STANDARDIZATION OF TECHNOLOGY FOR MICROGREEN PRODUCTION

By

ARYA K. S.

(2019-12-038)

DEPARTMENT OF VEGETABLE SCIENCE

COLLEGE OF AGRICULTURE

KERALA AGRICULTURAL UNIVERSITY

VELLANIKKARA, THRISSUR- 680 656

KERALA, INDIA

2021

STANDARDIZATION OF TECHNOLOGY FOR MICROGREEN PRODUCTION

By

ARYA K. S.

(2019-12-038)

THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Horticulture

(VEGETABLE SCIENCE)

Faculty of Agriculture

Kerala Agricultural University, Thrissur

DEPARTMENT OF VEGETABLE SCIENCE

COLLEGE OF AGRICULTURE

KERALA AGRICULTURAL UNIVERSITY

VELLANIKKARA, THRISSUR- 680 656

KERALA, INDIA

2021

DECLARATION

I, hereby declare that this thesis entitled **"Standardization of technology for microgreen production"** is a bonafide record of research work done by me during the course of research and that it has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of other University or Society.

Vellanikkara
Date: 07.02.2022

Arya K. S.
(2019-12-038)

CERTIFICATE

Certified that this thesis, entitled **"Standardization of technology for microgreen production"** is a bonafide record of research work done independently by **Ms. Arya K. S. (2019-12-038)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship, or associateship to her.

Vellanikkara

Date: 07.02.2022

Dr. M. Sangeeta Kutty
(Chairperson, Advisory Committee)
Assistant Professor
Department of Vegetable Science
College of Agriculture,
Vellanikkara, Thrissur

CERTIFICATE

We, the undersigned members of the advisory committee of **Ms. Arya K. S. (2019-12-038)**, a candidate for the degree of **Master of Science in Horticulture** with major field in **Vegetable Science,** agree that this thesis entitled **"Standardization of technology for microgreen production"** may be submitted by **Ms. Arya K. S.** in partial fulfilment of the requirement for the degree.

7/02/2022

Dr. M. Sangeeta Kutty
(Chairperson of Advisory Committee)
Assistant Professor
Department of Vegetable Science
College of Agriculture,
Vellanikkara, Thrissur

Dr. T. Pradeepkumar
(Member, Advisory Committee)
Professor and Head
Department of Vegetable Science
College of Agriculture,
Vellanikkara, Thrissur

7/02/2022

Dr. Beena V. I.
(Member, Advisory Committee)
Assistant Professor and Head
Radiotracer laboratory
College of Agriculture,
Vellanikkara, Thrissur

7/02/2022

Dr. Aswini A.
(Member, Advisory Committee)
Assisitant Professor
Department of Fruit Science
College of Agriculture,
Vellanikkara, Thrissur

ACKNOWLEDGEMENT

*First and foremost, I would like to thank **God Almighty** for giving me the strength, knowledge, ability and opportunity to undertake this research studies and to preserve and complete the thesis work satisfactorily. Without his blessings, this achievement would not have been possible.*

*With immense pleasure I avail this opportunity to express my deep sense of whole hearted gratitude and indebtedness to my major advisor **Dr. M. Sangeeta Kutty**, Assistant Professor, Department of Vegetable Science, chairperson of my advisory committee for her expert advice, valuable guidance, practical suggestions, constant patience, inspiring encouragement, friendly approach and timely help at various stages of my research work and thesis preparation and will be remembered forever.*

*I express my heartfelt gratitude to **Dr. T. Pradeepkumar**, Professor and Head, Department of Vegetable Science and member of my Advisory Committee for his scientific advice, valuable suggestions, constant support and cooperation throughout the course of study.*

*I would like express my heartiest gratitude to **Dr. Beena V. I.**, Assistant Professor & Head, Radiotracer laboratory, College of Agriculture and member of my advisory committee for her ever willing help, valuable guidance and creative suggestions throughout the period of my study.*

*I sincerely thank Dr. **Aswini A.**, Assistant Professor, Department of Fruit Science and member of my Advisory committee for her unwavering encouragement, timely support and constructive criticism towards me during the past two years.*

*It is with my heartfelt feelings, I wish to express my deep gratitude and sincere thanks to **Dr. P. Anitha, Dr. Dicto Jose M., Dr. Reshmi Dr. Rekha C. R. and Dr. Flemine Xavier** for their encouragement, valuable help and advice rendered during the course of study.*

I am thankful to the research associates and farm staff of Department of Vegetable Science especially ***Dr. Anu Kurian, Varun chettan, Veni chechi and Geethu chechi*** *for their*

whole hearted cooperation and timely assistance. I also cordially acknowledge the assistance extended by all office and field staff for timely and sincere help, especially ***Sunitha chechi, Surabhi, Jisna, Joel, Vijaya chechi, Lailitha*** *and all.*

I cherish the friendship I had and take this opportunity to thank each one of them. It gives me great pleasure in acknowledging the love, support and help of my dear classmates and friends ***Arya and Anulatha, Parvathy, Anila, Nanda, Athira, Priya, Seethal, Ammu, Swathy and Anjitha*** *which could never be forgotten.*

I have infinite pleasure to express whole hearted thanks to my dear seniors ***Alphy chechi, Nidhin chettan, Divya chechi, Remzeena chechi, Athulya chechi, Anju chechi, Malavika chechi and Ansaba chechi*** *for their encouragement, moral support and timely assistance.*

Special word of thanks to my juniors ***Aparna, Mintu, Jayalakshmi and Kousthubha*** *for their prompt help and co-operation during the entire period of study.*

I take this opportunity to extend my gratitude to ***Dr. A. T. Francis****, Librarian of the KAU central library.*

I acknowledge the whole- hearted co- operation and gracious help rendered by each and every member of ***College of Agriculture*** *during the period of study.*

I am in dearth of words to express my love towards ***my beloved parents and brother*** *for their boundless affection, moral support, deep concern, prayers and personal sacrifices, without which this endeavour would never have become a reality.*

For the whole journey, my head bows to Kerala Agricultural University for letting my dreams come true...

Arya K. S.

Dedicated to my parents……

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Introduction

1. INTRODUCTION

Microgreens are an introduced genre of edible greens recently visible in luxurious restaurants and markets, they have gained a wide acceptance as culinary vegetable since the last few years. It is an emerging technology for providing food crops well adapted to global climate change, urbanization and changing food habits among the population. Despite the fact that the term "microgreens" has been around for a while, more research on the subject has emerged in the twenty-first century. It is now gaining popularity in India, especially in five- star hotels as an urban food item. According to Global Hunger Index 2021, India had secured 101 st position out of 116 countries whereas in 2020 it was in the 94 th position among a total of 107 countries. This implies that Indian population is suffering from serious level of hunger and malnutrition, necessitating the provision of highly nutritious food to all segments of society in an easily accessible manner. Thus, microgreens are gaining more importance as it is nutritionally rich as well as easily cultivatable even in indoor spaces (Weber, 2016).

Microgreens are "tender immature greens produced from seeds of vegetables and herbs having fully developed cotyledons with or without the emergence of a rudimentary pair of first true leaves"(Xiao *et al*., 2012). They are tender immature seedlings of vegetables and herbs known for several health benefits and are mostly used as salad crop, harvested within 10-12 days of planting (Senevirathne *et al*., 2019; Murphy and Pill, 2010). The most valuable benefits of traditional leafy vegetables are their high concentration of vitamins, minerals, fiber and other micronutrients essential for human health. Microgreens represent a new class of vegetables that can be considered as "functional foods", which possess particular health benefiting or disease preventing properties in addition to their normal nutritional values (Xiao *et al*., 2012). Different parts of microgreens include a central stem, cotyledonary leaves and a pair of true leaves (Tiwari*et al*., 2015). Their bioactive properties include higher levels of antioxidant compounds such as polyphenols, β carotene, and ascorbic acid, than their mature plants, thus qualifying them as functional foods (Xiao *et al*., 2012). Microgreens are larger than sprouts, but

are smaller than baby greens and therefore their harvesting stages are also in between those two stages (Xiao *et al*., 2012; Ebert, 2013).

Demand for these products is growing rapidly (Janovska *et al*., 2010; Samuoliene *et al*., 2012) due to their unique colour, rich flavour and significant content of bioactive substances (Brazaityte *et al*., 2013; Kou *et al*., 2013; Brazaityte *et al*., 2015). Different types of vegetables can be used to produce microgreens, with wide range of tastes-mellow, spicy, tangy, earthy, nutty and crisp. Commonly grown microgreens include amaranth, basil, beet, cabbage, celery, chervil, chinese kale, cilantro, fennel, mustard, parsley, radish, swiss chard *etc*. Crops from the Brassicaceae and Amaranthaceae families contribute the most species and varieties used in microgreen production (Kyriacou *et al*., 2016; Xiao *et al*., 2016). Even though the microgreens are very small sized, they are abundant in many of the nutrients. Many metabolic processes are involved during the process of germination, seedling emergence and plant development, which leads to increase in the content of protein and the essential amino acids (Deepa and Malladadavar, 2020). They are found to be rich source of vitamin C, vitamin E, vitamin K and beta carotene and also minerals like Ca, Mg, Fe, Mn, Se and Mo (Pinto *et al*., 2015). Microgreens are reported to be 4-6 times more nutrient dense than their mature counterparts (Xiao *et al*., 2012).

The production cycle of these crops are very quick, so their maintenance is very easy (Kopsell *et al*., 2012; Virsile and Whittas, 2013). It requires only limited inputs as their life cycle is very small, while their nutrient content is much higher than the mature plants. The seed to biomass ratio is reported to be very high in microgreens (Dalal *et al*., 2020). Microgreens are well suited for indoor vegetable production, and are a part of the global movement towards controlled environmental agriculture (CEA) (Riggio, Jones, & Gibson, 2019). As they have the shortest growing period with little space requirement, less nutrient supply and year-round suitability for planting, they can be raised in the open as well as balcony, window sides or spaces inside the house. These practices can ensure food availability to the most vulnerable members of society, as well as serve as a good source of several vital nutrients and resist certain dietary deficiencies, thus playing an important role in the nutritional security of rural and urban populations. During the off seasons,

it is a profitable entrepreneurial activity for farmers which help to maintain their economic status (Ghoora and Srividya, 2018).

In recent days, the availability of fresh and pesticide free vegetables is decreasing especially with regard to the vegetarians in our country. As a culinary vegetable, microgreens are well known and served as an edible garnish or a new salad ingredient in many countries (Frank and Richardson, 2009; Hedges and Lister, 2009; Chandra *et al*., 2012; Xiao *et al*., 2012; Kou *et al*., 2013; Pinto *et al*., 2015). It is not readily available in the market due to its high cost as well as its low shelf life. Another problem observed is the incidence of biological agents which interrupt the normal growth of microgreens. Microgreens are becoming more popular as a result of their potential to improve farmers economic, food, and nutritional security. It is a new technology well suited for urban areas for planting crops in indoor areas with minimum input and maximum output (Dalal *et al*., 2020). The present study is undertaken with the aim to standardize the production technology for microgreens in terms of seed treatment, growing media, seed density and to compare the nutritional value and yield of microgreens under different growing conditions.

Review of literature

2. REVIEW OF LITERATURE

Microgreen cultivation is a novel idea to develop highly nutritious tender greens from seeds of vegetables or herbs with cotyledonary leaves and a pair of true leaves in a small area with a limited supply of light and temperature. It is currently gaining more interest due to its potential for enhancing economic, food and nutritional security among the growing population. In order to make these highly nutritious greens available to every section of society it is very important to standardize its growth by utilizing the available local conditions and resources in remote areas instead of adopting multilayer unit for its growth. Still, many more research studies are required in this area to be focused. Literature on present study entitled "Standardization of technology for microgreen production" is reviewed below

1. Seed treatment
2. Media
3. Germination
4. Seed density
5. Light requirement
6. Harvest
7. Nutritional value
8. Shelf life
9. Microbial growth

2.1. Seed treatment

Seed treatment has found to play a major role in protecting the seeds from several seed, soil borne diseases and insect infestations for the healthy emergence of seedlings. These include the application of physical, chemical or biological agents to seeds in advance to sowing in the field (Sharma *et al*., 2015). Seed treatments are normally done prior to sowing in order to free the seeds from any kind of fungal or bacterial spores. For this the seeds can be treated with 3% hydrogen peroxide before planting then washed well using tap water to remove the excess solution (Kaur and Singh, 2020).

Pernezny (2002) conducted a study using lettuce seeds which was treated with 3% hydrogen peroxide for 5 minutes and were found to be free from bacterial infections while chard or beet seeds when soaked in water at 20^0C for 48 hrs, hydrogen peroxide @ 0.3% for 48 hrs, hydrogen chloride @ 0.3 M at 20^0C for 2 hrs and sodium hypochlorite @ 4% for 3 hrs and by matric priming (fine grade vermiculite), maximum seedling emergence was gained by germinating seeds in fine grade vermiculite than soaking in hydrogen peroxide (Lee *et al*., 2004). Szopinska *et al*. (2017) treated the carrot seeds with 3, 6, 9 and 12 % hydrogen peroxide solutions for 10, 30 and 60 minutes in order to check the germination, vigour , health and plant emergence and revealed that it had an important role in preventing the incidence of seed infestation with *A. alternata, A. radicina* and *Fusarium* spp. The seed treatment with 3% hydrogen peroxide for 30 minutes was found to exhibit more plant emergence in majority of the samples.

Similarly, acetic acid can also be used for controlling many seed borne diseases as well as to promote proper germination and seedling emergence. The effect of seed treatment with acetic acid on carrot seed was assessed by treating contaminated seeds with 5%, 10% and 20% of solution. The seeds treated with 5 % concentration of acetic acid, reported 48% of normal seedlings. At 10% acetic acid, 38% of normal seedlings were recorded. (Benothem *et al*., 2019).

Seed soaking is an important factor that encourages the seed germination, vigour and proper development of seedling. In microgreen production soaking has a tremendous role for initiating sprouting of seeds, which further enhances the growth of seedlings. For larger seeds, a soaking period of 8 hours favours their rapid germination while small seeds require less soaking time for their proper seedling development (Moran, 2017). The water should be drained out after soaking the seeds up to required period otherwise it may lead to fungal contaminations (Parida, 2020).

2.2. Media

Selection of suitable growing media has a crucial role in supporting the plant growth as well as act as pillars for well developed and strong development of the root

system. The characteristics of an ideal growing media include, it should provide well physical support to root system, good aeration, porosity and proper water holding capacity (Landis *et al*., 1990). The growing media for microgreens should possess a pH range of 5.5-6.5, low electrical conductivity (<500 µS/cm), optimum water holding ability with a range of 55 to 70% v/v and aeration in a range of 20 to 30% v/v for their proper germination (Abad *et al*., 2001; Bewley, 1997; Kyriacou *et al*., 2016).

Generally, media used for microgreens production should be inert one like cocopeat, vermiculite or in combination (3:1) of both the media (Kumar *et al*., 2018). It should be most preferably standard, sterile, loose, soilless germinating media with a mixture of peat, vermiculite, perlite and coconut fibre *etc.* and the tray should be filled with appropriate medium to a depth of ½ inches to 1 or 2 inches (Treadwell *et al*., 2010). Media with neutral pH and loose soil are best suited for microgreen production and among the soils used black soil was found to be ideal for proper growth as well producing good quality microgreens (Lau *et al*., 2019).

Murphy and Pill (2010) conducted an experiment by filling peat- lite medium up to depth of 2.5 cm for microgreen production and seeds were uniformly broadcasted and covered with medium of thickness 1-2 mm. The height of media used for microgreen production lies at a range of 0.5 to 2 inches, according to the irrigation facilities used. Similarly, Bulgari *et al*., (2021) evaluated the influence of growing media on yield and growth parameters in microgreen production with vermiculite, coconut fibre, and jute fabric media in green and red basil varieties. It was reported that the media mainly influence only the yield while all other quality parameters may vary according to different species used in the study.

Mohanty *et al*., (2020) studied the nutritional value of three different microgreens in two different growing conditions like soil and water and concluded that the growth of microgreens grown in water was higher than that grown in soil.

2.3. Seed germination

Seed germination is one of most important basic step involved in the period of plant growth. The availability of seeds with good germination is one of the chief factor for maintaining a good crop production. It is the emergence of a radicle through

the seed coat of seed (Copeland and Mc Donald, 2001). For maintaining good seed viability, it should be stored at low temperature ranging from 1 to 5°C and relative humidity of 3 to 10 percent at dark room and the seeds are not let to rehydrate during storage period as the viability of seed could be affected. For enhancing rapid germination, the seeds should be soaked in water or acid solution prior to sowing.

The germination rates of different microgreens can be different for fenugreek, green radish lettuce, mustard and sesame seeds, it was found to be fast within 2 days; moderate in kale and green peas within 2-3 days; slow in carrot within 7 days and very slow in finger millet and red amaranth which germinate within 14 days (Polash *et al*., 2019).

2.4. Seed density

As seed rate increase, there will be competition among plants for water, nutrients, and light, so maintaining an optimum seed density is an important criterion while sowing the seeds. The seed rate may vary according to the seed size used for planting, 10-12 seeds per square inch for small seeded crops and 6-8 seeds for large seeds are appropriate for cultivating healthy microgreens otherwise it may result in soft elongated and smaller leaves with shorter shelf life (Koley, 2016).

Murphy and Pill (2010) reported that while using argula (*Eruca vesicaria* sub sp.sativa) seeds the economic yield of microgreen was found to be high when seeds were sown at high seed rate of 55 g m^{-2} as it results in a greater shoot fresh weight (FW) m–2 at 10 day after planting (DAP) than low density planting. Ghoora and Srividya,(2018) optimized seed density for other crops such as 188 gm^{-2} per tray for carrot microgreens and 500 gm^{-2} per tray for fennel microgreens. For spinach, onion and french basil microgreens the seeds are sown at a seed rates of 250 g m^{-2}, for roselle, fenugreek and sunflower microgreens 375 gm^{-2} and 313 gm^{-2} were used for radish for raising ideal microgreen production. Nolan (2019) reported that the seed densities of all the crops experimented had a mean density of 59.2 $g \cdot m^{-2}$, ranging from 19.5 $g \cdot m^{-2}$ to 129.4 $g \cdot m^{-2}$ for each seed.

2.5. Light requirement

Light is one of the major external factor that influence the energy building process in green plants, photosynthesis and also acts as a source to sense the environment around it (Murchie and Niyogi, 2011; Fortunato *et al*., 2015). For well-established microgreen production, high light for a period of 12-16 hours is essential along with low humidity and good air circulation (Kumar*et al*., 2016); Lau *et al*., (2019) reported sunlight as the most preferred source of light as it showed a fast growth period ranging from 8 to 11 days and also produced high yield as well as high quality microgeens much better than the ones raised under artificial light. For the ideal growth and development of microgreens, a temperature range of 18-25^{0}C, light intensity of 12000-16000 lux and relative humidity of about 60-70% are required. Some seeds may not germinate under low temperature and also seedlings may show bending and stunty growth at low light condition whereas growth will be retarded at low relative humidity and there may incidence of fungal infection at high humidity condition (Singh *et al*., 2020).

In borage (*Borago officinalis* L.) microgreens LED illumination at 440 µ mol m^{-2} s^{-1} is the desirable light intensity for optimum growth and to gain good nutrient status for the crop while, an increased nitrates content as well as decrease in hypocotyl length and fresh weight was observed at lower light intensity (Virsile and Sirtautas, 2013) similarly, when britton (*Perilla frutescens* (L.)) was grown under 638- red light for a short period before harvest, increased the main antioxidant concentrations such as anthocyanins and ascorbic acid while reduced the nitrate content in them (Brazaityte *et al*., 2013).

In the microgreens of the Brassicaceae family, light intensity and its quality help to accumulate more total carotenoids at a wavelength of 330–440 µ mol m^{-2} s^{-1} than that in the normal case at 220 µ mol m^{-2} s^{-1} (Brazaityte *et al*., 2015). The use of artificial lights like fluorescent lamps, halogen light, LED light with a spectral range of 250 nm to 750 nm has shown a good effect on nutritional quality of microgreens (Zhang *et al*., 2020).

2.6. Harvest

Microgreens are ready for harvest when they reach the first true leaf stage, usually at about 2 inches tall. Harvesting time usually vary according to the crops selected, some crops may take 5 days and it may go up to 21 days for some crops to reach their harvesting stage (Danielle *et al*, 1999; Murphy and Pill, 2010; Senevirathne *et al*., 2019).

Microgreens are harvested by cutting the stem portion along with leaves just above the roots using a sharp scissors which is sterilized with ethanol (Murphy and Pill, 2010). Ghoora and Srividya (2018) reported that harvesting stage is attained after the complete opening of cotyledonary leaves and development of first true leaf nearly at a height of 2-3 inches. After harvest they are properly cleaned to eliminate the dirt and seed husk. It is washed in deionised water and dried by keeping it under the fan to avoid any contamination (Ghoora *et al*., 2020).

2.7. Nutritional value

Microgreens contain a large amount of phytonutrients like ascorbic acid, β-carotene, α-tocopherol and phylloquinone, many minerals such as calcium, magnesium, iron, manganese, zinc, selenium and molybdenum and also possess low amount of nitrate compared to their mature vegetables (Xiao *et al*., 2012). When consumed, microgreens are a good source of fibre, vitamins and minerals, and they have valuable antioxidant capabilities that help to protect the body against cancer and cardiovascular disease (Brazaityte *et al*., 2015). It was reported that compared to the baby and mature greens, microgreens contain low amount of nitrates, mostly in swiss chard and arugula (Bulgari, *et al.*, 2017; Pinto, *et al*., 2015). Dalal (2020) reported that the amount of the carotenoid, ascorbic acid, total phenol and antioxidant content progressively decline with increase in storage time. It was also found that presence of organic acids shows a positive effect on shelf life of microgreens.

The microgreens of red cabbage are highly sufficient in vitamin C, while daikon radish to be rich in Vitamin E (Xio *et al*., 2014) and protein, iron, vitamin C, beta carotene, lutein and violaxanthin content in *Amaranthus tricolor* microgreens were found to be higher compared to their sprouts (Ebert *et al*., 2013). Weber (2016)

compared the nutritional value of lettuce and cabbage microgreens grown hydroponically and in vermicompost and showed that crops raised in vermicompost possessed more nutrients like P, K, S, Mg, Mn, Cu, Zn, Fe and Na than that in hydroponics.

Antony and Radha (2019) reported that minerals such as P, K, Ca, Mg, Fe, Na, and Zn to be rich in broccoli microgreens, so that they are nutritionally rich than their mature ones. Nair and Lekshmi (2019) evaluated ten microgreens and the vitamin C content was found to be high in all the microgreens, thiamine, riboflavin and niacin were more concentrated in *Amaranthus viridis, Vigna radiata* and *Allium cepa* microgreens. *Vigna radiata* and *V. unguiculata* microgreens were reported to be best with high amount of carbohydrate, protein and vitamin.

Niroula*et al*. (2019) reported that the carotenoid concentration in wheat and barley microgreens increased up to a growth period of 16 days, a slight decrease was recorded in 7 and 10 days of wheat and 10 and 13 days of barley. The kale microgreens showed lower concentration of carotenoid content than their mature crop, while in broccoli and cauliflower microgreens the amount of carotenoid was found to be higher than their mature crops (Xiao *et al*., 2019). The calcium and magnesium content were reported to be maximum in *Lactuca sativa* L. var. capitata cultivars (green and red Salanova) microgreens while phosphorous, potassium, chlorophyll, lutein, β-carotene and nitrate concentration were found to increase as the plant matures (El-Nakhel *et al*., 2020) . The nutrient content of three crops *Vigna radiata L., Brassica nigra* L. and *Trigonella foenum graecum L.* were compared and showed that the iron and calcium concentration to be larger in fenugreek microgreens compared to others (Mohanty *et al*., 2020).

The macro and micro nutrient content of fenugreek, mustard and coriander microgreens were reported to be much higher than their mature greens. Microgreens of red cabbage, garnet amaranth and radish microgreens contain high amount of Vitamin C, K, 1 and E (Parida, 2020) while, fennel microgreens comprise elements like calcium, potassium and sodium, spinach microgreens are rich source of magnesium, similarly roselle microgreens had high amount of phosphorus, zinc and selenium content (Ghoora *et al*., 2020).

2.8. Shelf life

Harvested microgreens are highly perishable and should be washed and cooled as quickly as possible. The factors affecting shelf life of microgreens include temperature, relative humidity, packaging film type and microbial load. The methods used to increase their shelf life are by the adjusting storage temperature and storage atmospheric conditions (Hodges and Toivonen, 2008). Microgreens are usually packed in small, plastic clamshell packages and cooled to recommended temperatures for the crops in the mix. (Danielle *et al*., 2011).

The temperature of storage had an immense role in delaying the shelf life of microgreens. Xiao *et al*. (2014) reported that at a temperature of 1ºC the radish microgreens were found to be best stored than at other storage temperature while Paradiso *et al*., (2018) evaluated the shelf life of six different microgreens of Asteraceae and Brasssicaceae family and it extended up to ten days when stored at a temperature of 5 ^{0}C.

The use of live microgreens are the most preferred freshest and nutritious form for consumption and they can be stored up to 14 days in refrigerator or stored at room temperature for 4-6 days with daily watering (Kumar *et al*., 2016). During harvesting there is chance of mechanical damage which can affect the shelf life of microgreens during storage, so adequate measures should be taken to reduce injuries and immediately after cutting it should be rinsed in chilling water in order to stop the exudates coming out from the cut ends (Kaur and Singh, 2020).

Berba and Uchanski (2012) reported that on visual analysis, the storage period of arugula and red cabbage extended up to two weeks and for radish it was found to be three weeks when stored at a temperature of 4°C and can be reduced to one week when stored at 10°C. The storage life of microgreens will depend on the age of the seedlings at harvest. Kou *et al*. (2013) reported highest quality and maximum shelf life of microgreens when stored at a temperature of 5 and 10 °C with moderately high oxygen (14.0 to 16.5 k Pa) and moderately low carbon dioxide (1.0 to 1.5 k Pa) level.

Kou *et al*., (2013) observed the effect of pre-harvest calcium treatment on the quality of broccoli microgreens. The postharvest quality and the shelf life were

increased to 21 days in the treated microgreens while the untreated microgreens can be stored only for up to 14 days.

2.9. Organoleptic evaluation

Sensory evaluation of microgreens is done in order to identify its overall acceptance regarding taste and appearance. Six microgreens such as arugula, broccoli, bull's blood beet, red cabbage, red garnet amaranth and tendril pea were evaluated by Michell *et al*. (2020) for sensory perception and acceptability by consumers and obtained high mean scores for acceptability with slightly different flavours and overall acceptability.

The effect of sensory attributes and visual appearance of twelve microgreens were checked and found that every microgreen to be superior in appearance but the texture and flavour depends on its consumer acceptance. Coriander and swiss chard showed the maximum score while mibuna and cress ranked the least acceptance by consumers (Caracciolo *et al*., 2020). Similarly, consumer's perception of broccoli microgreens which is grown by commercial hydroponics, local hydroponics and local soil grown were recorded and the produce from local farm recorded maximum sensory score than the commercially grown microgreens (Chen *et al*., 2020).

2.10. Microbial growth

Generally, at low temperature all the physiological activities such as respiration in fruits and vegetables will be suppressed and at the same time it is a favourable condition for the microorganism to cause spoilage to the produce (Nunes and Emond, 2005). So, it is very important to check for the presence of any microorganism in the harvested microgreens.

Chandra *et al*., (2012) evaluated the role of different sanitizers on quality and microbial populations in chinese cabbage microgreens. Tap water, chlorinated water, mix of citric acid and ascorbic acid and solution of citric acid and ethanol were used for washing it and among them chlorine water treated samples shown minimum number of microbial count than others.

Ebert (2013) observed that the microgreens raised in the soil or other medium such as peat moss or other fibrous materials, the growth of bacterial contamination was comparatively less.

Bergspica *et al*., (2020) checked the presence of *Escherichia coli* (STEC), *Salmonella* spp., and *Listeria* spp. in microgreens, sprouts, and seeds in retail market in Riga, Latvia. *Listeria* spp. were observed in two samples, three samples of dried sprouts showed the presence of *Escherichia coli* genes and *Salmonella* spp. were also reported in one of the seed samples. The results reveal that the seeds and microgreens from the market are safe to use other than the dried sprouts.

Materials and methods

3. MATERIALS AND METHODS

The present experiment on Standardization of technology for microgreen production was conducted in the Department of Vegetable Science, College of Agriculture, Kerala Agricultural University during the period of 2019-2021.

3.1. Experimental materials

The experiment was conducted using seeds of six different crops collected from local market. They are listed in the Table 1 below

Table 1. Crops used in the study

Sl. No.	Crops	Scientific name	Family	1000 seed weight (g)	Source
1.	Wheat	*Triticum aestivum*	Poaceae	46	Local market
2.	Ragi	*Eleusine coracana*	Poaceae	5	Local market
3.	Green gram	*Vigna radiate*	Leguminoseae	50	Local market
4.	Horse gram	*Macrotyloma uniflorum*	Leguminoseae	30	Local market
5.	Amaranthus	*Amaranthus tricolor*	Amaranthaceae	0.35	Dept. of Vegetable Science
6.	Mustard	*Brassica juncea*	Brassicaceae	5.4	Local market

3.2. Experimental methods

The study was divided into four experiments *viz,* standardization of seed treatment, standardization of media, standardization of seed density and to analyse the nutritional value and yield under different growing conditions. The trays used for

study had dimensions of 27×21×5.5 cm. The experiments were laid out in a completely randomized design. For the last experiment t- test was carried out to compare their growth and yield under two growing conditions. The days to harvest for the six species of microgreens were as follows (Table2).

Table 2. Days to harvest for the microgreens

Crops	Days to harvest	Harvest index
Wheat	6-7 days	Prior to yellowing of foliage/ development of fibrous texture
Ragi	7-8 days	Prior to yellowing of foliage
Green gram	4-5 days	Prior to toppling of seedlings
Horse gram	6-7 days	Prior to toppling of seedlings
Amaranthus	10-12 days	Prior to toppling of seedlings
Mustard	4-5 days	Prior to toppling of seedlings

The above shown duration was derived from the preliminary experiments conducted in the department.

Experiment 1

First experiment was carried out to identify the best seed treatment for raising healthy microgreens. For this, the seeds were treated with two chemicals *viz,* hydrogen peroxide at different concentration of 0.5%, 1% and 2% and vinegar at concentrations 2%, 5% and 7%. Seed soaking in distilled water was used as a control in this experiment. For each treatment three replications were worked out.

Experiment 2

In the second experiment, standardization of media was done using five different growing media- sterilized sand, cocopeat, coir mat, tissue paper, and newspaper. For each treatment four replications were carried out in each of the six crops. In the trays the different growing media were filled up to height of 1 inch for

sowing seeds. The microgreens were irrigated with distilled water, which was sprayed on the plants three to four times a day. The experiment was repeated twice at an interval of one month.

Experiment 3

Third experiment was conducted to standardize seed density used for microgreen production. The seeds were sown at three densities low, medium and high to find the optimum one. For each treatment five replications were done. The density used for planting will vary for each crop depending on the size of the seeds as shown in Table 3.

Table 3. Seed density for the microgreens

Crops	Low density (g/ m^2)	Medium density (g/ m^2)	High density (g/ m^2)
Wheat	246	440	705
Ragi	264	388	520
Green gram	670	850	1128
Horse gram	352	617	881
Amaranthus	35	70	120
Mustard	211	440	529

Experiment 4

In this experiment, microgreens were raised in two different growing conditions room

condition and rain shelter. The growth and nutritional characteristics of six species of microgreens under both conditions were recorded. Observations on organoleptic analysis and shelf life of microgreens were also noted in this experiment.

3.3. Observations recorded

The morphological observations were recorded by selecting five plants per replication. The morphological characters were recorded at the time of harvest and

biochemical characters like nutritional data of microgreens were also recorded and an average was calculated for the analysis.

3.3.1. Morphological characters

3.3.1.1. Germination percentage

The germination percentage of seeds was determined in the first experiment. According to Nandi*et al*. (2017) the trays were filled with cocopeat up to a height of 1 inch and are sprinkled with distilled water. Sixty seeds of each crop were sown in each tray after proper seed treatment with different concentrations of acetic acid and hydrogen peroxide. The seedlings were evaluated at 7 days after sowing according to ISTA, 2012 and it is calculated as

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds on tray}} \times 100$$

3.3.1.2. Seedling vigour

For vigour test, root and shoot length as well as dry weight of randomly taken 5 seedlings per each replication were measured at the day of harvest. This observation was also recorded in the first experiment. The seedling vigour was determined by the following formula (AOSA, 1983)

Vigour index 1 = (Mean root length+ Mean shoot length) × Germination (%)

Vigour index 2 = Germination (%) × Seedling dry weight

3.3.1.3. Seedling height

The seedling height of five plants from each replication of six different crops was recorded and the average was calculated. It was determined in second and third experiments.

3.3.1.4. Yield

The microgreens of six crops were harvested and the yields of each replication were noted and the average was taken. The data regarding yield was noticed in all the experiments.

3.3.2. Biochemical characters

The biochemical analysis of iron, calcium, beta carotene, vitamin C, crude protein, crude fibre, chlorophyll, oxalates and nitrates of microgreens from six crops were analysed according to Sadasivam and Manickam (1992). These observations were recorded in the second and fourth experiments. Oxalates and nitrates concentration were determined only for amaranthus and mustard microgreens.

3.3.2.1. Iron and calcium

The plant sample was dried and powdered, 0.2 g of it was digested for the analysis. Sample was digested with di- acid mixture (HNO_3: $HClO_4$ in 9:4). Dried sample (0.2g) was taken in digestion tubes along with 10 ml of di-acid mixture. The digestion was continued till the contents in digestion tubes become colourless. The contents were cooled and the extract was diluted with 50 ml of distilled water. The absorbance was measured in ICP- OES and standard graph was plotted and standard curve was prepared. (Piper, 1996).

3.3.2.2. Betacarotene

Beta- carotene in microgreen sample was estimated using n butanol (AOAC, 1970).

Stock solution: Prepared by adding 10 mg of beta- carotene to 100 ml n butanol.

Working standard: 10 ml of stock solution was transferred and made up to 100 ml.

Five grams of dried plant sample was taken in conical flask and 50 ml of water saturated butanol (8:2 n butanol: distilled water) was added. It was kept overnight in darkness and next day the content was filtered using Whatman no. 1 filter paper. Pipette out 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1 ml from working standard and 0.5 ml

supernatant from sample into test tubes and added 10 ml of water saturated butanol to each test tubes. The absorbance was recorded at 435.8 nm in spectrophotometer. A standard graph was plotted with the values obtained.

$$\text{Amount of beta carotene (mg/ 100 g)} = \frac{\text{Absorbance of sample} \times \text{concentration of standard}}{\text{Absorbance of standard} \times \text{volume of sample} \times 100}$$

3.3.2.3. Vitamin C

Vitamin C in plant sample was determined by method suggested by Sadasivam and Manickam (1992).

Reagents used: 4% oxalic acid, ascorbic acid and 2, 6- dichlorophenol-indo-phenol dye.

Dye solution: Sodium bicarbonate (42 mg) was mixed with 52 mg 2, 6- dichloro phenol-indo-phenol dye and made up to 200 ml with distilled water.

Standard solution: Ascorbic acid, 100 mg was weighed and dissolved with 4% oxalic acid solution in standard flask.

Working standard: Stock solution 10 ml is diluted with 4% oxalic acid to 100 ml. Collect 5 ml of stock solution and it was dissolved with 10 ml 4% oxalic acid. Titrated against dye (V1 ml) till the end point *i.e.*, appearance of pink colour and the amount of dye in buirette will be noted.

Five millilitres of working sample were taken, 10 ml of 4% oxalic acid was added to it and titrated against dye till pink colour appears. The sample was extracted with 4% oxalic acid and centrifuged. From the supernatant, 5 ml was collected and 10 ml 4% oxalic acid was added to it and titrated against dye.

$$\text{Amount of vitamin C (mg/100g sample)} = \frac{0.5 \times V2 \text{ ml} \times 100 \text{ ml} \times 100}{V1 \text{ ml} \times 5 \text{ ml} \times \text{Wt. of the sample}}$$

3.3.2.4. Crude protein

Protein content in microgreens sample was estimated by Lowry's method described by Sadasivam and Manickam (1992).

Reagents:

Reagent A: Mix 2% sodium carbonate in 0.1N sodium hydroxide.

Reagent B: 0.5 % copper sulphate is mixed in 1% potassium sodium tartarate.

Alkaline copper solution (Reagent C): 50 ml of reagent A was mixed with 1 ml of reagent B before use.

Folin- cioalteau reagent (Reagent D): A mixture of 100 g sodium tungstate, 25 g sodium molybdate, 700 ml water, 50 ml 85% phosphoric acid and 100 ml concentrated hydrochloric acid was refluxed gently for 10 hours. Again 150 g lithium sulphate, 50 ml water and a few drops of bromine water was added and boiled for 15 minutes to remove excess bromine. The mixture was cooled and diluted with 1 L distilled water and filtered. Stock standard: 50 mg bovine serum was dissolved in distilled water and made up to 50 ml.

Working standard: 10 ml of stock solution was transferred and diluted with 50 ml distilled water.

From working standard, 0.2, 0.4, 0.6, 0.8 and 1 ml was pipetted out. For the extraction of protein from sample 500 mg of sample was grind well in 5-10 ml buffer, then centrifuged and supernatant was collected in test tubes. Made up the volume to 1ml and mixed well by adding 5 ml reagent C and kept for 10 minutes. Reagent D, 0.5 ml was mixed and incubated in dark for 30 minutes till blue colour appear. The absorbance was noted in spectrophotometer at 660 nm and protein content in sample was recorded from the standard graph.

$$\text{Amount of protein (g/100 g)} = \frac{\text{Absorbance of sample} \times \text{concentration of standard}}{\text{Absorbance of standard} \times \text{volume of sample} \times 100}$$

3.3.2.5. Crude fibre

Crude fibre content was estimated according to method suggested by Sadasivam and Manickam (1992).

Two grams of dried plant sample was taken in conical flask, 200 ml of 1.25 per cent sulphuric acid was added and boiled for 30 min. The mixture was filtered using muslin cloth and washed with hot water. It was boiled again by adding 200 ml of 1.25 per cent sodium hydroxide solution for 30 minutes. Filtered again through

muslin cloth and washed with 25 ml 1.25% H_2SO_4, 50 ml water and 25 ml alcohol. The residue was collected and transferred to pre-weighed ashing dish (W_1) and dried for 2 h at 130^0C. It was cooled and weight was noted (W_2), again ignited for 30 minutes at 600^0 C. The residue was cooled in desiccator and weight was noted (W_3).

$$\% \text{ Crude fibre in ground sample} = \frac{\text{Loss in weight on ignition } (W_2\text{-}W_1) - (W_3\text{-}W_1)}{\text{Weight of the sample}} \times 100$$

3.3.2.6. Chlorophyll

Chlorophyll content in plant sample was estimated according to the method suggested by Sadasivam and Manickam (1992).

One gram fresh plant sample was weighed and grinded to fine pulp using 20 ml of 80% acetone. The content was centrifuged at 5000 rpm for 5 minutes and supernatant was transferred to 100 ml volumetric flask. The residue was again grinded with 20 ml 80% acetone, centrifuged and transferred to volumetric flask it was continued until the residue become colourless. Then the volume was made up to 100 ml with 80% acetone and the absorbance of the solution was recorded at 645 and 663 nm.

$$\text{Chlorophyll content in sample (mg/g tissue)} = \frac{20.2(A_{645}) + 8.02(A_{663}) \times V}{1000 \times W}$$

3.3.2.7. Oxalates

Oxalate content of microgreens sample was analysed by a method suggested by Marderosian *et al*. (1979).

Two grams of plant sample was taken in 250 ml volumetric flask, 190 ml distilled water and 10 ml of 6N HCl was added to it. The content was boiled in water bath, volume was made up and filtered. The precipitate was washed and collected and diluted to 125 ml. After that 3-4 drops of methyl red and ammonia was added to the

solution till a faint yellow colour develops. The content was heated at 90-100^{0}C and filtered in Whatsman No. 41 filter paper, washed properly to remove impurities. Then 10 ml of 5 percent calcium chloride was added to filtrate and is kept for 24 hrs. Again, filtered through Whatsman No.41filter paper and washed many times with hot water to remove calcium ions. The precipitate was washed with distilled water and taken in a beaker. Till the precipitate completely dissolve diluted sulphuric acid was added and heated to a temperature of 70 ^{0}C. The content was titrated against N/20 $KMnO_4$ till the end point.

$$\text{Oxalate content (g/100g)} = \text{N/20 } KMnO_4 \text{ used (ml)} \times 0.00225 \times \frac{250}{50} \times \frac{100}{2}$$

3.3.2.8. Nitrates

The nitrate content in microgreen sample was determined using phenol di-sulphonic acid suggested by Bharghava and Raghupati (1993).

The plant sample was dried and powdered, 0.5 g of it was extracted using 50 ml water and filtered. Transferred two ml of the aliquot in to a porcelain dish and evaporated to dryness. Phenol disulphonic acid (3 ml) and 15 ml water was added to it and then cooled and washed down in to 100 ml volumetric flask. Ammonia 1:1 was added till the colour turns to yellow and the absorbance was measured at 420 nm. A standard graph was plotted and nitrate content was estimated on dry weight basis.

3.3.3. Organoleptic analysis

A panel of 15 judges were selected for the evaluation using triangle test (Jellineck, 1985) and organoleptic qualities were analysed using 9-point hedonic scale.

The samples of six different microgreens raised under room condition and rain shelter were investigated using 9- point hedonic scale to examine the appearance, texture, taste, flavour, aroma and overall acceptability (Michell *et al*., 2020). The analysis was performed using Kendall's coefficient of concordance test and the superior one was selected by evaluating the mean scores.

3.3.4. Shelf life

Shelf life of microgreens was compared under room condition and low temperature condition. The ranking was given according to the visual qualities of samples. The shelf life was noted every day till the plant sample showed rotting symptoms or any fungal growth. This observation was recorded only for experiment 4.

The evaluation of visual quality of microgreen was performed on the basis of a 5-point scale according to Berba and Uchanski (2012) as shown in Table 4.The number of days for which a species maintained the visual score of '4' or '5' was taken as its shelf life.

Table 4. Visual quality assessment using 5- point scale

Score	Description
5	Essentially free from defects, freshly harvested - No profound visible defects
4	Minor defects, not objectionable - Some (<10%)physical damage (*i.e*., creased cotyledons) - Product is turgid (not wilted)
3	Moderately objectionable defects, marketability threshold -Slight chlorosis (yellowing) -Areas of dry and wilted microgreens(<25%)
2	Excessive defects, not saleable -Discoloured hypocotyls (blue, black) -Cotyledon chlorosis (>25%) -Dry and wilted (>50%)
1	Unusable, degraded product -100% chlorotic -Mould present, foul odour -Extensive rooting -Physical degradation apparent (liquid present)

3.3.5. Microscopic observation of fungal growth

The microscopic contaminations were recorded according to method suggested by Aneja, 2007.

This observation was recorded on the day of harvest of microgreens and also on the day till it survives without any physical damage. The microbial infections in microgreens were checked by the microscopic observation of samples after preparing slides. A clean slide was taken and a small drop of lactophenol cotton blue dye was poured at the centre. A piece of tape was cut in a way that sticky side held towards downside and it is gently pressed towards the shoots where the contamination is to be noted. Then the tape is slowly removed and placed in the lactophenol dye taken in the slide. The slides were observed under low and high resolution of compound microscope and the fungal growth were identified if any. The presence of fungal growth on microgreens were checked in first, second and third experiments.

Agar plate method was also conducted for detection of any fungal or bacterial contamination. Transferred one ml of plant washings to the sterile petriplates. For fungal detection potato dextrose agar (20 ml) and similarly for bacterial detection nutrient agar (20 ml) was poured to sterilized petriplates and moved it in clockwise and anticlock wise direction 3 to 4 times and allowed to solidify. Incubated the plates in room temperature at 28^0C. The observation on bacterial contamination was recorded after 2 days and fungal contamination was noted after 5 to 7 days.

3.3.6. Statistical analysis

The results obtained in the experiments are represented as the means of replicated observations and critical difference, standard error and coefficient of variation are also calculated. Different parameters recorded during the experiments were analyzed using Analysis of variance test. In the fourth experiment, t- test was carried to compare the growth of microgreens under two growing condition. All the statistical analysis were carried out using GRAPES software.

Results

4. RESULTS

The present study entitled “Standardization of technology for microgreen production” was performed with the intention to standardize seed treatment, media, seed density for microgreen cultivation and to compare their growth and nutrient content under two growing conditions. The results recorded in the study are demonstrated below.

4.1. Standardization of seed treatment

4.1.1. Germination (%)

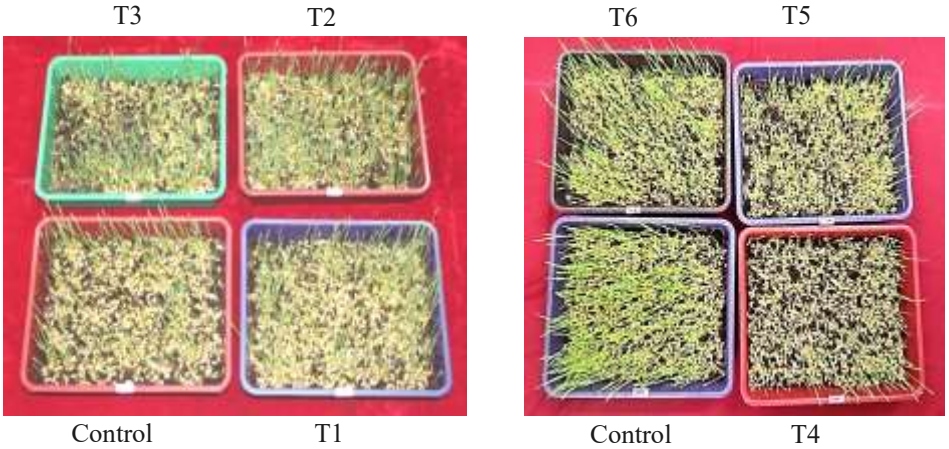
The observations on seed germination revealed that for each of the six crops the germination percentage was noticed to be on par, on treating the seeds with hydrogen peroxide and vinegar at different concentrations compared to control. For wheat it ranged from 88.33 % to 93.33 %, ragi 76.66 % to 86.66, green gram 88.33 % to 93.33%, horse gram 86.66 % to 91.66 %, amaranthus 88.33 % to 93.33 % and mustard recorded 76.66 % to 85 % germination percentage (Table5 to Table 10).

4.1.2. Seedling vigour index

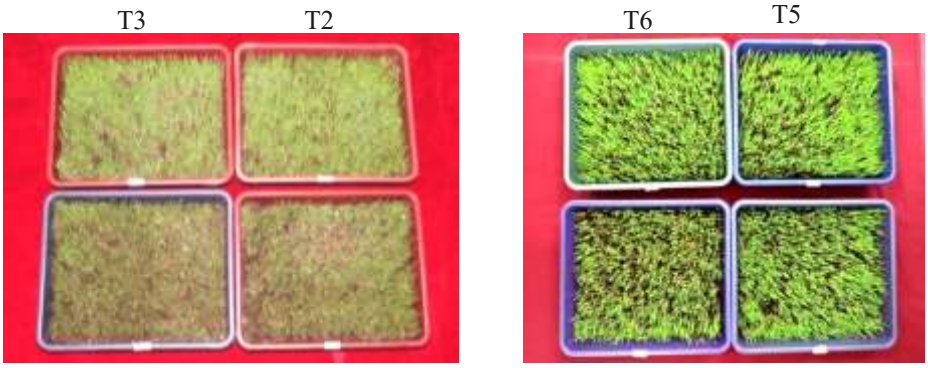
The seedling vigour index 1 and 2 was calculated for the six crops treated with two chemicals hydrogen peroxide and vinegar at different concentrations and were found to be on par for each crop. The highest vigour index 1 and 2 was recorded for green gram which ranged from 1650 to 1857 for vigour index 1 and 1.34 to 1.43 for vigour index 2 followed by wheat with a range of 1221.83 to 1640.33 for vigour index 1 and 0.55 to 0.61 for vigour index 2 and least was observed for ragi microgreens with vigour index 1 in a range of 509.33 to 612.5 and vigour index 2 in a range of 0.14 to 0.17 (Table 5 to Table 10).

4.1.3. Yield (kg/m^2)

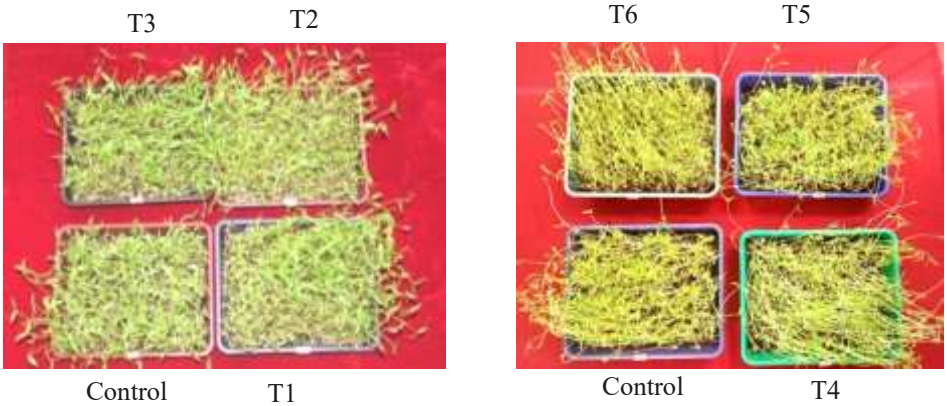
The fresh yield of microgreens recorded for the crops after treating the seeds with two chemicals at different concentrations, showed no significant difference, compared to the control. The maximum yield was recorded for green gram microgreens and least was for amaranthus microgreens. Similarly dry yield of microgreens noted for each crop also showed no significant difference when treated with chemicals and among the crops it was observed to be maximum in green gram microgreens and lowest weight was noted for amaranthus microgreens (Table 5 to Table10).



a) Wheat microgreens



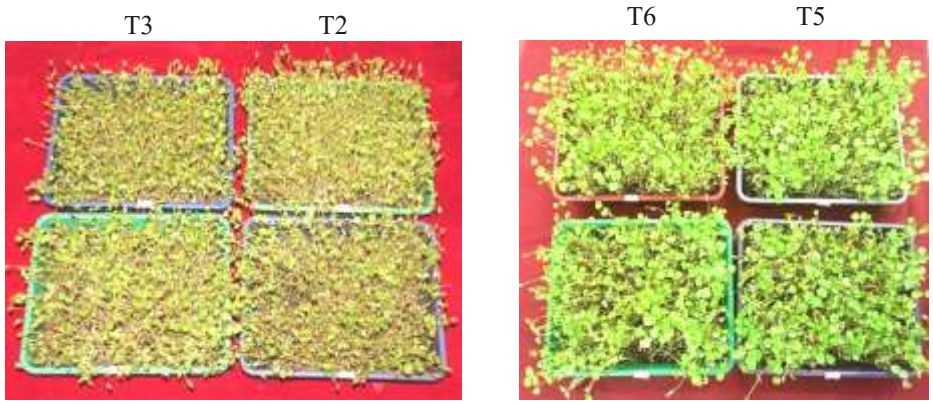
b) Ragi microgreens



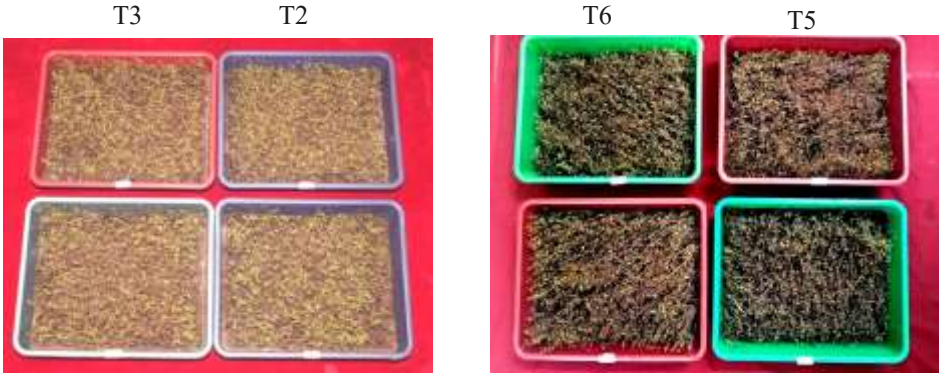
c) Green gram microgreens

T1- Hydrogen peroxide @ 0.5%, T2- Hydrogen peroxide @ 1%, T3- Hydrogen peroxide @ 2%, T4- Vinegar @ 2%, T5- Vinegar @ 5% and T6- Vinegar @ 7%

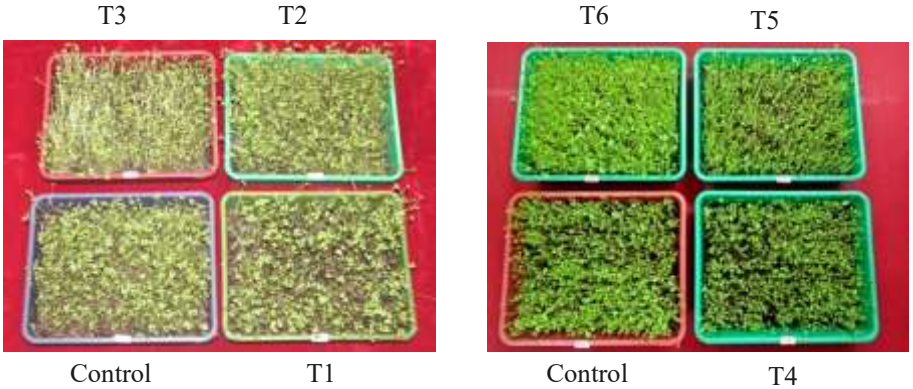
Plate 1. Wheat, ragi and green gram microgreens raised after seed treatment



a) Horse gram microgeens



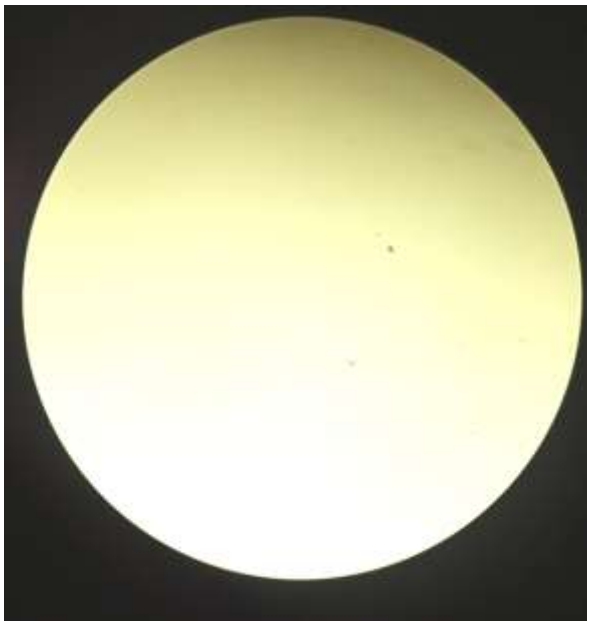
b) Amaranthus microgreens



c) Mustard microgreens

T1- Hydrogen peroxide @ 0.5%, T2- Hydrogen peroxide @ 1%, T3- Hydrogen peroxide @ 2%, T4- Vinegar @ 2%, T5- Vinegar @ 5% and T6- Vinegar @ 7%

Plate 2. Horse gram, amaranthus and mustard microgreens raised after seed treatment



a) Microscopic observation for fungal contamination

b)

c)

d)

e)

f)

g)

C) Agar plating technique done in microgreens

Plate 3. Observations on fungal contamination

Table 5. Influence of seed treatment on wheat microgreens

Treatments	Germination percentage (%)	Vigour index 1	Vigour index 2	Fresh yield (kg/m^2)	Dry yield (kg/m^2)
Hydrogen peroxide @ 0.5%	91.66	1570.16	0.55	0.57	0.06
Hydrogen peroxide @ 1%	93.33	1554.66	0.55	0.58	0.05
Hydrogen peroxide @ 2%	93.33	1640.33	0.59	0.60	0.05
Vinegar @ 2%	88.33	1469.66	0.60	0.56	0.05
Vinegar @ 5%	90.00	1398.00	0.58	0.58	0.05
Vinegar @ 7%	91.66	1221.83	0.60	0.57	0.05
Control	90.00	1313.16	0.60	NS	0.05
CD	NS	NS	NS	NS	NS
SE	1.99	143.03	0.02	0.02	0.002
CV	2.67	17.05	4.73	8.60	4.87

Table 6. Influence of seed treatment on ragi microgreens

Treatments	Germination percentage (%)	Vigour index 1	Vigour index 2	Fresh yield (kg/m^2)	Dry yield (kg/m^2)
Hydrogen peroxide @ 0.5%	83.33	596.33	0.17	0.66	0.06
Hydrogen peroxide @ 1%	86.66	592.66	0.16	0.62	0.06
Hydrogen peroxide @ 2%	86.66	612.50	0.17	0.61	0.06
Vinegar @ 2%	83.33	589.33	0.16	0.61	0.05
Vinegar @ 5%	76.66	560.50	0.14	0.61	0.05
Vinegar @ 7%	83.33	579.66	0.17	0.58	0.05
Control	78.33	509.33	0.15	0.57	0.05
CD	NS	NS	NS	NS	NS
SE	2.52	25.95	0.009	0.02	0.003
CV	5.28	7.78	9.27	7.12	7.61

Table 7. Influence of seed treatment on green gram microgreens

Treatments	Germination percentage (%)	Vigour index 1	Vigour index 2	Fresh yield (kg/m^2)	Dry yield (kg/m^2)
Hydrogen peroxide @ 0.5%	88.33	1699.33	1.34	1.52	88.33
Hydrogen peroxide @ 1%	90.00	1719.00	1.36	1.56	90.00
Hydrogen peroxide @ 2%	93.33	1857.00	1.43	1.71	93.33
Vinegar @ 2%	91.66	1731.66	1.39	1.10	91.66
Vinegar @ 5%	88.33	1722.16	1.37	1.42	88.33
Vinegar @ 7%	93.33	1730.16	1.39	1.28	93.33
Control	88.33	1650.00	1.37	1.41	88.33
CD	NS	NS	NS	NS	NS
SE	1.54	54.42	0.03	0.13	1.54
CV	2.95	5.44	4.23	16.11	2.95

Table 8. Influence of seed treatment on horse gram microgreens

Treatments	Germination percentage (%)	Vigour index 1	Vigour index 2	Fresh yield (kg/m^2)	Dry yield (kg/m^2)
Hydrogen peroxide @ 0.5%	86.66	1347.00	0.95	1.02	0.14
Hydrogen peroxide @ 1%	91.66	1451.66	0.95	1.11	0.14
Hydrogen peroxide @ 2%	91.66	1513.50	1.008	1.05	0.15
Vinegar @ 2%	86.66	1288.83	0.95	0.97	0.12
Vinegar @ 5%	90.00	1461.00	0.99	1.07	0.14
Vinegar @ 7%	91.66	1416.83	0.96	0.97	0.12
Control	88.33	1230.33	0.97	1.09	0.14
CD	NS	NS	NS	1.09	NS
SE	1.54	63.36	0.02	0.07	0.009
CV	2.98	7.91	3.08	11.56	10.83

Table 9. Influence of seed treatment on amaranthus microgreens

Treatments	Germination percentage (%)	Vigour index 1	Vigour index 2	Fresh yield (kg/m^2)	Dry yield (kg/m^2)
Hydrogen peroxide @ 0.5%	88.33	724.33	0.16	0.37	0.03
Hydrogen peroxide @ 1%	88.33	733.16	0.17	0.36	0.03
Hydrogen peroxide @ 2%	88.33	785.33	0.21	0.38	0.03
Vinegar @ 2%	90.00	726.00	0.18	0.36	0.03
Vinegar @ 5%	91.66	739.00	0.18	0.35	0.029
Vinegar @ 7%	93.33	715.83	0.19	0.36	0.03
Control	90.00	749.66	0.19	0.36	0.03
CD	NS	NS	NS	NS	NS
SE	2.35	4.49	0.009	0.013	0.001
CV	4.53	19.19	8.51	6.19	6.19

Table 10. Influence of seed treatment on mustard microgreens

Treatments	Germination percentage (%)	Vigour index 1	Vigour index 2	Fresh yield (kg/m^2)	Dry yield (kg/m^2)
Hydrogen peroxide @ 0.5%	76.66	1134.16	0.31	0.72	76.66
Hydrogen peroxide @ 1%	76.66	1144.16	0.35	0.77	76.66
Hydrogen peroxide @ 2%	85.00	1234.50	0.38	0.90	85.00
Vinegar @ 2%	78.33	1181.83	0.32	0.90	78.33
Vinegar @ 5%	81.66	1081.50	0.37	0.94	81.66
Vinegar @ 7%	78.33	1202.83	0.34	0.96	78.33
Control	78.33	1135.50	0.35	0.80	78.33
CD	NS	NS	NS	NS	NS
SE	1.89	53.67	0.02	0.06	1.89
CV	4.12	8.02	8.57	11.98	4.13

4.1.4. Microscopic observation for fungal growth

Microscopic observation by slide preparation had also shown absence of fungal contamination in the harvested produce (Table 11). Agar plate technique conducted for observation of fungal growth revealed absence of fungal contamination in the freshly harvested microgreens.

Table11. Observations on fungal contamination in seed treated microgreens

Crops	Hydrogen peroxide @ 0.5%	Hydrogen peroxide @ 1%	Hydrogen peroxide @ 2%	Vinegar @ 2%	Vinegar @ 5%	Vinegar @ 7%	Control
Wheat	Not present	Not present	Not present	Not present	Not present	Not present	Not present
Ragi	Not present	Not present	Not present	Not present	Not present	Not present	Not present
Green gram	Not present	Not present	Not present	Not present	Not present	Not present	Not present
Horse gram	Not present	Not present	Not present	Not present	Not present	Not present	Not present
Amaranthus	Not present	Not present	Not present	Not present	Not present	Not present	Not present
Mustard	Not present	Not present	Not present	Not present	Not present	Not present	Not present

4.2.Standardization of media

4.2.1. Yield (kg/m^2)

The yield of microgreens of six crops grown on five different media was recorded. The results indicate that microgreens grown on cocopeat media yielded significantly higher for all the species. Among the crops green gram recorded the highest yield ranged from 1.03 to 1.49 kg/m^2 in different media. The least yield was recorded in amaranthus microgreens which ranged from 0.28 to 0.48 kg/m^2. Similarly, the dry yield recorded for microgreens also exhibited significantly higher yield when grown on cocopeat media. Among the crops, highest dry weight was noted for green gram microgreens (0.230 to 0.333 kg/m^2) and amaranthus microgreens recorded the lowest dry weight (0.027 to 0.046 kg/m^2) (Table 12 to Table 17).

4.2.2. Seedling height (cm)

The height of microgreens grown on different media were observed to be on par for all the six crops raised on five media, except for ragi and mustard where the microgreens raised on cocopeat recorded maximum height and least was noted in newspaper raised microgreens. The seedling height was found to vary according to different crop species used for microgreen production (Table 8 to Table 13).

Table 12. Influence of growing media on wheat microgreens

Treatments	Fresh yield (kg/m^2)	Dry yield (kg/m^2)	Seedling height (cm)
Sterile sand	0.41^{b}	0.04^{b}	8.26
Cocopeat	0.49^{a}	0.05^{a}	8.25
Coir mat	0.38^{bc}	0.04^{bc}	8.27
Tissue paper	0.39^{b}	0.04^{b}	7.97
Newspaper	0.35^{c}	0.03^{c}	8.26
CD	0.04	0.004	NS
SE	0.013	0.001	0.15
CV	6.59	6.59	3.67

Table 13. Influence of growing media on ragi microgreens

Treatments	Fresh yield (kg/m^2)	Dry yield (kg/m^2)	Seedling height (cm)
Sterile sand	0.66[b]	0.07[b]	3.76[a]
Cocopeat	0.73[a]	0.07[a]	3.79[a]
Coir mat	0.62[b]	0.06[b]	3.70[a]
Tissue paper	0.64b	0.06[b]	3.56[a]
Newspaper	0.60[b]	0.06[b]	3.25[b]
CD	0.06	0.007	NS
SE	0.02	0.002	0.08
CV	6.75	6.75	4.37

Table 14. Influence of growing media on green gram microgreens

Treatments	Fresh yield (kg/m^2)	Dry yield (kg/m^2)	Seedling height (cm)
Sterile sand	1.33[b]	0.29[b]	10.26
Cocopeat	1.49[a]	0.33[a]	10.50
Coir mat	1.19[b]	0.26[b]	10.23
Tissue paper	1.22[b]	0.27[b]	10.41
Newspaper	1.03[c]	0.23[c]	10.54
CD	0.15	0.03	NS
SE	0.05	0.01	0.13
CV	8.33	8.33	2.61

Table 15. Influence of growing media on horse gram microgreens

Treatments	Fresh yield (kg/m^2)	Dry yield (kg/m^2)	Seedling height (cm)
Sterile sand	1.12^{b}	0.13^{b}	8.49
Cocopeat	1.33^{a}	0.15^{a}	8.58
Coir mat	0.96^{b}	0.13^{bc}	8.42
Tissue paper	0.59^{c}	0.11^{cd}	8.53
Newspaper	0.58^{c}	0.11^{d}	8.03
CD	0.21	0.007	NS
SE	0.072	0.007	0.08
CV	15.60	10.17	1.87

Table 16. Influence of growing media on amaranthus microgreens

Treatments	Fresh yield (kg/m^2)	Dry yield (kg/m^2)	Seedling height (cm)
Sterile sand	0.39[b]	0.03[b]	4.77
Cocopeat	0.48[a]	0.04[a]	4.90
Coir mat	0.38[b]	0.03[b]	4.73
Tissue paper	0.37[b]	0.03[b]	4.54
Newspaper	0.28[c]	0.023[c]	3.92
CD	0.08	0.008	NS
SE	0.03	0.003	0.15
CV	14.22	14.22	6.63

Table 17. Influence of growing media on mustard microgreens

Treatments	Fresh yield (kg/m^2)	Dry yield (kg/m^2)	Seedling height (cm)
Sterile sand	1.12^{b}	0.07^{b}	5.38^{ab}
Cocopeat	1.28^{a}	0.08^{a}	5.72^{a}
Coir mat	1.08^{bc}	0.06^{b}	5.23^{ab}
Tissue paper	0.94^{cd}	0.03^{c}	4.93^{bc}
Newspaper	0.90^{d}	0.04^{c}	4.28^{c}
CD	0.054	0.01	0.69
SE	0.054	0.005	0.23
CV	10.17	15.60	9.07

4.2.3. Nutrient content of microgreens

4.2.3.1. Iron (mg/100g)

While comparing the iron content, there was no significant difference found among the microgreens grown on different media. Within the crops maximum iron content was found in amaranthus microgreens (1.42 to 1.62 mg/100g) and minimum content was recorded in wheat microgreens (0.19 to 0.22 mg/100g) (Table 18 to Table 23).

4.2.3.2. Calcium(mg/100g)

There was no significant difference in the calcium content of microgreens grown on different media and among the species highest calcium content was noted in ragi microgreens ranging from 272.57 to 286.65 mg/100g and lowest was found in wheat microgreens ranging from 90.67 to 101.95 mg/100g in five different media (Table 18 to Table 23).

4.2.3.3. Beta carotene (mg/100g)

Beta carotene content in microgreens was not showing significant difference when grown on five different media. In all the crops it was found to be present in very small quantity, maximum was noticed in mustard (2.70 to 2.92 mg/100 g) and lowest concentration was recorded in wheat microgreens (0.88 to 0.90 mg/100 g) (Table 18 to Table 23).

4.2.3.4. Vitamin C (mg/100g)

The vitamin C content in microgreens showed no significant difference when raised in different media. It was highest in wheat and amarathus microgreens which ranged from 40.42 to 42.25 mg/100g and lowest content

were noted in ragi microgreens within a range of 21.12 to 22.88 mg/100g (Table 18 to Table 23).

4.2.3.5. Crude Protein (g/100g)

The amount of crude protein in microgreens was not showing significant difference when grown in different media. It was recorded to be high in mustard (1.57 to 1.86 g/100g) and it was observed to be least in ragi microgreens (0.38 to 0.40 g/100g) (Table 18 to Table 23).

4.2.3.6. Crude Fibre (%)

Fibre content noted in microgreens were not showing significant difference when raised in five media. The maximum content was noted in wheat (22.50 to 23.45 %) followed by ragi (17.79 to18.08%) microgreens and lowest content was observed in amaranthus microgreens (3.32 to 3.57 %) (Table 18 to Table 23).

4.2.3.7. Chlorophyll (mg/g)

Chlorophyll content in microgreens does not show significant difference when grown on different media. The amount of chlorophyll varied among the crops it was found to be high in green gram (1.40 to1.43 mg/g) and lowest was recorded in amaranthus microgreen which ranged from 0.16 to 0.17 mg/g (Table 18 to Table 23).

4.2.3.8. Oxalates (mg/100g)

The oxalate content was recorded only for amaranthus and mustard microgreens. There was no significant difference when microgreens were raised in five media. It was reported to be higher in amaranthus microgreens

(75.65 to 77.40 mg/100g) than mustard microgreens (35.60 to 37.67 mg/100g) (Table 22 and Table 23).

4.2.3.9. Nitrates (mg/100g)

The nitrate content in microgreens did not showed significant difference when grown in different media. Amaranthus microgreens recorded highest nitrate content ranging from 52.72 to 54.62 mg/100g followed by mustard microgreens ranging from 25.05 to 26.52 mg/100g (Table 22 and Table 23).

Table 18. Influence of growing media on biochemical characters of wheat microgreens

Treatments	Iron(mg/100g)	Calcium(mg/100g)	Beta carotene (mg/100g)	Vitamin C(mg/100g)	Crude protein (g/100g)	Crude fibre(%)	Chlorophyll (mg/g)
Sterile sand	0.22	101.95	0.87	40.49	0.95	22.60	0.87
Cocopeat	0.20	95.95	0.90	40.49	1.08	22.50	0.90
Coir mat	0.21	99.20	0.89	38.73	0.94	23.45	0.89
Tissue paper	0.19	90.67	0.88	40.49	0.95	23.07	0.88
Newspaper	0.20	96.07	0.88	38.73	1.01	23.25	0.88
CD	NS	NP	NS	NS	NS	NS	NS
SE	0.01	4.99	0.02	2.61	0.04	2.11	0.02
CV	15.19	10.32	5.25	13.12	8.23	18.38	5.25

Table 19. Influence of growing media on biochemical characters of ragi microgreens

Treatments	Iron(mg/100g)	Calcium(mg/100g)	Beta carotene (mg/100g)	Vitamin C(mg/100g)	Crude protein (g/100g)	Crude fibre(%)	Chlorophyll (mg/g)
Sterile sand	0.36	286.65	1.77	21.12	0.40	17.47	0.37
Cocopeat	0.38	284.92	1.65	22.88	0.40	17.46	0.37
Coir mat	0.37	282.92	1.55	22.88	0.40	17.40	0.35
Tissue paper	0.37	272.57	1.65	22.88	0.38	17.36	0.35
Newspaper	0.37	275.47	1.45	22.88	0.39	17.40	0.35
CD	NS	NS	NS	NS	NS	NS	NS
SE	0.01	7.90	0.13	2.72	0.013	0.56	0.01
CV	8.57	5.63	16.82	24.20	6.355	6.46	7.83

Table 20. Influence of growing media on biochemical characters of green gram microgreens

Treatments	Iron(mg/100g)	Calcium(mg/100g)	Beta carotene (mg/100g)	Vitamin C (mg/100g)	Crude protein (g/100g)	Crude fibre(%)	Chlorophyll (mg/g)
Sterile sand	0.57	163.30	1.75	35.19	1.14	13.65	1.40
Cocopeat	0.56	160.72	1.62	36.95	1.18	13.90	1.43
Coir mat	0.56	160.35	1.75	35.19	1.03	14.01	1.43
Tissue paper	0.56	160.67	1.60	38.70	1.09	13.67	1.41
Newspaper	0.57	161.15	1.72	36.94	0.98	13.60	1.40
CD	NS	NS	NS	NS	NS	NS	NS
SE	0.02	3.63	0.14	2.32	0.05	0.29	0.04
CV	7.27	4.50	17.52	12.69	10.01	4.31	6.15

Table 21. Influence of growing media on biochemical characters of horse gram microgreens

Treatments	Iron(mg/100g)	Calcium(mg/100g)	Beta carotene (mg/100g)	Vitamin C (mg/100g)	Crude protein (g/100g)	Crude fibre(%)	Chlorophyll (mg/g)
Sterile sand	0.68	163.30	1.87	35.21	1.42	17.35	1.37
Cocopeat	0.70	160.72	2.00	35.21	1.51	16.92	1.37
Coir mat	0.69	160.35	1.82	35.21	1.55	15.80	1.39
Tissue paper	0.68	160.67	1.80	33.44	1.46	16.27	1.36
Newspaper	0.68	161.15	1.77	35.20	1.49	16.92	1.31
CD	NS	NS	NS	NS	NS	NS	NS
SE	0.02	3.63	0.12	2.98	0.06	1.24	0.05
CV	7.05	4.50	13.79	17.11	8.55	14.90	7.77

Table 22. Influence of growing media on biochemical characters of amaranthus microgreens

Treatments	Iron(mg/100g)	Calcium(mg/100g)	Beta carotene (mg/100g)	Vitamin C (mg/100g)	Crude protein (g/100g)	Crude fibre(%)	Chlorophyll (mg/g)	Nitrates (mg/100g)	Oxalates (mg/100g)
Sterile sand	1.52	145.60	1.95	42.25	0.76	3.47	0.17	53.9	75.95
Cocopeat	1.42	146.32	1.85	40.49	0.74	3.47	0.17	53.85	77.02
Coir mat	1.55	149.32	1.95	40.49	0.76	3.57	0.17	52.72	75.65
Tissue paper	1.62	151.27	1.97	40.49	0.72	3.32	0.17	54.62	76.50
Newspaper	1.62	145.05	1.87	42.2	0.74	3.35	0.16	53.47	77.40
CD	NS	NS	NS	NS	NS	NS	NS	NS	NS
SE	0.1	4.96	0.21	2.91	0.02	0.30	0.01	2.24	3.30
CV	12.90	6.72	21.62	14.12	6.44	17.50	14.86	8.37	8.63

Table 23. Influence of growing media on biochemical characters of mustard microgreens

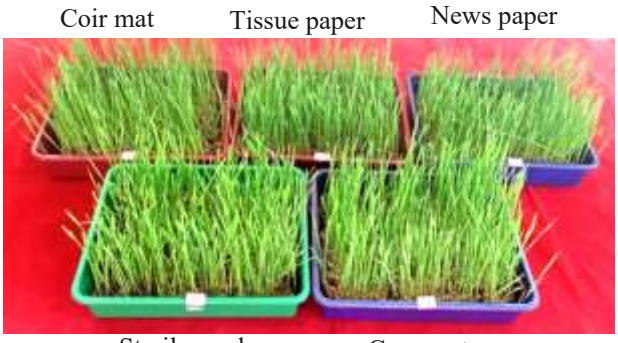
Treatments	Iron(mg/100g)	Calcium (mg/100g)	Beta carotene (mg/100g)	Vitamin C(mg/100g)	Crude protein (g/100g)	Crude fibre(%)	Chlorophyll (mg/g)	Nitrates (mg/100g)	Oxalates (mg/100g)
Sterile sand	0.28	129.35	2.85	35.20	1.85	5.92	0.79	26.52	35.60
Cocopeat	0.29	128.45	2.92	35.20	1.86	6.00	0.78	26.35	37.67
Coir mat	0.27	129.47	2.90	35.20	1.71	6.47	0.82	25.05	37.10
Tissue paper	0.30	127.07	2.85	33.44	1.66	6.350	0.78	25.57	37.32
Newspaper	0.27	126.00	2.70	35.20	1.57	5.92	0.73	25.55	35.97
CD	NS	NS	NS	NS	NS	NS	NS	NS	NS
SE	0.03	3.57	0.15	3.72	0.1	0.34	0.06	1.24	1.852
CV	20.29	5.58	10.69	21.35	11.573	11.16	16.54	9.61	10.08

4.2.4. Microscopic observation for fungal growth

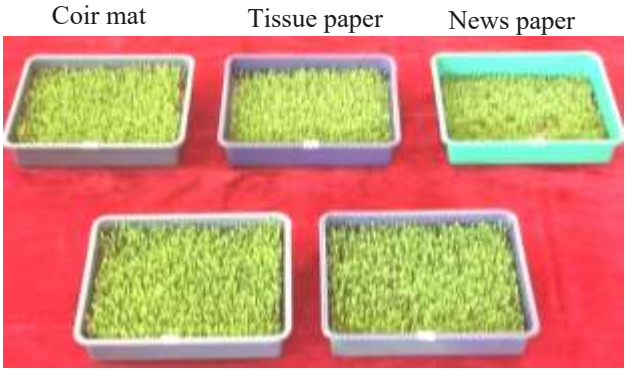
The microscopic observations were recorded at the time of harvest for each species and no microbial contamination was observed in the fresh produce. The microgreens were well suited for consumption (Table 24).

Table 24. Observations of fungal contamination in microgreens grown on five media

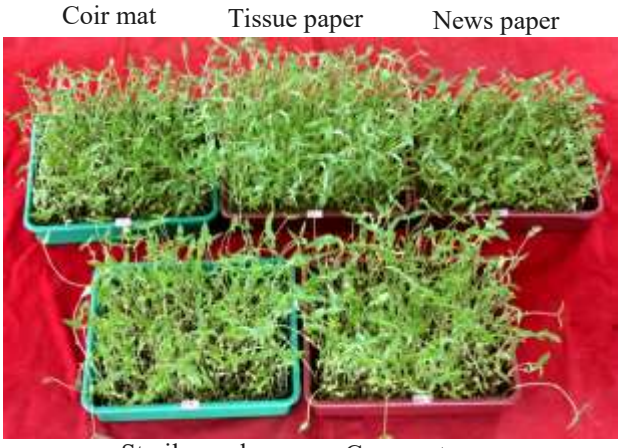
Crops	Sterile sand	Cocopeat	Coir mat	Tissue paper	Newspaper
Wheat	Not present	Not present	Not present	Not present	Not present
Ragi	Not present	Not present	Not present	Not present	Not present
Green gram	Not present	Not present	Not present	Not present	Not present
Horse gram	Not present	Not present	Not present	Not present	Not present
Amaranthus	Not present	Not present	Not present	Not present	Not present
Mustard	Not present	Not present	Not present	Not present	Not present



a) Wheat microgreens

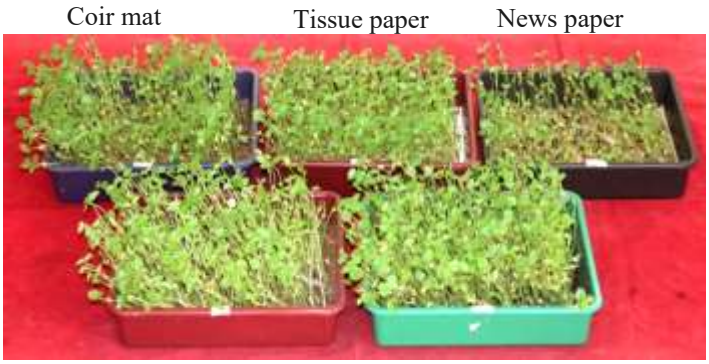


b) Ragi microgreens

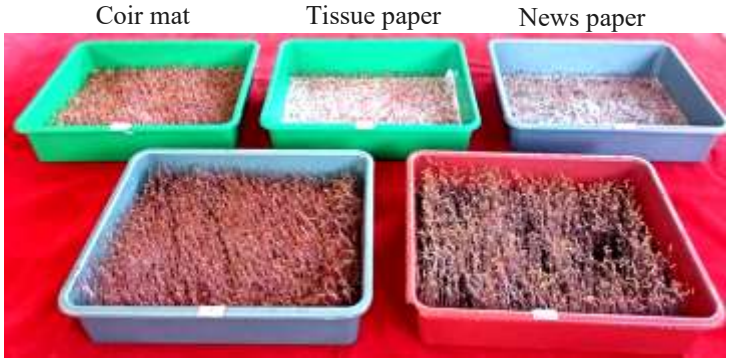


c) Green gram microgreens

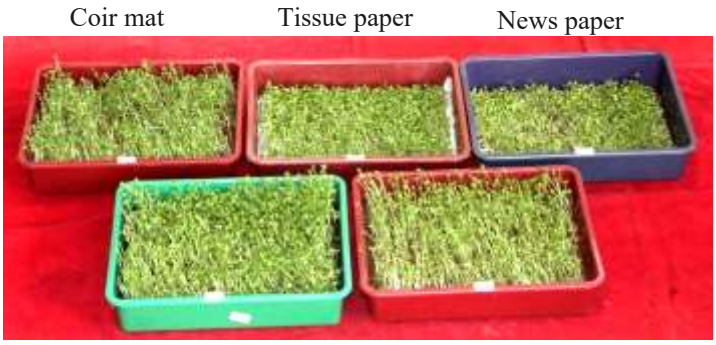
Plate 4. Wheat, ragi and green gram microgreens grown on different growing media



a) Horse gram microgreens



b) Amaranthus microgreens



c) Mustard microgreens

Plate 5. Horse gram, amaranthus and mustard microgreens grown on different growing media

4.3 Standardization of seed density

4.3.1 Yield

Microgreens were sown at three different seed densities *viz.* low, medium and high. For each crop the yield was found to vary when sown at different densities. Yield of wheat (0.59 kg/m^2), ragi (0.61 kg/m^2) and amaranthus (0.58 kg/m^2) microgreens were found to be highest in high density planting,while it was highest in medium density planting for green gram (1.75 kg/m^2), horse gram (1.47 kg/$m^{2)}$ and mustard (1.11 kg/m^2). The dry yield of microgreens recorded also showed significantly higher yield when sown at different seed density. Dry yield of crops was maximum in high density for crops like wheat (0.06 kg/m^2), ragi (0.06 kg/m^2) and amaranthus (0.07 kg/m^2) and medium density sowing showed significantly higher dry yield for green gram (0.17 kg/m^2), horse gram (0.15 kg/m^2) and mustard (0.08 kg/m^2) microgreens. (Table 26 to Table 31). The seed density used for each crop may vary, for wheat microgreens an optimum growth and yield was exhibited at a seed density of 705 g/m^2, for ragi it was 520g/m^2, green gram it was 850 g/m^2, for horse gram it was 617 g/m^2, for amaranthus 120 g/m^2 and for mustard 440 g/m^2 was observed to be ideal (Table 25).

Table 25. Optimum seed density for microgreens

Crop	Seed rate (g/ m^2)	Seed density (seeds/cm^2)
Wheat	705	6
Ragi	520	13
Green gram	850	4
Horse gram	617	5
Amaranthus	120	16
Mustard	440	8

4.3.2 Seedling height

The seedling height of crops grown in three densities were observed and no significant difference for seedling height was observed among the three densities used, except for green gram and horse gram where maximum height was observed in medium density (10.77cm) and least in low density planting (9.35 cm) (Table 26 to Table 31).

Table 26. Mean performance of wheat microgreens under different seed densities

Treatments	Fresh yield (kg/m^2)	Dry yield (kg/m^2)	Seedling height (cm)
Low density	0.23^c	0.02^c	8.65
Medium density	0.46^b	0.05^b	8.89
High density	0.59^a	0.06^a	8.93
CD	0.05	0.005	NS
SE	0.02	0.002	0.13
CV	9.19	8.01	3.45

Table 27. Mean performance of ragi microgreens under different seed densities

Treatments	Fresh yield (kg/m^2)	Dry yield (kg/m^2)	Seedling height (cm)
Low density	0.19^c	0.02^c	3.72
Medium density	0.30^b	0.03^b	3.51
High density	0.61^a	0.06^a	3.64
CD	0.06	0.007	NS
SE	0.02	0.002	0.09
CV	12.11	12.11	5.72

Table 28. Mean performance of green gram microgreens under different seed densities

Treatments	Fresh yield (kg/m^2)	Dry yield (kg/m$^{2)}$	Seedling height (cm)
Low density	0.68^c	0.11^c	10.07^c
Medium density	1.75^a	0.17^a	10.77^a
High density	1.05^b	0.15^b	9.35^b
CD	0.12	0.01	0.55
SE	0.03	0.005	0.18
CV	7.36	8.02	4.02

Table 29. Mean performance of horse gram microgreens under different seed densities

Treatments	Fresh yield (kg/m^2)	Dry yield (kg/m^2)	Seedling height (cm)
Low density	0.80^c	0.12^c	8.28^b
Medium density	1.47^a	0.15^a	8.77^a
High density	0.96^b	0.13^b	8.11^b
CD	0.13	0.01	0.27
SE	0.04	0.004	0.09
CV	8.939	6.525	2.382

Table 30. Mean performance of amaranthus microgreens under different seed densities

Treatments	Fresh yield (kg/m^2)	Dry yield (kg/m^2)	Seedling height (cm)
Low density	0.21^c	0.03^c	4.10
Medium density	0.41^b	0.04^b	4.14
High density	0.58^a	0.07^a	4.27
CD	0.05	0.006	NS
SE	0.02	0.002	0.06
CV	9.32	9.18	3.35

Table 31. Mean performance of mustard microgreens under different seed densities

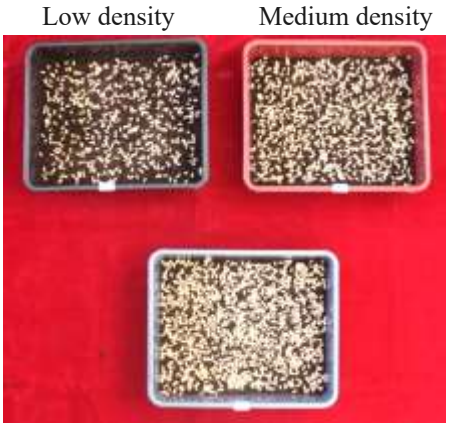
Treatments	Fresh yield (kg/m^2)	Dry yield (kg/m^2)	Seedling height (cm)
Low density	0.71^c	0.05^c	5.64
Medium density	1.11^a	0.08^a	5.97
High density	0.81^b	0.06^b	5.58
CD	0.08	0.005	NS
SE	0.02	0.002	0.13
CV	7.06	5.94	5.35

4.3.3 Microscopic observation for fungal growth

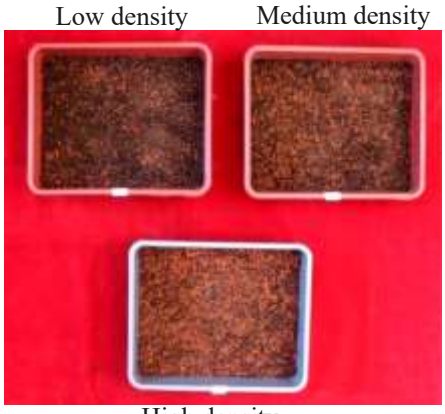
The microscopic observation for fungal growth was done by preparing slides on the day of harvest. No fungal structures or spores were found in the harvested greens (Table 32).

Table 32. Observation of fungal contamination on different seed density sowing

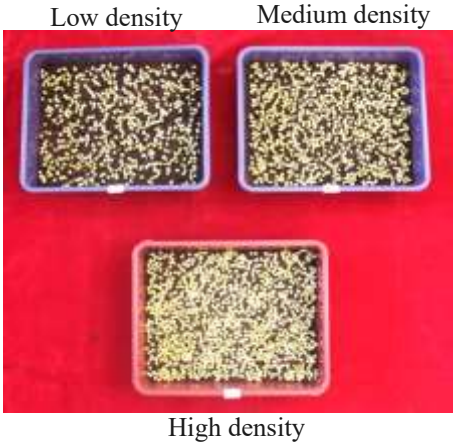
Treatments	Low density	Medium density	High density
Wheat	Not present	Not present	Not present
Ragi	Not present	Not present	Not present
Green gram	Not present	Not present	Not present
Horse gram	Not present	Not present	Not present
Amaranthus	Not present	Not present	Not present
Mustard	Not present	Not present	Not present



a) Wheat seeds sown at different seed density



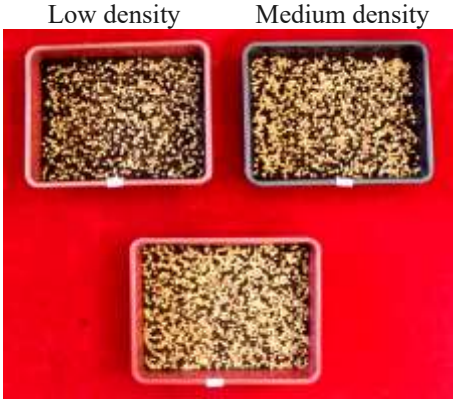
b) Ragi seeds sown at different seed density



c) Green gram seeds sown at different seed density

Plate 6. Wheat, ragi and green gram seeds sown at different seed density

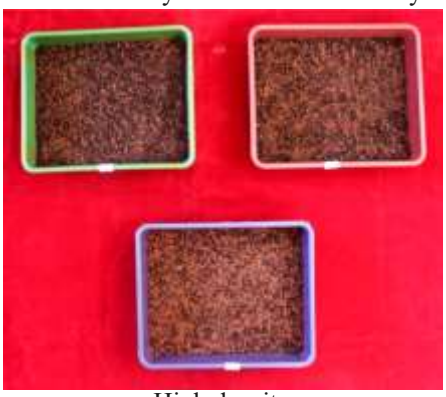
Low density Medium density



High density

a) Horse gram seeds sown at different seed density

Low density Medium density



High density

b) Mustard seeds sown at different seed density

Low density Medium density

High density

c) Amaranthus seeds sown at different seed density

Plate 7. Horse gram, mustard and amaranthus seeds sown at different seed density

4.4 Nutritional value and yield under different growing conditions

4.4.1 Yield (kg/ m^2)

The fresh yield data of microgreens grown under rain shelter condition had shown a significantly higher value compared to the crops raised in room condition. A higher yield of 2.261 kg/m^2 was recorded from green gram microgreens, when grown under rain shelter when compared to room condition where it yielded 1.4 kg/m^2. The yield was observed to be least in the amaranthus microgreens, under rain shelter it was 0.49 kg/m^2 and 0.29 kg/m^2 High density condition. The observations on dry weight of microgreens also showed significantly higher value for rain shelter raised crops. The maximum weight was noted for green gram microgreens (0.31 kg/m^2) and least was noted for amaranthus microgreens (0.05 kg/m^2) (Table33 to Table 38).

4.4.2 Seedling height (cm)

The observations on seedling height indicated that the microgreens raised in room condition and rain shelter showed significant difference for the character. In general, there was a slight reduction in height of seedlings when planted under rain shelter. It was found to be maximum for green gram microgreens grown under room condition with 10.71 cm while rain shelter raised ones had 8.36 cm height. The seedling height was noticed to be least in ragi microgreens raised under rain shelter (3.35 cm) and about 3.83 cm in room condition (Table 33 to Table 38).

Table 33. Mean performance of wheat microgreens under two growing conditions

Treatments	Fresh yield (kg/m^2)	Dry yield (kg/m^2)	Seedling height (cm)
Room condition	0.63	0.05	8.76
Rain shelter	0.68	0.07	7.07
t value	-2.84**	-16.26**	8.84**

Table 34. Mean performance of ragi microgreens under two growing conditions

Treatments	Fresh yield (kg/m^2)	Dry yield (kg/m^2)	Seedling height (cm)
Room condition	0.39	0.04	3.83
Rain shelter	0.64	0.06	3.35
t value	-17.36**	-16.15**	5.17**

Table 35. Mean performance of green gram microgreens under two growing conditions

Treatments	Fresh yield (kg/m^2)	Dry yield (kg/m^2)	Seedling height (cm)
Room condition	1.40	0.22	10.71
Rain shelter	2.26	0.31	8.36
t value	-12.552**	-2.01**	13.17**

Table 36. Mean performance of horse gram microgreens under two growing co

Treatments	Fresh yield (kg/m^2)	Dry yield (kg/m^2)	Seedling height (cm)
Room condition	1.01	0.14	8.65
Rain shelter	1.59	0.20	5.28
t value	-7.03**	-5.34**	-15.67**

Table 37. Mean performance of amaranthus microgreens under two growing conditions

Treatments	Fresh yield (kg/m^2)	Dry yield (kg/m^2)	Seedling height (cm)
Room condition	0.29	0.05	5.55
Rain shelter	0.49	0.08	3.98
t value	-13.23**	-8.92**	9.86**

Table 38. Mean performance of mustard microgreens under two growing conditions

Treatments	Fresh yield (kg/m^2)	Dry yield (kg/m^2)	Seedling height (cm)
Room condition	1.17	0.04	5.41
Rainshelter	1.87	0.06	4.25
t value	-10.91**	-16.15**	8.69**

4.4.3 Nutrient content of microgreens

4.4.3.1 Iron (mg/100 g)

The iron content analysed was observed to be significantly high in the microgreens raised under rains helter than that in room condition. Among different species used, the maximum iron content was seen in amaranthus microgreens grown under rain shelter with 1.928 mg/100 g whereas under room condition the value was 1.62 mg/100g and least was observed in wheat microgreens under rain shelter it was recorded to be 0.35 mg/100 g and in room condition it was 0.22 mg/100 g (Table 39 to Table 44).

4.4.3.2 Calcium (mg/100 g)

In general, the calcium content was observed to be significantly high in rain shelter cultivated microgreens. Microgreens of ragi grown under rain shelter shown the maximum calcium content of 305.74 mg/100 g whereas rain shelter grown ragi had 294.32 mg/100 g of calcium and least was seen in wheat microgreen under rain shelter (102.13 mg/100 g) and room condition (113.39 mg/100 g) (Table 39 to Table 44).

4.4.3.3 Beta carotene (mg/100 g)

The amount of beta carotene was significantly high in microgreens raised under rain shelter condition and among the crops it was maximum in mustard microgreens

raised under rain shelter (3.46 mg/100 g) followed by room condition raised ones (3.29 mg/100 g) and least amount was obtained in green gram microgreens when raised under rain shelter (1.83 mg/100g) and under room condition (1.11 mg/100 g) (Table 39 to Table 44).

4.4.3.4 Vitamin C (mg/100 g)

The highest amount of vitamin C content was noted in rain shelter grown microgreens than the ones grown in room condition. Maximum vitamin C content was noted in amaranthus microgreens when raised under rain shelter 47.94 mg/100 g whereas amaranthus raised in room condition had 45.09 mg/100g of vitamin C and least was observed in ragi microgreens when raised under rain shelter (33.09 mg/100 g) and under room condition it was 29.57 mg/100g (Table 39 to Table 44).

4.4.3.5 Crude Protein (g/100g)

The amount of crude protein showed significant difference when grown under two conditions. The protein content was observed to be high in microgreens when raised under rain shelter condition and it was noted to be maximum in mustard microgreens when raised under rain shelter (2.38 g/100 g) followed by room condition cultivated mustard (1.61 g/100g) and the lowest protein content was noted in ragi microgreens, under rain shelter it was 0.79 g/100 g and in room condition it was 0.56 g/100 g (Table 39 to Table 44).

4.4.3.6 Crude Fibre (%)

The fibre content was observed to be significantly high in wheat microgreens when raised under rain shelter it was noted to be 23.98 % followed by wheat microgreens when raised in room condition (20.88 %) and low content was found in amaranthus microgreens raised under rain shelter (3.32 %) and room condition (1.79 %) (Table 39 to Table 44).

4.4.3.7 Chlorophyll (mg/g)

The chlorophyll content was reported to be very high in the microgreens raised under rain shelter condition. It was observed to be maximum in green gram microgreens raised in rain shelter (1.58 mg/ g) followed by room condition (0.97 mg/g). The least chlorophyll content was recorded in ragi microgreens with 0.55 mg/g under rain shelter and 0.46 mg/g under room condition (Table 39 to Table 44).

4.4.3.8 Oxalates (mg/100 g)

While comparing the two conditions, the oxalate content was observed to be significantly high in rain shelter grown microgreens. The maximum amount of oxalate was noticed in amaranthus microgreens, where it was 72.99 mg/100 g and 68.57 mg/100 g in rain shelter and room condition respectively followed by mustard microgreens which recorded 35.70 mg/100 g under rain shelter and 32.84 mg/100 g in room condition (Table 43and Table 44).

4.4.3.9 Nitrates (mg/100 g)

The amount of nitrates was also found to be significantly higher when grown under rain shelter. It was recorded to be higher in amaranthus microgreens with a value of 65.21 mg/100 g in rain shelter and 58.45 mg/100 g in room condition, followed by mustard microgreens, 28.81mg/100 g in rain shelter and 26.11 mg/100 g in room condition (Table 43 and Table 44).

Table 39. Mean performance of wheat microgreens for biochemical characters

Treatments	Iron (mg/100g)	Calcium (mg/100g)	Beta carotene (mg/100g)	Vitamin C (mg/100g)	Crude protein (g/100g)	Crude fibre (%)	Chlorophyll (mg/g)
T1	0.22	102.13	2.67	41.14	0.91	20.88	0.74
T2	0.35	113.39	3.47	45.77	1.24	23.98	0.95
t value	-5.50**	-8.52**	-13.91**	-3.11**	-9.33**	-8.75**	-9.17**

Table 40. Mean performance of ragi microgreens for biochemical characters

Treatments	Iron (mg/100g)	Calcium (mg/100g)	Beta carotene (mg/100g)	Vitamin C (mg/100g)	Crude protein (g/100g)	Crude fibre (%)	Chlorophyll mg/g)
T1	0.33	294.32	1.24	29.57	0.56	18.02	0.46
T2	0.38	305.74	3.05	33.09	0.79	19.94	0.55
t value	-2.34**	-3.18**	0.85**	-3**	-7.49**	8.14**	-6.48**

Table 41. Mean performance of green gram microgreens for biochemical characters

Treatments	Iron (mg/100g)	Calcium (mg/100g)	Beta carotene (mg/100g)	Vitamin C (mg/100g)	Crude protein (g/100g)	Crude fibre (%)	Chlorophyll mg/g)
T1	0.44	159.22	1.11	29.57	1.02	14.09	0.97
T2	0.54	181.32	1.83	37.32	1.21	16.58	1.58
t value	-6.12**	-11.06**	-14.57**	-11.00**	-5.91**	-6.05**	-25.77**

Table 42. Mean performance of horse gram microgreens for biochemical characters

Treatments	Iron (mg/100g)	Calcium (mg/100g)	Beta carotene (mg/100g)	Vitamin C (mg/100g)	Crude protein (g/100g)	Crude fibre (%)	Chlorophyll mg/g)
T1	0.76	228.05	1.42	30.98	1.45	14.13	0.93
T2	1.09	251.97	2.45	34.50	1.68	16.08	1.49
t value	-12.07**	-9.76**	-13.78**	-2.23**	-2.85**	-5.45**	-8.10**

Table 43. Mean performance of amaranthus microgreens for biochemical characters

Treatments	Iron (mg/100g)	Calcium (mg/100g)	Beta carotene (mg/100g)	Vitamin C (mg/100g)	Crude protein (g/100g)	Crude fibre (%)	Chlorophyll mg/g)	Oxalates (mg/100g)	Nitrates (mg/100g)
T1	1.62	149.45	1.21	45.09	0.99	1.79	0.64	68.57	58.45
T2	1.92	178.28	2.58	47.94	1.20	3.32	0.93	72.99	65.21
t value	-5.72**	-13.02**	-8.55**	-2.45**	-1.97**	-14.01**	-6.35**	-3.29**	-11.45**

Table 44. Mean performance of mustard microgreens for biochemical characters

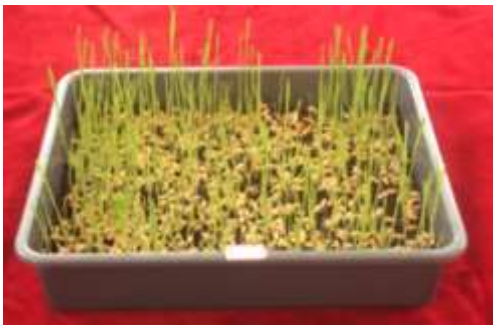
Treatments	Iron (mg/100g)	Calcium (mg/100g)	Beta carotene (mg/100g)	Vitamin C (mg/100g)	Crude protein (g/100g)	Crude fibre (%)	Chlorophyll mg/g)	Oxalates (mg/100g)	Nitrates (mg/100g)
T1	0.26	125.09	3.29	37.32	1.61	12.18	0.87	32.84	26.11
T2	0.43	146.03	3.46	38.73	2.38	16.20	1.20	35.70	28.81
t value	-10.77**	-17.95**	-2.88**	-1	-15.76**	-27.70**	-8.92**	-2.51	-5.72**

a) Room condition

b) Rain shelter

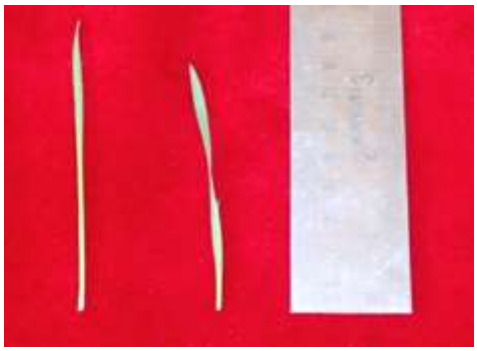
Plate 8. Microgreens grown under room condition and rain shelter

Room condition



Rain shelter

a) Wheat microgreens



b) Seedling height of wheat microgreens grown under room and rain shelter condition

Room condition

Rain shelter

c) Ragi microgreens

d) Seedling height of ragi microgreens grown under room and rain shelter condition

Plate 9. Wheat and ragi microgreens grown under two conditions

Room condition

Rain shelter

a) Green gram microgreens

b) Seedling height of green gram microgreens under room and rain shelter condition

Room condition

Rain shelter

c) Horse gram microgreens

d) Seedling height of horse gram microgreens grown under room and rain shelter condition

Plate 10. Green gram and horse gram microgreens grown under two conditions

Room condition

Rain shelter

a) Amaranthus microgreens

b) Seedling height of amaranthus microgreens grown under room and rain shelter condition

Room condition

Rain shelter

c) Mustard microgreens

d) Seedling height of mustard microgreens grown under room and rain shelter condition

Plate 11. Amaranthus and mustard microgreens grown under two conditions

4.4.4 Organoleptic analysis

The sensory qualities of six species of microgreens grown under room condition and rain shelter were evaluated on the basis of scores obtained in 9- point hedonic scale. The characters such as appearance, texture, taste, flavour, aroma and overall acceptability were ranked and statistical analysis were carried out using Kendall's coefficient of concordance and the mean rank obtained for the organoleptic characters of the six species under two growing conditions are represented in Table 45.

Table 45. Sensory qualities of microgreens grown under two growing conditions

Crop	Treatments	Appearance	Texture	Taste	Flavour	Aroma	Overall acceptability	Total score
Wheat	Room condition	5.20	3.07	4.47	4.87	6.57	3.87	28.05
	Rain shelter	4.80	3.17	4.43	4.40	5.63	4.17	26.6
Ragi	Room condition	6.00	5.20	7.00	6.97	5.20	6.43	36.8
	Rain shelter	5.10	4.43	6.20	6.20	5.77	5.70	33.4
Green gram	Room condition	8.40	6.47	8.27	8.70	11.00	8.73	51.57
	Rain shelter	7.00	7.13	7.73	7.43	10.03	8.63	47.95
Horse gram	Room condition	8.90	5.27	2.50	2.27	3.50	4.10	26.54
	Rain shelter	8.40	4.60	1.50	1.47	3.93	3.70	23.6
Amaranthus	Room condition	7.13	9.70	8.17	7.60	6.07	7.70	46.37
	Rain shelter	9.03	10.20	8.07	7.97	6.43	8.47	50.17
Mustard	Room condition	9.73	9.83	8.80	9.97	7.53	8.70	54.56
	Rain shelter	10.03	8.93	7.87	8.43	6.33	8.90	50.49

4.4.4.1 Appearance

The mean rank was observed to be high in mustard microgreens grown under rain shelter condition (10.03) followed by mustard microgreens grown under room condition (9.73) and then amaranthus grown in rain shelter (9.03). Lowest rank was obtained for wheat microgreens grown under rain shelter (4.80).

4.4.4.2 Texture

The highest mean rank for texture was observed for amaranthus microgreens raised in rain shelter (10.20) followed by mustard microgreens under room condition (9.83) and amaranthus microgreens under room condition (9.70). The least mean rank was obtained for wheat microgreens grown under room condition (3.07).

4.4.4.3 Taste

The microgreens of mustard grown under room condition (8.80) ranked the highest position followed by green gram grown under room condition (8.27) and amaranthus grown under room condition (8.17). The least rank was observed for horse gram grown under rain shelter (1.5).

4.4.4.4 Flavour

The highest mean rank for flavour was obtained for mustard microgreens grown under room condition (9.97) followed by mustard microgreen raised in rain shelter (8.43) and green gram grown under room condition (8.70). The microgreens of horse gram under rain shelter showed the least value (8.70).

4.4.4.5 Aroma

The highest mean rank was obtained for green gram microgreens grown under room condition (11.00) followed by green gram grown under rain shelter (10.03) and mustard microgreens raised in room condition (7.53). Lowest rank was observed for horse gram microgreens raised in rain shelter (3.93).

4.4.4.6 Overall acceptability

The overall acceptability was recorded to be high in mustard microgreens raised in rain shelter (8.90) followed by green gram grown in room condition (8.73) and mustard raised in room condition (8.70).The lowest rank was for horse gram grown in rain shelter (3.70).

4.4.4.7 Total score

The sum of mean rank score was observed to be highest in mustard microgreens raised under room condition followed by rain shelter raised ones. The least mean rank score was obtained for horse gram microgreens grown under rain shelter.

4.4.5 Shelf life under room and low temperature storage

The shelf life of six microgreens in two conditions *viz,* room temperature (28^0C) and low temperature (4^0C) were evaluated by visual scoring using 5-point scale. The mean scores were calculated using two-factor repeated measures ANOVA for each crop stored under each condition. The highest score was observed for wheat microgreens and horse gram microgreens (4.87) when stored in zip lock PPE bag under low temperature condition. It is followed by ragi and green gram microgreens (4.75) when stored in zip lock PPE bag under low temperature and least score was observed for all the six crops when stored in container at room temperature (3.00). The mean scores obtained are shown in the Table 46.

Table 46. Shelf life of microgreens under two storage conditions

Crops	Storage conditions	Storage package	Mean score
Wheat	Room temperature	Aluminium foil container	3.00[i]
		Zip lock PPE bag	3.87[e]
	Low temperature	Aluminium foil container	3.12[h]
		Zip lock PPE bag	4.87[a]
Ragi	Room temperature	Aluminium foil container	3.00[i]
		Zip lock PPE bag	3.87[e]
	Low temperature	Aluminium foil container	3.12[h]
		Zip lock PPE bag	4.75[b]
Green gram	Room temperature	Aluminium foil container	3.00[i]
		Zip lock PPE bag	3.87[e]
	Low temperature	Aluminium foil container	3.12[h]
		Zip lock PPE bag	4.75[b]
Horse gram	Room temperature	Aluminium foil container	3.00[i]
		Zip lock PPE bag	3.87[e]
	Low temperature	Aluminium foil container	3.12[h]
		Zip lock PPE bag	4.87[a]
Amaranthus	Room temperature	Aluminium foil container	3.00[i]
		Zip lock PPE bag	3.66[f]
	Low temperature	Aluminium foil container	3.12[h]
		Zip lock PPE bag	4.29[d]
Mustard	Room temperature	Container	3.00[i]
		Standing pouch	3.41[g]
	Low temperature	Aluminium foil container	3.12[h]
		Zip lock PPE bag	4.37[c]

The number of days for which the shelf life was retained for each species of microgreens under two conditions are mentioned in Table 47.

Table 47. Shelf life of microgreens under two storage conditions

Crops	Storage temperature	Storage container	Shelf life (days)
Wheat	Room temperature	Aluminium foil container	1
		Zip lock PPE bag	5
	Low temperature	Aluminium foil container	2
		Zip lock PPE bag	9
Ragi	Room temperature	Aluminium foil container	1
		Zip lock PPE bag	5
	Low temperature	Aluminium foil container	2
		Zip lock PPE bag	8
Green gram	Room temperature	Aluminium foil container	1
		Zip lock PPE bag	5
	Low temperature	Aluminium foil container	2
		Zip lock PPE bag	8
Horse gram	Room temperature	Aluminium foil container	1
		Zip lock PPE bag	5
	Low temperature	Aluminium foil container	2
		Zip lock PPE bag	9
Amaranthus	Room temperature	Aluminium foil container	1
		Zip lock PPE bag	4
	Low temperature	Aluminium foil container	2
		Zip lock PPE bag	6
Mustard	Room temperature	Container	1
		Standing pouch	3
	Low temperature	Aluminium foil container	2
		Zip lock PPE bag	7

The shelf life was observed to be maximum for wheat and horse gram microgreens when stored in zip lock PPE bag under low temperature storage for 9 days followed by ragi and green gram microgreens whose shelf life was extended up to 8 days and mustard microgreens which is stored up to 7 days and least shelf life was observed in amaranthus microgreens.

Discussion

5 DISCUSSION

In the present study production technology of microgreens were standardized using six species *viz.* wheat, ragi, green gram, horse gram, amaranthus and mustard. The protocols were standardized for seed treatment, growing media and seed density for the cultivation of microgreens. Further the growth of microgreens under room condition and rain shelter was also evaluated. The statistical analysis of observations recorded was conducted to draw conclusions regarding the various aspects of microgreen cultivation.

5.1 Evaluation of seed treatments in microgreens

The six species used for the study were subjected to seed treatment using two chemicals, hydrogen peroxide and vinegar at different concentrations. The results on germination percentage (Figure 1), vigour index (Figure 2 and Figure 3) and yield (Figure 4) revealed that there was no adverse effect of seed treatment with either of the two chemicals in comparison to the control (soaking seeds in sterile water). On microscopic observation, no fungal contamination was observed in treated as well as control seeds. In a study conducted by Hong and Kang (2016) reported that seed treatment with hydrogen peroxide on alfalfa seeds reduced the incidence of *Salmonella Typhimurium* attack and enhanced the seed germination. Similarly, Szopinska (2014) reported that the treatment of zinnia seeds with hydrogen peroxide effectively improved the germination of pathogen attacked seeds.

Acetic acid is a cheap naturally occurring biodegradable substance which is less hazardous to human beings and a cost-effective method for seed treatment. It is reported that the incidence of barley leaf stripe disease was well controlled when the seeds were treated with 20 ml/kg acetic acid and no effect on germination and vigour was noted (Borgen and and Nielsen, 2001). Similarly, the acetic acid treated seeds of mung bean were not exhibiting reduced germination and also no contamination was observed when viewed under microscope (Delaquis*et al*., 1999). Acetic acid treated seeds had shown positive effect on germination parameter in carrot seeds (Benothmen *et al*., 2019). The results obtained from our experiment manifest that the seed treatment did not adversely or positively influence the germination percentage, vigour

and yield. There was no microbial contamination in any of the treatments including control which indicate that the seed lot used in the experiments were free of pathogens. Hence it can be inferred that if the seed lot is pathogen free then seed treatment is not a prerequisite for production of disease free and clean microgreens.

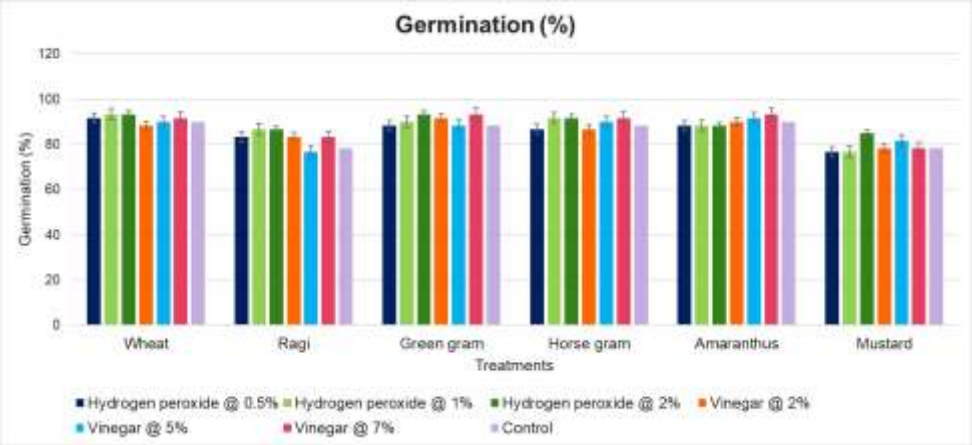


Figure 1. Germination percentage of microgreens under seed treatments

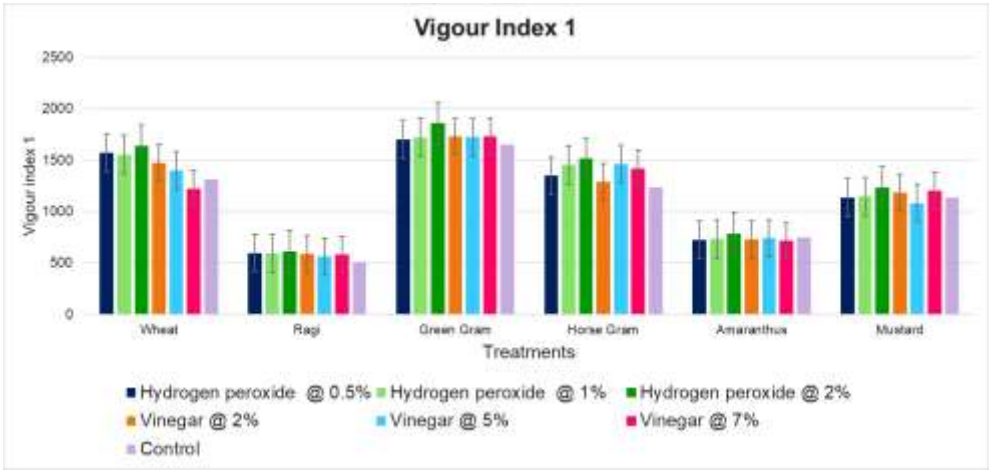


Figure 2. Vigour index 1 of microgreens under seed treatments

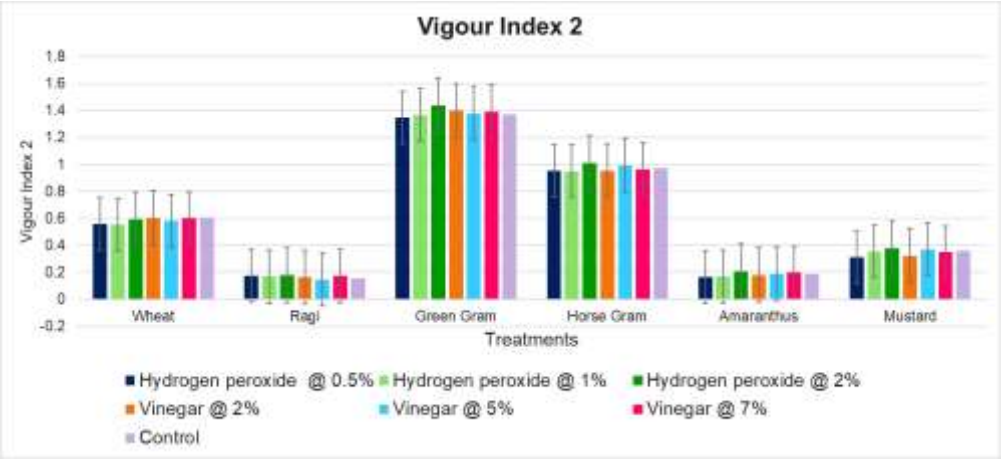


Figure 3. Vigour index 2 of microgreens under seed treatments

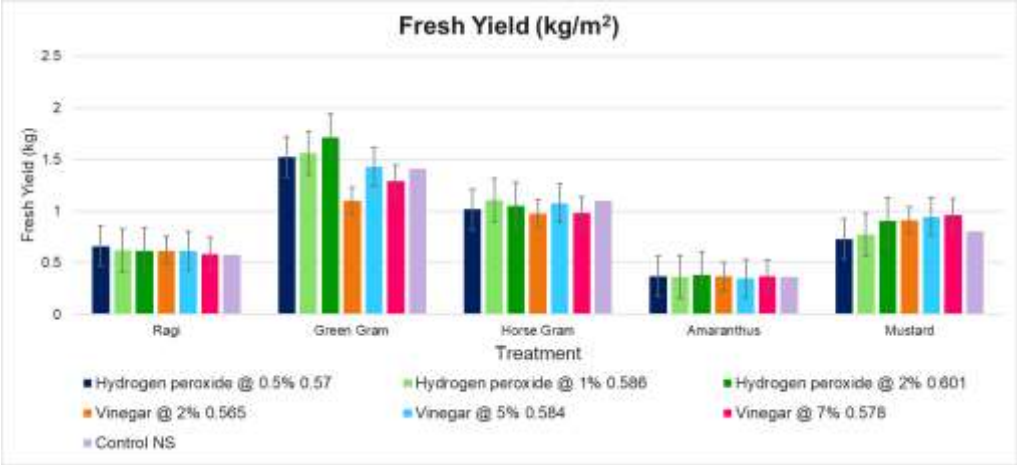


Figure 4. Fresh yield of microgreens under seed treatments

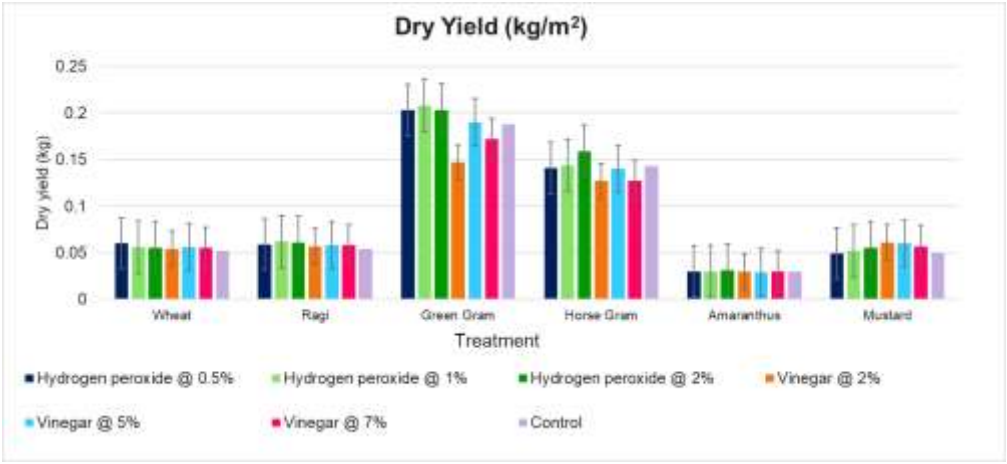


Figure 5. Dry yield of microgreens under seed treatments

5.2 nfluence of growing media on microgreen production

Six species of microgreens were planted using five different media to identify the ideal one for growing microgreens. The parameters recorded in this experiment were yield, seedling height, nutrient content and microscopic observation for fungal growth. The fresh yield and dry yield of microgreens was significantly different among different media used. The highest yield was recorded in cocopeat media and least yield was recorded when newspaper was used as growing media. The reason for this may be due to the desirable qualities of cocopeat such as optimum water holding capacity, good drainage, absence of weeds and pathogens, slow decomposition, an acceptable range of pH, cation exchange capacity and electrical conductivity (Awang, 2009). The cocopeat is cheap, easily available and almost similar to peat which is the common growing medium used for microgreen production (Landis *et al*., 1990). In the present experiment, the pH and electrical conductivity of cocopeat was 5.4 and 357 µS/cm respectively which is within the permissible limit for growing microgreens. Similarly, Tiwari (2015) reported cocopeat to be an excellent growing media especially for soil less production of vegetable with its physiochemical properties which promote the growth and development of vegetables.

The lowest yield was reported in newspaper media which may be due to poor seedling emergence and root penetration in this media (Figure 6 and Figure 7) Chrysargyris *et al*. (2020) reported that when seedlings are grown in printed paper medium their growth will be decreased, which results in reduction of plant height, number of leaves and fresh weight due to reduced aeration and available water in plant root.

The observations on seedling height were not significantly different across the different media used, except for mustard where seedling height was significantly higher in cocopeat, sterile sand and coir mat media and seedlings were small and stunted in newspaper raised seedlings (Figure 8).

The nutrient content analysis revealed that microgreens are immense source of nutrients such as vitamin C, beta carotene, protein, iron and calcium. However, there was no influence of media on the nutrient content of microgreens since the nutrient

content of a microgreen species did not significantly vary with the difference in the media used for production, although the nutrient content varied among different crops used for the study. The selection of media can affect the fresh yield and dry matter content in microgreens whereas the nutrient content may vary according to different species used (Bulgari *et al.*, 2021). All the six species of microgreens were observed to be good source of nutrients such as vitamin C, protein, beta carotene, iron and calcium. The iron content in the six species of microgreens was in the range of 0.19 to 1.7 mg/100 g, calcium content ranged from 91.83 to 287.06 mg/100 g, beta carotene content ranged from 1.5 to 3.06 mg/100 g, the amount of vitamin C ranged from 21.12 to 42.25 mg/100 g, protein content was in a range of 0.37 to 1.32 mg/100 g, fibre content ranged from 2.46 to 22.4 %, chlorophyll content was in the range of 0.16 to 1.4 mg/g, oxalate content in amaranthus and mustard microgreens ranged from 35.33 to 79.46 mg/100 g and nitrate content ranged from 22.6 to 54.23 mg/100 g (Figure 9 to Figure 17). Similar results were reported by Ghoora *et al*. (2020) who assessed the nutrient content of ten microgreens, among which mustard was found rich in beta carotene (7.4 mg/100 g), calcium (51.2 mg /100 g) and iron (2.4 mg/100 g). Mohanty *et al.* (2020) reported green gram to be the rich source of elements like calcium (29.93 %), potassium (27.49%) and iron (1.28 %) and mustard to be rich in elements like sulphur (26.32 %), calcium (24.37 %) and iron (0.56 %). Renna *et al.* (2017) assessed the nutritional value of Brassica species of microgreens, and reported to show protein content ranging from 2.34 g/100 g to 2.5 g/100 g and beta carotene content ranging from 4.05mg/100 g to 5.3 mg/100 g. Xiao *et al*. (2014) evaluated vitamin C and beta carotene content of twenty five microgreens and it ranged from 20.4 to147.0 mg/ 100 g and 0.6 to 12.1 mg/100 g. Microbial contamination was not observed when planted in different media.

Since all the microgreens produced highest yield in cocopeat media and considering the superior plant root growth promoting properties such as aeration, water holding capacity *etc.* cocopeat was selected to be the best media among the five.

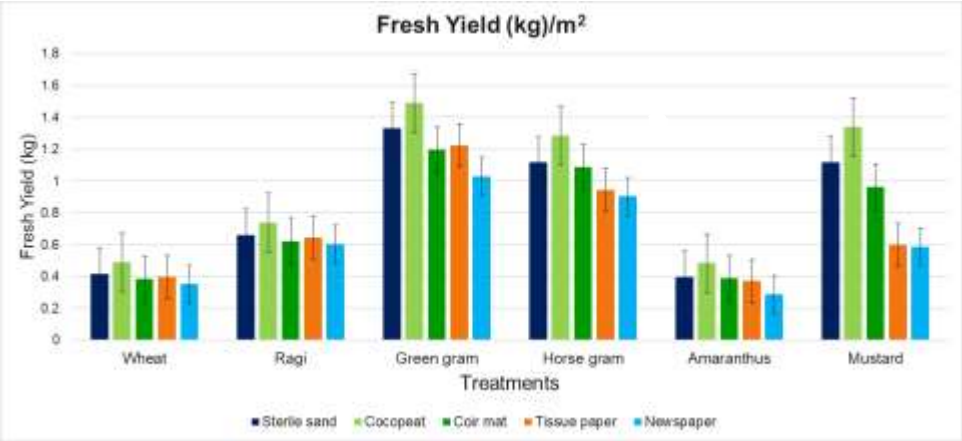


Figure 6.Fresh yield of microgreens grown on different media

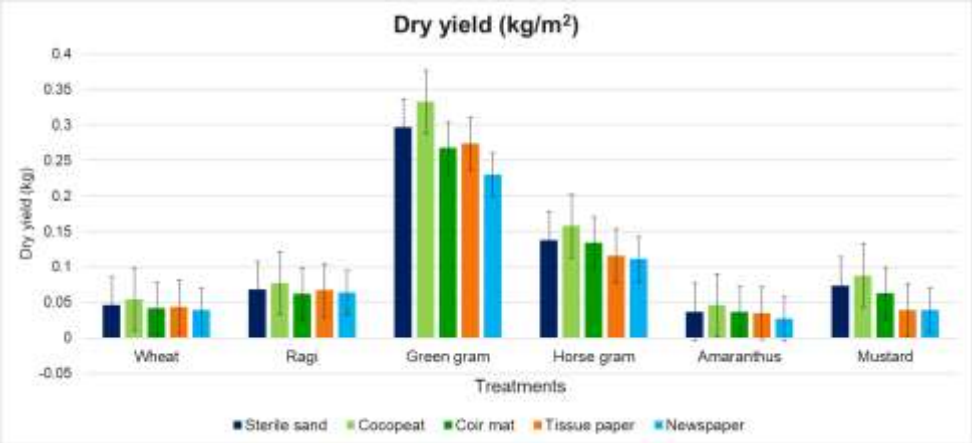


Figure 7. Dry yield of microgreens grown on different media

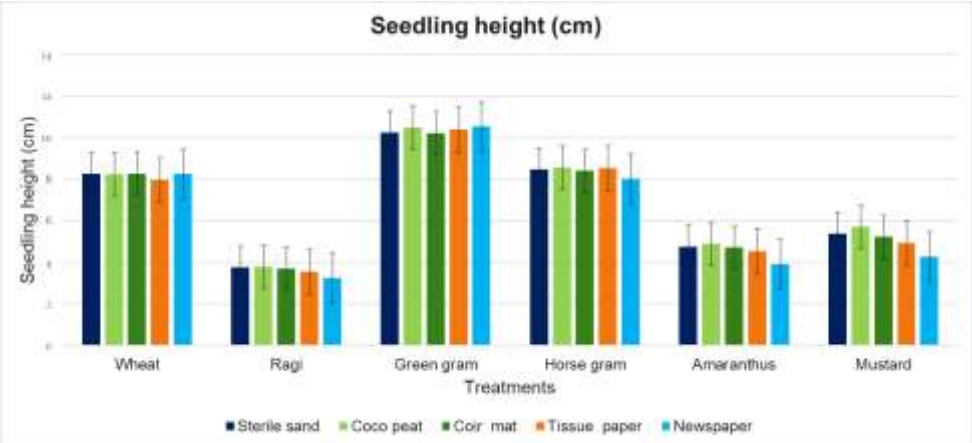


Figure 8. Seedling height of microgreens grown on different media

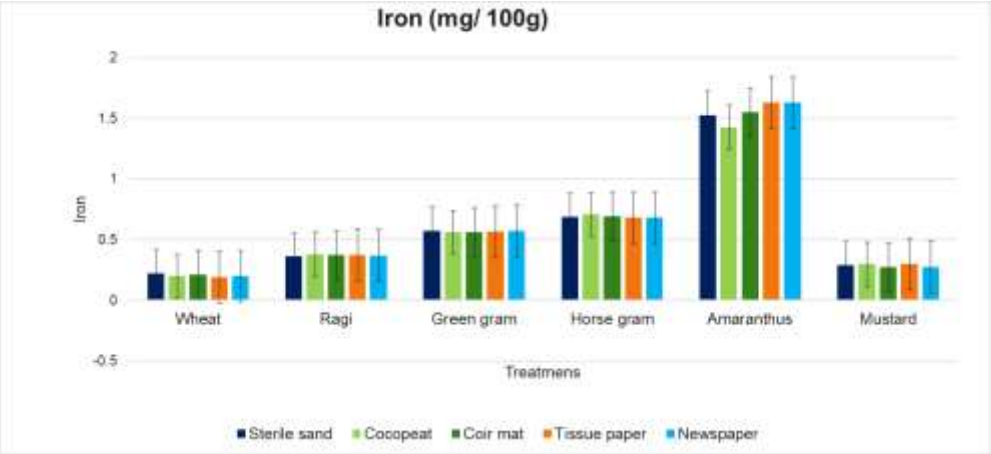


Figure 9. Iron content in microgreens grown on different media

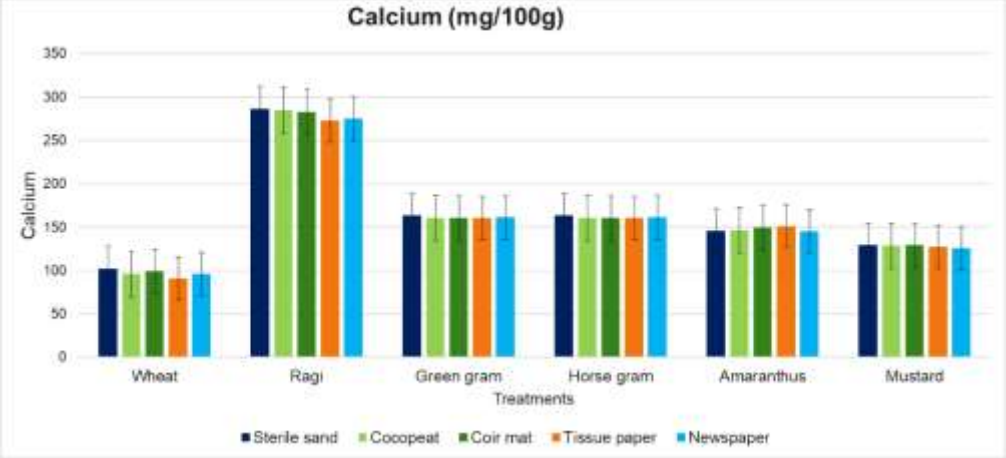


Figure 10. Calcium content in microgreens grown on different media

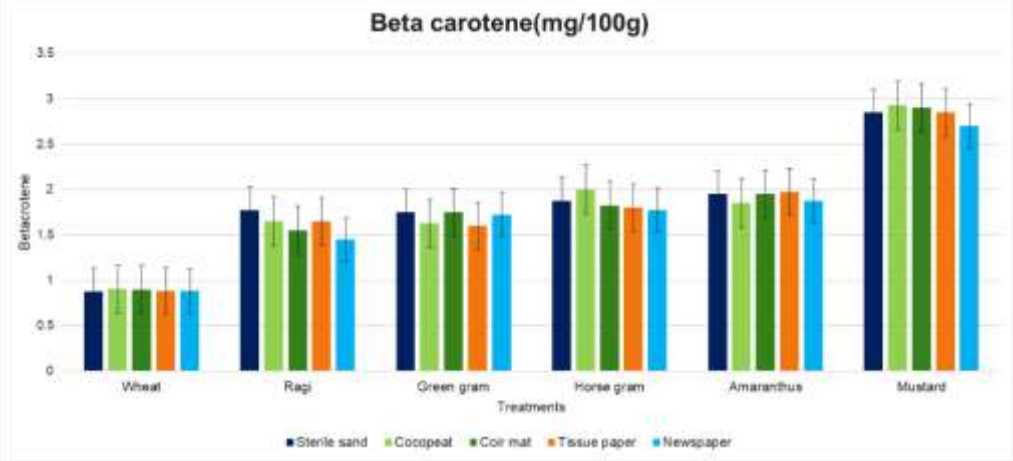


Figure 11. Betacarotene content in microgreens grown on different media

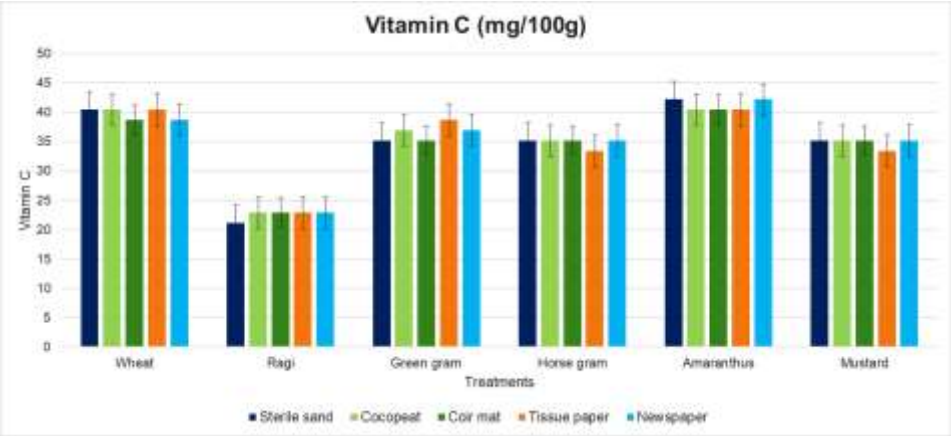


Figure 12. Vitamin C content in microgreens grown on different media

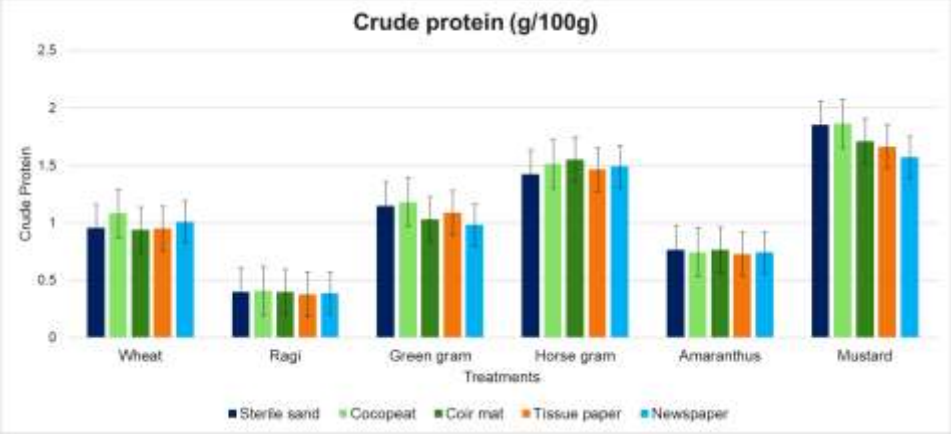


Figure 13. Crude protein content in microgreens grown on different media

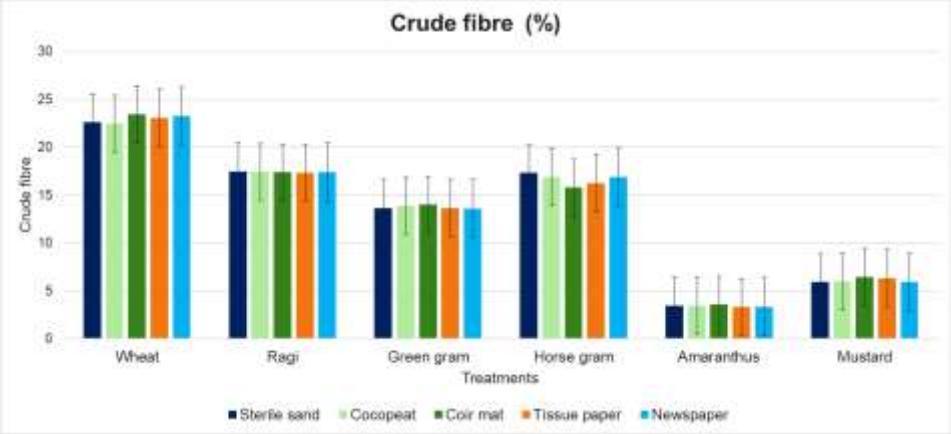


Figure 14. Crude fibre content in microgreens grown on different media

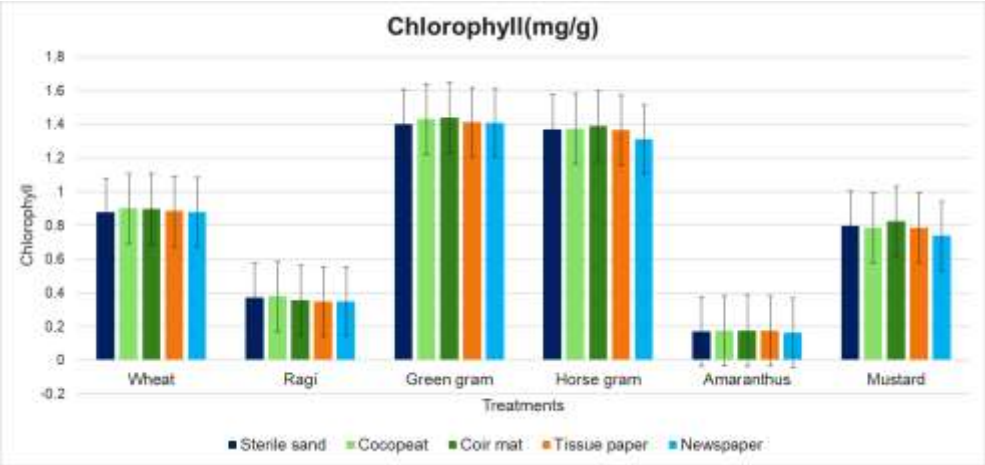


Figure 15. Chlorophyll content in microgreens grown on different media

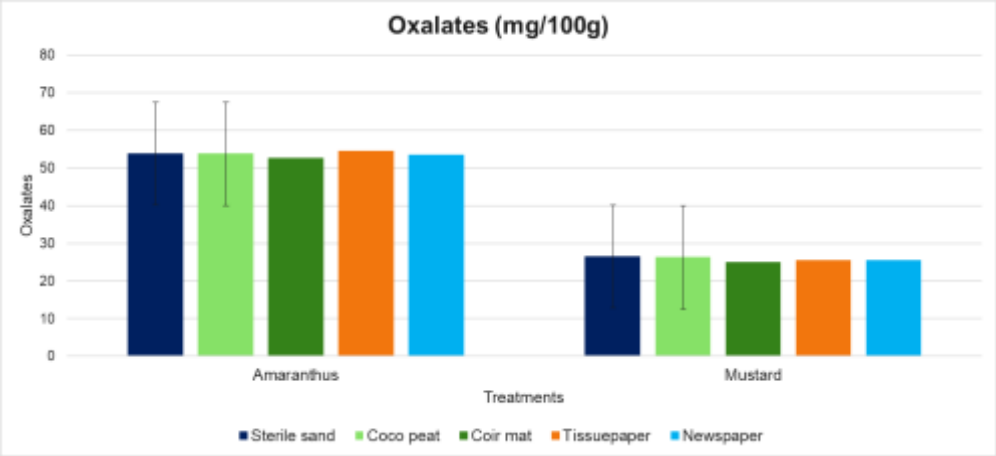


Figure 16. Oxalate content in microgreens grown on different media

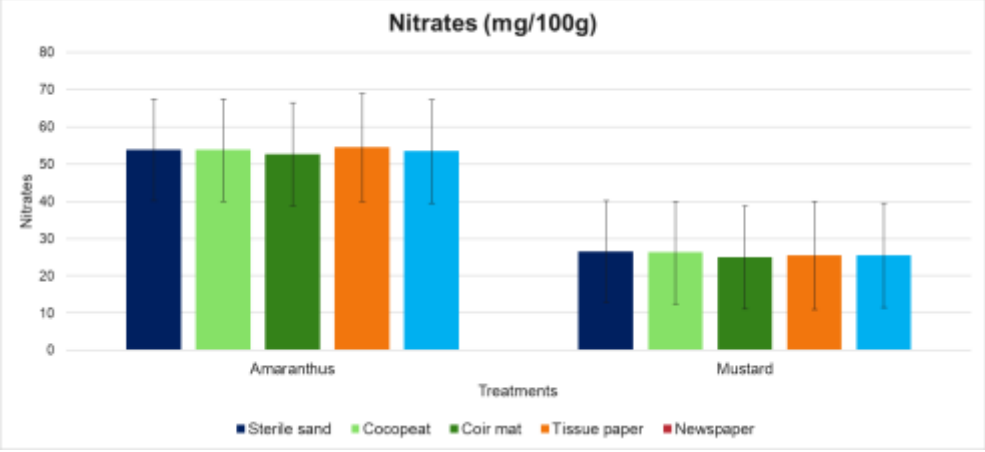


Figure 17. Nitrate content in microgreens grown on different media

5.3 Selection of Seed density

The seeds of six different species sown at three seed densities revealed that seed densities of 705 g/m^2, 520 g/m^2, 850g/m^2, 617 g/m^2, 120 g/m^2 and 440 g/m^2 for wheat, ragi, green gram, horse gram, amaranthus, and mustard respectively, was ideal for obtaining optimum growth and yield (Figure 18 and Figure 19). There was significant difference in yield for each crop when it was planted at different densities. Similarly, Ghoora and Srividya (2018) optimized seed density for different crops such as 188 gm^{-2} for carrot microgreens and 500 gm^{-2} for fennel microgreens. For spinach, onion and french basil microgreens the seeds are sown at a seed rate of 250 g m^{-2}, for roselle, fenugreek and sunflower microgreens the seeds were sown at 375 gm^{-2} and 313 gm^{-2} was used for sowing radish. For radish microgreens, a seed density of 439 gm^{-2} was reported to be more economical, a seed rate higher or lower than this may cause decreased fresh yield (Storey, 2017). As the seed rate for planting increases, there will be competition among the crops for space, water and light which further leads to stunted growth of seedlings or overcrowding and thus reducing the yield.

The observations on seedling height did not show significant difference while sowing in three seed densities, except for green gram and horse gram whose seedling height was best in medium density sowing (Figure 20). When these seeds were planted at high density, it leads to overcrowding of seedling resulting in suppressed and stunted growth.

Microbial contamination was not observed in the freshly harvested microgreens from any of the three seed densities tested, when viewed under microscope.

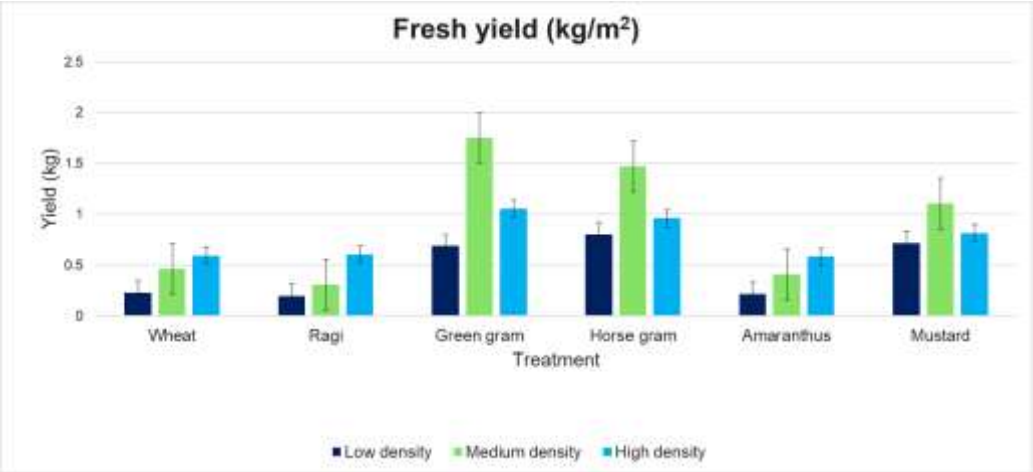


Figure 18. Fresh yield of microgreens sown at different densities

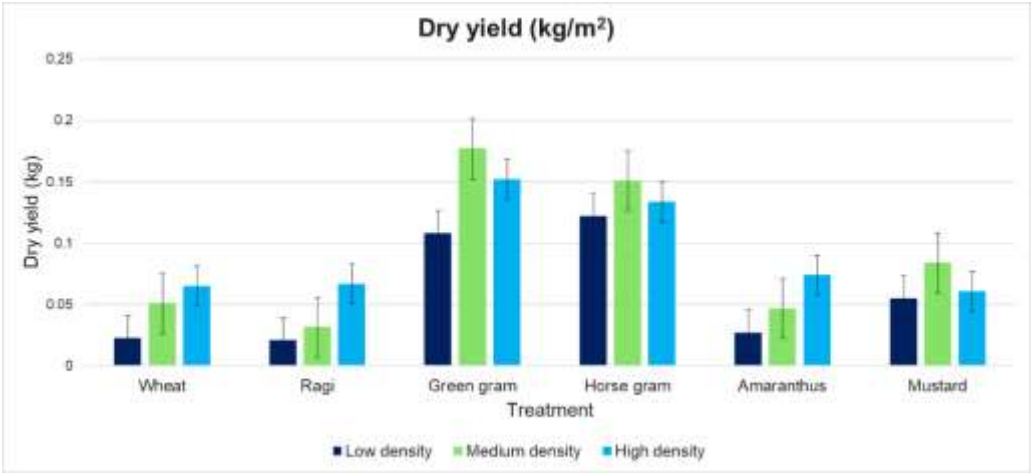


Figure 19. Dry yield of microgreens sown at different densities

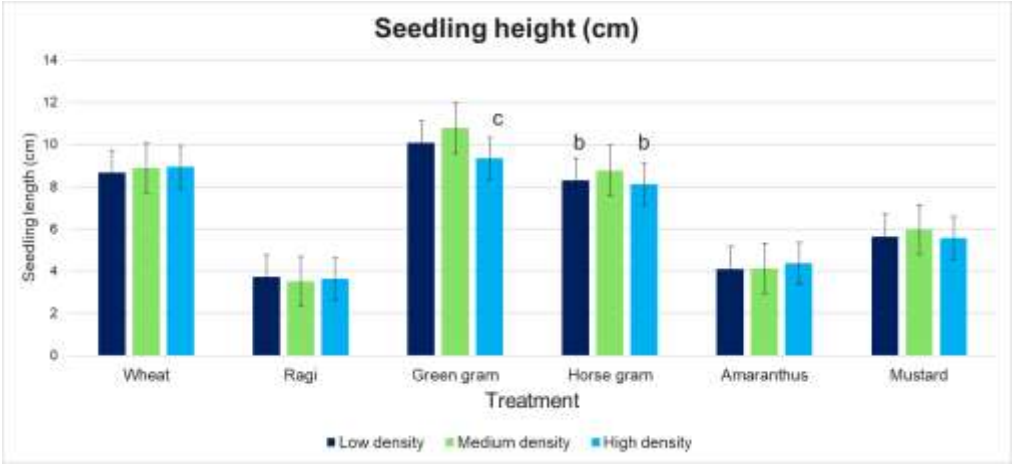


Figure 20. Seeding height of microgreens sown at different densities

5.4 Microgreen production under two growth conditions.

In the last experiment, microgreens were planted under two environments *viz*, room condition and rain shelter. The observations on seedling height revealed that there is slight reduction in seedling height for all microgreens when raised under rain shelter (Figure 23). The difference in height was readily visible for green gram and horse gram microgreens. The main reason for this may be due to the wide variation in light intensity in two growth conditions. In room condition, the light intensity was 8.78 µ mol m^{-2} s^{-1} and in rain shelter the light intensity was much higher 117.82 µ mol m^{-2} s^{-1}. When the light intensity is low, it may cause the plant to increase specific leaf area and plant height in order to capture maximum light from the environment for performing proper photosynthesis. Similarly, when the light intensity increases the stem diameter and leaf thickness of plants were reported to increase due to the growth of palisade tissue to avoid the injuries caused by excess sunlight and thus promoting photosynthesis (Steinger *et al*., 2003). Wang *et al*. (2017) reported that the light has a major role in designing the morphological characters of plant, under red light the length of seedlings was found to be higher and blue light promoted stem diameter.

The results on yield recorded revealed that the yield was significantly higher for the microgreens raised under rain shelter than under room condition (Figure 21 and Figure 22). The growth of the seedlings was very vigorous under rain shelter which can be attributed to the prevalence high temperature (32.2 ^{0}C in rain shelter and 29.1^{0}C in room condition) and light intensity (117.8 µ mol m^{-2} s^{-1} in rain shelter and 8.78 µ mol m^{-2} s^{-1} in room condition). The growth and photosynthetic activity in plants are reported to be high when light availability increases (Holt, 1995). Similarly Zavala and Ravetta, (2001) reported that the low light intensity may reduce the plant growth as well as productivity by affecting the gas exchange and high light intensity will enhance the photosynthesis process thus growth will be very fast and plants will be vigorous (Lichtenthaler *et al*., 2007). The amaranthus microgreens grown under natural sunlight produced highest fresh and dry weight than that grown under artificial light source and the days to harvest for microgreens under natural light source was less compared to other one, as the high light intensity favours higher photosynthetic rate in plants (Mortensen and Grimstad , 1990). When the microgreens are cultivated

at a light intensity from 105 to 315 μ mol m^{-2} s^{-1} the fresh yield as well as dry yield were increased (Gerovac *et al*., 2016).

The analysis of nutrient content in the microgreens grown under two conditions revealed that the microgreens raised under rain shelter had significantly higher nutrient content compared to that raised in room condition (Figure 24 to Figure 32). In rain shelter grown microgreens, better light availability and higher temperature may have resulted in enhanced metabolic activity resulting in higher levels of phytochemicals like vitamin C, beta carotene, crude protein and chlorophyll. Craver *et al*. (2017) reported that the chlorophyll, anthocyanin and phenolic content to be higher in *Brassica* microgreens when grown under higher light intensity. Lau *et al*. (2019) reported that microgreens cultivated under natural sunlight had shown quick growth rate and good yield with better quality and antioxidant content compared to that grown under artificial light. Delian *et al*. (2015) reported predominant influence of light on the morpho-physiology and accumulation of phytochemicals in microgreens.

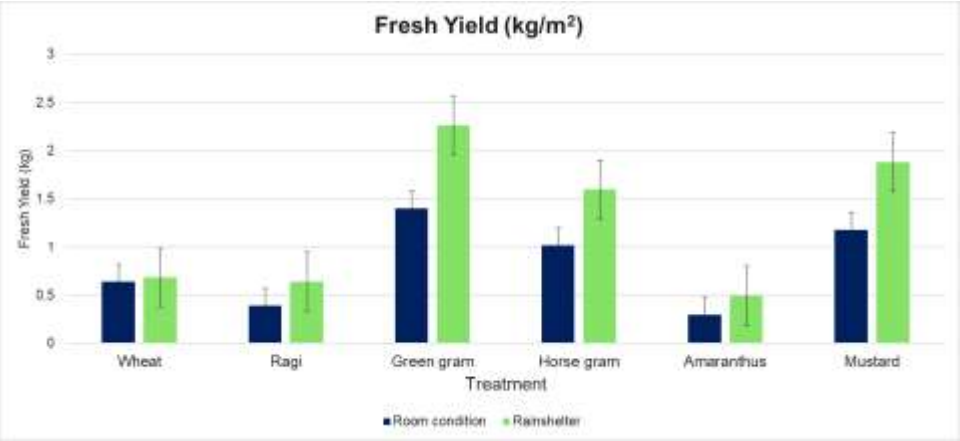


Figure 21. Fresh yield of microgreens grown under two growth conditions

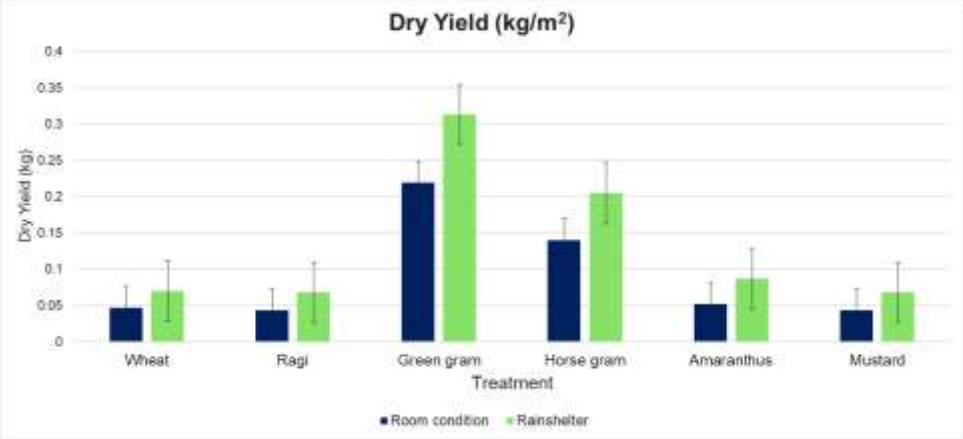


Figure 22. Dry yield of microgreens grown under two growth conditions

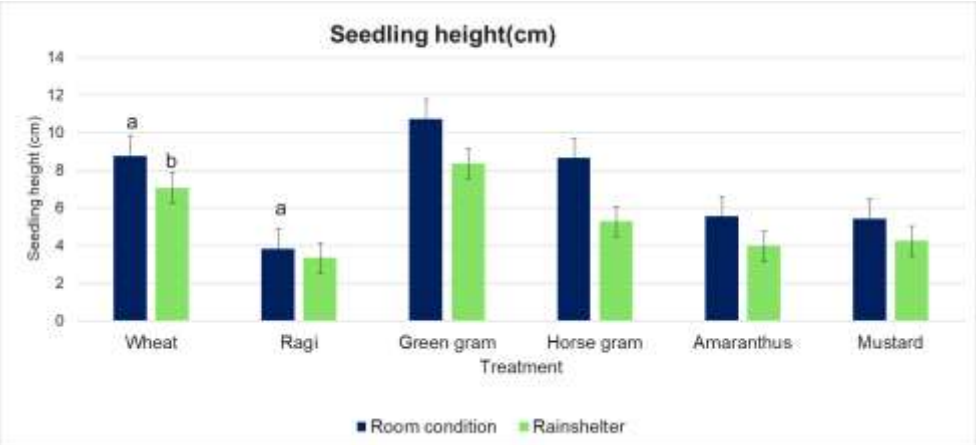


Figure 23. Seedling height of microgreens grown under two conditions

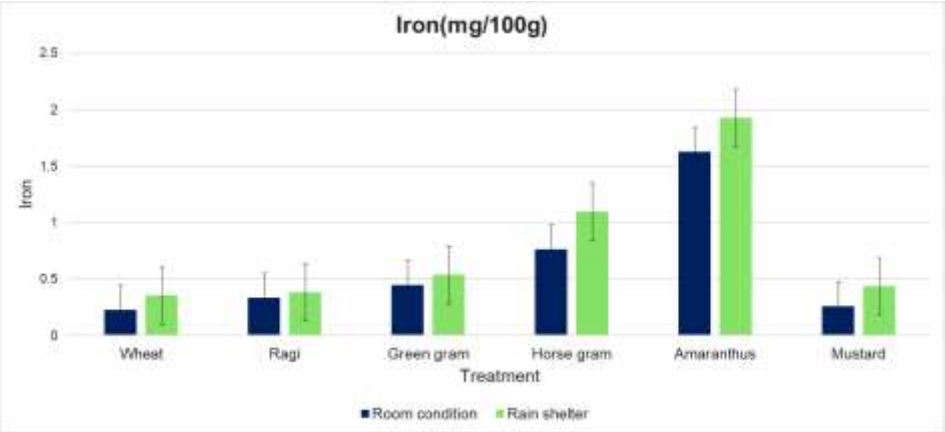


Figure 24. Iron content of microgreens grown under two growth conditions

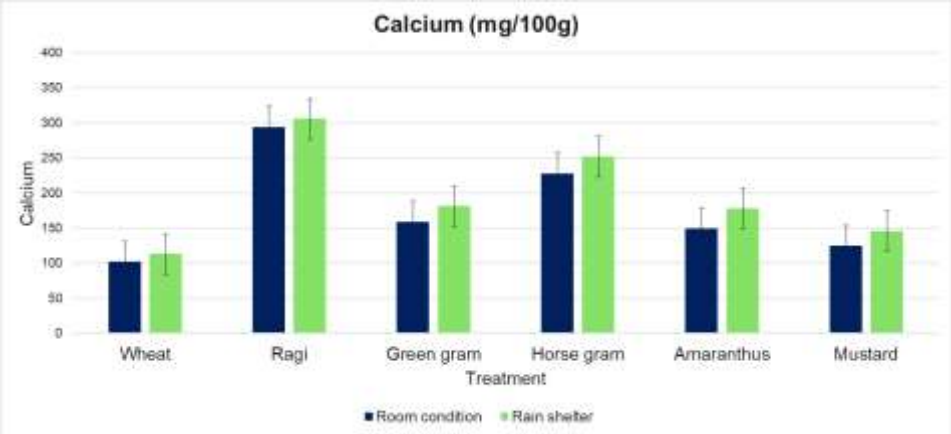


Figure 25. Calcium content of microgreens grown under two growth conditions

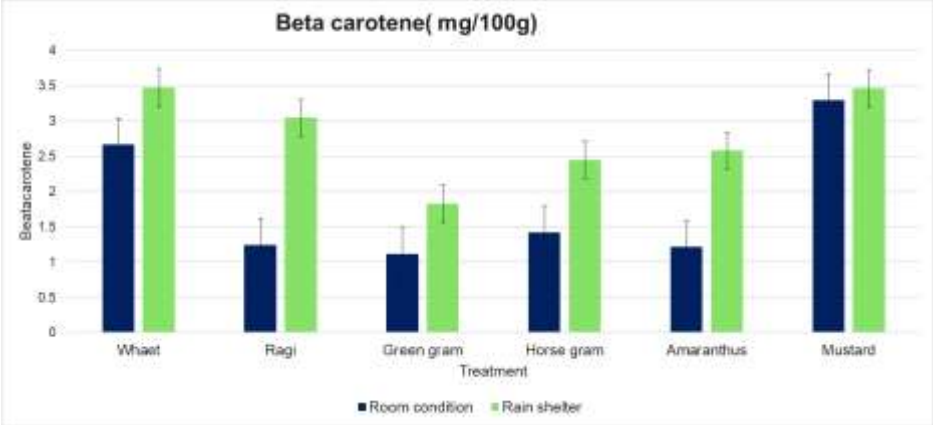


Figure 26. Beta carotene content of microgreens grown under two growth conditions

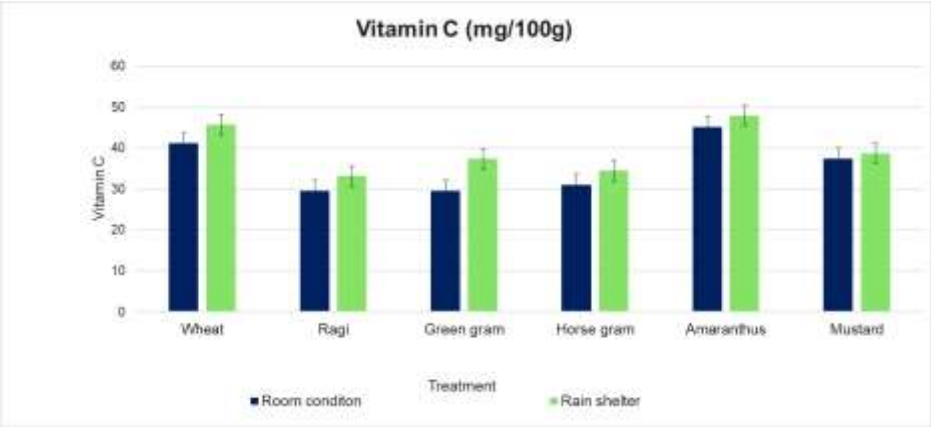


Figure 27. Vitamin C content of microgreens grown under two growth conditions

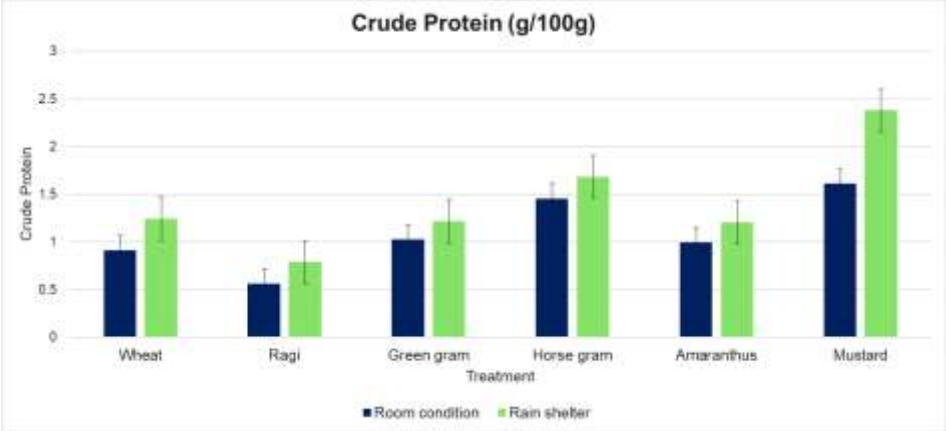


Figure 28. Crude protein content of microgreens grown under two growth conditions

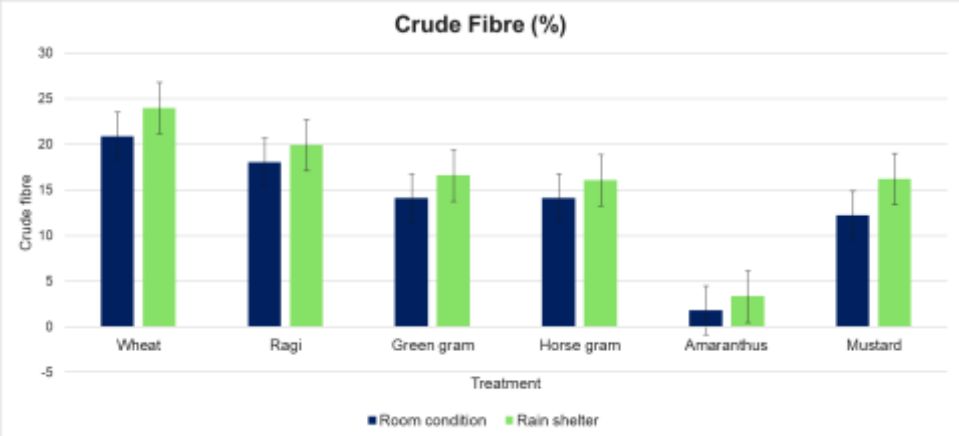


Figure 29. Crude fibre content of microgreens grown under two growth conditions

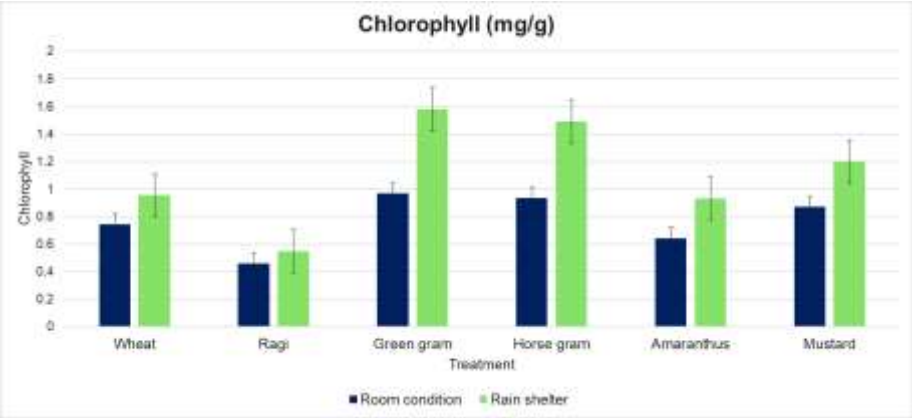


Figure 30. Chlorophyll content of microgreens grown under two growth conditions

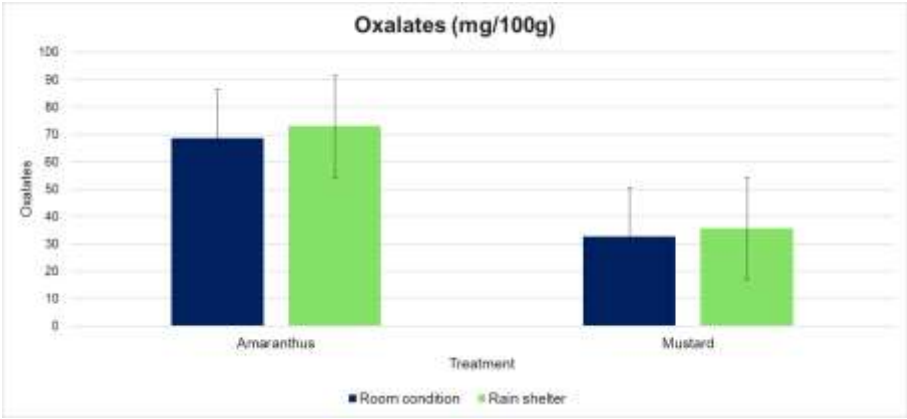


Figure 31. Oxalate content of microgreens grown under two growth conditions

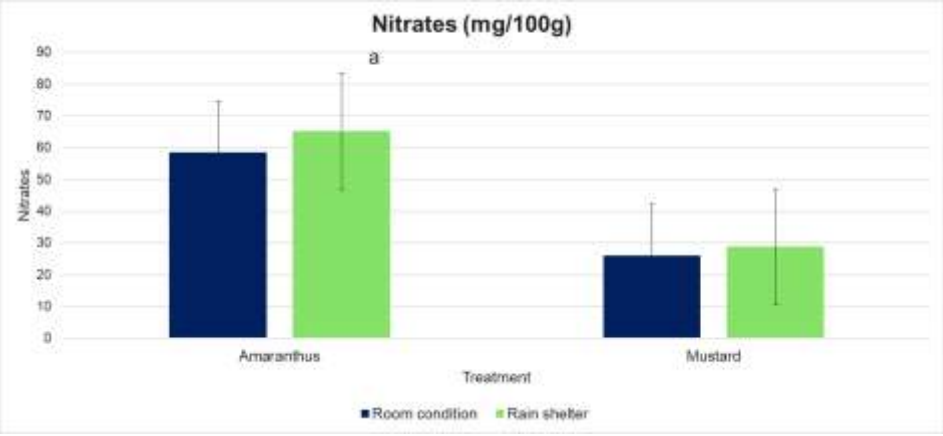


Figure 32. Nitrate content of microgreens grown under two growth conditions

5.5 Organoleptic evaluation

Microgreens are emerging food with sustainably diversifying global food system, adaptations to urbanization, global climate change and enhancing human health. Evaluation of consumer acceptance of microgreen is very important for better reception into the global food system and increased per capita consumption (Michell *et al*., 2020).The organoleptic analysis of six microgreens grown under room condition and rain shelter were compared to evaluate the consumer acceptance. The score was given according to the 9- point hedonic scale for the characters *viz*. appearance, texture, taste, flavour, aroma and overall acceptability. Similarly, the sensory qualities of sunflower microgreens were studied by Dalal *et al*. (2020) using 9-point Hedonic scale. The mustard microgreens grown under room condition had the highest total score followed by green gram microgreens raised under room condition followed by mustard microgreens raised under rain shelter. The lowest score was obtained for horse gram microgreen raised under rain shelter. The highest mean rank for appearance was recorded for mustard microgreens grown under rain shelter condition (10.03). The amaranthus microgreens raised under rain shelter obtained maximum mean score for texture and the highest rank for taste was noted for mustard grown under room condition (8.80). The maximum rank for flavour was recorded for mustard microgreens grown under room condition and for aroma highest rank was obtained for green gram microgreens raised under room condition. The overall acceptability ranked highest for mustard microgreens grown under rain shelter. Similarly, the culinary uses of three species of microgreen were assessed by Renna *et al*. (2017) which included red mustard.

5.6 Shelf life evaluation

The shelf life of six microgreens under room temperature and low temperature storage was evaluated in two packages. For each day scoring was done based on 5-point scale for respective treatment. The scores were statistically analysed and the maximum score was obtained for wheat and horse gram microgreens with a score of 4.87 when packed in zip lock PPE bag under low temperature (4^0C). The lowest score was observed for all the microgreens packed in aluminium foil container stored at

room temperature. This implies that wheat and horse gram microgreens when packed in zip lock PPE bag and stored under low temperature exhibited the maximum storage period of 9 days. Similarly, the effects of storage temperature, modified atmosphere packaging (MAP) on shelf life of buckwheat microgreens were studied by Kou *et al.* (2013). This study clearly indicates that storage life was greatly influenced by packaging material as well as temperature while storing microgreens. Since the aluminium foil container was not possessing proper air tight condition, the dehydration and wilting of products was very fast in it, whereas the microgreens in zip lock PPE bag showed more storage life as it was in complete air tight condition, which limits moisture loss. When the microgreens were stored in low temperature (4^0C) shelf life was found to extend twice that of room temperature storage (28^0C). Similarly, Paradiso *et al*., (2018) reported that in microgreens of Asteraceae and Brassicaceae family the shelf life was observed to prolong up to ten days when stored under 5^0C. The storage period may also vary for different crops, here maximum shelf life was observed for wheat microgreens and least was for mustard microgreens. The fresh microgreens reported to be stored for 14 days in refrigerator and up to 4-6 in room condition by daily irrigation (Kumar *et al*., 2016). From this shelf life study, it is manifested that the maximum storage period was obtained when the microgreens were packed in zip lock PPE bag and stored under low temperature.

5.7 Crop wise summary of microgreen production

The six crops under study were showing several good qualities to be used as microgreens, which has been summarized in Table 48. The study indicates that seed treatment is not adversely affecting the growth parameters of microgreens so it is recommended only if the seeds used for microgreen production is of poor quality or contaminated. Cocopeat was found to be the best growing medium for the commercial production of all the six species of microgreens. The other media used in the study can also be utilised for the production, however the yield of microgreens will be compromised to a certain extent. Green gram microgreens ranked first in yield, followed by mustard and horse gram in both the growing conditions. The nutrient content in microgreens varied according to different species used. The organoleptic ranking showed mustard microgreens to be the best with superior sensory qualities

followed by green gram microgreens. Shelf life was observed to be maximum for wheat and horse gram microgreens (9 days) followed by green gram and ragi microgreens (8 days). The average temperature and light intensity under room condition was 29.1^{0}C and 8.78 µ mol m^{-2} s^{-1} respectively and under rain shelter it was 32.2 ^{0}C and 117.8 µ mol m^{-2} s^{-1} respectively. The microgreens can be recommended to be grown under both room and rain shelter condition but in rain shelter, the yield and nutrient content was found to be higher for all the microgreens, hence rain shelter cultivation can be preferred over indoor conditions if possible. The shelf life of microgreens was observed to be maximum when it was stored in zip lock PPE bag at low temperature (4^{0}C).

Table 48. Crop wise summary of microgreens

Crop	Best growing media	Seed rate (g/ m^2)	Yield under room condition (kg/m^2)	Yield under rain shelter (kg/m^2)	Prominent nutrients	Organoleptic mean scores (room condition)	Optimum storage temperature	Shelf life (days)	Recommended package
Wheat	Cocopeat	705	0.63	0.68	Vitamin C, beta carotene and protein	28.05	4^0C	9	Zip lock PPE bag
Ragi	Cocopeat	520	0.39	0.64	Calcium and beta carotene	36.80	4^0C	8	Zip lock PPE bag
Green gram	Cocopeat	850	1.40	2.26	Protein, vitamin C and calcium	51.57	4^0C	8	Zip lock PPE bag
Horse gram	Cocopeat	617	1.01	1.59	Iron, calcium, vitamin C, and protein	26.54	4^0C	9	Zip lock PPE bag
Amaranthus	Cocopeat	120	0.29	0.49	Iron, calcium, beta carotene and vitamin C	50.17	4^0C	6	Zip lock PPE bag
Mustard	Cocopeat	440	1.17	1.87	Protein and beta carotene, iron and vitamin C	54.56	4^0C	7	Zip lock PPE bag

Summary

6. SUMMARY

The study entitled “Standardization of technology for microgreen production” was carried out in the Department of Vegetable Science, College of Agriculture, Vellanikkara during January 2021. The study was carried out with the main objective of standardization of production technology for microgreens. The salient findings and important conclusions from the study are summarized below.

Six species of crops *viz*, wheat, ragi, green gram, horse gram, amaranthus and mustard were used for the study. Seed treatment using hydrogen peroxide and vinegar at different concentrations did not adversely affect the seed germination, seedling vigour and yield of microgreens compared to control. The microscopic observation on freshly harvested microgreens revealed that fungal contamination was absent in the harvested produce. This indicates that the seed lot used for the study was of good quality and free from any contamination. Thus, we can conclude that if the seed lot used for microgreen production is free from contamination, then there is no requirement for seed treatment. However, if the seeds are infested with any pathogens, then seed treatment will be effective as reported by several authors.

The microgreens of six species were grown on five growing media to identify the best one. The observations on yield showed that it was highest when grown in cocopeat media, than on other four media. The cocopeat media has good water holding capacity, provides adequate aeration and maintains moisture for longer period which promotes the healthy root growth of microgreens, thereby resulting in maximum shoot growth in this media. Among the six crops, green gram recorded the highest fresh (1.03 to 1.49 kg/m^2) and dry weight (0.230 to 0.333 kg/m^2) and the least yield was recorded for amaranthus microgreens (fresh weight- 0.28 to 0.48 kg/m^2 and dry weight- 0.027 to 0.046 kg/m^2). The seedling height of microgreens, was observed to be same except for ragi and mustard where it was maximum for cocopeat raised microgreens and least was noted for newspaper grown ones.

The nutritional value of microgreens assessed revealed that it was not influenced by different growing media, it varied only with different species used for microgreen production. The iron content was reported to be maximum in amaranthus microgreens (1.43 to 1.7 mg/100 g), calcium content was recorded to be highest in ragi microgreens (279.13 to 287.06 mg/100 g), beta carotene was noticed to be maximum in mustard microgreens (2.63 to 3.06 mg/100 g), vitamin C was maximum in amaranthus microgreens (35.2 to 42.25 mg/100 g), crude protein was recorded to be highest in (1.5 to 2 g/100 g), wheat reported maximum fibre content (21.8 to 22.4%) and chlorophyll content was highest in green gram microgreens (1.31 to1.4 mg/g). Nitrates and oxalates were estimated only for amaranthus and mustard microgreens, it was also not influenced by the different growing media but among the species amaranthus reported highest oxalate content (76.26 to 79.46 mg/100 g) and nitrate content (51.13 to 54.23 mg/100 g). The freshly harvested microgreens did not exhibit any fungal contamination.

The seed density for the six species of microgreens were standardized. The yield recorded was highest in high density planting for wheat (0.59 kg/m^2), ragi (0.61 kg/m^2) and amaranthus (0.58 kg/m^2) microgreens while it was maximum in medium density planting for green gram (1.75 kg/m^2), horse gram(1.47 kg/m^2) and mustard (1.11 kg/m^2) microgreens. Seedling height was on par in all the densities, except for green gram (10.77 cm) and horse gram microgreens (9.35 cm) where it was highest when sown in medium density planting. The fungal contamination was absent in the freshly harvested microgreens sown in all the three densities. Thus, it is concluded that for wheat (705 g/m^2), ragi (520 g/m^2) and amaranthus microgreens (120 g/m^2) high density planting, while for green gram (850 g/m^2), horse gram and mustard (440 g/m^2) medium density planting is recommended as optimum seed density for their healthy growth and higher yield.

The yield and nutritional value of microgreens under two growing conditions were evaluated. The yield was recorded to be significantly higher for microgreens grown in rain shelter condition than room condition. The temperature and light intensity widely varied in both conditions *viz*, in room condition mean temperature and light intensity was 29.1 ^{0}C and 8.78 µ mol m^{-2} s^{-1} respectively and in rain shelter

condition it was 32.2 ^{0}C and 117.8 µ mol m^{-2} s^{-1} respectively. The higher light intensity and temperature may have resulted in increased photosynthetic rate and vigorous plant growth. Among the crops, green gram yielded highest under rain shelter (2.261 kg/m^2) while in room condition the yield was 1.4 kg/m^2. The yield was least for amaranthus microgreens grown under rain shelter (0.49 kg/m^2) while in room condition it was 0.29 kg/m^2. The seedling height was recorded to be higher in microgreens grown in room condition compared to rain shelter raised microgreens. In room condition, the lower light intensity may have induced lanky growth of the seedlings thereby resulting in higher seedling height. The nutritional value of microgreens assessed under two conditions revealed that rain shelter grown microgreens had higher nutrient content. In rain shelter grown microgreens, better light availability and higher temperature may have resulted in enhanced metabolic activity resulting in higher levels of phytochemicals like vitamin C, beta carotene, crude protein and chlorophyll. Iron content was maximum for amaranthus microgreens grown in rain shelter (1.928 mg/100g), calcium content was highest for ragi microgreens (294.32 mg/100g), beta carotene was maximum for mustard microgreens (3.46 mg/100g), vitamin C was highest for amaranthus microgreens (47.94 mg/100g), mustard microgreens were exhibiting highest crude protein content (2.38 g/100g), fibre content was maximum in wheat microgreens (23.98 %) and chlorophyll content was highest in green gram microgreens (1.58 mg/ g). The antinutrient content was also noted to be higher in rain shelter grown microgreens this may be due to the higher temperature under the rain shelter. Oxalates and nitrates content were highest in amaranthus microgreens raised in rain shelter condition, the values being 72.99 mg/100g and 65.21 mg/100g respectively. The organoleptic evaluation of microgreens revealed that mustard microgreens grown in room condition had maximum sensory qualities followed by green gram microgreens grown in room condition and mustard microgreens grown in rain shelter . The shelf life of six microgreens recorded showed that the storage life can be extended to the maximum when stored in zip lock PPE bag under low temperature storage (4^0C). Among the crops shelf life was observed to be maximum for wheat and horse gram microgreens (9 days). As a conclusion, seed treatment is recommended only if the seeds used for microgreen production are infested with pathogens. Both the chemicals can be used

for seed treatment as neither of them adversely affected the seed germination or vigour when compared to control. Cocopeat was selected as the best media for commercial production of microgreens among the five media used in the study, however other media used in the study can also be used for raising microgreens in households. Seed densities were standardized for each crop. Microgreens can be recommended to be grown in room condition as well as rain shelter condition. For commercial production of microgreens rain shelter cultivation is preferred due to higher yield. All the six species of microgreens studied are nutritionally superior and can be popularized as a functional food to all classes of society, thereby ensuring nutritional security to everyone.

References

REFERENCES

Abad. M., Noguera, P. and Bures, S. 2001. National inventory of organic wastes for use as growing media for ornamental potted plant production: Case study in Spain. *Biores. Technol*. 77: 197-200.

Aneja, K. R. 2007. *Experiments in Microbiology Plant Pathology and Biotechnology*. New Age International Limited, New Delhi. 88p.

Antony, V and Radha, R. 2019. Broccoli microgreens: A crop that can diversify the food systems. *Int. J. Appl. Home Sci*. 6 (9): 312-314.

AOAC [Association of Official Analytical Chemists]. 1970. *Official methods of the analysis of official analytical chemists.* Washington, DC. 1015p.

AOSA [Association of Official Seed Analysts]. 1983. *Seed Vigor Testing Handbook: Contribution to the Handbook on Seed Testing.* Association of Official Seed Analysts, Lincoln, NE, USA. 230p.

Awang, Y., Shaharom, A. S., Mohamad, R. B. and Selamat, A. 2009. Chemical and physical characteristics of cocopeat-based media mixtures and their effects on the growth and development of Celosia cristata. *Am. J. Agric. Biol. Sci.* 4 (1): 63-71.

Benothmen, S., Elimem, M., Abir, H., Abbes, K. and Chermiti, B. 2019. The effect of acetic acid treatment on germination parameters of carrot seeds infected with "Candidattus Liberibacter solanacearum". *J. New Sci. Sustain. Livestock Manag.* 10 (1): 207-212.

Berba, K. J. and Uchanski, M. E. 2012. Post-harvest physiology of microgreens. *J. Young Investig*. 24(1) : 5.

Bergspica, I., Ozola, A., Miltiņa, E., Alksne, L., Meistere, I., Cibrovska, A. and Grantiņa-Ieviņa, L. 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(7): 420-428.

Bewley. J. D. 1997. Seed germination and dormancy. *Plant Cell*. 9: 1055-1060.

Bharghava, B. S. and Raghupati, H. B. 1993. Analysis of plant materials for macro and micro nutrients. In: Tandon, H. L. S. (ed.), *Methods of Analysis of Soil, Plants, Water and Fertilizers.* Fertilizer Development and consultation Organization, New Delhi, 82p.

Borgen, A. and Nielsen, B. 2001. Effect of seed treatment with acetic acid in control of seed borne diseases. In: *Proceedings of the BCPC Symposium No. 76, Seed Treatment: Challenges & Opportunities,* 26- 27 February, 2001, UK [On-line].

Brazaityte, A., Jankauskiené, J. and Novickovas, A. 2013. The effects of supplementary short term red LEDs lighting on nutritional quality of *Perilla frutescens* L. microgreens. *Rural Dev*. 54-57.

Brazaityte, A. S. Sakalauskiene, G. Samuoliene, J., Jankauskiene, A., Virsile, A. Novickovas, R. Sirtautas, J., Miliauskiene, V., Vastakaite, L., Dabasinskas, V., and Duchovskis, P. 2015. The effects of LED illumination spectra and intensity on carotenoid content in Brassicaceae microgreens. *Food Chem*. 173: 600-606.

Bulgari, R., Baldi, A., Ferrante, A., and Lenzi, A. 2017. Yield and quality of basil, Swiss chard, and rocket microgreens grown in a hydroponic system. *New Zealand J. Crop Hortic. Sci.* 45(2): 119-129.

Bulgari, R., Negri, M., Santoro, P. and Ferrante, A. 2021. Quality Evaluation of Indoor-Grown Microgreens Cultivated on Three Different Substrates. *Horticulturae*. 7(5): 96.

Caracciolo, F., El-Nakhel, C., Raimondo, M., Kyriacou, M. C., Cembalo, L., De Pascale, S. and Rouphael, Y. 2020. Sensory attributes and consumer acceptability of 12 microgreens species. *Agron*. 10 (7): 1043.

Chandra, D., Kim, J. G., and Kim, Y. P. 2012. Changes in microbial population and quality of microgreen treated with different sanitizers and packaging films. *Hort. Environ. Biotechnol.* 53: 32-40.

Chen, H., Tong, X., Tan, L. and Kong, L. 2020. Consumers acceptability and perceptions towards the consumption of hydroponically and soil grown broccoli microgreens. *J. Agric. Food Res.* 2: 100051.

Chrysargyris, A., Xylia, P., Akinci, G., Moustakas, K. and Tzortzakis, N. 2020. Printed Paper Waste as an Alternative Growing Medium Component to Produce Brassica Seedlings under Nursery Conditions. *Sustainability*. 12 (15): 5992.

Copeland, L. O. and Mc Donald, M. B. 2001. Seed germination. In: *Principles of Seed Science and Technology. Springer*. 72-123.

Craver, J. K., Gerovac, J. R., Lopez, R. G. and Kopsell, D. A. 2017. Light intensity and light quality from sole-source light-emitting diodes impact phytochemical concentrations within Brassica microgreens. *J. Am. Society Hortic. Sci.* 142(1): 3-12.

Dalal, N., Siddiqui, S., and Neeraj. 2020. Sensory Attributes of Sunflower Microgreens with storage under Ethanol vapour and Organic acid treatments. *Int. J. Microbial. App. Sci.* (902): 208-214.

Danielle, D. T., Hochmuth, R., Landrum, L., and Laughlin, W. 1999. Microgreens: A new specialty crop. *J. Hortic.* 3: 16-20.

Danielle, D., Treadwell, Hochmuth, R., Landrum, L. and Wanda Laughlin. 2011. *Microgreens: A New Specialty Crop.* University of Florida IFAS Extension. 6-13.

Deepa, N. and Malladadavar, D. 2020. Microgreens: The treasure of nutrients. *Int. J. Curr. Microbiol. Appl. Sci.* 9(2): 18-23.

Delaquis, P. J., Sholberg, P. L. and Stanich, K. 1999. Disinfection of mung bean seed with gaseous acetic acid. *J. Food Protection*. 62(8): 953-957.

Delian, E., Chira, A., Badulescu, L., and Chira, L. 2015. Insight into microgreens. *Hortic*. 59: 447-454.

Ebert, A. 2013. Sprouts, microgreens, and edible flowers: The potential for high value specialty produce in Asia. *SEAVEG*. 216-227.

El-Nakhel, C., Pannico, A., Graziani, G., Kyriacou, M.C., Giordano, M., Ritieni, A., De Pascale, S. and Rouphael, Y. 2020. Variation in macronutrient content, phytochemical constitution and in vitro antioxidant capacity of green and red butterhead lettuce dictated by different developmental stages of harvest maturity. *Antioxidants*. 9(4): 300p.

Fortunato, A. E., Annunziata, R., Jaubert, M., Bouly, J. P. and Falciatore, A. 2015. Dealing with light: The widespread and multitasking cryptochrome/photolyase family in photosynthetic organisms. *J. Plant Physiol*. 172: 42- 54.

Gayathree, I. S., Gama-Arachchige, N. S., and Anjani, M. K. 2019. Germination, harvesting stage, antioxidant activity and consumer acceptance of ten microgreens. *Ceylon J. Sci*. 48 (1): 91-96.

Gerovac, J.R., Craver, J.K., Boldt, J.K. and Lopez, R.G., 2016. Light intensity and quality from sole-source light-emitting diodes impact growth, morphology, and nutrient content of Brassica microgreens. *Hort. Sci.* 51(5): 497-503.

Ghoora, M. D and Srividya, N. 2018. Micro-farming of greens: A viable enterprise for enhancing economic, food and nutritional security of farmers. *Int. J. Nutr. Agric. Res.* 5(1): 10-16.

Ghoora, M. D., Babu, D. R. and Srividya, N. 2020. Nutrient composition, oxalate content and nutritional ranking often culinary microgreens. *J. Food Composition Anal.* 91: 103495p.

Ghoora, M. D., Halidipur, A. C. and Srividya, N. 2020. Comparative evaluation of phytochemical content, antioxidant capacities and overall antioxidant potential of selected culinary microgreens. *J. Agric. Food Res*. 2 (100046):1-7.

Hedges, L. J. and Lister, C. E. 2009. Nutritional attributes of some exotic and lesser known vegetables. *Plant Food Res. Con. Rep*. 2325: 22-4.

Hodges, D. M. and Toivonen, P. M. A. 2008. Quality of fresh-cut fruits and vegetables as affected by exposure to abiotic stress. *Postharvest Bio. Tech*. 48: 155-162.

Holt, J. S. 1995. Plant responses to light: A potential tool for weed management. *Weed Sci.* 43(3): 474-482.

Hong, E.J. and Kang, D.H., 2016. Effect of sequential dry heat and hydrogen peroxide treatment on inactivation of *Salmonella Typhimurium* on alfalfa seeds and seeds germination. *Food microbiol*. 53:9-14.

International Rules for Seed Testing. 2012. *Seed Testing International*. International Seed Testing Association (ISTA), Bassersdorf, Switzerland. 60p.

Janovska D., Stockova L., and Stehno Z. 2010. Evaluation of buckwheat sprouts as microgreens. *Acta Agriculturae Slovenica*. 95(2): 157-162.

Jellinek, G. 1985. Sensory evaluation of food- Theoryand Practice. Ellis Horwood limited Chichester, England. 596p.

Kaur, A. and Singh, A. K. H. 2020. High value low volume vegetable confetti: Microgreens. *Indian Farmer*. 630p.

Koley, T.K., Maurya, A. and Singh, B. 2016. Microgreens from vegetables: more nutrition for better health. *Training Manual on "Advances in Genetic Enhancement of Underutilized Vegetable Crops";* Indian Institute of Vegetable Research: Varanasi, India. pp.18-27.

Kopsell, D. A., Pantanizopoulos, N. I., Sams, C. E. and Kopsell, D. E. 2012. Shoot tissue pigment levels increase in 'Florida Broadleaf' mustard (*Brassica juncea* L.) microgreens following high light treatment. *Sci. Hortic*. 140: 96-99.

Kou, L., Luo, Y., Yang, T., Xiao Z., Turner, E. R., Lester, G. E., Wang, Q. and Camp, M. J. 2013. Pos tharvest biology, quality and shelf life of buckwheat microgreens. *Food Sci. Technol*. 51: 73-78.

Kumar, S., Jasmin, L. B. and Golakiya, P. 2016. Microgreens cultivation. *Kerala Karshakan*. 4 (6): 22-29.

Kumar, S., Lathiya Jasmin, B. and Saravaiya, S.N., 2018. Microgreens: A new beginning towards nutrition and livelihood in urban-peri-urban and rural continuum. 246p.

Kyriacou, M. C., Rouphael, Y., Di Gioia, F., Kyratzis, A., Serio, F., Renna, M., De Pascale, S. and Santamaria, P. 2016. Micro-scale vegetable production and the rise of microgreens. *Trends Food Sci. Technol*. 57: 103-115.

Landis, T. D., Jacobs, D. F., Wilkinson, K. M., and Luna, T. 1990. Growing media. *The container tree nursery manual*. 2: 41-85.

Lau, T. Q., Tang, V. T. H. and Kansedo, J. 2019. Influence of Soil and Light Condition on the Growth and Antioxidants Content of Amaranthus Cruentus (Red Amaranth) Microgreen. In: *IOP Conference Series: Materials Science and Engineering.* 26-28.

Lee, J. S., Pil, W. G., Cobb, B. B. and Olszewski, M. 2004. Seed treatments to advance greenhouse establishment of beet and chard microgreens. *J. Hortic. Sci. Biotechnol.* 79: 565-570.

Lichtenthaler, H. K., Marek, M. V., Kalina, J., and Urban, O. 2007. Differences in pigment composition, photosynthetic rates and chlorophyll fluorescence images of sun and shade leaves of four tree species. *J. Plant Physiol. Biochem*. 45: 577-585.

Marderosian, A.D., Beutler, J., Pfender, W., Chambers, J., Yoder, R, Weinstriger, E. and Senft, J. 1979. *Nitrate and Oxalate Content of Vegetable Amaranth*. Rodale Press, Emmaus, pp. 31-40.

Michell, K. A., Isweiri, H., Newman, S. E., Bunning, M., Bellows, L. L., Dinges, M. M., Grabos, L. E., Rao, S., Foster, M. T., Heuberger, A. L. and Prenni, J. E. 2020. Microgreens: Consumer sensory perception and acceptance of an emerging functional food crop. *J. Food Sci.* 85(4): 926-935.

Mohanty, A., Mahahlik, G. and Parida, S. 2020. Nutritional Analysis of Few Edible Microgreens in Variable Growth Medium using XRF Technique. *Asian J. Biol. Life Sci.* 9(3): 361.

Moran, N. 2017. Managing diseases in microgreens. *Produce Grower*. 9: 45-49.

Mortensen, L. M., and Grimstad, S.O. 1990. The Effect of Lighting Period and Photon Flux Density on Growth of Six Foliage Plants. *Scientia Horticulturae.* 41 (4): 337-342.

Murchie, E. H. and Niyogi K. K. 2011. Manipulation of photoprotection to improve plant photosynthesis. *Plant Physiol*. 155: 86-52.

Murphy, C. J. and Pill, W. G. 2010. Cultural practices to speed the growth of microgreen arugula. *J. Hortic. Sci. Biotechnol.* 85 (3): 171-176.

Nair, B. R. and Lekshmi, G. P. 2019. Nutritional and anti-nutritional analysis of some selected microgreens. *Applied Biol. Res*. 21(2): 35-38.

Nandi, M., Pervez, Z., Alam, M. S., Islam, M. S. and Mahmud, M. R. 2017. Effect of hydrogen peroxide treatment on health and quality of chilli seed. *Int. J. Plant Path.* 8(1): 1-6.

Niroula, A., Khatri, S., Timilsina, R., Khadka, D., Khadka, A., and Ojha, P. 2019. Profile of chlorophylls and carotenoids of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) microgreens. *J. Food Sci. Tech.* 56: 2758-2763.

Nolan, D. A. 2019. Effects of seed density and other factors on the yield of microgreens grown hydroponically on burlap. Project Report 2018. Virginia. 44p.

Nunes, M. C. N. and Emond, J. P. 2005. Storage temperature. In: J.A. Bartz and J.K. Brecht (eds.). *Postharvest physiology and pathology of vegetables.* Marcel Dekker Inc., New York, USA. pp. 226-249.

Paradiso, V. M., Castellino, M., Renna, M., Gattullo, C. E., Calasso, M., Terzano, R., Allegretta, I., Leoni, B., Caponio, F. and Santamaria, P. 2018. Nutritional characterization and shelf-life of packaged microgreens. *Food function*. 9(11): 5629-5640.

Parida, S. 2020. *Innovative farming of edible micro greens at home and their nutritional composition.* The Mattingley Publishing Co. pp: 17630-17640.

Pernezny, K., Nagata, R., Raid, R. N., Collins, J. and Carroll, A. 2002. Investigation of seed treatments for management of bacterial leaf spot of lettuce. *Plant Dis*. 86(2): 151-155.

Pinto, E., Almeida, A. A., Aguir, A. A. and Ferreira I. M. P. L. V. O. 2015. Comparison between the mineral profile and nitrate content of microgreens and mature lettuces. *J. Food Comp. Anal.* 37: 38-43.

Piper, C. S. 1996. *Soil and Plant Analysis.* Hans Publishers, Bombay. 368p.

Polash, M. A. S., Sakil, M. A., Sazia, S and Hossain, M. A. 2019. Selection of suitable growing media and nutritional assessment of microgreens. *Agric. Res. J.* 56: 752p.

Renna, M., Di Gioia, F., Leoni, B., Mninni, C and Santamaris, P. 2017. Culinary assessment of self- produced microgreens as basic ingredients in sweet and savory dishes. *J. Culinary Sci. Technol.* 15 (2):126-142.

Riggio, G. M., Jones, S. L. and Gibson, K. E. 2019. Risk of human pathogen internalization in leafy vegetables during lab-scale hydroponic cultivation. *Hortic.* 5: 1-22.

Sadasivam, S. and Manickoukam, A. 1992. *Biochemical Methods*. New Age International Publishers, New Delhi. 250p.

Samuoliene, G., Brazaityte, A., Sirtautas, R., Sakalauskiene, S., Jankauskiene, J., Duchovskis, P. 2012. The impact of supplementary short-term red LED lighting on the antioxidant properties of microgreens. *Acta. Hort*. (ISHS) 956: 649-656.

Senevirathne, G. I., Gama-Arachchige, N. S. and Karunaratne, A. M. 2019. Germination, harvesting stage, antioxidant activity and consumer acceptance of ten microgreens. *Ceylon J. Sci*. 48(1): 91-96.

Sharma, K. K., Singh, U. S., Sharma, P., Kumar, A. and Sharma, L. 2015. Seed treatments for sustainable agriculture-A review. *J. Appl. Natural Sci.* 7(1): 521-539.

Singh, N., Rani, S. and Chaurasia, O. P. 2020. Vegetable Microgreens Farming in High-Altitude Region of Trans-Himalayas to Maintain Nutritional Diet of Indian Troops. National Acad. Sci. 90(4): 743-752.

Sirtautas, R. and Samuolienė, G. 2013. The effect of red-LED lighting on the antioxidant properties and nitrates in red baby leaf lettuces. Rural Development. 237-240.

Steinger, T., Roy, B. A. and Stanton, M. L. 2003. Evolution in stressful environments II: adaptive value and costs of plasticity in response to low light in Sinapis arvensis. *J. Evol. Biol.* 16: 313-323.

Storey, A. 2017. 6 ways to grow better microgreens. Crops & Growing Science, Upstart University, Bright Agrotech, Plenty [On-line]. Available: https://university.upstartfarmers.com/blog/6-ways-to-grow-better-microgreens

Szopinska, D., 2014. Effects of hydrogen peroxide treatment on the germination, vigour and health of *Zinnia elegans* seeds. *Folia Hort*. 26(1): 19-29.

Szopinska, D., Jarosz, M. and Sławińska, B. 2017. The effect of hydrogen peroxide on seed quality and emergence of carrot (*Daucus carota* L.). *Acta Sci. Pol. Hortorum Cultus*. 16:21-33.

Tiwari, P. 2015. Coco peat: a new era of soil less urban farming. *Int. J. Res. Biosci. Agric. Tech*. 2(7): 287-289.

Treadwell, D., Hochmuth, R., Landrum, L. and Laughlin, W. 2010. *Microgreens: A New Specialty Crop.* Horticultural Sciences Department, Florida. 32p.

Virsile, A. and Sirtautas, R. 2013. Light irradiance level for optimal growth and nutrient contents in borage microgreens. *Rural Devel*. 272-275.

Wang, Y., Tong, Y., Chu, H., Chen, X., Guo, H., Yuan, H., Yan, D. and Zheng, B. 2017. Effects of different light qualities on seedling growth and chlorophyll fluorescence parameters of *Dendrobium officinale*. *Biologia*. 72(7): 735-744.

Weber, C.F. 2016. Nutrient content of cabbage and lettuce microgreens grown on vermicompost and hydroponic growing pads. *J. Hortic*. 3(4): 1-5.

Xiao Z. L., Nou X.W., Luo Y.G., and Wang Q. 2014. Comparison of the growth of Escherichia coli O157: H7 and O104: H4 during sprouting and microgreen production from contaminated radish seeds. *Food Microbiol*. 44: 60-63.

Xiao, Z., Codling E. E., Luo, Y., Nou, G. E. Lester, and Wang, Q. 2016. Microgreens of Brassicaceae: Mineral composition and content of 30 varieties. *J. Food Comp. Anal*. 49: 87-93.

Xiao, Z., Lester, G.E., Luo, Y. and Wang, Q. 2012. Assessment of vitamin and carotenoid concentrations of emerging food products: edible microgreens. *J. Agric. Food Chem*. 60: 7644-7651.

Xiao, Z., Luo, Y., Lester, G.E., Kou, L., Yang, T. and Wang, Q. 2014. Postharvest quality and shelf life of radish microgreens as impacted by storage

temperature, packaging film, and chlorine wash treatment. *Food Sci. Technol*. 55(2): 551-558.

Xiao, Z., Rausch, S. R., Luo, Y., Sun, J., Yu, L., Wang, Q., and Stommel, J. R. 2019. Microgreens of Brassicacae: Genetic diversity of phytochemical concentrations and antioxidant capacity. *Food Sci. Technol*. 101: 731-737.

Ying, Q., Kong, Y. and Zheng, Y. 2020. Growth and appearance quality of four microgreen species under light-emitting diode lights with different spectral combinations. *Hort. Sci.* 55(9): 1399-1405.

Zavala, J. A. and Ravetta, D. A. 2001. Allocation of photoassimilates to biomass, resin and carbohydrates in *Grindelia chiloensis* as affected by light intensity. *J. Field Crop Res*. 69: 143-149.

Zhang, X., Bian, Z., Yuan, X., Chen, X. and Lu, C. 2020. A review on the effects of light-emitting diode (LED) light on the nutrients of sprouts and microgreens. *Trends Food Sci. Technol.* 99: 203-216.

Appendices

Appendix-I

Meteorological data during each experiment

Experiments		Mean temperature (^{0}C)	Mean relative humidity (%)	Mean light intensity (µ mol m^{-2} s^{-1})
Experiment 1		30.93	63.96	8.50
Experiment 2		38.62	47.9	8.12
Experiment 3		32.91	62.2	7.89
Experiment 4	Room condition	30.22	73.23	8.78
	Rain shelter	32.31	65.03	117.82

STANDARDIZATION OF TECHNOLOGY FOR MICROGREEN PRODUCTION

By

ARYA K. S.

(2019-12-038)

ABSTRACT OF THE THESIS

Submitted in partial fulfillment of the requirement for the degree of

Master of Science in Horticulture

(Vegetable Science)

Faculty of Agriculture

Kerala Agricultural University, Thrissur

DEPARTMENT OF VEGETABLE SCIENCE

COLLEGE OF AGRICULTURE

KERALA AGRICULTURAL UNIVERSITY

VELLANIKKARA, THRISSUR- 680656

KERALA, INDIA

2021

ABSTRACT

Microgreens are new class of vegetables that are gaining popularity in the recent years due to their attractive appearance coupled with vivid flavors. They are young immature greens produced from seeds of vegetables or herbs which are harvested at its true leaf stage. Most of the research works in this area are carried out with temperate species of crops whose seeds are very expensive. Hence there is a need to popularize the microgreens of tropical species at an affordable price with minimum inputs, so that it is easily accessible to common people. The study entitled "Standardization of technology for microgreen production" was conducted at the Department of Vegetable Science, College of Agriculture, Vellanikkara during January to October 2021.

The study was experimented with wheat, ragi, green gram, horse gram, amaranthus and mustard whose seeds were collected from the local market. It was conducted as four experiments (standardization of seed treatment, standardization of media, standardization of seed density and to assess the nutritional value and yield under different growing conditions). The first experiment was to standardize seed treatment done using two chemicals hydrogen peroxide and vinegar treated at different concentrations. The observations on germination percentage, seedling vigour and yield were recorded. The seed treatment did not show any effect on growth of microgreens when compared to the control on the parameters recorded. The microscopic observation of freshly harvested microgreens for fungal contamination indicated absence of any contamination in the fresh microgreens.

The experiment on standardizing growing media was carried out using five media *viz*, sterile sand, cocopeat, coir mat, tissue paper and newspaper. The observation on yield, seedling height, nutritional value of microgreens and microscopic observation on fungal growth were recorded. Yield was observed to be highest in microgreens grown on cocopeat media and lowest was recorded in microgreen grown on newspaper media and among the crops it was highest for green gram microgreens (1.03 to 1.49 kg/m^2). The seedling height showed a slight decrease in ragi and mustard microgreens grown on newspaper and comparing the crops, it was

observed to be maximum in green gram microgreens (10.23- 10.54 cm). The nutritional parameters recorded were not showing any difference when sown in different media. The wheat microgreens were observed to rich source of vitamin C, beta carotene and protein, ragi microgreens were showing more calcium and vitamin C and beta carotene content, green gram microgreen were rich in protein, vitamin C and calcium, horse gram microgreens were rich in vitamin C, chlorophyll, beta carotene and crude protein, amaranthus microgreens possess high iron, calcium, beta carotene and vitamin C content and mustard microgreens were rich in protein and beta carotene, iron and vitamin C. Microscopic observation on freshly harvested produce revealed that there is absence of fungal contamination in microgreens.

The seed density for microgreen production was standardized in the third experiment. The seeds were sown at three densities *viz*, low, medium and high density for each crop. The observations recorded included yield, seedling height and microscopic observation on fungal growth. The yield was recorded to be highest when sown at high density for wheat (705 g seeds/m^2), ragi (520g seeds/m^2) and amaranthus(120g seeds/m^2) microgreens and medium density planting yielded highest for green gram (850g seeds/m^2), horse gram (617 g seeds/m^2) and mustard (440 g seeds/m^2) microgreens. The seedling height recorded was observed to be on par except for green gram and horse gram microgreens, where highest seedling height was found in medium density planting. No fungal contamination was observed in microscopic observation of fresh produce.

Nutritional value and yield of microgreens grown under two conditions (room condition and rain shelter) were studied in fourth experiment. The parameters recorded were yield, seedling height, nutrient content under two conditions, organoleptic evaluation and shelf life of microgreens. The yield was observed to be significantly high when raised under rain shelter condition than room condition. Among the crops it was highest for green gram microgreens (2.261 kg/m^2). The seedling height was observed to reduce under rain shelter condition. Nutritional content was also observed to be high when planted under rain shelter for all parameters *viz*, iron, calcium, beta carotene, vitamin C, crude protein, crude fibre, chlorophyll, oxalates and nitrates. Organoleptic evaluation revealed highest total mean

rank for mustard microgreens (54.56) raised under room condition. The shelf life study revealed that the storage period of microgreens can be extended when it is stored in ziplock PPE bag under low temperature condition. In this study, several aspects of microgreens production *viz*, seed treatment, growing media, seed density, growth conditions and shelf life were standardized. The results indicate that microgreen cultivation can be recommended both as a commercial and household venture.