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EVALUATION OF ASEPTIC AND NON-ASEPTIC SYSTEMS AND SUBSTRATE EFFECTS ON SORREL MICROPLANTS CULTIVATED UNDER LED IRRADIATION

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Abstract

This study investigates the impact of aseptic (AS) and non-aseptic (NAS) cultivation systems, using various substrates, on the germination capacity and quality of sorrel microplants. The AS conditions significantly improved germination rates, achieving over 90% capacity compared to lower rates in NAS. Morphological parameters, such as hypocotyl length, were also significantly enhanced under AS. Biochemical analysis showed notable differences in dry matter (9.94±0.97% in AS vs. 6.31±0.14% in NAS), macro- and microelement content, antioxidant activity (up to 11836.28±1065.06 mg TE/100 g FW), total polyphenols (623.01±88.51 mg GAE/100 g FW), and flavonoid levels, highlighting the importance of substrate choice in improving microplant quality. NAS systems exhibited a higher risk of microbial contamination in all substrate types, whereas the AS showed Aspergillus and Penicillium contamination only when organic waste of banana peel was used in high proportions (75 to 100%) as germination substrate. The results suggest that AS, especially with 100% agar substrate (MV2), offers a more effective and safer method for producing high-quality, edible sorrel microplants.

Key words*: sorrel, production technology, quality, microplants (microgreens/sprouts), substrate.*

INTRODUCTION

Sorrel is a perennial plant from the *Polygonaceae* family (Li et al., 2022). It is resistant to various biological stress factors, such as plant diseases, as well as physical factors like low winter temperatures, and can be consumed fresh or cooked (Barbu et al., 2023). Sorrel is considered one of the functional foods with therapeutic potential (Bello et al., 2019). In recent years, due to its flavor and nutritional content (Puccinelli et al., 2021), sorrel has been increasingly used as microplants (microgreens/sprouts).

Microplants are typically grown in greenhouses through seed germination under warm, humid conditions. Various growing substrates can be used, including soil, peat moss, perlite, vermiculite, mineral wool, fibrous materials, or through hydroponic culture (Xiao et al., 2015). However, traditional methods of cultivating microplants carry the risk of microbial contamination, which can compromise the quality of the product and pose health risks to consumers (Xiao et al., 2015; Riggio et al., 2019; Deng et al., 2021). Pathogenic bacteria, such as *Escherichia coli* O157:H7 and *Salmonella enterica*, have been identified on raw vegetables (Xiao et al., 2015; Riggio et al., 2019). Fortunately, these contaminants can be accurately detected (Al-Zaidi et al., 2024), preventing the contaminated batches from being released onto the market.

Reducing the risk of pathogenic contamination requires appropriate disinfection methods, controlled production technologies, and safe packaging (Dueck et al., 2016; Carillo et al., 2020).

Modern plant-growing technologies, such as LED lighting (Tong et al., 2023), have been

shown to enhance the nutritional value of plants (Lone et al., 2024) and improve the quality of sprout production, as demonstrated by recent research (Livadariu et al., 2024b; Barbu et al., 2023).

The objective of this study is to evaluate the impact of aseptic (AS) and non-aseptic (NAS) cultivation systems on the germination efficiency, biochemical profile, and overall quality of sorrel microplants. Specifically, the research aims to assess the influence of various substrate compositions on key physiological and biochemical parameters, including antioxidant activity, total phenolic content, flavonoid content, and mineral composition, in both cultivation systems. Additionally, the study investigates the prevalence of microbial contamination in each system, with the goal of identifying optimal cultivation practices that enhance the yield, quality, and safety of sorrel microplants for consumption.

MATERIALS AND METHODS

Biological material

In this study, organic certified seeds of red-veined sorrel were used, from a commercial source (Italian Sprout Srl, Italy, Lot: 0202/HG6501).

Cultivation conditions

The lighting was maintained on a 16/8 h photoperiod using white LEDs (Lee et al., 2014; Enache & Livadariu, 2016; Raiciu et al., 2020; Livadariu et al., 2023). These LEDs emitted white light with a continuous tunable color temperature ranging 2000 to 6500 K, with a light flux of 1140 lm, a power consumption 12.7 W, and operating at 220V (Livadariu et al., 2024a). Within the growth chamber, microplants were placed under artificial light, achieving a measured photosynthetic photon flux density (PPFD) of 281±5.5 μmol $m^{-2} \cdot s^{-1}$, with carbon dioxide levels at 350 ppm, ambiental temperature of 21±2°C, and relative humidity maintained between 50-70% (Barbu et al., 2023).

Experimental variants

The experimental studies were conducted using two production technologies. The first was the cultivation system performed *in vitro* under aseptic conditions (AS) (Patent application No. A/00039 on 30.01.2023). Therefore, the seeds were surface washed using a 10% sodium hypochlorite (NaOCl) solution. After disinfection, the seeds were rinsed with distilled water. Also, the substrates and containers were autoclaved (Vuguziga et al., 2020). The second production technology was a non-aseptic system (NAS) carried out *ex vitro* under similar growing conditions but without the aseptization steps.

In both cultivation systems (AS and NAS), seeds were germinated in recyclable transparent glass containers with a diameter of 63 mm and a capacity of 300 mL, using four substrate types. The substrates for germination and rooting included perlite, edible agar, banana peels (food waste), and a conventional peat. These were combined in 13 different experimental variants (Table 1) each of which was replicated in triplicate.

Table 1. Substrate variants for sorrel microplants cultivation

Experimental variants	Substrate types			
	Perlite	Edible agar	Banana peels	Peat
MV1	100%	-	-	-
MV2	-	100%	-	-
MV3	-	-	100%	-
MV4	75%	25%	-	-
MV5	50%	50%	-	-
MV6	25%	75%	-	-
MV7	75%	-	25%	-
MV8	50%	-	50%	-
MV9	25%	-	75%	-
MV10	-	-	-	100%
MV11	75%	-	-	25%
MV12	50%	-	-	50%
MV13	25%	-	-	75%

Biometric measurements

The biometric analyses focused on evaluating germination capacity (GC), hypocotyl length, and the number of leaves.

Hypocotyl length (HL) and the number of leaves (NL) for each experimental variant were measured using an EPSON Expression 11000XL scanner in conjunction with WinFOLIA™ software (Livadariu et al., 2024b).

Biochemical analyses

Biochemical analyses were conducted on fresh sorrel microplants using the following methods:

The dry matter content (DM) was determined according to the procedures outlined in the European Pharmacopoeia (2010), with results expressed as a percentage by mass (g/100 g fresh weight (FW), %).

Total phenolic content (TPC) was measured using the Folin-Ciocâlteu reagent, as described by Ion et al. (2020). Absorbance was recorded at 760 nm using a Specord 210 Plus UV-VIS spectrophotometer, and results were quantified based on a gallic acid calibration curve, expressed as mg gallic acid equivalents per 100 g FW (mg GAE/100 g FW).

Antioxidant activity (AA) was evaluated using the DPPH radical scavenging method (Irimescu et al., 2021). Absorbance was measured at 515 nm (Gulcin & Alwasel, 2023), with results expressed as mg trolox equivalents per 100 g FW (mg TE/100 g FW).

Total flavonoid content (TFC) was determined using a spectrophotometric method adapted from Manole et al. (2017) and Dobrin et al. (2018). The results were calculated from a rutin calibration curve and expressed as mg rutin equivalents per 100 g FW (mg RE/100 g FW).

Mineral composition was analyzed to quantify macronutrients (Na, Mg, P, K, and Ca) and micronutrients (Mn, Cu, and Zn) following Dobrin et al. (2020). Fresh samples were ground and digested using nitric acid and hydrogen peroxide via microwave digestion. Quantification was performed with inductively coupled plasma mass spectrometry (ICP-MS) using a multi-element calibration curve (Lenzi et al., 2019), analyzed with an Agilent ICP-MS 7700 and the MassHunter software (Bashdar Abuzed et al., 2023).

Microbiological analyses

The spoilage fungi present on inedible microplants were examined on Potato Dextrose Agar (PDA) using slide cultures placed in wet chamber. Incubation was carried out at 25°C, in the dark for 5, 7, or 10 days, depending on the microorganism. Fungal slides were examined under a light microscope at 10x or 40x optical magnification. Some samples were stained with cotton blue to highlight the sporulation forms. Species identification was performed through classical microbial analysis based on the fungal microscopic characteristics (Cighir et al., 2023).

Statistical Procedures

Measurements and analyses were conducted in triplicate for each experimental variant in both AS and NAS. The recorded data were statistically analyzed and expressed as mean with standard deviation (± SD).

RESULTS AND DISCUSSIONS

The influence of different growing substrates on sorrel microplants produced using AS and NAS was analyzed. The results revealed various biometric, biochemical, and microbiologic differences, highlighting the impact of the substrate and the benefits of AS compared to NAS.

Germination capacity and biometry of sorrel microplants

When comparing the GC of sorrel in both production technologies, it was observed that the AS positively influenced seed germination across all substrate types compared to NAS. Notably, in the MV3, MV7, MV8, and MV9 variants in NAS, germination of sorrel seeds was completely inhibited (Table 2).

Table 2. Sorrel germination on different substrate types in AS and NAS

Experimental variant	AS	NAS
	GC (%)	
MV1	97.53±1.63	91.13±2.38
MV2	97.17±1.15	93.03±1.95
MV3	16.43±2.71	-
MV4	90.33±2.57	87.07±2.45
MV5	89.20±3.79	84.67±2.76
MV6	88.73±2.90	85.23±3.19
MV7	42.53±4.58	-
MV8	23.23±2.86	-
MV9	22.67±3.60	-
MV10	10.77±2.81	8.63±4.80
MV11	31.73±2.62	8.1±2.84
MV12	22.27±2.88	5.63±3.43
MV13	13.77±3.29	9.37±4.66

Legend: AS = aseptic system; NAS = non-aseptic system; GC = germination capacity; MV1 to MV13 = substrate types. Note: Values are presented as Mean ± Standard deviation.

Given that seeds are the central component in microplant production and represent a significant cost (Di Gioia et al., 2015), establishing optimal germination conditions is crucial.

In the current study, the highest germination percentages were obtained on 100% perlite (97.53±1.63%) and 100% edible agar (97.17±1.15%) in AS. While these substrate types also produced favorable results in NAS, the germination percentages were significantly lower than those observed in AS. Variants MV4 to MV6 also demonstrated good GC in both AS and NAS. However, the combinations containing banana peel and peat negatively impacted the GC of sorrel seeds in both production systems (Table 2).

Previous studies have investigated the GC of sorrel under various conditions, highlighting the influence of the substrate, temperature, humidity, and scarification on sorrel seeds (Yazdi et al., 2013). Additionally, Hintikka (1990) revealed a wide range of GC values, from 46% to 99%, for *Rumex acetosella* L. (sour sorrel), depending on the germination protocol applied.

Hypocotyl length was significantly higher in sorrel microplants grown on peat-containing substrates compared to the other substrate types in both the AS and NAS (Table 3). These results show an inverse correlation with seed germination capacity, as the low GC percentages reduced microplant density per container, allowing for better growth in these experimental variants. A similar inverse correlation between hypocotyl length and seed germination capacity was also noted in the 100% perlite (MV1) and 100% edible agar (MV2) substrates, as well as in their combinations (MV4 to MV6).

In the AS system, substrates containing banana peels (MV3, MV7 to MV9) negatively influenced hypocotyl growth, as well as seed germination. In the NAS system, no biometric analyses could be performed on these experimental variants with banana peels as substrates due to the lack of seed germination (Table 2).

The experimental results on the hypocotyl length of sorrel confirm that substrates significantly influence the biometry of microplants. Similar findings were demonstrated for other microplants species, such as:

- ✓ *Basella alba* Linn.: The best results were obtained with the substrate containing coconut coir dust and peat (1:1), followed by coconut coir dust and vermicompost (1:1), peat, coconut coir dust, vermicompost, coconut coir dust, sugarcane filter cake (1:1), sugarcane filter cake, sand (Muchjajib et al., 2015);
- ✓ *Brassica juncea* Czern. & Coss., *Raphanus sativus* var. *caudatus* Linn., *Ipomoea aquatica* Forsk., *Leucaena leucocephala* de Wit.: For these species, the best results were achieved with a substrate containing coconut coir dust and sugarcane filter cake (1:1), while the lower results were obtained with sand (Muchjajib et al., 2015);
- ✓ *Ocimum bassilicum* L.: The best results for microplants biometry were obtained on peat containing substrates (Livadariu et al., 2024b).

Table 3. Sorrel microplants biometry on different substrate types in AS and NAS

Experimental variants	AS		NAS	
	HL (cm)	LN	HL (cm)	LN
MV1	1.59±0.05	3.25±0.02	1.51±0.10	3.11±0.20
MV2	1.82±0.64	3.31±0.05	1.77±0.57	3.19±0.05
MV3	0.91±0.50	2.98±0.10	N.A.	N.A.
MV4	2.31±0.09	3.69±0.03	2.27±0.19	3.63±0.12
MV5	2.08±0.14	3.52±0.13	2.03±0.83	3.41±0.19
MV6	2.85±0.33	3.73±0.06	2.62±0.06	3.82±0.15
MV7	1.35±0.36	3.19±0.14	N.A.	N.A.
MV8	0.81±0.75	2.83±0.23	N.A.	N.A.
MV9	1.16±0.79	3.05±0.17	N.A.	N.A.
MV10	3.41±0.49	4.26±0.12	2.17±0.26	3.62±0.24
MV11	3.33±1.42	4.14±0.13	3.50±0.58	4.63±0.05
MV12	3.59±0.37	4.63±0.04	3.44±1.28	4.55±0.07
MV13	3.76±0.31	4.51±0.10	3.33±0.47	4.34±0.25

Legend: AS = aseptic system; NAS = non-aseptic system; HL = hypocotyl length; LN = number of leaves; MV1 to MV13 = substrate types. N.A. = not available

Note: Values are presented as Mean ± Standard deviation.

Similar to the hypocotyl length, the number of leaves in sorrel microplants was greater in variants with lower germination capacity, attributable to the reduced density of microplants within the growth containers. However, substrates containing banana peels (MV3, MV7 to MV9) adversely affected the number of leaves in sorrel microplants in the AS (Table 3). Given the detrimental impact of banana peel-containing substrates on both seed germination and microplant growth in both AS and NAS, the experimental variants MV3 and MV7 to MV9 were excluded from dry mass content or biochemical analysis. The AS combination of eco-innovative cultivation techniques and specific substrate types (MV1, MV2, MV4, MV5, MV10, MV12, and MV13)

demonstrated higher biometric parameters compared to NAS and similar substrates.

Biochemical properties of sorrel microplants

Dry matter content

The highest dry matter content (9.94±0.97%) was observed in the experimental variant MV6, under NAS conditions. A similar value was recorded for MV2 (9.90±0.46%), in NAS. However, NAS conditions displayed considerable variability in dry matter content, ranging from 9.94±0.97% in MV6 to 6.31±0.14% in MV11, depending on the substrate type. In contrast, AS conditions exhibited reduced variability (Table 4).

Table 4. Influence of the substrate type and cultivation systems on biochemical parameters of sorrel microplants

Experimental variant	System	DM (%)	TPC (mg GAE/100 g FW)	AA (mg TE/100 g FW)	TFC (mg RE/100 g FW)
MV1	AS	7.74±0.27	516.48±39.87	10320.93±90.94	1.26±0.04
	NAS	8.28±0.32	506.50±11.78	10689.89±46.83	1.35±0.04
MV2	AS	7.65±0.44	503.23±20.67	11103.03±412.27	1.24±0.01
	NAS	9.90±0.46	623.01±88.51	11836.28±1065.06	1.55±0.09
MV4	AS	7.83±0.09	543.81±52.38	10365.71±873.44	1.22±0.01
	NAS	7.92±0.23	474.90±31.76	9701.26±116.12	1.22±0.01
MV5	AS	6.30±0.32	568.97±24.06	11500.97±813.82	1.27±0.06
	NAS	8.40±0.12	489.66±56.11	10672.41±1488.02	1.32±0.10
MV6	AS	6.59±0.20	548.58±23.34	10879.16±212.21	1.18±0.07
	NAS	9.94±0.97	577.79±25.32	11068.41±306.59	1.44±0.10
MV10	NAS	-	71.35± 6.30	2171.38±207.59	0.39±0.02
MV11	NAS	6.31±0.14	100.05± 3.85	3790.62±517.79	0.50±0.01
MV12	NAS	7.43±0.20	121.05± 4.49	4344.88±210.18	0.56±0.02
MV13	NAS	6.52±0.15	80.69± 9.41	2356.07±320.54	0.42±0.01

Legend: DM (%) = dry matter content; TPC = total phenolic content; GAE = gallic acid equivalents; FW = fresh weight; AA = antioxidant activity; TE = Trolox Equivalents; TFC = total flavonoid content; RE = rutin equivalents; AS = aseptic system; NAS = non-aseptic system; N.A. = not available; MV1 to MV13 = substrate types.
Note: Values are presented as Mean ± Standard deviation.

Studies on the effects of specific chemical elements on microplant growth have shown that sodium selenate (Na_2SeO_4) reduced fresh biomass while increasing dry matter content in microgreens such as *Rumex acetosa* L., *Plantago coronopus* L., and *Portulaca oleracea* L. (Puccinelli et al., 2021).

Total polyphenolic content

The analysis reveals a distinct trend in TPC when comparing the aseptic (AS) and non-aseptic (NAS) systems. For example, in the MV2 variant, the TPC in NAS is higher at 623.01 mg GAE/100 g FW, compared to 503.23 mg GAE/100 g FW in AS. This represents an increase of approximately 23.7%, indicating that the NAS system may enhance the polyphenolic content for specific substrate combinations. Conversely, variants like MV1, MV4, and MV5 show higher TPC values in the AS system, with MV1 recording 516.48 mg GAE/100 g FW compared to 506.50 mg GAE/100 g FW in NAS, a difference of about 1.9%.

In particular, MV6 demonstrates a higher TPC in NAS (577.79 mg GAE/100 g FW) than in AS (548.58 mg GAE/100 g FW), indicating a potential advantage of the non-aseptic approach for certain substrate types. This variant showcases an increase of approximately 5.3% in TPC when grown in NAS conditions. However, in MV4, the TPC drops in NAS (474.90 mg GAE/100 g FW) compared to AS (543.81 mg GAE/100 g FW), illustrating the variability in response to different growing conditions.

Overall, the AS system generally produced higher TPC values for several variants, specifically MV1, MV4, MV5, and MV6, suggesting that controlled conditions may enhance polyphenolic content. In contrast, the NAS system particularly benefits MV2, where a remarkable increase in TPC was observed.

The TPC values exhibit substantial variability across different experimental variants, especially in the NAS system. For instance, the highest TPC was recorded in MV2 at 623.01 mg GAE/100 g FW, while the lowest value was

observed in MV10 at only 71.35 mg GAE/ 100 g FW. This contrast of 551.66 mg GAE/ 100 g FW emphasizes the profound influence of substrate type and environmental conditions on polyphenolic accumulation. The majority of our results surpass those reported by Puccinelli et al. (2021), who achieved a total phenolic content (TPC) of 3.43 mg GAE/g FW in sorrel microplants cultivated with a 1.5% selenium (Se) supplementation. In a separate study that examined the leaves of various species within the *Rumex* genus, *R. acetosa* demonstrated the lowest concentration of phenolic compounds, approximately 23 mg GAE/g dry weight (DW), while *R. crispus* revealed the highest content of TPC, of approximately 131 mg GAE/g dry DW as reported by Feduraev et al. (2022).

Antioxidant activity

The analysis of antioxidant activity (AA) in sorrel microplants reveals notable trends and variability across different experimental variants and cultivation systems. For instance, the highest AA was observed in variant MV2 under NAS conditions, with a remarkable value of 11,836.28±1,065.06 mg TE/100 g FW, compared to 11,103.03±412.27 mg TE/100 g FW in the AS system. This increase of approximately 6.6% suggests that the NAS system may enhance the antioxidant properties of certain substrates. Similarly, MV5 exhibited a higher AA in AS (11,500.97±813.82 mg TE/100 g FW) compared to NAS (10,672.41±1,488.02 mg TE/100 g FW), showcasing a decrease of around 7.2% when transitioning to the non-aseptic environment.

In contrast, variants such as MV4 and MV1 presented higher antioxidant activity in the AS system (10,365.71±873.44 mg TE/100 g FW and 10,320.93±90.94 mg TE/100 g FW, respectively) than in NAS, with MV4 decreasing to 9,701.26±116.12 mg TE/100 g FW. This pattern suggests that while some substrates benefit from controlled conditions, others may thrive under a more dynamic environment.

Total flavonoid content

The assessment of total flavonoid content (TFC) in sorrel microplants revealed significant discrepancies between the aseptic (AS) and non-aseptic (NAS) cultivation systems across various experimental variants. Notably, variant MV2 exhibited the highest TFC in the NAS environment, recording 1.55±0.09 mg RE/ 100 g FW, which represents a 24.8% increase compared to its AS counterpart (1.24±0.01 mg RE/100 g FW). This observation suggests that NAS conditions may enhance the biosynthesis of flavonoids, thereby improving the nutritional profile of the microplants.

Similarly, both variants MV1 and MV6 demonstrated elevated TFC levels in NAS (1.35±0.04 mg RE/100 g FW and 1.44± 0.10 mg RE/100 g FW, respectively) compared to their AS equivalents (1.26±0.04 mg RE/ 100 g FW and 1.18±0.07 mg RE/100 g FW), yielding increases of 7.1% and 22.0%, respectively. This trend indicates that, while NAS generally promotes flavonoid accumulation, the response to environmental factors is variant-specific.

In contrast, the TFC values for variants MV4 and MV5 remained consistent across both systems, with MV4 measuring 1.22±0.01 mg RE/100 g FW in both AS and NAS conditions, suggesting a stable flavonoid concentration regardless of the cultivation method. Conversely, NAS variants MV10, MV11, MV12, and MV13 exhibited the lowest TFC values, measuring 0.39±0.02 mg RE/100 g FW, 0.50±0.01 mg RE/100 g FW, 0.56±0.02 mg RE/100 g FW, and 0.42±0.01 mg RE/100 g FW, respectively. However, the total flavonoid content (TFC) observed in our microplants was lower than the values reported by Puccinelli et al. (2021), which ranged between 1.09 mg catechin/g and 2.11 mg catechin/g.

This significant disparity underscores the critical role of substrate selection in modulating flavonoid levels, as these variants displayed markedly lower TFC compared to those cultivated under AS conditions. A lower concentration of flavonoids was also observed in the leaves of the wild species *R. confertus* (approximately 38 mg RE/g DW) and *R. acetosa* (approximately 18 mg RE/g DW), as reported by Feduraev et al. (2022).

The data shows variability in TPC, AA, and TFC between AS and NAS systems. For example, while some variants such as MV2 show improved TPC and AA in NAS conditions, others like MV10 exhibit significantly lower values across all three parameters. This

variability highlights the impact of cultivation methods on the accumulation of beneficial phytochemicals, suggesting that optimizing growth conditions could enhance these properties.

Macro and microelements content

The mineral content of sorrel microplants encompasses both macroelements and microelements. The macroelements analyzed include sodium (Na), magnesium (Mg), phosphorus (P), potassium (K), and calcium (Ca), while the microelements focus on manganese (Mn), copper (Cu), and zinc (Zn).

For sodium (Na), the highest concentration observed was 138.76±12.52 mg Na/100 g FW, found in sorrel microplants cultivated on the MV1 substrate under non-aseptic (NAS) conditions. In contrast, the lowest sodium content of 10.20±0.88 mg Na/100 g FW was recorded in NAS when the microplants were grown on the MV10 substrate (Table 5).

Table 5. Influence of the substrate type and cultivation systems on the macro- and microelements content in sorrel microplants

Experimental variant	System	Na (mg/100 g)	Mg (mg/100 g)	P (mg/100 g)	K (mg/100 g)	Ca (mg/100 g)	Mn (mg/Kg)	Cu (mg/Kg)	Zn (mg/Kg)
MV1	AS	80.73±7.01	24.34±0.70	71.72±3.77	97.52±0.11	17.97±0.13	10.87±0.76	0.52±0.00	6.73±0.63
	NAS	138.76±12.52	31.94±2.29	79.09±2.59	114.54±3.58	26.14±1.11	14.21±0.94	0.48±0.01	8.46±0.45
MV2	AS	33.63±2.23	11.42±0.16	68.08±2.21	100.45±5.69	13.90±0.89	5.39±0.06	0.50±0.04	5.33±0.44
	NAS	43.65±0.03	25.50±1.49	94.43±4.60	143.79±6.64	23.54±1.60	11.06±0.64	0.45±0.02	13.64±0.16
MV4	AS	69.17±3.40	20.62±0.89	62.44±1.78	104.67±6.58	22.04±1.69	9.32±0.18	0.32±0.02	7.02±0.18
	NAS	92.94±4.64	36.75±0.96	73.30±3.13	126.88±11.15	38.87±2.12	14.68±0.27	0.51±0.03	9.40±0.10
MV5	AS	77.76±0.05	21.30±0.34	66.67±3.15	103.23±7.25	12.06±0.20	10.49±0.77	0.61±0.01	5.64±0.46
	NAS	82.51±0.21	32.99±1.48	72.40±2.65	124.07±6.21	25.13±0.83	14.40±1.22	0.33±0.01	7.91±0.52
MV6	AS	55.32±0.31	17.77±0.14	71.02±6.04	120.93±11.18	18.14±1.66	8.46±0.53	0.40±0.04	7.06±0.02
	NAS	72.43±2.74	31.52±2.53	73.14±4.79	131.38±7.67	33.36±2.04	13.50±1.32	0.52±0.04	9.93±0.21
MV10	NAS	10.20±0.88	27.24±1.57	48.81±4.08	237.44±5.93	95.09±8.91	111.56±9.29	0.65±0.06	8.12±0.80
MV11	NAS	32.70±2.31	21.18±1.36	48.96±1.23	174.57±19.66	43.31±1.80	77.75±4.00	0.29±0.02	3.84±0.28
MV12	NAS	76.66±5.81	30.48±2.80	66.08±4.26	233.82±22.36	92.18±5.60	79.96±1.05	0.84±0.01	8.05±0.05
MV13	NAS	16.45±0.86	20.47±1.80	40.79±3.39	230.90±6.62	59.18±4.62	102.70±8.10	0.49±0.02	4.27±0.32

Legend: Na = sodium; Mg = magnesium; P = phosphorus; K = potassium; Ca = calcium; Mn = manganese; Cu = copper; Zn = zinc; MV1 to MV13 = substrate types, AS = aseptic system; NAS = non-aseptic system.
Note: Values are presented as Mean ± Standard deviation.

Regarding magnesium (Mg), the maximum concentration of 36.75±0.96 mg Mg/100 g FW was achieved in NAS using the MV4 substrate, while in the aseptic system (AS) with the MV2 substrate, a lower value of 11.42±0.16 mg Mg/100 g FW was observed.

Phosphorus (P) content was highest in sorrel microplants grown on the MV2 substrate in NAS, measuring 94.43±4.60 mg P/100 g FW. The lowest phosphorus concentration was found in NAS for microplants grown on MV10, which recorded 40.79±3.39 mg P/100 g FW.

For potassium (K), the highest value recorded was 237.44±5.93 mg K/100 g FW, obtained from sorrel microplants grown on MV10 in NAS conditions. Conversely, only 97.52±0.11 mg K/100 g FW was noted in AS using the MV1 substrate.

Calcium (Ca) content was measured at 95.09±8.91 mg Ca/100 g FW in sorrel grown on the MV10 substrate in NAS. In the AS with MV5 substrate, a significantly lower calcium concentration of 12.06±0.20 mg Ca/100 g FW was recorded (Table 5).

Microbiological evaluation

The microbial contamination of sorrel microplants was assessed in both aseptic (AS) and non-aseptic (NAS) production technologies. The AS method produced healthy sorrel microplants, with only sporadic contamination observed in some replicates of the MV3 and MV9 experimental variants. The disinfection

procedures implemented for the seeds, substrates, and jars, along with stringent phytosanitary hygiene measures during the *in vitro* production process, effectively minimized microbial contamination of the microgreens (Table 6).

Among the opportunistic contaminants identified, *Aspergillus* sp. black mold and *Penicillium* sp. blue mold were detected. The identification was based on the distinctive microscopic characteristics (Figure 1).

Table 6. Microbial contaminants on sorrel microplants

Experimental variant	Aseptic system (AS)	Non-aseptic system (NAS)
MV1	Uncontaminated	*Phoma* sp.
MV2	Uncontaminated	*Aspergillus* sp.
MV3	*Aspergillus* sp.	*Cladosporium* sp.
MV4	Uncontaminated	Rod bacteria
MV5	Uncontaminated	Rod bacteria
MV6	Uncontaminated	*Aspergillus* sp.
MV7	Uncontaminated	*Aspergillus* sp. and *Phoma* sp.
MV8	Uncontaminated	Rod bacteria
MV9	*Penicillium* sp.	*Penicillium* sp.
MV10	Uncontaminated	Fungal and bacterial community
MV11	Uncontaminated	*Fusarium* sp.
MV12	Uncontaminated	*Fusarium* sp.
MV13	Uncontaminated	*Fusarium* sp. and *Phoma* sp.

The non-aseptic system (NAS) exhibited high contamination levels across the replicates of all experimental variants (Table 6). Among the identified phytopathogenic fungi, genera such as *Phoma*, *Cladosporium*, and *Fusarium* were present. Notably, *Fusarium* sp. was detected exclusively in microplants grown on peat-containing substrates within the NAS system.

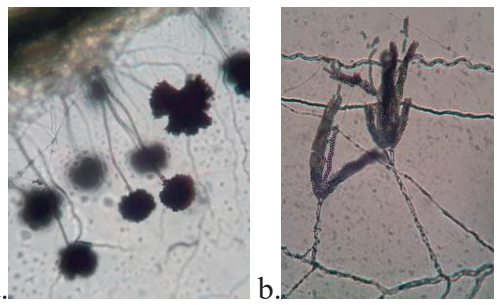

Figure 1. Fungal contaminants of sorrel microplants - (a) *Aspergillus* sp. and (b) *Penicillium* sp. conidiophores and conidia

The presence of *Phoma* sp. is commonly observed in microplants (source: https://homemicrogreens.com), while infections from *Cladosporium* sp. and *Fusarium* sp. can occur asymptomatically in seeds without posing phytosanitary issues in subsequent crop cycles (source: www.syngenta.ca).

The experimental results obtained from combining the eco-innovative cultivation system (AS) with a 100% agar substrate (MV2) indicate that no additional technical interventions, such as mechanical scarification (as recommended by Yazdi et al., 2013), are necessary to enhance the germination of *Rumex acetosella* seeds. This approach aligns with the recommendations of the European Sprouted Seeds Association (ESSA) (source: http://sproutedseeds.eu/list-of-members/), providing a solution that achieves a significant germination capacity of over 90%. This capacity allows for the production of sorrel microplants at an optimal quantity-to-quality ratio.

CONCLUSIONS

The germination capacity and morphological parameters of sorrel microplants demonstrated a significant improvement when cultivated using the aseptic system (AS) compared to the non-aseptic system (NAS), regardless of the substrate type employed.

In addition, biochemical parameters, including dry matter content, assimilatory pigments, macro and microelement concentrations, antioxidant activity, total polyphenol levels, and flavonoid content, exhibited notable variations influenced by substrate type in both AS and NAS, indicating the importance of substrate selection in optimizing the quality of sorrel microplants.

Microbial contamination assessments revealed that the NAS significantly increased the risk of infection across all substrate types. In contrast, the AS exhibited a lower contamination risk, primarily associated with the use of banana peel substrates. Based on a comprehensive analysis of all results, AS is strongly recommended for the production of sorrel microplants. This method is particularly advantageous when utilizing a 100% agar substrate (MV2), as it is not only effective in enhancing germination and

growth but is also edible, allowing for consumption alongside the rooted microplants.

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