 9.31±.247ab | 9.11±.318b | 8.99±.181b |
| | D6 | 7.36±.410c | 7.49±.376d | 7.33±.437d | 7.30±.239d |
| (c) Mustard | | | | | |
| PDZM31 | D1 | 9.47±.237a | 9.09±.155b | 9.24±.342ab | 9.26±.206ab |
| | D4 | 9.09±.146b | 9.01±.181b | 8.86±.333bc | 8.90±.120bc |
| | D6 | 5.43±.342c | 5.24±.232d | 4.71±.290d | 5.16±.118d |
| PM28 | D1 | 9.70±.256a | 9.40±.355a | 9.61±.467a | 9.46±.607a |
| | D4 | 9.03±.167b | 9.14±.232ab | 8.77±.212c | 8.61±.352c |
| | D6 | 5.57±.373c | 5.61±.318c | 4.76±.362d | 5.30±.185d |

Where values are expressed as mean±SD (n = 7) and different letters indicate a significant difference (P≤.05).

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contamination of the growing microgreens [55]. More scientific information should be generated for various microgreens to eliminate the possibility of microbial contamination through seed, grow-media, grow-trays, and harvesting implements. In addition, post-harvest care such as harvesting at an optimum stage, proper sanitation, and maintenance of optimum temperature and humidity will help in longer storage and reduced risk of human pathogen contamination [12, 18, 56]. Thus, if grown and stored properly, there is no major risk of microbial illness from any kind of microgreens consumption. The success of microgreens technology will largely depend on the collective and collaborative efforts from the industry and researchers in the food-chemistry, biochemistry, genetics, and human nutrition working to enhance the yield and quality. This is the first such study from India, which included the microgreens of mungbean, lentil, and Indian mustard. Interestingly seeds of studied crops are readily available in any Indian kitchen, and we hope that the results will help in the popularization of these microgreens even at household levels.

Supporting information

S1 Fig. The difference in the growth pattern of mungbean microgreens (a) 4th day of sowing and, (b) 9th day after sowing (At a later stage the plants become lanky and of poor marketable quality).

(TIF)

S2 Fig. Representative figure showing microgreens of mungbean lentil, and Indian mustard stored for different durations at 4°C.

(TIF)
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References

1. Cohen MJ, Garrett JL (2010) The food price crisis and urban food (in)security. Environ Urbanization 22:467–482. https://doi.org/10.1177/0956247810380375
2. Sun J, Xiao Z, Lin L, Lester GE, Wang Q, Hamly JM, et al. (2013) Profiling polyphenols in five Brassica species microgreens by UHPLC-PDA-ESI/HRMS®. J Agril Food Chem 61:10960–10970. https://doi.org/10.1021/jf401802n PMID: 24144328
3. Priti, Mishra GP, Dikshit HK, Vinutha T, Mechiya T, Stobdan T, et al. (2021) Diversity in phytochemical composition, antioxidant capacities, and nutrient contents among mungbean and lentil microgreens when grown at plain-altitude region (Delhi) and high-altitude region (Leh-Ladakh), India. Front in Plant Sciences, 12:710812. https://doi.org/10.3389/fpls.2021.710812 PMID: 34497624
4. Treadwell D, Hochmuth R, Landrum L, Laughlin W (2010) Microgreens: A new specialty crop. University of Florida, IFAS Extension. EDIS 2010(3). https://journals.flvc.org/edis/article/view/118552.
5. Globe Newswire Report (2020) "United States Microgreens Market—Growth, Trends and Forecast (2020–2025)" https://www.reportlinker.com/p05916379/?utm_source=GNW. https://www.globenewswire.com/news-release/2020/07/02/2057048/0/en/United-States-Microgreens-Market-Growth-Trends-and-Forecast-2020-2025.html (Accessed on 30th June 2021)
6. https://www.datamintelligence.com/research-report/microgreens-market
7. www.researchandmarkets.com
8. Puccinelli M, Malorgio F, Rosellini I, Pezzarossa B (2019) Production of selenium-biofortified microgreens from selenium-enriched seeds of basil. J Sci Food Agric 99:5601–5605. https://doi.org/10.1002/jsfa.9826 PMID: 31149731
9. Di Gioia F, Petropoulos SA, Ozores-Hampton M, Morgan K, Rosskopf EN. Zinc and Iron Agronomic Biofortification of Brassicaceae Microgreens. *Agronomy*. 2019; 9(11):677. https://doi.org/10.3390/agronomy9110677

10. Ebert AW, Chang CH, Yan MR et al (2017) Nutritional composition of mungbean and soybean sprouts compared to their adult growth stage. *Food Chem* 237:15–22. https://doi.org/10.1016/j.foodchem.2017.05.073 PMID: 28763980

11. Bergspica I, Ozola A, Mitlinia E, Alksne L, Meistere I, Cibrovska A, et al. (2020) Occurrence of pathogenic and potentially pathogenic bacteria in microgreens, sprouts, and sprouted seeds on retail market in Riga, Latvia. *Foodborne Pathogens and Disease* 17(6):1–9. https://doi.org/10.1089/foodp.2019.2733 PMID: 31895586

12. Riggio GM, Wang Q, Kniel KE, Gibson KE (2019) Microgreens—A review of food safety considerations along the farm to fork continuum. *Int J Food Microbiol* 290:76–85. https://doi.org/10.1016/j.ijfoodmicro.2018.09.027 PMID: 30308448

13. CFIA-Canadian Food Inspection Agency (2018a) Lufa Farms Inc. brand Arugula Microgreens recalled due to *Salmonella*. Recalls and Safety Alerts. http://healthycanadians.gc.ca/recall-alert-rappel-avis/inspection2018/67156r-eng.php

14. Clark B (2017) *Salmonella* test prompts microgreens recall. *Food Poison Journal*. https://www.foodpoisonjournal.com/food-recall/salmonella-test-prompts-microgreens-recall/

15. CFIA-Canadian Food Inspection Agency (2018b) Food recall warning—Goodleaf brand Daikon Radish microgreens recalled due to *Listeria monocytogenes*. Recalls and Safety Alerts. https://inspection.gc.ca/about-the-cfia/newsroom/food-recallwarnings/complete-listing/2018-06-28/eng/1530237479767/1530237483085

16. CFIA-Canadian Food Inspection Agency (2019) Pousses et Cie brand Mix Spicy Microgreens recalled due to Listeria monocytogenes. Recalls and Safety Alerts. https://inspection.gc.ca/food-recall-warnings-and-allergy-alerts/2019-05-22/eng/1558549526741/1558549527573

17. Whole Foods Market (2018) Updated food recall warning—Certain Greenbelt microgreens brand microgreens recalled due to *Listeria monocytogenes*. https://www.wholefoodsmarket.com/content/updated-food-recall-warning-certain-greenbelt-microgreens-brand-microgreens-recalled-du

18. Turner ER, Luo Y, Buchanan RL (2020) Microgreen nutrition, food safety, and shelf life: A review. *J Food Sci* 85(4):870–882. https://doi.org/10.1111/1750-3841.15049 PMID: 32144769

19. Callejón RM, Rodríguez-Naranjo MI, Ubeda C, Horedo-Ortega R, Garcia-Parrilla MC, Troncoso AM (2015) Reported foodborne outbreaks due to fresh produce in the United States and European Union: Trends and causes. *Foodborne Pathogen Dis* 12:32–38. https://doi.org/10.1089/fpd.2014.1821 PMID: 25587926

20. Watanabe Y, Ozasa K, Mermin JH, Griffin PM, Masuda K, Imashuku S, et al. (1999) Factory outbreak of *Escherichia coli* O157:H7 infection in Japan. *Emerg Infect Dis* 5:424–428. https://doi.org/10.3201/eid0503.990315 PMID: 10341179

21. Buchholz U, Bernard H, Werber D, Böhmer MM, Remschmidt C, Wilking H, et al. (2011) German outbreak of *Escherichia coli* O104:H4 associated with sprouts. N Engl J Med 365:1763–1770. https://doi.org/10.1056/NEJMoa1106482 PMID: 22029753

22. Smith A, Moorhouse E, Monaghan J, Taylor C, Singleton I (2018) Sources and survival of *Listeria monocytogenes* on fresh, leafy produce. *J Appl Microbiol* 125:930–942. https://doi.org/10.1111/jam.14025 PMID: 30039586

23. Buchanan RL, Gorris LGM, Hayman MM, Jackson TC, Whiting RC (2017) A review of *Listeria monocytogenes*: An update on outbreaks, virulence, dose-response, ecology, and risk assessments. *Food Control* 75:1–13. https://doi.org/10.1016/j.foodcont.2016.12.016

24. Di Gioia F., Renna M, Santamaria P (2017a) Sprouts, Microgreens and “Baby Leaf” Vegetables. In: Yildiz F., Wiley R. (eds) Minimally processed refrigerated fruits and vegetables. *Food Engineering Series*. Springer, Boston.

25. Reed E, Ferreira CM, Bell R, Brown EW, Zheng J (2018) Plant microbe and abiotic factors influencing *Salmonella* survival and growth on alfalfa sprouts and Swiss chard microgreens. *Appl Environ Microbiol* 84:e02814–e02817. https://doi.org/10.1128/AEM.02814-17 PMID: 29432627

26. Kyriacou MC, Rouphael Y, Di Gioia F, Kyratzis A, Serfo R, Renna M, et al. (2016) Micro-scale vegetable production and the rise of microgreens. *Trends Food Sci Technol* 57:103–115. https://doi.org/10.1016/j.tifs.2016.09.005

27. Paradiso VM, Castellino M, Renna M, Gattullo CE, Calasso M, Terzano R, et al. (2018) Nutritional characterization and shelf-life of packaged microgreens. *Food Funct* 9:5629–5640. https://doi.org/10.1039/c8fo01182f PMID: 30298894
28. Berba KJ, Uchanski ME (2012) Postharvest physiology of microgreens. Journal of Young Investigators 24:1–5.
29. Chandra D, Kim JG, Kim YP (2012) Changes in microbial population and quality of microgreens treated with different sanitizers and packaging films. Horticulture, Environment, and Biotechnology 53:32–40.
30. Kou L, Luo Y, Yang T, Xiao Z, Turner ER, Lester GE, et al. (2013) Postharvest biology, quality and shelf life of buckwheat microgreens. LWT–Food Science and Technology 51:73–78. https://doi.org/10.1016/j.lwt.2012.11.017
31. Xiao Z, Luo Y, Turner ER, Lester GE, Kou L, Yang T, Wang Q (2014) Postharvest quality and shelf life of radish microgreens as impacted by storage temperature, packaging film, and chlorine wash treatment. LWT–Food Science and Technology 55:551–558. https://doi.org/10.1016/j.lwt.2013.09.009
32. Yadava DK, Yashpal, Vasudev S, Singh N, Saini N, Prabhu KV, et al. (2019) Indian mustard: Variety Pusa Double Zero Mustard-31 (PDZM-31). Indian Journal of Genetics and Plant Breeding 79(3):636–637.
33. Sauer DB, Burroughs R (1986) Disinfection of seed surfaces with sodium hypochlorite. Phytopathology 76:745–749.
34. Luo Y, McEvoy JL, Wachtel MR, Kim JG, Huang Y (2004) Package atmosphere affects postharvest biology and quality of fresh-cut cilantro leaves. HortSci 39:567–570. https://doi.org/10.21273/HORTSCI.39.3.567
35. Allende A, Luo Y, McEvoy JL, Artés F, Wang CY (2004) Microbial and quality changes in minimally processed baby spinach leaves stored under super atmospheric oxygen and modified atmosphere conditions. Postharvest Biol Technol 33:51–59. https://doi.org/10.1016/j.postharvbio.2004.03.003
36. Sharma P, Sharma A, Rasane P, Dey A, Choudhury A, Singh, et al. (2020) Optimization of a process for microgreen and fruit-based functional beverage An Acad Bras Cienc 92(3):e20190596. https://doi.org/10.1590/0001-3765202020190596 PMID: 33111819
37. Kim JG, Luo Y, Gross KC (2004) Effect of packaging film on the quality of fresh-cut salad savoy. Postharvest Biol Technol 32:99–107. https://doi.org/10.1016/j.postharvbio.2003.10.006
38. Das BK, Kim JG (2010) Microbial quality and safety of fresh-cut broccoli with different sanitizers and contact times. J Microbiol Biotechnol 20(2):363–369. https://doi.org/10.4014/jmb.0907.07009 PMID: 20208442
39. Choe U, Yu LL, Wang TTY (2018) The science behind microgreens as an exciting new food for the 21st century. J Agric Food Chem 66:11519–11530. https://doi.org/10.1021/acs.jafc.8b03096 PMID: 30343573
40. Benincasa P, Falcinelli B, Lutts S, Stagnari F, Galieni A (2019) Sprouted grains: A comprehensive review. Nutrients 11:421. https://doi.org/10.3390/nu11020421 PMID: 30781547
41. Abad M, Nogueira P, Burés S (2001) National inventory of organic wastes for use as growing media for ornamental potted plant production: case study in Spain, Bioresour Technol 77:197–200. https://doi.org/10.1016/s0960-8524(00)00152-8 PMID: 11272028
42. Di Gioia F, Mininni C, Santamaria P (2015) How to grow microgreens. In Di Gioia F., Santamaria P. (Eds.), Microgreens: Novel fresh and functional food to explore all the value of biodiversity (pp. 51–79). Italy: ECO-logicas rl Bari
43. Murphy CJ, Pill WG (2010) Cultural practices to speed the growth of microgreen arugula (roquette; Eruca vesicaria subsp. sativa). J Hortic Sci Biotechnol 85:171–176. https://doi.org/10.1080/14620316.2010.11512650
44. Moura A, Tissot B, Lavina M, Thouvenot P, Huang L, Leclercq A, et al. (2019) Typical hemolytic Listeria innocua isolates are virulent, albeit less than Listeria monocytogenes. Infect Immun 87:e00758–18. https://doi.org/10.1128/IAI.00758-18 PMID: 30670551
45. Chandra D, Choi AJ, Kim YP, Kim JG (2015) Physicochemical, microbial and sensory quality of fresh-cut red beetroots in relation to sanitization method and storage duration. Italian Journal of Food Sciences 27(2):80–92. https://doi.org/10.14674/1120-1770/jifs.v188
46. Xiao Z, Bauchan G, Nichols-Russell L, Luo Y, Wang Q, Nou X (2015) Proliferation of Escherichia coli O157:H7 in soil-substitute and hydroponic microgreen production systems. Journal of Food Protection 78:1785–1790. https://doi.org/10.4315/0362-028X.JFP-15-063 PMID: 26408126
47. Park HK, Kushad MM, Feng H (2013) Survival of Escherichia coli O157:H7 strain87-23 on arugula, kale, lettuce and mizuna microgreens, and comparison of leaf surface morphology for mature greens and microgreens. Presented at Poster session, IAFP Annual Meeting, Charlotte, NC.
48. Di Gioia F, De Bellis P, Mininni C et al (2017b) Physicochemical, agronomical and microbiological evaluation of alternative growing media for the production of rapini (Brassica rapa L.) microgreens. J Sci Food Agric 97:1212–1219. https://doi.org/10.1002/jsfa.7852 PMID: 27311947
49. Wright KM, Holden NJ (2018) Quantification and colonisation dynamics of *Escherichia coli* O157:H7 inoculation of microgreen species and plant growth substrates. International Journal of Food Microbiology 273:1–10. https://doi.org/10.1016/j.ijfoodmicro.2018.02.025 PMID: 29554556

50. Warriner K, Ibrahim F, Dickinson M, Wright C, Waites WM (2003) Internalization of human pathogens within growing salad vegetables. Biotechnology and Genetic Engineering Reviews 20:117–136. https://doi.org/10.1080/02648725.2003.10648040 PMID: 14997849

51. Brecht JK (1995) Physiology of lightly processed fruits and vegetables. Hortscience, 30(1):18–22. https://doi.org/10.21273/HORTSCI.30.1.18

52. Watada AE, Ko NP, Minott DA (1996) Factors affecting quality of fresh-cut horticultural products. Post-harvest Biology and Technology, 9(2):115–125. https://doi.org/10.1016/S0925-5214(96)00041-5

53. Deza-Durand KM, Petersen MA (2011) The effect of cutting direction on aroma compounds and respiration rate of fresh-cut iceberg lettuce (*Lactuca sativa* L.). Postharvest Biol Technol 61(1):83–90. https://doi.org/10.1016/j.postharvbio.2011.02.011

54. Wang H, Feng H, Luo Y (2004) Microbial reduction and storage quality of fresh-cut cilantro washed with acidic electrolyzed water and aqueous ozone. Food Res Intl 37:949–956. https://doi.org/10.1016/j.foodres.2004.06.004

55. USFDA- U.S. Food and Drug Administration Center for Food Safety and Applied Nutrition [US FDA- SAN]. (1999). Guidance for Industry: Reducing microbial food safety hazards for sprouted seeds. Federal Register 64(207):57893–57902. https://www.govinfo.gov/content/pkg/FR-1999-10-27/pdf/99-28016.pdf

56. Mir SA, Shah MA, Mir MM (2017) Microgreens: Production, shelf life and bioactive components. Critical Reviews in Food Science and Nutrition 57(12):2730–2736. https://doi.org/10.1080/10408398.2016.1144557 PMID: 26857557