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An Investigation into Sustainable Strawberry Production using Alternative Growing Systems

Thesis presented by

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Abstract

Strawberry is a fruit propagated by runners who avoid direct seed plantation due to its low germination rate. The study aimed to explore the germination rate of strawberry seeds by considering two varieties, 'Temptation' and 'Florian,' in different growing substrates (rockwool with and without nutrients and compost). The outcomes revealed that the change in substrate positively affects seed germination. Rockwool with and without nutrients depicted better germination. The study aimed to explore the growth and development of strawberry runners in aeroponic towers and the effect of different concentrations of nutrients (A and B) on the growth and yield of strawberry plants. Tower 1 (50% nutrient A and B) showed the survival of three plants out of seven, tower 2 (70%nutrient A and 30% nutrient B) depicted the survival of two plants, and tower 3 (30%nutrient A and 70% nutrient B) showed the survival of six plants out of seven. The leaves taken from Tower 3 (Nutrient A 30% and Nutrient B 70%) were the largest among all. The effect of different light (red, dark, and white) temperatures and Ethephon on seed germination was observed for Temptation and Florian seeds. The statistical analysis revealed that light has no difference in seed germination in Temptation and Florian strawberry seeds. The temperature and ethephon treatment did not show any effect on Florian and temptation strawberry seed germination. Tissue culturing of Calypso strawberry plants revealed that tissue culturing in rockwool was difficult in comparison to agar gel medium, whereas charcoal can be used efficiently to reduce fungal contamination. This study's results indicated that Temptation strawberry seeds had higher germination rates compared to Florian seeds. Red light, temperature and ethephon showed no

significant impact on seed germination. Tissue culture techniques for Calypso strawberry plants were effective, with shoot multiplication medium (SMM) and shoot propagation medium (SPM) media supporting plant growth. Charcoal significantly reduced contamination in tissue culture. Aeroponic towers positively impacted strawberry plant growth, as evidenced by improved leaf size and fruit yield. Healthy growth, flowering, and fruiting were observed in strawberry plants grown in aeroponic towers, with no fungal or bacterial diseases recorded. Data showed a total of 270 flowers and 193 fruits in parent plants and 245 flowers and 164 fruits in runners, with a total dry weight yield of 191.73 grams for parent plants and 186.32 grams for runners. Overall, the study was designed to examine how different factors (rockwool substrate, aeroponic towers, tissue culture, light, temperature and ethephon) have a significant influence on strawberry seed germination and overall plant growth.

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List of Abbreviations

No.	Number
Shoot multiplication medium	SMM
Shoot propagation medium	SPA
Apple latent spherical virus	ALSV
virus-induced flowering	VIF

Declaration

"This was to certify that the work I am submitting was my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources were acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism and intellectual property."

Student's Signature | Date

Dec' 2025

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Chapter 1: Literature Review

1.1. Introduction and Background

Strawberry (*Fragaria ananassa*) is a berry-like fruit produced in almost all regions of the world. The rising demand and advancements in production technology have led to a higher yield globally (Kafkas, 2016). Strawberries, most commonly used as a fresh commodity, are preferred because of their nutritional value; however, they are also consumed as juices, jams, jellies, chocolate ice creams, and beverages (Giampieri et al., 2013). According to the Food and Agriculture Organisation, the worldwide production of strawberries reached more than 8 million tons in 2019, where China is leading in its production, followed by the USA (Oğuz, Oğuz & Kafkas, 2022).

Strawberry is a soft fruit that requires great care throughout its growing phase. Because of its softness, it is often affected by different diseases (*Botrytis* rot and powdery mildew) and insects (spider mites and weevils) (Lahiri et al., 2022; Swett et al., 2020). Due to its direct consumption, there are several limitations to the use of pesticides, which leads to the reliance on modern technology, including genetic modification, in some regions, of several trades and resistant varieties to deal with the loss of yield due to pest infestation and viral and fungal diseases (Torrico et al., 2018). The strawberry can also be affected by poor harvest and post-harvest handling; therefore, modern technology implications, including strawberry robots, are used to reduce the harvest and post-harvest losses. Machine and deep learning are also being implemented, and hydroponic and field systems are used to identify diseases, monitor plants, and estimate possible yield. Technological implications have modernized strawberry production, which has resulted in positive outcomes in meeting the increasing demand (Samtani et al., 2019).

1.2. Varieties used for commercial production

Strawberries are classified as short-day, long-day, and day-neutral plants according to their photoperiodic requirement (plants’ response to flower bud formation). Currently, short-day strawberries are being cultivated, especially in areas having high latitude; however, there has been an increasing trend in using day-neutral cultivars. Day-neutral cultivars of strawberries do not require specific photoperiodic conditions for flowering as long as the temperature is in the moderate range, usually 30 ºC for the day and 26 ºC for the night. For commercial strawberry production, day-neutral strawberry cultivars were used, which were planted in early spring and usually bear fruit at the end of June while continuing fruiting throughout the summer and fall months in highland areas (Lizalo & Demirsoy, 2020). Because of its increased economic importance and demand, breeders continuously develop new varieties to meet changing climatic conditions and production systems. Currently, one focus of the breeders is the production of varieties with extended harvesting seasons. Countries and regions are now adopting various varieties and cultivars to boost strawberry production and satisfy global demand. The main strawberry varieties used worldwide include Albion, Aromas, Diamante, Everest, Chandler, Torrey, Selva, Pajaro, Red cost, Florida, Darselect, Earligrow, and Elsanta (Lantz et al., 2011). According to Teagasc, the main cultivar of strawberry in Ireland is Malling Centenary, with an annual worth of €47m (Teagasc, 2017). Another notable name in strawberry production is Keelings, which produces about 50% of the total strawberries in Ireland. . Keelings is also involved in the production of different fruits including pineapples, blueberries, raspberries, blackberries, apples, pears, melons, grapes and cherries. Food Central St., Margarets, Co, and Dubline Ireland are major distribution sectors of Keelings. Keelings is engaged in producing 100% tabletop strawberries, ensuring the fruit remains of high quality (Keelings, 2025).

1.3. Systems for Strawberry Production

1.3.1. Matted Row Traditional System

The matted row is a traditional production system and one of the most popular methods for growing strawberries, where the strawberries are planted in rows as shown in **Figure 1** (Strawberry Production Systems Comparison, 2020). This is one of the most commonly used methods in colder climates (Kelly et al., 2007). This system utilises short varieties and focuses on producing strawberries with a concentrated harvest season in late spring or early summer. The varieties used for this system depend on their daughter plants for crop production instead of the crown on the parent plants that are originally planted. This has been considered one of the most reliable systems because of its continuous usage over a long time. It is also an efficient system for the areas where the strawberry is freshly consumed, making it easier for local markets to access fresh fruit. Strawberry plants set out on fertile land, planted in regularly spaced rows. In the initial phases, the strawberry plants were allowed to grow, where they started intertwining with reduced plant spacing, leading to the creation of an intertwined network (Wilson & Dixon, 1988). However, this practice requires increased preparation, as farmers must prepare the land every year to remove mother plants from previous seasons or any other varieties. This system also comes with various other issues, where the primary concern is weed control (Weber, 2021). Moreover, an increased infestation of soil-borne pathogens and insects requires soil treatment. The matted row system has various challenges because of its limitations regarding using other practices, e.g., strawberries require tending for the whole year, and the matted row system makes production more laborious. As a result, the traditional matted row system tends to have a lower annual yield than other methods (i.e., hydroponics) designed to boost strawberry production and yield (Weber, 2021).

Figure 1. Matted Row Traditional System for Strawberry Production (Strawberry Production Systems Comparison, 2020).

1.4. Modern Systems for Strawberry Production

1.4.1. Hydroponics

Hydroponics is a modern agriculture technique that uses nutrient solutions instead of soil to produce crops (Khan, 2018). Hydroponics allows plants to grow with or without an inert medium, such as sawdust, coir dust, coconut fibre, peat moss, vermiculite, or gravel. Hydroponics is one of the significant examples of modern technological innovations that address different issues affecting crop yield, including lack of land resources and increased risk of diseases and insects. Hydroponics also helps provide a controlled environment where different technological implementations help manage different resources and other factors (light intensity, humidity, and temperature) that significantly impact crop yield (Sharma et al., 2018).

Hydroponics has emerged as one of the significant technologies improving strawberries' production (Wortman et al., 2016). A major challenge in strawberry production is managing pests and weeds. This issue is being tackled by growing strawberries in high tunnels, where nutrient solutions or other soilless substrates serve as the growing media (Wortman et al., 2016). High tunnels are also crucial in extending the growing season, which helps farmers achieve increased strawberry yield. The hydroponic system is further divided into vertical and horizontal hydroponic systems, which are used according to the availability of the space.

Vertical Hydroponic System: Vertical hydroponic systems are advantageous as they cover and utilise the vertical space of the greenhouse at a large scale. Vertical hydroponic systems involve stacking growth rooms or controlled environments where different factors are controlled to obtain maximum yield through indoor farming as shown in **Figure 2** (chhajed garden, 2023). Because of the increasing year-round demand for strawberries, greenhouses with vertical hydroponic systems are being used to meet the requirements of strawberries in the markets (i.e., America and Europe) (Ramirez-Arias et al., 2017).

Figure 2. Vertical hydroponic systems (chhajed garden, 2023).

Horizontal Hydroponic System: Horizontal systems usually lay on the ground but also have the potential to be stacked on top of each other. Horizontal systems are favoured for their convenience in harvesting and other tasks, making the plants more accessible at breast or neck height as shown in **Figure 3** (Hydroponic systems international, 2020). Various pots, bags, or containers were employed, with PVC pipes being commonly used. The materials chosen to hold the plants varied based on availability, while wire or other support structures were used to ensure the plants grew upright. (Touliatos et al., 2016; Oğuz et al., 2022).

Figure 3. Horizontal Hydroponic technique (Hydroponic systems international, 2020).

1.4.2. Aeroponics

Aeroponics is the process of growing plants in air or mist environments without using aggregate material, growing medium, or soil as shown in **Figure 3** (Agrotonomy, 2018). Different aeroponics techniques are being used in which different field crops are produced by planting on a firm pinnacle or tower by suspending the growing plants in closed and semi-closed environments where the dangling roots of the plants are atomized or sprayed with the water solution containing essential nutrients for optimum plant growth (Akon et al., 2018). Strawberries are effectively grown in vertical towers using aeroponic methods, making this system notable for its water use efficiency. According to Mohammed et al. (2022), utilising aeroponics improves water productivity by up to 82%. Moreover, the controlled environment is also used for better production with less pesticide use than the current use. This technique also minimizes compatibility issues with fertilizers, resulting in optimal yields by using a combination of fertilizers in the ideal quantities to promote growth (Akon, 2019).

Figure 4. Aeroponic technique (Agrotonomy, 2018).

The aeroponic tower system supports strawberry growth vertically. These vertical towers are fed with nutrients required for optimum production of strawberry, allowing plants to grow without soil. In this innovative method, a nutrient-rich mist is continuously sprayed onto the plant's suspended roots, delivering essential nutrients and oxygen. The towers are planted at different levels, maximizing space and enabling efficient growth in confined areas. This system improves nutrient uptake and accelerates growth, leading to greater yields of large, flavorful strawberries (Abdullah, 2016). Besides, the controlled climate limits the risk of soil-borne illnesses and considers the exact administration of supplement levels, guaranteeing ideal circumstances for strawberry plants. The upward growth amplifies space use and improves harvesting, as the strawberries are effectively open at various levels. Aeroponic towers offer a supportable and water-productive arrangement, as they utilise fundamentally less water with customary soil-based strategies (Sengodan, 2022). This way of dealing with strawberry development embodies the capability of aeroponics in changing horticultural works, giving a brief look into the future of effective, space-saving, and Eco-friendly crop production.

The vertical aeroponic technique enables strawberry cultivation both indoors and outdoors. For this purpose, companies have introduced vertical towers, e.g. Tower Garden®. With Tower Garden's LED light system, strawberries may be grown indoors all year; even better, they can be grown in a greenhouse. It takes roughly six to eight weeks for strawberries to sprout and become suitable for planting in the towers. Although strawberries can be intercropped with other plants, our experience shows that planting strawberries alone in a monoculture on the tower leads to higher crop yields. Using Tower Garden® aeroponic technology, it is possible to grow up to fifty-two strawberry plants per square meter (Chen et al., 2019).

1.5. Use of LED Lights in Commercial Greenhouses

Plant growth and increased food production depend on several external and internal factors, such as temperature and light, which contribute significantly to the growth and development of the plant. Several alternatives to conventional agriculture have resulted in better profits (Dueck et al., 2016). Commercially, strawberry transplants are propagated through runners, and the plant propagation ratio and quality are affected by various environmental conditions, including temperature, photoperiod, and diseases. Artificial lighting has positively impacted the vegetative propagation of plants in controlled environments. Zheng et al. (2019) have observed that the transplants produced in a growth chamber with artificial lighting (PFAL) have resulted in increased productivity compared to conventional propagation methods. A light-emitting diode (LED) is generally used as a light source to alter the photoperiod and adjust light intensity. Photoperiod has a noticeable impact on the formation and elongation of runners. Ever-bearing strawberry plants exhibit improved performance when grown under longer photoperiods, particularly when the day length is extended to 15 hours using fluorescent lighting. Strawberries cultivated in greenhouses yield higher qualities and better-quality fruit, resulting in greater profits compared to those grown in the field.

LEDs can be considered an innovative tool and approach that can be used to fine-tune the light conditions for plants according to their requirements. Modifying the photoperiod and light intensity can have a major effect on fruit quality and yield, potentially leading to more efficient and productive crops. This approach can enhance both output and cost-effectiveness, particularly when compared to conventional agricultural methods, especially for horticultural plants (Folta & Carvalho, 2015). Strawberries grown in the growth chamber have a significant plant biomass ratio and higher fruit weight when treated with blue light. Choi et al. (2015) observed an increase in the growth of strawberry plants where higher vegetative growth of leaflet length was observed when the strawberry plants were exposed to blue LED light. Blue LED light has a significant impact on increasing the stem length or leaf area. Nadalini et al. (2017) have observed the beneficial impact of blue LED on strawberry growth and fruit quality in soilless hydroponic systems. It has been observed that LED blue has resulted in a 25% increase in fruit set, leading to higher yields than conventional or red LED-treated plants. The quality of food was also maintained, and it was observed that there was no decrease in its quality. A study by Díaz-Galián (2021) also showed that the combination of blue and red LEDs has increased strawberry flowering by up to 300%. On comparison between blue and red light, it was confirmed that blue light significantly promotes flowering as compared to red. This underscores the critical role of a controlled environment, lighting, and greenhouse conditions in strawberry production. The study's findings demonstrate that using blue light in a regulated greenhouse setting can significantly boost yield, especially when the strawberry plants are exposed to blue light without compromising fruit quality (Díaz-Galián et al., 2021).

1.6. Remote Sensing and Machine Learning

Remote sensing is an evolving technique which allows sensors to have a clear image of the field. Various types of sensors are installed on different platforms, enabling remote data collection. These platforms may include satellites, ground-based vehicles, and unmanned aerial vehicles (UAVs)/drones. These sensors are widely used in remote sensing agricultural applications, performing different functions that lead to precision agriculture (Zhang et al., 2020). Strawberry differs from other economic crops as it is vegetatively propagated and is relatively small compared to other agricultural or fruit plants. The majority of the farmers use manual counting means to estimate the yield of strawberries. However, the use of remote sensing technologies can facilitate real-time measurement and monitoring of plant growth and environmental conditions, aiding in the accurate estimation of crop yield (Zhang et al., 2020).

Different types of sensors are required to monitor the needs and requirements of the strawberry plants; for accurate monitoring and identification of the needs of the plants, higher spatial resolution imagery is required, which can review the canopy structure and identify the fruit. Various types of sensors are employed, including handheld, UAV-mounted, and ground-based platforms. Additionally, multispectral and hyperspectral sensors were used to detect diseases, such as identifying powdery mildew (Zheng, Abd-Elrahman & Whitaker, 2021). Wireless sensor technologies (WST) are widely being used, including radio frequency identification and wireless sensor networks (WSN) for disease detection. Different customized wireless sensor networks are being produced according to the crop and requirement. In strawberry production, a customized WSN is used to detect fungal diseases, where a combination of different sensors equipped with data on relative humidity, temperature and leaf wetness sensors resulted in disease detection (Martos et al., 2021).

Machine learning has also emerged as a significant way to analyse and monitor crops using remote sensing techniques. Machine learning has been used to identify plant diseases. By employing support vector machines and neural networks, the early detection of plant diseases and weeds is made possible, enabling the effective implementation of control strategies and preventive actions. Recent advancements in agriculture have seen the application of deep learning, particularly in advanced image processing for data analysis. A faster version of region-based convolutional neural networks (R-CNN) has been utilised to accurately count the number of flowers and categorise strawberries as mature or immature, achieving an average precision of 84.1%. This system has been used to accurately count strawberry flowers, which has been very helpful in forecasting strawberry yields (Chen et al., 2019).

1.7. In-vitro Culture System

In vitro culture systems have been widely used for the production of different fruits, including strawberries. Explants (node culture, leaf, sepal and petiol explants) are being used for optimum production and yield of strawberries (Naing et al., 2019). According to Debnath & Teixeira da Silva (2007) introduction of in-vitro strawberry cultivation techniques has facilitated growers from damage caused by microbes (especially soil fungi). In-vitro culture and micropropagation allowed the growers to grow more mother plants and runners in shorter periods of time in comparison to conventional methods. According to Bien et al. (2023), controlled conditions and environment for strawberry production results in increased plant growth with improved runner formation.

The strawberry cultivars are proliferated by runners and created from the mother plants' stolons. The traditional method of proliferation and production of runners from mother plants is unable to limit disease transformation. However, the use of in vitro culture can prevent disease transmission. Jan et al. (2013) highlighted the effectiveness of in vitro culture as an efficient method for sanitising strawberry micropropagation. Since the presence of microorganisms poses a significant risk to the sensitive strawberry plants, employing an effective sterilisation technique before propagation in an in vitro culture is crucial for reducing viral transmission. Surface sterilisation with mercuric chloride (0.1%) for 4 minutes increased the survival rate of the explants, thereby lowering the risk of disease transmission.

Surface sanitisation is a critical and essential step in establishing in vitro regeneration and propagation. In vitro, plant recovery and propagation methods are vital for producing plants that are free from infection. Different micropropagation techniques can be used for the elimination of viruses and other disease causing agents among plants. According to Yeasmin et al.

(2022), in vitro propagation of strawberry may lead to virus-free plants leading to improved fruit production.

Strawberries are consumed in light of their different medical advantages, taste, and fragrance, making it popular leading to increasing demands. Naing et al. (2019) proposed using different concentrations of kinetin (Kn) to enhance in vitro propagation through tissue culture. It was observed that cultures containing 0.5 mg/L of (Kn) were particularly effective in producing true-to-type plants of strawberry cultivars for successful propagation. Naing et al. (2019) suggested that at the commercial level, kinetin can enhance plant multiplication, especially under controlled or greenhouse conditions.

Modern techniques for strawberry production has resulted in improved yield and quality with further developed plant energy against any infection and disease. Li et al. (2019) observed the use of Apple latent spherical virus (ALSV) as a vector to introduce virus-induced flowering (VIF) for early flowering in strawberry plants through micropropagation. The results of the study has revealed that the use of in vitro techniques by utilising ASLV as a vector can induce early flowering and fruit generation improving yield.

1.8. Germination in Strawberry

The proper nutrients for both vegetative and regenerative development are essential for achieving optimal strawberry yield. The goal of Madhavi et al. (2021) was to examine how different growing media influence the growth characteristics and fruit yield of strawberries under controlled conditions. Four treatments were tested to assess their impact on growth and fruit yield across different growing media: control soil (CS), bio-compost (T1), bio-compost combined with other growing media (T2), synthetic nutrient-applied soil media (T3), and synthetic nutrient-applied integrated media (T4).

The goal was to determine the significant effects of these media on development and fruit production. The morphological parameters, including plant height, leaf area, fresh weight, and dry weight of roots, were measured at eight and four months during the reproductive stage, respectively. The yield ascribing boundaries, which incorporate the number of fruits per plant and the number of organic products per plant, were estimated towards the beginning and end of the conceptive stage. The effects of growing conditions on the development and production of strawberry plants were examined using variance analysis with a significance level of $p < 0.05$. Madhavi et al. (2021) indicated that synthetic fertilizers combined with bio-plus compost provide a better medium for strawberry growth and production under greenhouse conditions.

Garza et al. (2022) demonstrated that bio-stimulation enhances tolerance to both biotic and abiotic stress, also promoting greater nutrient absorption and accumulation, thereby supporting plant metabolism. Strawberries are highly valued not only for their excellent organoleptic and nutraceutical qualities, such as being rich in phenolic compounds, vitamins, and minerals but also for their significant commercial value.

Coastal California continues to be one of the world's leading strawberry-growing regions, producing fruit on around 15,000 hectares every year. The soil-borne infections *Verticillium dahliae*, *Fusarium oxysporum* f. sp. *fragariae, Macrophomina phaseolina*, and the perennial weed *Cyperus* *esculentus* have been the main causes of biotic stress production issues during the past ten years. Daugovish et al. (2021) outline cost-effective strategies for sustaining strawberry germination and production. These included non-fumigant approaches such as the development and use of resistant cultivars, soil solarisation (method of soil-disinfestation by covering soil with polyethylene sheet during summer to control soil pests), steam treatment, crop rotation, anaerobic disinfestation, soilless media, and the application of natural biocides.

1.9. Use of pesticides in strawberry production

Soil-borne pests, including diseases and insects, have significantly affected the yield of strawberries throughout history. Several management practices are introduced to deal with soil-borne pests. Despite their effectiveness, fumigants are recognised for their significant environmental impact, with methyl bromide being significantly identified as a direct contributor to ozone depletion. As a result, several alternative methods for soil treatment are introduced (Holmes et al., 2020). Spider mites are a major insect pest of strawberries, typically found in the emerging folded leaves where humidity is high. Most spider mites favour the underside of the leaves for laying their eggs, while during diapause, the females seek dark, sheltered areas close to the plant. Other potential pests for strawberries include thrips, tarnished plant bugs, beetles, and spotted wing drosophila. The overall pest management of strawberries includes an integrated system ranging from cultivation cultural practices to the use of chemical and biological controls (Lahiri et al., 2022). Other than insect pests, diseases are also responsible for causing losses in field production, while some diseases are harmful in the post-harvest period. These diseases are caused by fungus and grey mould (botrytis rot), which are usually managed by chemical fungicide spray (Fontana et al., 2021).

Anaerobic soil disinfestation (ASD) is an elective interaction that is utilised for soil treatment. This interaction involves anaerobic conditions during which soil disinfestation is carried out. It involves three main steps: adding a degradable carbon source to the soil, irrigating the soil to fill the pore spaces, and covering the treated area to prevent oxygen from reaching the soil, thereby creating anaerobic conditions (Muramoto et al., 2016). Steam is one more effective strategy for soil disinfestation. This technique incorporates sterilizing hardware, and the effective treatment needs to be at a 65°C temperature for 20 to 30 minutes (Fennimore & Goodhue, 2016). There have been limitations and restrictions regarding the cost of these techniques. Accordingly, strawberry cultivating in many parts of Europe is currently being driven toward soilless systems due to restrictions on the use of soil fumigants and increased microbial contamination, which has been exacerbated by rainfall that creates favourable conditions for infestations. (Holmeset al., 2020). Along with efficient strategies and methodology to treat the soil from soil-borne diseases, efficient methods include utilising host plant resistance, which is also considered to have great potential against soil-borne diseases (Holmes et al., 2020).

1.10. Conclusion

Strawberries are one of the essential fruits because of their various health implications for human beings, resulting in increased demand. Strawberries are being produced in many countries at the commercial level, where both conventional and modern cultivation techniques are being used. Different varieties, including drought-resistant and pest-resistant cultivars, have been introduced to meet the increasing demand for better yield and quality. Increased modernization has also resulted in the introduction of modern production technology, where the use of soilless culture in aeroponic systems has provided the growers with the option of a control system. There have been various improvements in the aeroponics system as most current aeroponic systems now utilise modern equipment that efficiently manages environmental conditions to provide optimum yield. Improvements in genetic technology have also resulted in the early production of flowers, leading to early and improved plant qualities, which have resulted in enhanced production. The combination of traditional and modern technologies to improve strawberry production has yielded productive and beneficial results, helping both researchers and growers meet the growing demand for the fruit.

1.11. Aims and Objectives

The main aims and objectives of this project are given below:

- Explore the impact of the vertical, aeroponic growing system on the growth of strawberries.

- Investigate the impact of different growth media (compost and rockwool) on the germination of strawberry seeds (cultivar Florian and Temptation).
- Optimise concentrations of Nutrient A and B (commercially formulated nutrient solutions) to monitor effects on growth.
- Explore in-vitro approaches to improve strawberry seed germination (Florian and Temptation).
- Investigate the performance of tissue culture-derived strawberry plants in an aeroponic system.

Chapter 2: An investigation of strawberry seed germination and plantlet growth in the aeroponic tower system

2. General Introduction

Aeroponics is a subset of hydroponics that was initially developed in the 1940s, but NASA's study into space missions' sustainable agriculture systems in the 1980s gave the technology a big boost. In contrast to conventional soil-based farming, aeroponic system grows plants in the air while misting them with nutrients. This technology allows for higher yields and quicker growth rates since it provides exact control over the water and fertilizer supply (Gurley, 2020). Aeroponics is a more appealing alternative for indoor and urban gardening because it lowers the danger of soil-borne illnesses and pests as it does not involve soil.

A vertical farming innovation, aeroponic towers maximize space efficiency by stacking multiple layers of plants. A closed-loop system to recirculate nutrient-rich water, minimize waste, and conserve resources is generally used (Behera et al., 2024). Researchers were among the first to adopt aeroponic towers, but the technology has since spread to commercial farming and urban agriculture. The towers have shown significant benefits in terms of crop productivity and water usage, making them a promising solution for challenges related to food security and sustainable agriculture in both urban and rural contexts (Mano et al., 2024).

Rockwool is especially popular among growers seeking to improve their systems. It has long been a widely used substrate in commercial horticulture for growing a wide range of crops, including tomatoes, melons, cucumbers, peppers, strawberries, herbs, and cut flowers. Melted basaltic rock is transferred into fine fibres, which are then shaped into various forms such as cubes, blocks, growth slabs, and granular products to create rockwool, a lightweight substrate used in aeroponic systems. While rockwool and other stone or mineral wools used in horticulture are often considered natural products due to their origin from root zone technology (basaltic rocks, which are processed to form water-absorbing rockwool), they provide excellent root zone technology (Fonteno & Nelson, 1990; Bussell & Mckennie, 2004). A series of experiments were conducted to monitor different growth substrates in terms of seed germination and transfer the germinated strawberry plantlets to aeroponic towers.

2.1. Experiment # 2.1: The impact of growth substrate (rockwool and compost) on the germination of strawberry (*Fragaria ananassa*) seed cultivar Florian

2.1.1. Introduction

The experiment was focused on observing the effect of growth substrates (rockwool and compost) on the germination, growth, and development of strawberry seeds. Does the change in growth substrate have any effect on seed germination, growth, and development?

2.1.2. Method

To conduct this experiment, strawberry (*Fragaria ananassa*) seeds (cultivar Florian), in compost and rockwool with and without nutrients, were sown. Nutrients are composed of nutrients A and B, a proprietary blend, i.e., nutrients by Future Growing Aeroponic Power-Gro dry mix A and B (Nitrates and inorganic minerals in powder form) dissolved in water with the 1:200 dilution. The rockwool with nutrients was soaked in diluted solution for 2 hours. The rockwool without nutrients was soaked in water for 2 hours to retain moisture before the seeds were sown. The concentrations of nutrients A and B are mentioned in Table 1. This research is carried out by the Sustainability Institute of UCC.

Table 1: Different concentrations of nutrient A and B

Different concentrations	Nutrient A	Nutrient B
Ammonia (mg N/L)	395	70
Nitrate (mg N/L)	77210	<1
Phosphate (mg P/L)	86	22949
Potassium (g K/L)	0.443	87.500
Calcium (g Ca/L)	78.0	0.010
Total Phosphorous (mg P/L)	101	24632
Total nitrogen (mg N/L)	104816	83

The seeds were moved to the growing substrate using forceps. Twenty seeds are in the compost tray, and 20 seeds are in rockwool, with and without nutrients. In each slot of rockwool, one seed was planted. Trays had small drainage holes and were covered with lids, i.e., large, high dome propagator covers. There were small holes in the lids for ventilation. HOBO data loggers were used in the greenhouses to track the humidity and temperature for experiments. The average temperature recorded during the experiment was 19.9 °C.

The strawberry seeds used in this experiment were sourced from "Unwin's strawberry (cultivar Florian)". The compost used throughout the experiments was sourced from Fruit Hill Farm (Klasmann peat-based compost for perennials). The rockwool was sourced from Greentowers, Ireland, and the experiment was initiated for 8 weeks. The compost tray was watered weekly with 100-150 ml of water.

2.1.3. Results- Experiment 2.1 Florian strawberry seed germination compost vs rockwool

Observations were recorded every week. The final observations were recorded in the 8th week. Out of the 20 strawberry seeds, 19 germinated in nutrient-rich rockwool, 19 in nutrient-free rockwool and 7 out of 20 seeds in compost. Compost showed increased and excessive weed growth, while bacterial growth was also observed in Rockwool with nutrients, as shown in **Figure 5**.

Figure 5. Weed growth in compost (upper left), grey arrows indicate bacterial growth in rockwool with nutrients (upper right), and strawberry seed germination in rockwool without nutrients (bottom).

Based on observation by week 10, 19 plants in rockwool without nutrients looked non-contaminated, so they were kept safe to be transferred to the aeroponic towers. Meanwhile, the rockwool with nutrients and the compost from experiment 2.1 were discarded because of increased bacterial and weed growth in rockwool with nutrients and compost, shown in **Figure 5**.

The experiment's outcomes revealed that Rockwool, with and without nutrients, was more efficient and promoted germination in Florian strawberry seeds than compost.

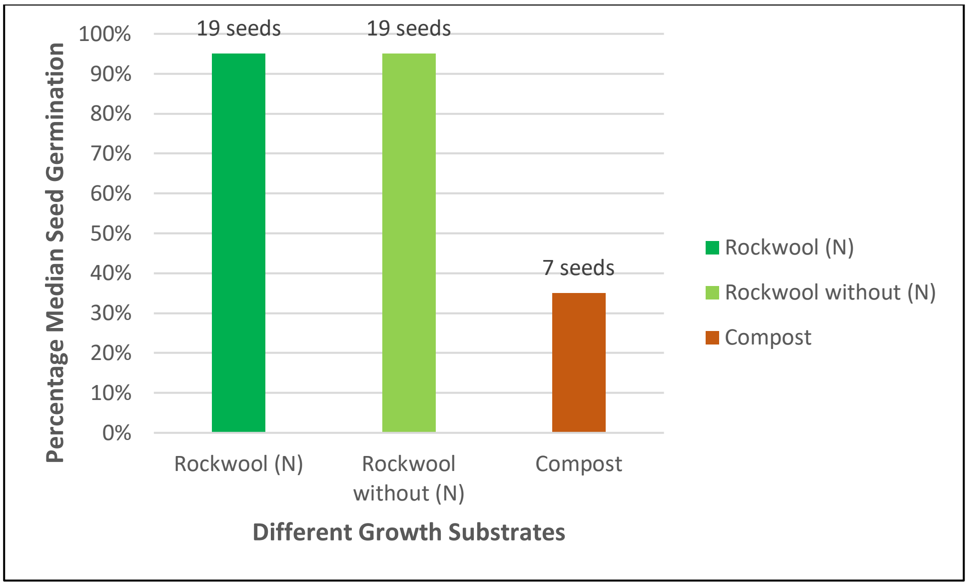

Figure 6. Percentage germination of Florian strawberry seeds in rockwool with nutrients, rockwool without nutrients, and compost.

The Statistical analysis (chi-square test) was done. Chi-square value 8.5 ($p \leq 0.05$) revealed that different growth substrates had a significant impact on strawberry seed germination (cultivar Florian). **Figure 6** shows an increased growth of seeds in rockwool in comparison to compost. Error bars are omitted in a few graphs due to a small sample size.

2.1.4. Discussion

The experiment demonstrated that rockwool is an effective substrate for germinating strawberry seeds, highlighting its significance and efficiency in facilitating seed germination. However, Reddy and Crohn (2018) used compost with 10% volume of rockwool and compost without the addition of rockwool for the growth of okra, tomato and chilli peppers. The results showed that the growth of all three crops was unaffected by the presence of 10% rockwool. Experiment 2.2 was designed because of the bacterial and weed growth in rockwool with nutrients and compost.

2.2. Experiment # 2.2: The germination rate of strawberry seeds in compost and rockwool

2.2.1. Introduction

Florian seed germination was delayed due to excessive bacterial growth in the rockwool with nutrients and excessive weed growth in compost in experiment 2.1. To observe the germination ratio of Florian seeds, experiment 2.2 was designed. The presence of different growth substrates such as rockwool and compost does or does not have a significant effect on the germination of strawberry seeds cultivar Florian.

2.2.2. Method

In experiment 2.2, 28 Florian strawberry seeds in each substrate were used in compost and rockwool without nutrients. The same methods from experiment 2.1 were followed here. In this experiment, the rockwool used lacked nutrients because the results of experiment 2.1 showed no significant difference in the seed germination of strawberries in rockwool. However, the rockwool, which had nutrients, did exhibit bacterial contamination.

2.2.3. Results- Experiment # 2.2: The germination rate of Florian strawberry seeds in compost and rockwool

Observations were recorded every week. Four out of 28 seeds germinated in compost over three weeks, while 11 seeds germinated in rockwool. The non-germinated seeds from both substrates were kept for further observation. However, no further germination was observed during the next 7 weeks in rockwool and compost, and thus substrates were discarded.

Figure 7. Discarded rockwool due to lack of germination.

The results of experiment 2.2 indicated that rockwool without nutrients was more effective in promoting the germination of strawberry seeds than compost, as shown in **Figure 8**. Statistical analysis (chi-square test) for experiment 2.2 revealed that the obtained value of chi-square was 7.78 ($p \leq 0.05$), indicating that there was a significant difference between the two treatments.

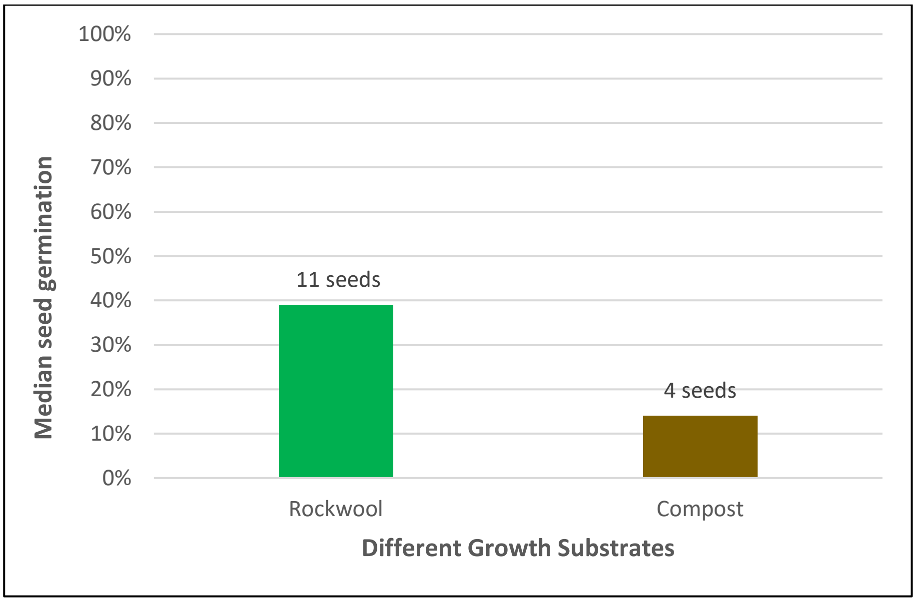

Figure 8. Percentage germination of strawberry seed in rockwool without nutrients and compost.

2.2.4. Discussion

This experiment was designed to observe the effect of different growth substrates on seed germination. For this purpose, seed germination in rockwool and compost was observed. The results of experiment 2.2 indicated that changing the substrates significantly affects seed germination. Campbell et al. (2020) conducted a study to check the impact of different parameters (temperature and substrate type) on the growth of butterfly peas in rockwool. The study's findings revealed that various other factors, including temperature, moisture, pH, etc, have significant effects.

The results from experiment 2.2 showed less germination in comparison to 2.1. The reasons may include seasonal differences in temperature, light, or other abiotic factors that can impact the reduced germination of seeds. Motsa et al. (2015) observed the impact of light and temperatures in spider flowers, non-heading Chinese cabbage, nightshade, pumpkin, tsamma melon, Jew's melon, and cowpea. Their study's findings indicated a significant difference in seed germination of leafy vegetables (i.e., the growth was observed) due to differences in light and temperature in different seasons.

2.3. Experiment 2.3: Transfer of strawberry plants from rockwool to aeroponic towers:

2.3.1. Introduction

This experiment was conducted to observe the growth of strawberry plantlets in the aeroponic tower and their performance and effectiveness. The

30 germinated strawberry plants from rockwool (without nutrients) were transferred to the aeroponic towers.

2.3.2. Method

The aeroponic towers were assembled following the instructions in the booklet by “Future Growing Aeroponic Power Gro”. The strawberry plants in rockwool without nutrients from experiments 2.1 and 2.2 were transferred to the aeroponic tower 1. The sheet of rockwool was broken into small square pieces, and each square piece of rockwool having one plant was transferred to each slot of the aeroponic towers using white clips, as shown in **Figure 9**. Each Aeroponic tower is 2.1m tall (about 7ft) with 9 sections; each section has 4 planting ports. Nineteen plants aged 13 weeks from experiment 2.1 and 11 plants aged 10 weeks from experiment 2.2 were used. All plants were treated identically from this point forward. The towers were equipped with a base pump that circulated water throughout the system. A small tube connected to a water reservoir served as both the water source and the return path for the water (Chowdhury et al., 2020). The towers were connected to timers set to turn on every 15 minutes within each hour, providing a regular and consistent watering schedule for the plants.

The parameters recorded throughout the experiment included the number of flowers, runners, and fruits on parent plants, the number of flowers and fruits on runners, as well as the dry weight of strawberry yield from both parent plants and runners, measured using a weighing scales.

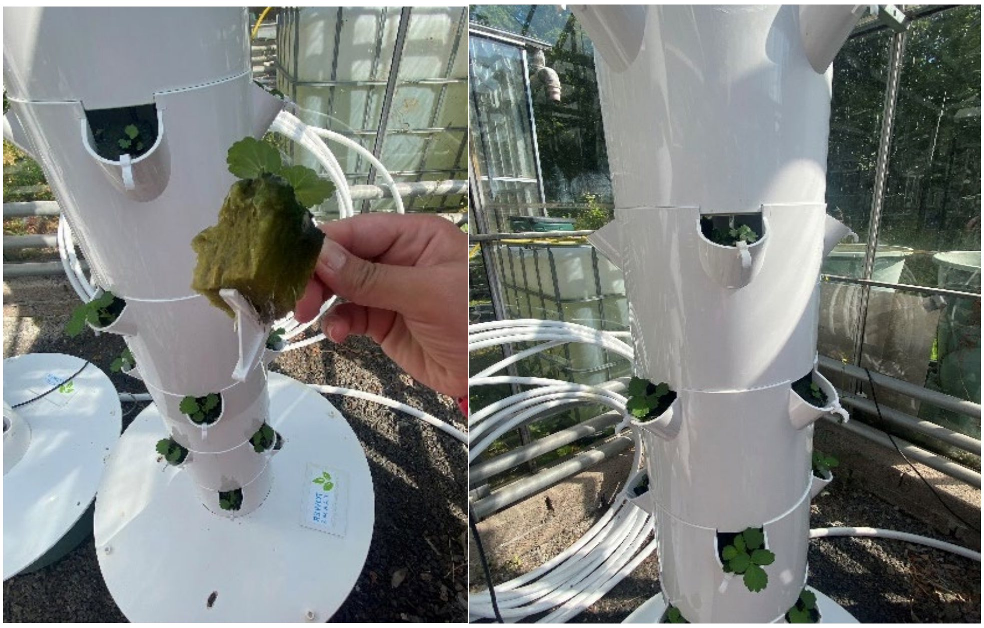

Figure 9. Strawberry plants in towers.

2.3.3. Results- Experiment # 2.3: Transferred strawberry plants to aeroponic tower

Observations were recorded weekly. By week 2, the plants were checked for any signs of pathology, such as fungal growth, as well as for general growth. No fungal growth was observed.

By week 8, some plants had begun to form runners, and no fungal or bacterial diseases were observed, as shown in **Figure 10**.

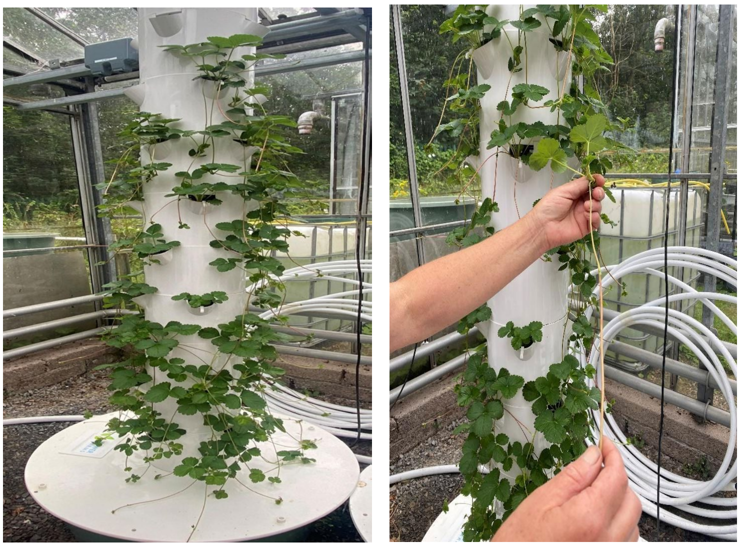

Figure 10. Strawberry plants in towers.

By week 9, the plants were labelled again so the data could be collected easily at the end. A few of the runners from parent plants 5, 9, 19, and 24 were transferred to the rockwool in the towers and labelled as runners. By week 10, runners from all parent plants were utilized to generate new plants, except for parent plants 4, 6, 30, 31, and 32, which did not produce any runners. By week 11, flowers appeared, and both green and red strawberries were formed on the parent plants. A few flowers and small green strawberries were observed on the runners, as shown in **Figure 11**.

Figure 11. (a) Growth of flowers and fruits in aeroponic towers, scale bar represents the length of one section of the tower, i.e 19cm with 4 planting ports. (b) growth of strawberry fruits on towers.

The towers were monitored to ensure optimal growth, and no signs of pests were detected until week 13. However, 2 to 3 bees were observed. No significant insect pests were detected. The aeroponic towers were designed with vertical slots stacked one above which indirectly promote self-pollination. In wind-pollinated or self-pollinating plants, the vertical layout of aeroponic systems enhances air circulation and conserves space, indirectly promoting healthier plants that are better suited for effective pollination.

By the 16th week, most of the strawberries had turned red, and nearly all of the flowers had developed into fruits. The fruit was subsequently harvested, as shown in **Figure 12**.

Figure 12. Fruits of strawberries. The scale bar represents the length of the tray and is 11cm long.

By week 17, the data was collected from the towers. The data obtained from the aeroponic tower experiment include observations from 32 plants. Among these plants median number of runners produced was 7. Additionally, the median dry yield recorded from the 32 parent plants was 6.38 grams.

The plants were subjected to drought; therefore, they were discarded. However, an infestation of spider mites was observed after data collection, which was controlled with miticide, as shown in **Figure 13**. After spraying, the leaves were observed under the microscope, and the spider mites were dead.

Figure 13. Spider mites' pesticide sprays and Drought plants.

2.3.4. Discussion

This experiment focused on observing the plant growth in aeroponic towers after the transfer of the strawberry plantlets. The outcomes of the experiment indicated improved plant growth and increased yield. The study of Peneyra and Peneyra (2024) also demonstrated that towers were useful in promoting plant growth by experimenting on *Lactuca sativa* L. The study supports the usefulness of towers in the experimental results. Although incorporating the supporting technologies, like root zone cooling systems, can enhance plant growth, their application should be tailored to meet specific plant requirements.

2.4. Experiment # 2.4: An investigation of lettuce seed germination in rockwool and compost

2.4.1. Introduction

Recognising the challenges in seed germination of strawberry cultivars, it was decided to focus on seeds with a shorter germination cycle. Experiment 2.4 was designed to investigate the germination rate of lettuce in two different growing substrates: compost and rockwool. Steps of experiment 2.1 were followed, ensuring that the methods were identical. This experiment, conducted with lettuce seeds, aimed to determine if the different growth substrates had a similar effect on seeds other than strawberry cultivars. Lettuce was selected for its rapid germination and growth. The experiment with lettuce was conducted to identify the role of rockwool in seed germination. The lettuce (*Lactuca sativa*) seeds, sourced from Demeter, were of the butterhead lettuce “Lucinde” variety.

2.4.2. Method

The same method was used for experiment 2.1 and experiment 2.2, but strawberry seeds were replaced with lettuce seeds. Three trays were used as replicates. Twenty-one lettuce seeds were used in each set of compost and rockwool with and without nutrients.

2.4.3. Results- Experiment # 2.4 Lettuce seed germination in rockwool and compost

By week 3, all of the lettuce seeds had germinated in each compost and rockwool set with and without nutrient rockwool, as shown in **Figure 14**.

Figure 14. Full germination of Lettuce seed in Compost from left to right, Rockwool with nutrients in the centre & rockwool without Nutrients from right to left. The scale bar represents the length of the green trays, and the length of scale bar is 35cm.

There was a 100% germination rate with lettuce seeds in compost and rockwool with and without nutrients. Statistical analysis (chi-square test) for experiment 2.4 revealed that the chi-squared value is 7($p \geq 0.05$,) which shows the change of substrates does not have a significant effect on the process of seed germination in lettuce.

2.4.4. Discussion

The experiment was focused on the impact of growing substrates on lettuce seed germination. The growth substrate was kept the same as 2.1 and 2.2, and the seeds were replaced by lettuce due to the low germination of strawberry seeds. The results showed that the growing substrate has no impact on the seed germination of lettuce. Rapid germination and growth of lettuce reduces the chances of contamination. These results can be supported by Choi et al. (2011), who state that using rockwool following moisture management can be efficient in the promotion of seed germination of lettuce.

These results indicate that different seed types react differently in different environments.

2.5. Experiment # 2.5: An investigation of the impact of two growing substrates, i.e., rockwool and compost, on the germination of an alternative strawberry cultivar.

2.5.1. Introduction

Due to the results obtained from experiment 2.4, experiment 2.5 was designed to monitor the impact of various growing substrates on a second strawberry cultivar, ‘Temptation’, seed germination and development processes in rockwool and compost. Strawberry (*Fragaria ananassa ‘Temptation’*) seeds were sourced from Verve and were bought from Co-Op. In this experiment, ‘Temptation’ seeds were used due to the increased germination time of ‘Florian’ seeds in previous experiments. Until this point, all the seeds used were ‘Florian’.

The experiment examines whether the change in the cultivars had a significant effect on seed germination, growth, and development (in compost and rockwool).

2.5.2. Method

For experiment 2.5, the same method as in experiment 2.1 was used, but with a change in the setup. Instead of compost and rockwool trays, six small pots of compost were used, each pot size measuring 5x5 cm^2 and was filled with 200 grams of compost; each pot contained five strawberry seeds (cultivar Temptation) and six pieces of rockwool (each piece consisted of 5 small squares and each square had one strawberry seed (cultivar Temptation). This change increased the sample sizes to ensure the accuracy of the results. The source of compost and rockwool is already mentioned in the experiment 2.1 method section. One pot of compost and one piece of rockwool was considered a replicate There were six sets, and a total of 30 seeds germinated in compost and 30 seeds in rockwool.

This experiment was designed to monitor the germination for 8-10 weeks.

2.5.3. Results- Experiment # 2.5 An investigation on the impact of two growing substrates, i.e., rockwool and compost, on the germination of an alternative strawberry cultivar

Observations were recorded every week. The final data recorded during the 10^{th} week showed germination in all six sets of rockwool and compost. Out of 30 seeds, 22 germinated in 6 sets of rockwool, and 14 seeds out of 30 germinated in compost, as shown in **Figure 15**.

Figure 15. Results of Seed Germination Week 10. The red arrow points towards rockwool, and the yellow arrow points towards compost. The scale bar is 5cm in length.

Based on observation and data collected at the end of the experiment, the seeds placed in rockwool show a higher germination rate than the compost substrate, as shown in **Figure 16**. A statistical analysis T-test was conducted, which shows the F-value 2.119 ($p \geq 0.05$). This suggests that there was no significant difference in germination of 'Temptation' seeds in rockwool compared to compost.

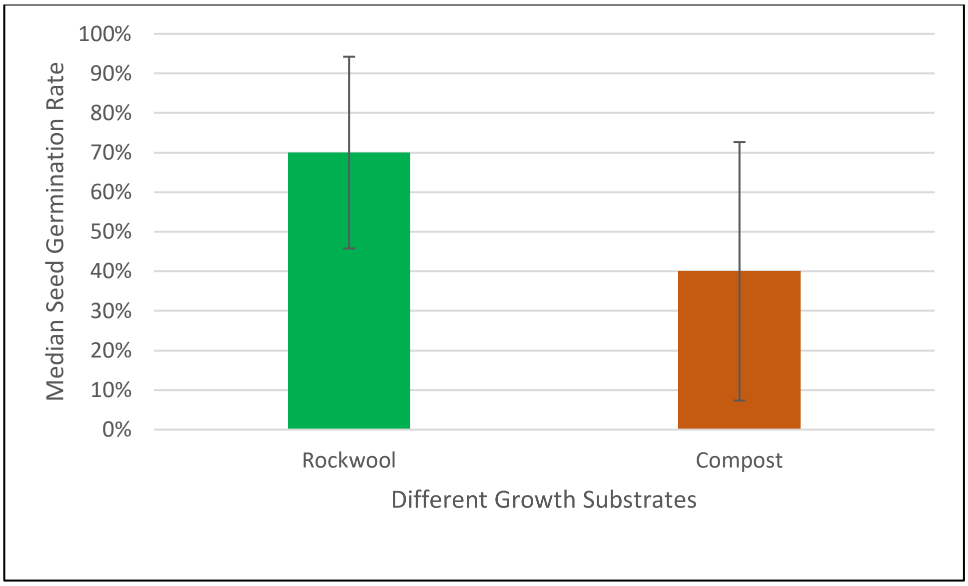

Figure 16. Median strawberry seed (cultivar Temptation) germination under different growth substrates.

2.5.4. Discussion

The results compared the germination rates of Temptation strawberry seeds in compost versus rockwool, indicating that while rockwool showed a higher germination rate overall, as shown in **Figure 16**, the statistical analysis revealed no significant difference between the two substrates ($p \geq 0.05$). According to Gallegos et al. (2024), the higher germination rates observed in rockwool could be attributed to their increased moisture retention and aeration properties compared to compost. This finding supports the notion that both rockwool and compost can be effectively used for the germination of Temptation strawberry seeds, providing flexibility in the choice of growing substrate based on other factors such as cost, availability, and sustainability. Ameri et al. (2012) analyzed the impact of different substrates (rice hull, sycamore pruning waste, cocopea and perlite (505:50 ratio), Vermicomposts, Perlite and Cocopeat (5:45:50), (15:40:45), (25:35:40) on different cultivars of strawberry (Mrak, Camarosa and Selva). The results revealed that Mrak had the highest ratio of production of malformed fruits in comparison to Camarosa and Selva.

2.6. Experiment # 2.6: Impact of different concentrations of nutrients in an aeroponic tower garden system on strawberry plants (Cultivar Temptation).

2.6.1. Introduction

This experiment aimed to observe the impact of different concentrations of nutrients A and B (mentioned already in experiment 2.1) in aeroponic towers having strawberry plants. The aim of this experiment was associated with the distribution of plants into three towers with different concentrations of nutrients A and B to observe the impact of nutrients.

Different concentrations of nutrients do have a significant effect on the growth of plants in the aeroponic towers.

2.6.2. Method

The same method from experiment 2.5 was followed. The only difference was that the towers were set with different concentrations of nutrients, as follows:

- Tower 1 was all set for CONTROL 50% nutrient A and 50% nutrient B following the concentration of nutrient dilution.
- 2nd tower 70% nutrient A 30% nutrient B
- 3rd tower 70% nutrient B 30 % nutrient A

Plants from experiment 2.5, grown in rockwool, were transferred to the aeroponic towers in the greenhouse, seven plants in each tower. So, three towers were set up with white lights due to the shortening of days (lights were turned on for 12 hours and were off for 12 hours during the day). The towers were set up with different concentrations of nutrients A and B, following the concentration of 1:200 dilution in water. The average plants from experiment 2.5 were transferred from the Rockwool to the new towers with light.

The following factors: Plant size, No. of petioles, No. of Runners and leaf size were observed among the plants in each tower.

Plant size was measured with a ruler in cm, and the Leaf area of one leaf in each plant was calculated in square millimetres using ImageJ (version 1.8.0).

2.6.3. Results- Experiment # 2.6 Impact of different concentrations of nutrients in aeroponic tower garden system in strawberry plants (Cultivar Temptation).

Observations were recorded weekly, and final data was collected after 6 weeks, which showed that 42% plants survived in the control tower. In tower 2, 28% of plants survived. In tower 3, 85% plants survived as shown in Fig 17.

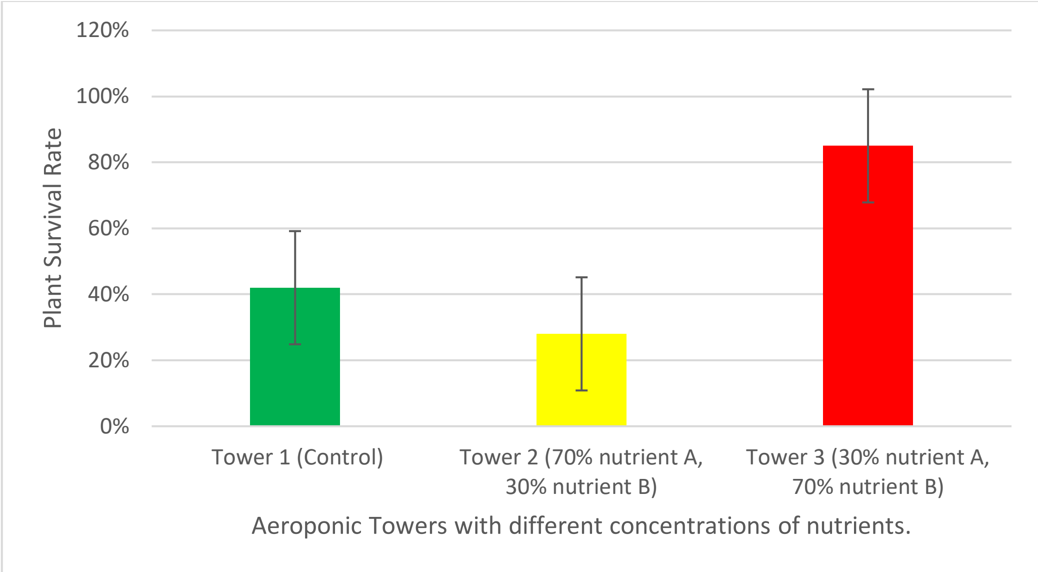

Figure 17. Plant survival rate (%) in Tower 1 (Control), Tower 2 (70% nutrient A and 30% B), and Tower 3 (30% nutrient A and 70% nutrient B).

Based on observation, more growth and large leaves were observed in tower 3 (30 % nutrient A, 70% nutrient B) compared to the control and tower 2, as shown in **Figure 18**.

Figure 18. Tower 1 from left to right (Control) Tower 2 in the centre (70% nutrient A and 30% B) and Tower 3 from right to left (30% nutrient A and 70% nutrient B).

Thus, few trials were made to observe the difference in (temptation) strawberry plants tower vise. The major difference between the plants in each tower was primarily the survival rate. Leaf and leaflet size. Further results of each tower are shown in the graphs below, following the factors mentioned in the method section above.

2.6.3.1. Impact of nutrients on strawberry plant height

The average height of every plant in each tower was calculated in cm. The height of the plants were measured with a measuring tape. The variation in the plant sizes across the towers was associated with the different concentrations of nutrients A and B, as shown in **Figure 19**. Statistical analysis ANOVA test was conducted, which indicated the F value of 2.7 ($p \geq 0.05$). This suggests that the different concentrations of nutrients do not significantly impact the plant size.

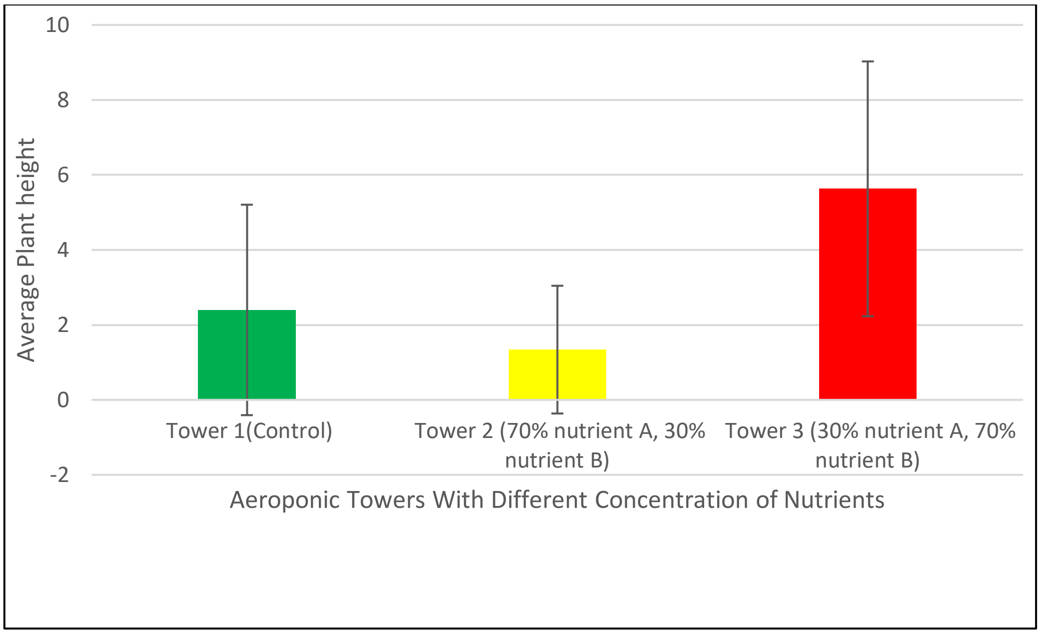

Figure 19. Average Plant height (cm) of every plant in tower 1 (control), tower 2(70% nutrient A,30%nutrient B), and tower 3 (30% nutrient A, 70% nutrient B).

2.6.3.2. Impact of nutrients on the number of Petioles in strawberry plants

The median no. of petioles of every plant in each tower was calculated by counting. The variation in the no. of petioles per plant in each tower was observed, as shown in **Figure 20** below. Statistical analysis ANOVA test was conducted, which indicated the F value of 4.48 ($p \leq 0.05$). This suggested that the different concentrations of nutrients significantly impact the no. of petioles in each tower.

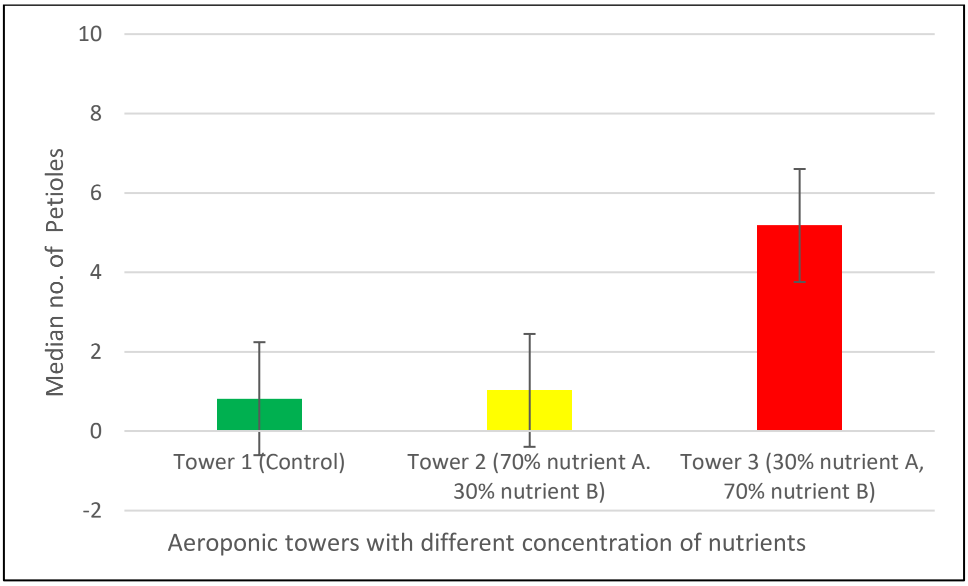

Figure 20. Median number of Petioles of every plant in tower 1 (control), tower 2 (70% nutrient A,30%nutrient B), and tower 3 (30% nutrient A, 70% nutrient B).

2.6.3.3. Impact of nutrients on the number of Runners

The median no. of runners in each tower was calculated simply by counting. **Figure 2**1 shows the difference observed in the no. of runners of each tower plant due to the different concentrations of nutrients. No runners production by strawberry plants in Tower 1 and 2 was observed. Statistical analysis Anova test was conducted, which indicated an F value of 8 ($p \leq 0.05$). This suggests that different concentrations of nutrients significantly affect the number of runners in each tower. Specifically, Towers 1 and 2 were significantly different from Tower 3, while Towers 1 and 2 did not show any significant differences from one another.

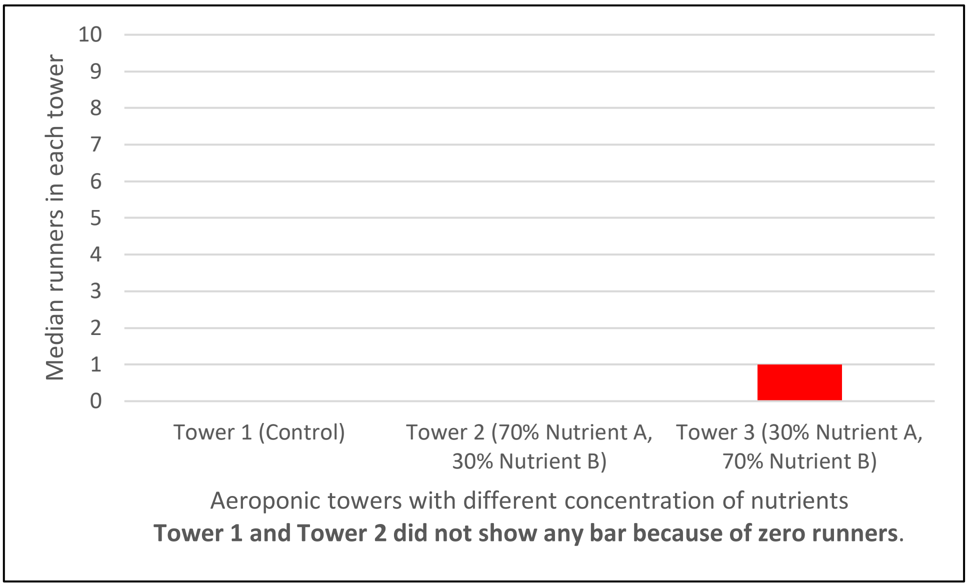

Figure 21. Median Runners in tower 1 (control), tower 2 (70% nutrient A,30%nutrient B), and tower 3 (30% nutrient A, 70% nutrient B).

2.6.3.4. Impact of nutrients on the Leaflet Area of the oldest leaf of each plant

One leaflet was taken from the oldest leaf of every plant of each tower. Image J was used to measure the leaflet area of each plant in towers 1, 2 and 3.

Figure 22. Leaflets taken from tower 1(left to right) (control left to right), tower 2 (70% A,30%nutrient B in the centre), and tower 3 (30% nutrient A, 70% nutrient B from right to left).

The median of each leaflet area was calculated and subjected to analysis. **Figure 23** shows the difference observed in the leaf size of each tower plant due to the different concentrations of nutrients. Statistical analysis Anova test was conducted, which indicated the F value of 11.4 ($p \leq 0.05$), depicting a significant difference. The outcomes showed that various concentrations of nutrients significantly differed in the plant's leaf area. Overall outcomes showed that tower 1 (control group) was not significantly different from tower 2. However, the outcomes of towers 1 and 2 significantly differed from those of tower 3.

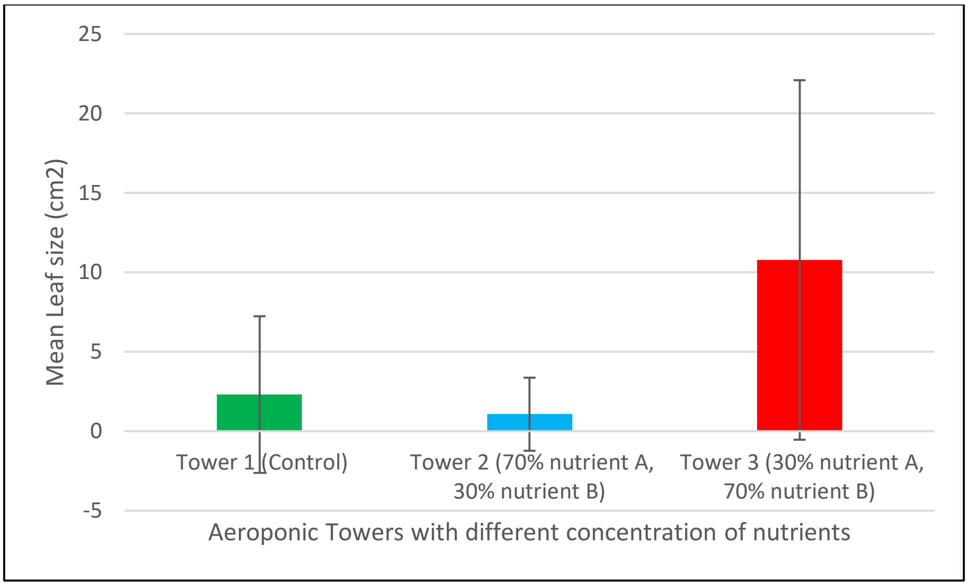

Figure 23. Average leaf area of plants in towers with different concentrations of nutrients.

2.6.4. Discussion

The findings show that different nutrient concentrations have a major effect on the development and growth of strawberry plants. In comparison to plants grown in Towers 1 (control) and 2, Tower 3, which had 70% nutrition B and 30% nutrient A, shows the strongest development, with bigger leaves and more runners. A considerable variation in leaf area between the towers was verified by statistical analysis ($p \leq 0.05$). Ahmad et al. (2017) revealed that phosphorus significantly influences the days to flowering of strawberry plants. Different increasing concentrations of phosphorus positively affect the growth, yield and fruit quality of strawberries. The results of increasing phosphorus concentration are consistent with the finding that improving phosphorus ratios (nutrient B, which has a higher phosphorus concentration than nutrient A) in aeroponic systems can improve strawberry growth. Due to delayed germination observed in strawberries, particularly Florian, in vitro experiments were designed to improve the germination process

Chapter 3: Enhancing in Vitro Strawberry Seed Germination: Strategies for Temptation and Florian Cultivars

3. General Introduction

Enhancing the germination of strawberry seeds in vitro is essential for increasing cultivation efficiency and promoting healthy plant growth. This chapter investigates various methods to optimise the germination of two strawberry cultivars, ‘Temptation’ and ‘Florian’. The significant reason for conducting these experiments lies in the delayed germination of strawberry seeds. Late germination was observed among strawberry seeds in previous experiments; therefore, a series of experiments were designed to monitor the effect of different treatments (below) on two different types of strawberry seeds in terms of seed germination (*in vitro*). By examining the impact of varying lighting conditions, temperature treatments, and chemical treatments, the aim was to identify the most effective strategies for promoting seed germination. These findings could significantly contribute to strawberry cultivation practices, offering insights into the optimal conditions required for the successful germination of these specific strawberry varieties. These experiments aimed to observe different parameters suitable for strawberry seed germination.

Three experiments were carried out to determine effective methods for enhancing the germination of strawberry seeds, specifically using the Temptation and Florian cultivars.

1. **Germination of strawberry seeds under different lighting conditions:** This includes red (630-750 nm), darkness, and white light.

2. **Pre-germination temperature treatments:** Seeds were stored at various temperatures for six weeks, including Cold 4 ºC, hot 25ºC, and a control monitored at room temperature.
3. **Soaking seeds in Ethephon before germination:** This experiment compared seeds soaked in Ethephon and contrasted them with non-Ethephon. Ethephon, an ethylene-releasing compound, when absorbed by plant tissues, is effective in overcoming seed dormancy imposed by the embryo, seed coat, adverse environmental conditions, or inhibitors (Weaver and R.J., 1972). Ethephon can be successfully used as a pre-harvest treatment to induce flowers, increase the proportion of pistillate flowers and improve yield and quality in various crops (Khan et al ., 2025).

   The experiment was based on the study of Sharpies (1973) and Qu et al. (2004) to determine the impact of ethephon on seed germination**.**

3.1. Experiment 3.1: Impact of Different Light Conditions on Strawberry Seeds Germination.

3.1.1. Introduction:

This experiment was designed to monitor the effect of different wavelengths of light on the germination of ‘Temptation’ and ‘Florian’ strawberry seeds over 8 weeks. The study aimed to determine how varying light conditions—specifically dark, red, and white light—affected the germination process. Red light, with wavelengths between 630-750 nm, was known to influence seed germination by promoting phytochrome activation, which can enhance germination rates and seedling growth. White light, encompassing a broad spectrum of wavelengths, provides a balanced light environment that supports overall plant development. The results could provide valuable insights for improving strawberry cultivation practices and enhancing germination success rates under controlled conditions. Based on the study by Nyachiro et al. (2002), a light treatment experiment tested the effects of different light conditions (white light, dark, and red light) on seed germination.

3.1.2. Methods:

The experiment was designed to place 10 Petri dishes of ‘Temptation’ and 10 Petri dishes of ‘Florian’ in each light, i.e., dark, red, and white light. A total of 150 Temptation’ and 150 ‘Florian’ strawberry seeds were used for the light experiment. HOBO data loggers were initiated using the HOBOware app. They were utilised to monitor the temperature in the red-light box, white-light tray, and dark environment, with the recorded median temperature being 20° for all three settings.

Five seeds of ‘Temptation’ and ‘Florian’ were placed in each petri dish. A set of 10 Petri dishes containing ‘Temptation’ and another set of 10 Petri dishes with ‘Florian’ were placed inside the wooden box, which featured a hole in the lid to put the Heliospectra LED light (HelioCore 2.0 Automated Light Control System). All of the Petri dishes lined with the filter paper were moist and sealed with parafilm. The Heliospectra was connected to a laptop, and the IP address 169.254.1.1 was used to set the Heliospectra on the required wavelength. The wavelength was set at 660nm. The light hours were set up for 16 hours on and 8 hours dark. The Heliospectra was kept at a 1-metre distance from the petri dishes, as shown in **Figure 24**. The data logger was placed inside the box to monitor the temperature every 30 minutes.

The same number of Petri dishes for white light was placed for both Temptation and Florian strawberry seeds. They were placed in the tray with the white light. The white light trays with ‘Temptation’ and ‘Florian’ seeds were connected to the timers (APT plug-in timers) for 16 hours of light and 8 hours of dark.

The same number of Petri dishes having ‘Temptation’ and ‘Florian’ strawberry seeds were placed for the dark light experiment, i.e., 10 Petri dishes, each having 5 seeds, were wrapped in aluminium foil to block light in the Petri dishes.

Figure 24. Chamber for strawberry seed germination in red light.

Figure 25. Strawberry seeds germinated in Petri dishes under white light.

3.1.3. Results

3.1.3.1. Impact of Different Light Conditions on Strawberry Seeds Germination 'Temptation'.

In order to analyse the impact of different lights on germination, observations were recorded every week. The final results were recorded, and statistical analysis was conducted. The graph in **Figure 26** and the outcomes of statistical analysis where the F value was 1.145 ($p \geq 0.05$) revealed that in the light experiment using 'Temptation' strawberry seeds, there was no significant difference in seed germination under different light conditions.

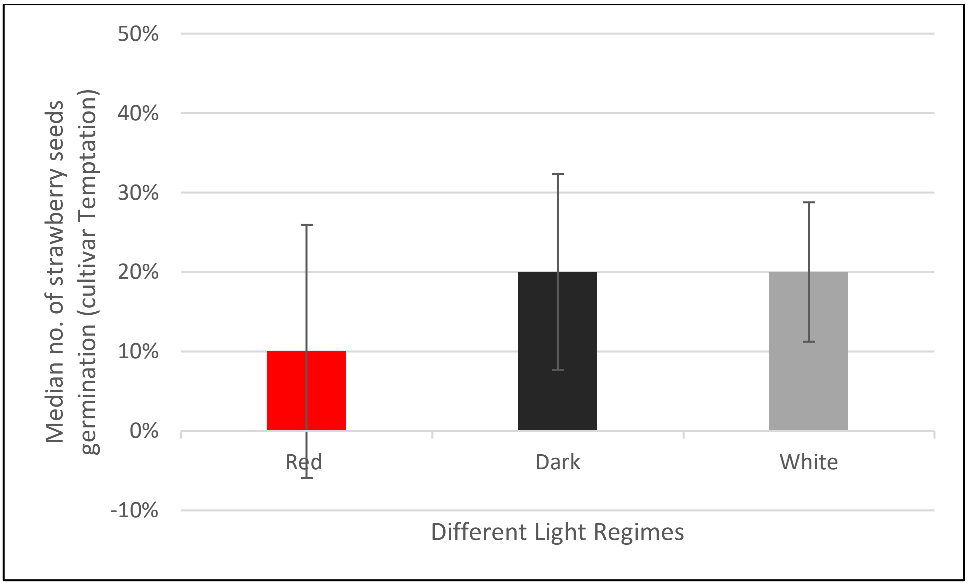

Figure 26. Percentage of strawberry seed germination 'Temptation' in different light regimes.

At the end of the experiment, under red light conditions, 10% seed germination across 10 Petri dishes, as shown in **Figure 27**. Under the dark light experiment, there was 20% seed germination, as shown in **Figure 28**. In white light, there was germination in all of the Petri dishes. 20% strawberry seeds germinated, as shown in **Figure 29.**

Figure 27. 10% Strawberry seed germination in red light. The scale bar is 9cm long and represents the diameter of pertri dish

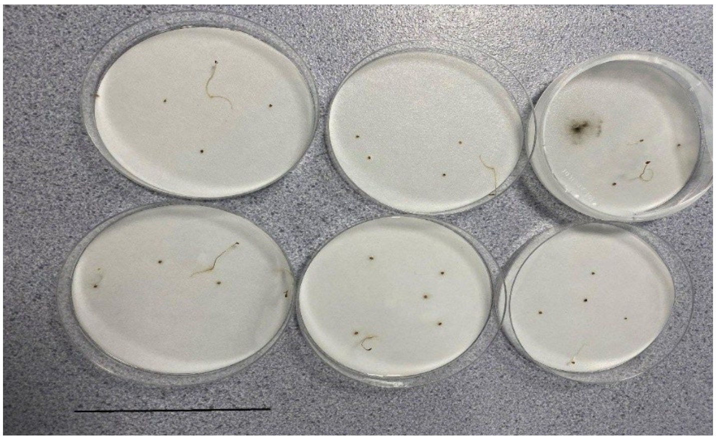

Figure 28. 20% Strawberry seed germination in a dark. The scale bar is 9cm long and represents the diameter of the petri dish

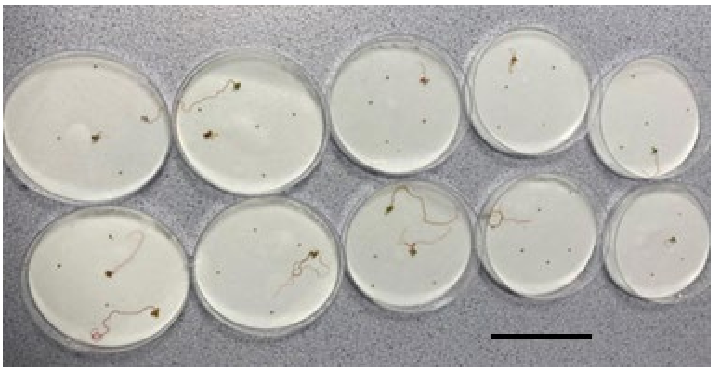

Figure 29. 20% Strawberry seed germination in white light. The scale bar is 9cm long and represents the diameter of the petri dish.

3.1.3.2. Results: Impact of Different Light Conditions on Strawberry Seeds cultivar Florian

This experiment was aimed to analyse the impact of different Light conditions on strawberry seeds cultivar florian therefore, observations were recorded every week. The final results were recorded, and statistical analysis was conducted. The graph in **Figure 30** and the outcomes of statistical analysis where the F value was 16.97 ($p \leq 0.05$) indicated that red light showed a statistical difference in germination rates compared to both dark and white because there was zero germination in red light. At the same time, there was no difference in germination between dark and white light conditions.

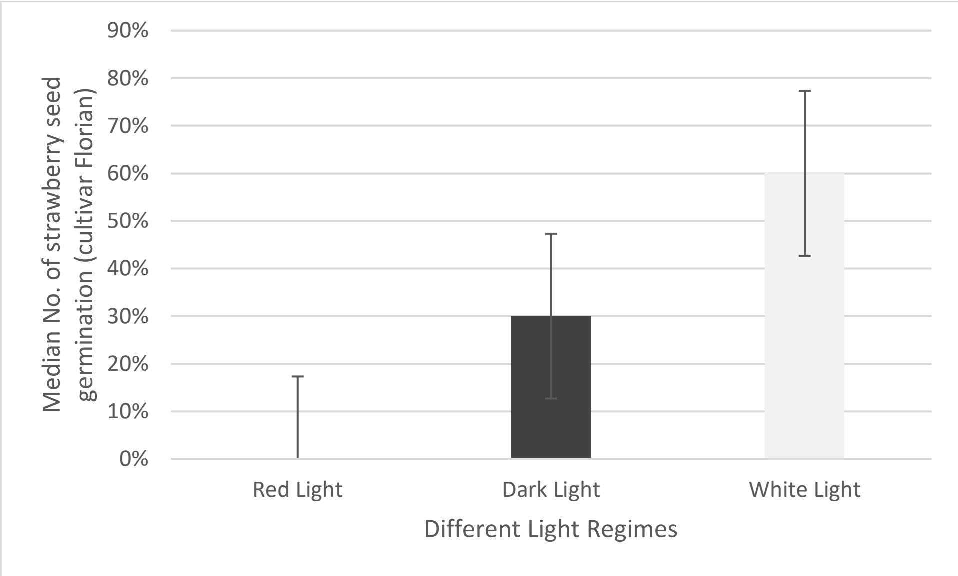

Figure 30. Median no. of strawberry seed germination 'Florian' in different light regimes.

At the end of the experiment, no germination was observed in any of the Petri dishes exposed to red light, as shown in **Figure 31**. In the dark, 30% of strawberry seeds germinated, as shown in **Figure 32** below. In the white light experiment, 60% seeds germinated, as shown in **Figure 33**.

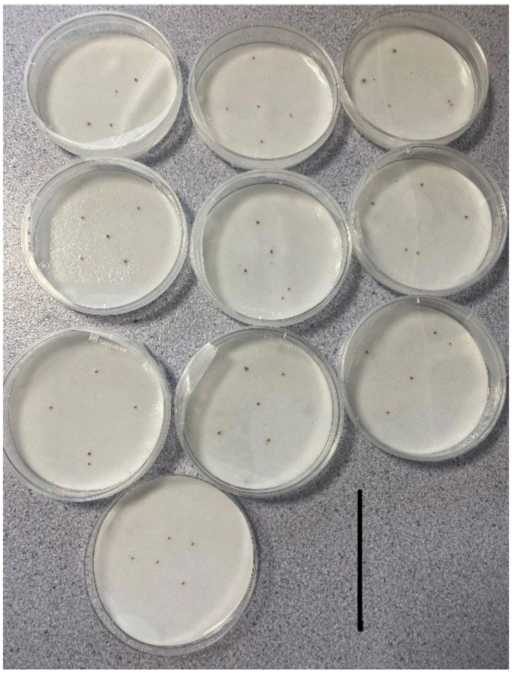

Figure 31. 0% of Strawberry seed germination in red light. The length of the scale bar is 9cm, representing the diameter of petri dishes.

Figure 32. 30% of Strawberry seed germination in dark. The length of the scale bar is 9cm, representing the diameter of petri dishes

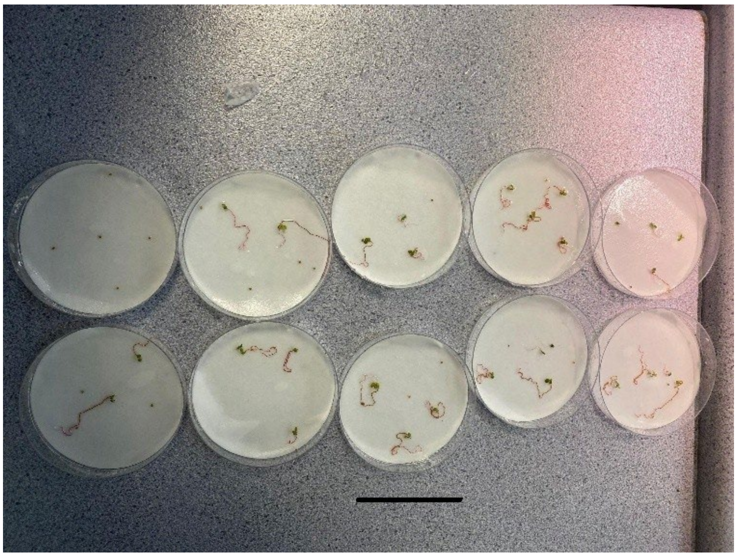

Figure 33. 60% of the Strawberry seed germination in white light. The length of the scale bar is 9cm, representing the diameter of the petri dishes

After the results were obtained, the Petri dishes that had been exposed to red light were subjected to white light. The seeds started germinating within two weeks in white light, as shown in **Figure 34**.

Figure 34. Strawberry seeds cultivar Florian in white light.

3.1.4. Discussion

The experiment revealed that light conditions affected germination significantly in terms of 'Florian' and non-significantly in terms of 'Temptation' strawberry seeds. In the case of 'Temptation' strawberry seeds, there was less germination in red light compared to white, i.e. 20%. Meanwhile, in 'Florian' strawberry seeds, no germination was observed in red light throughout the entire duration, indicating that red light (660 nm) was not conducive to seed germination. In contrast, seeds in dark conditions showed a moderate germination rate, with 30% seeds germinating by the end of the experiment. White light conditions were the most effective, with 60% seeds germinating, showing a consistent increase over the weeks. The statistical analysis confirmed significant differences in germination rates between the light conditions, with red light proving ineffective compared to both dark and white light, suggesting that white light is more beneficial for the germination of 'Florian' strawberry seeds. Regarding 'Temptation' strawberry seeds, there was no significant difference in the seed germination process under various light conditions. Tang et al. (2024) examined the impact of red light on seed germination of weeds (*Amaranthus retroflexus, Echinocloa crus-galli,* and

Digitaria adscendens). Their results indicated that seed germination of *Digitaria adscendens* was increased by red light, which is in contrast to the results found here. The study also included the impact of nitrogenous compounds, and the outcomes showed that the combined effects of red light and nitrogen had an increased germination rate. Solano et al. (2020) observed the impact of red light (640-670 nm) LED treatment. The results of the study revealed that red light did not affect the germination ratio or percentage of pea (*Pisum sativum* L.) and melon seeds (*Cucumis melo* L.)

3.2. Impact of Temperature Variations on Strawberry Seed Germination

3.2.1. Introduction

This experiment was designed to monitor the effect of pre-germination temperature on the strawberry seed cultivars ‘Temptation’ and ‘Florian’. Seeds were kept at different temperatures before germination.

Temperature is a key factor in seed germination, affecting metabolic processes and enzyme activity. For both cultivars, ‘Temptation’ and ‘Florian’ strawberry seeds, for germination, maintaining a consistent temperature of 20 ºC in the growth room was essential to ensure uniform conditions. This temperature is optimal for strawberry seed germination, promoting consistent seedling growth (Hammami et al., 2005). The temperature treatment experiment involved the exposure of seeds to high temperatures in an incubator and cold temperatures in a walk-in cold room. The control group was kept at room temperature. The temperatures chosen for the experiment are based on the work by Mallik and Gimingham (1985) as well as Budelsky and Galatowitsch (2002).

3.2.2. Method

This experiment was designed to monitor the effect of different temperatures on strawberry seed cultivars ‘Temptation’ and ‘Florian’. The seeds were treated before seed germination for 6 weeks. All the seeds were stored in brown paper bags at different temperatures for six weeks, after which they were transferred to the Petri dishes to germinate for 8 weeks. Each group of ‘Temptation’ strawberry seeds had 12 Petri dishes, having five ‘Temptation’ strawberry seeds in each Petri dish, and each group of ‘Florian’ strawberry seeds had 10 Petri dishes, having five ‘Florian’ strawberry seeds in each Petri dish as shown in **Figure 35** and **36**.

For the cold treatment, the strawberry seeds of ‘Temptation’ and strawberry seeds of ‘Florian’ were placed at 4°C in a cold room. For the heat treatment, seeds were placed in an incubator at 25°C. The third group served as a control; the seeds were kept at room temperature. HOBO data loggers were initiated and operated through the laptop using the HOBO ware app. For the control group, the temperatures were recorded every 30 minutes, and the average temperature was 22.5 ºC for the strawberry seeds control group cultivar ‘Temptation’ and ‘Florian’.

Upon transfer to the petri dishes, the seeds were placed in a growth room in white light under controlled conditions. The temperature was consistently maintained between 20-21°C, and the light was regulated to be on for 16 hours and off for 8 hours. These measures were taken to ensure the seeds were subjected to consistent and optimal growth conditions, thereby enhancing the reliability and validity of the experiment's results.

Figure 35. 'Temptation' strawberry seeds in Petri dishes in 3 groups: (a) cold from left to right, (b) control in the centre, (c) heat from right to left.

Figure 36. 'Florian' strawberry seeds in Petri dishes in 3 groups: (a) cold from left to right, (b) control in the centre, (c) heat from right to left. The length of the scale bar is 9cm.

3.2.3. Results

3.2.3.1. Impact of Temperature Variations on Strawberry Seeds Germination Cultivar 'Temptation'.

To observed the impact of temperature variations on strawberry seeds germination observations were recorded every week. The final results were recorded, and statistical analysis was conducted. The graph in **Figure 37** and the outcomes of statistical analysis where the F value was 1.31 ($p \geq 0.05$) revealed that in the temperature base experiment using 'Temptation' strawberry seeds, there was no significant difference in the seed germination process.

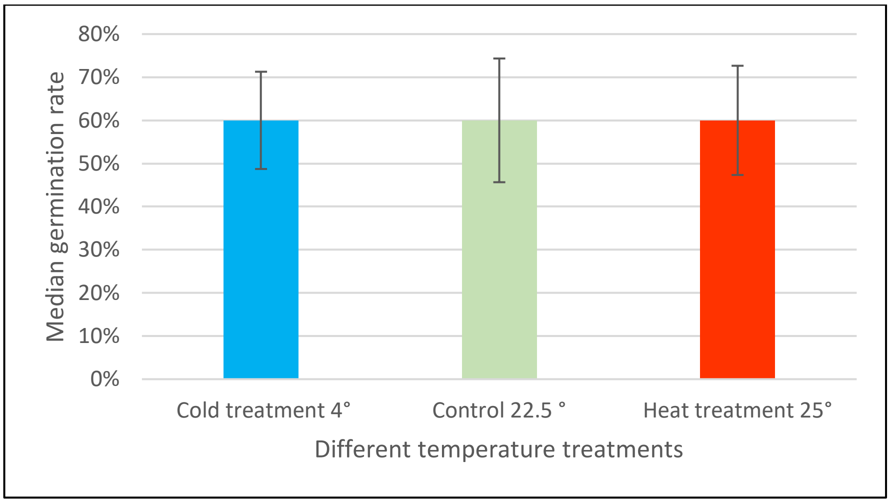

Figure 37. Median % seed germination cultivar Temptation under different temperatures.

Temperature variation observations were recorded every week. At the end of the experiment, there was germination in all petri dishes; in the cold, control and heat treatments, 60% seeds germinated, as shown in **Figure 38**, **Figure 39** and **Figure 40**.

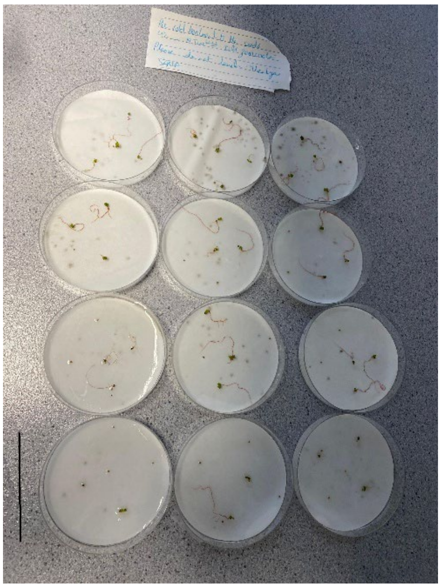

Figure 38. 60% of the Strawberry seeds germinated in the cold treatment. The scale bar represents the diameter of petri dishes i.e 9cm.

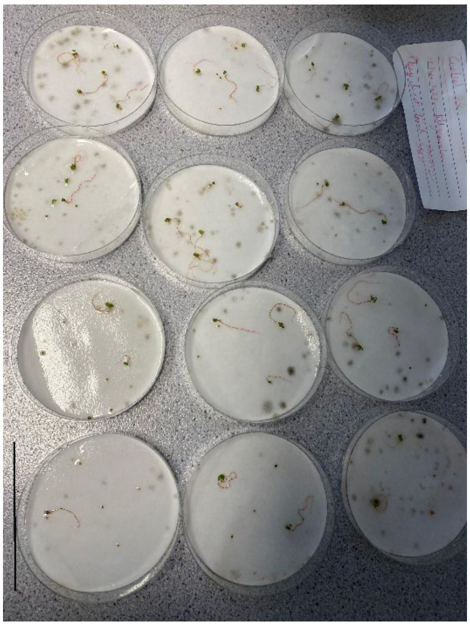

Figure 39. Strawberry seeds germinated in the control group. The scale bar represents the diameter of petri dishes i.e 9cm.

Figure 40. Strawberry seeds germinated in heat treatment. The scale bar represents the diameter of petri dishes i.e 9cm.

3.2.3.2. Results- Impact of Temperature Variations on Strawberry Seeds Germination Cultivar Florian

Observations were recorded every week. The graph in **Figure 41** shows some differences between different temperature treatments with 'Florian' strawberry seeds, but the outcomes of statistical analysis (ANOVA) where the F value was 0.685 ($p \geq 0.05$) revealed that in the temperature base experiment using 'Florian' strawberry seeds, there was no significant difference in the seed germination process.

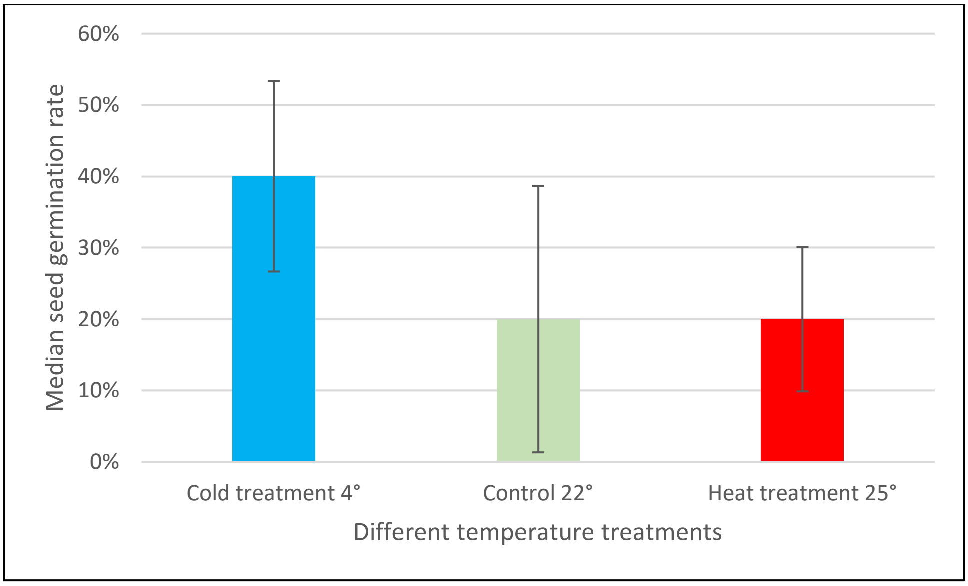

Figure 41. Median % seed germination cultivar Florian under different temperatures.

The final results were documented in the 8th week, while observations were recorded every week. In the cold treatment, 40% of the seeds germinated, as shown in **Figure 42**. In the control and heat group, there was 20% seed germination, as shown in **Figure 43** and **Figure 44**

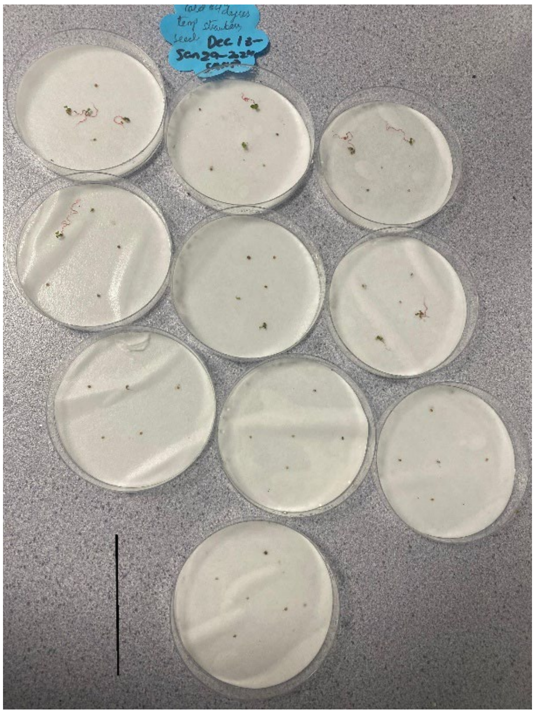

Figure 42. 40% of the Strawberry seeds germinated in the cold treatment. The scale bar represents the diameter of petri dishes, i.e 9cm in length.

Figure 43. 20% of the Strawberry seeds germinated in the control group. The scale bar represents the diameter of petri dishes, i.e 9cm in length.

Figure 44. 20 % of the Strawberry seeds germinated in heat treatment. The scale bar represents the diameter of petri dishes, i.e 9cm in length.

3.2.4. Discussion

The analysis of seed germination under different temperature treatments, cultivar Temptation and cultivar Florian strawberry seeds revealed no differences. For ‘Temptation’ seeds, no significant effect was observed across the temperature treatments. Germination rates were consistent across pre-cold, control, and pre-heat treatments, with no statistically significant differences. This indicates that temperature variations did not impact seed germination in cultivars ‘Temptation’ and ‘Florian’. Conversely, ‘Florian’ seed exhibited lower germination rates across treatments. However, these differences were not statistically significant. Overall, temperature treatments did not significantly influence germination rates of the strawberry variety cultivar Temptation and cultivar Florian. Khaeim et al. (2022) studied the impact of temperature on maize seedlings. The outcomes of the study showed that temperature has a significant effect on seed germination. These outcomes conflict with the outcomes of the current research, as temperature did not show any impact on strawberry seed germination. Liu et al. (2021) examined the impact of temperature on different grass species (*Festuca arundinacea, Bromus inermis, and Elymus breviaristatus*). The results of the study revealed that *F. arundinacea* did not show any significant difference under different temperature regimes, supporting the findings of the experiment. The study indicates the results of the current experiment, showing no significant effects of temperature on seed germination.

3.3. Impact of Ethephon on Strawberry Seed Germination Cultivars' Temptation and Florian

3.3.1. Introduction

The experiment was designed to monitor the effects of ethephon on strawberry seed germination in cultivars Temptation and Florian. The experiment was based on the study of Sharpies (1973) and Qu et al. (2004) to determine the impact of ethephon on seed germination. Seeds were soaked in an ethephon solution and germinated under controlled light and temperature conditions to assess the chemical's effectiveness. These experiments aimed to observe how different chemical treatments affect the germination of both ‘Temptation’ and ‘Florian’ strawberry seeds. Białecka and Kępczyński (2009) found that the application of ethephon significantly enhanced the germination of *Amaranthus caudatus* compared to the control group.

3.3.2. Method

This experiment was monitored for 6-8 weeks. Ethephon (0.14) grams was mixed in 1 litre of distilled water. Strawberry seeds cultivar Temptation was soaked in the solution for 10 minutes. Sixty strawberry seeds cultivar Temptation were treated with 1mM ethephon solution. The seeds were then transferred to the petri dishes containing filter paper moist with distilled water and sealed with parafilm, with five seeds in each petri dish. A total of 12 petri dishes were prepared. Sixty seeds of Temptation were directly transferred to petri dishes without any treatment and labelled as a control group. All the Petri dishes containing ethephon-treated and the control group were transferred to the growth room, where the light was on for 16 hours and off for 8 hours, with a constant temperature of 22°C. Similar method was used against Florian seeds; however, due to shortage of seeds on 50 Florian seeds were used.

3.3.3. Results

3.3.3.1. Impact of Ethephon on Strawberry Seeds Germination Cultivar Temptation.

At the end of the experiment, the final results were recorded, and statistical analysis (ANOVA) was conducted. The graph in **Figure 45** and statistical analysis where the F value was 0.542 ($p \geq 0.05$) revealed that in the ethephon-treated experiment using cultivar Temptation, there was no significant difference in the seed germination.

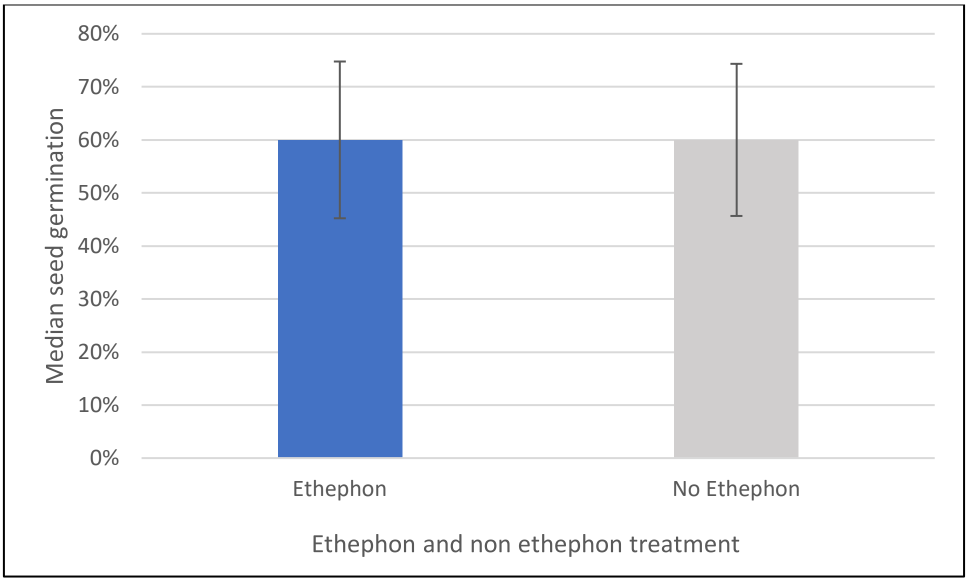

Figure 45: The median percentage seed germination of cultivar Temptation compared between ethephon-treated and untreated groups.

60% of seeds germinated in the ethephon-treated and in the control group experiment, as shown in **Figure 46**.

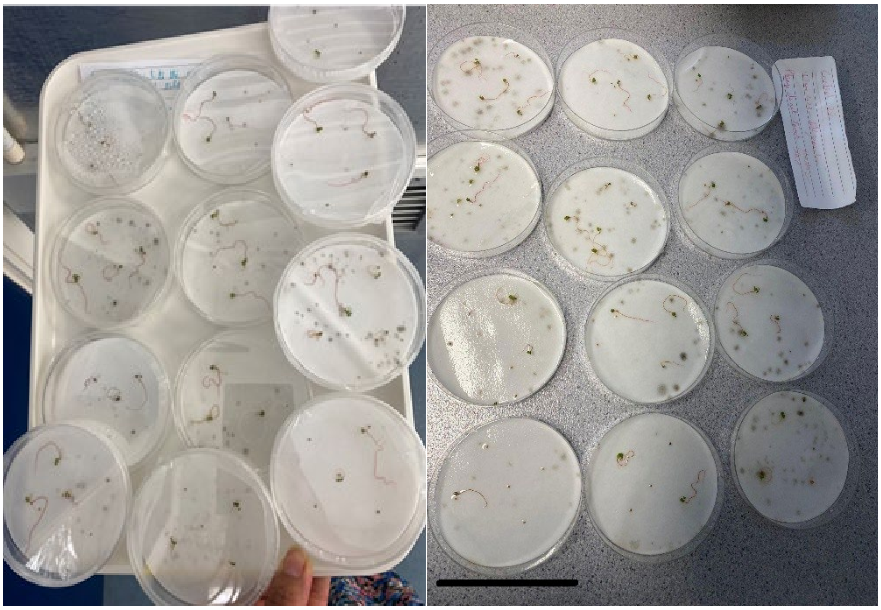

Figure 46. 60% of Strawberry seed Germination ethephon (left) and non-ethephon (right). The length of the scale bar is 9cm.

3.3.3.2. Results: Impact of Ethephon on strawberry seeds germination cultivar Florian

At the end of the experiment, the final results were recorded, and statistical analysis was conducted. The graph in **Figure 47** and statistical analysis (ANOVA) where the F value was 2.76 ($p \geq 0.05$) revealed that in the ethephon-treated experiment using the cultivar Florian, there was no significant difference in the seed germination process.

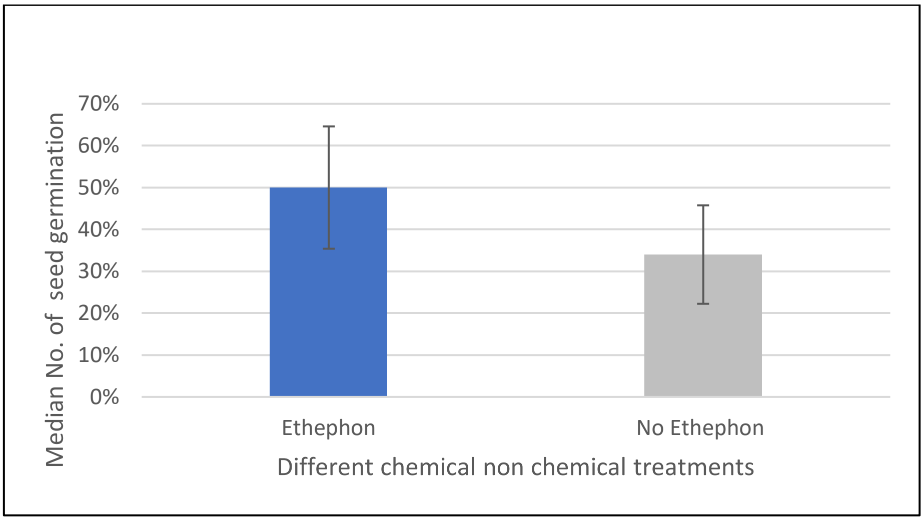

Figure 47. The median % seed germination rate of cultivar Florian compared between ethephon-treated and untreated groups.

50% of seeds germinated in the Ethephon-treated experiment, and 35% of the seeds germinated in the control group, as shown in **Figure 48**.

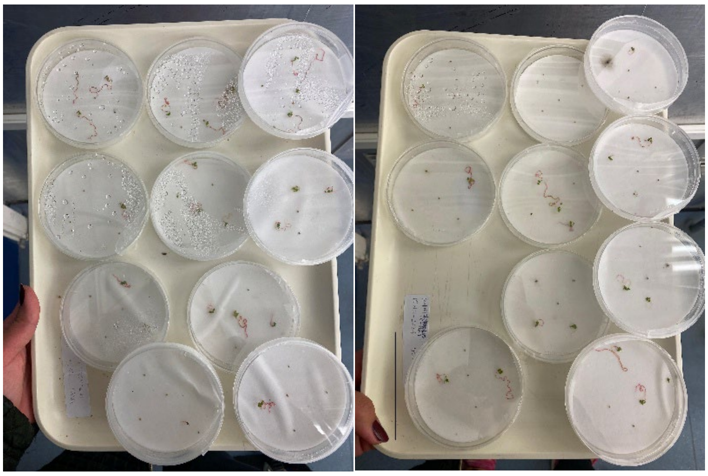

Figure 48. 50% of the Strawberry seeds germinated in ethephon-treated (left) and 35% of the strawberry seeds germinated in the control (right). The length of the scale bar is 9cm.

3.3.4. Discussion

The results from the experiments on strawberry seed cultivars Temptation and Florian treated with ethephon showed that ethephon did not significantly impact seed germination. Despite some variations in initial germination rates, the overall impact of ethephon on both strawberry seed types was minimal and not statistically significant. Based on observation, seeds treated with ethephon germinated more rapidly than those not treated with ethephon in both cultivars, Temptation and Florian. The study's outcomes were supported by the study conducted by De and De (2003). The study showed that ethephon application on *Trigonella foenum-graecum* can affect peroxidase activities and diosgenin content; however, ethephon did not show any significant difference in seed germination. At the same time, the research conducted by Qu et al. (2004) demonstrated that ethephon treatment significantly influenced the seed germination of *E.angustifolia* and *E. pallida*. Due to the statistically insignificant results from the Chapter 2 experiments and the complete lack of germination of the strawberry seeds cultivar Florian under red light, it was decided to use tissue culture techniques to obtain disease-free plants. This approach enables the transfer to aeroponic towers while skipping the seed germination process.

Chapter 4: Tissue Culture of Strawberries (cultivar Calypso): A Pathway to Sustainable Aeroponic Growth

4. Introduction

Tissue culture is a general term encompassing cultivating various plant parts, including cells or tissues, in artificial media while ensuring a controlled environment. Tissue culture techniques require the maintenance of sterile conditions and a consistent and optimal supply of nutrients and plant growth regulators to achieve the desired outcomes (Loyola-Vargas & Ochoa-Alejo, 2018). The study aimed to assess the effect of tissue culturing techniques on the strawberry plant (*Fragaria x ananassa)* cultivar Calypso. The tissue culture technique was utilised for the strawberry seed because the results from the previous experiments with 'Temptation' and 'Florian' were statistically insignificant, and the cultivar Calypso was already cultured in this laboratory. This chapter presents a series of experiments aimed at exploring the multiplication of 'Calypso' strawberry plantlets in different tissue culture media and the use of rockwool to identify any changes in the growth of plants during tissue culturing and, after the transfer of strawberry plantlets to the aeroponic tower.

General Methods

Tissue culturing is utilized for the rapid propagation of disease-free plants and the conservation of endangered species. The strawberry plant cultivar Calypso underwent tissue culture every four weeks in Shoot propagation media (SPM) and, once in shoot multiplication media (SMM). The preparation methods for both growth media are detailed below. For SMM growth media, Murashige and Skoog (MS) 4.4g/L, Sucrose (30g), Benzylaminopurine-6 (BAP) 0.5 mg/L, and agar (Daishin 9g/L) were used at 5.8 pH. For SPM, MS (4.4g), Sucrose (30g), BAP 0.1 mg/L, Indole-3-butyric acid (IBA) 0.1 mg/L, and agar (9g/L) were used at 5.8 pH (Schaart, 2014). The difference between SMM and SPM involves using IBA in SPM, which enhances root growth (Schaart, 2014). The tissue culturing technique was applied in rockwool to ease the transportation of plantlets in aeroponic towers.

4.1. Experiment # 4.1. Effective Multiplication of Strawberry Plants in SPM and SMM Media

4.1.1. Introduction

For experiment 3.1, the aim was to multiplicate strawberry plantlets cultivar Calypso. The tissue culturing was conducted every 4 weeks, once in SPM growth media and the next time in SMM growth media.

4.1.2. Methods

The experiment was monitored for 4 weeks. Each time, all equipment and the growth media were sterilized using an autoclave. The sterilized equipment, Magenta vessels, and growth media were transferred to the laminar flow hood. The growth media was transferred from a borosilicate glass bottle to 14 Magenta vessels (approximately 5ml per Magenta vessel). The prepared Magenta vessels were left at room temperature for the next 7 days to monitor if there was any contamination.

If there was no contamination, the laminar flow hood, with all sterilised equipment (forceps, blades, Petri dishes, and glass bead sterilizer), was prepared and turned on UV for 15 minutes.

The strawberry plants from SMM growth media were transferred to SPM growth media and vice versa.

The scalpel blade and forceps were used to cut the shoots of strawberry plants from SMM growth media and transfer them to SPM growth media vessels. The roots were removed, and the crown was trimmed into small plant shoots in a "V", ensuring that each had at least one leaf. A total of 14 Magenta vessels were prepared from 5 Magenta vessels (5-6 plants in each vessel). All the Magenta vessels were labelled and transferred to the plant tissue culture growth room, as shown in **Figure 49.** The temperature of the growth room was maintained at 21-22 ℃ with 16 hours of light on and 8 hours of light off.

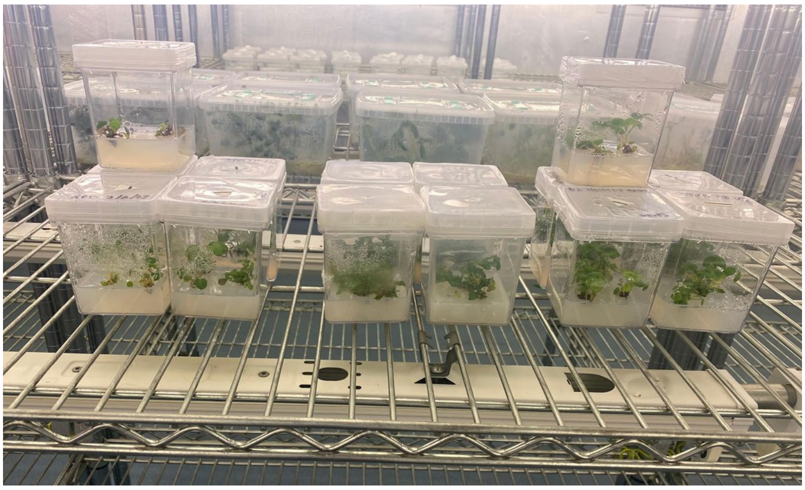

Figure 49: Tissue culture strawberry plantlets in SPM growth media.

4.1.3. Results: Effective Multiplication of Calypso Strawberry Plants in SPM and SMM Media

Observations were recorded daily to monitor any contamination. There was contamination in SPM edia. 6 out of 14 Magenta vessels were contaminated by the end of the experiment, as shown in **Figure 50**.

In the SMM media at the end of the experiment, 5 out of 14 Magenta vessels were contaminated, as shown in **Figure 51.**

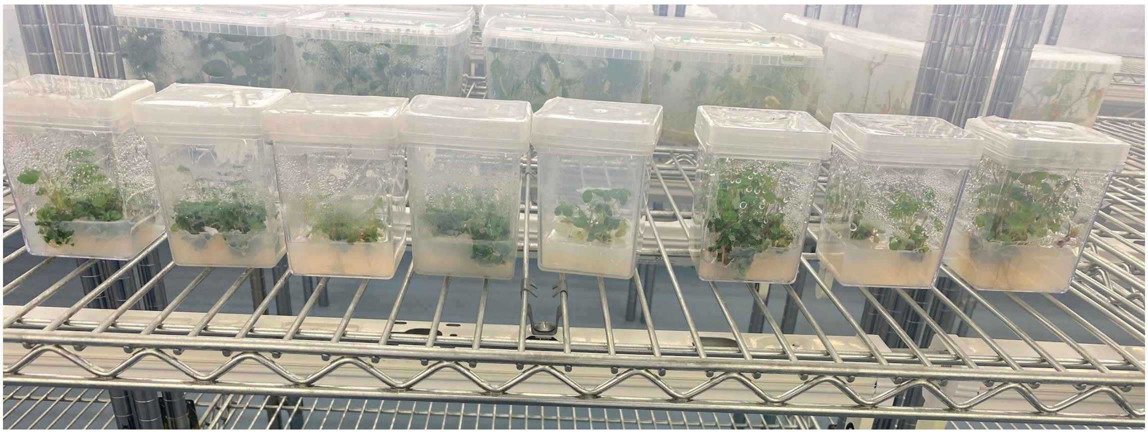

Figure 50. 8 Uncontaminated tissue cultured strawberry plants in vessels in SPM growth media.

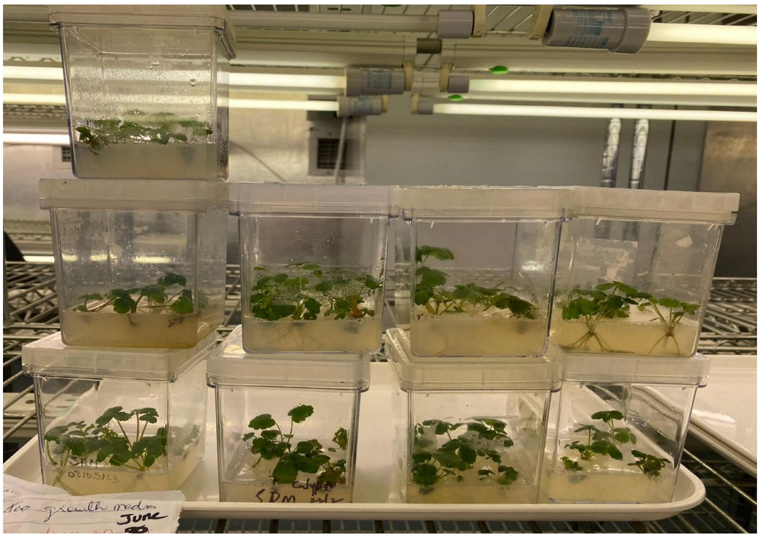

Figure 51. Uncontaminated tissue-cultured strawberry plants in SMM growth media.

4.1.4. Discussion

While many healthy, uncontaminated plants were produced, some of the Magenta vessels exhibited contamination. To reduce this issue, additional experiments were conducted to determine if charcoal could help minimise contamination. Furthermore, rockwool was utilised to facilitate the transfer of plantlets from the tissue culture system to aeroponic towers.

4.2. Experiment # 4.2: Exploring Rockwool as a Substrate for Strawberry Tissue Culture

4.2.1. Introduction

Plants produced through tissue culturing techniques encounter several challenges, with acclimatization and susceptibility to diseases being major concerns. Initially, the plants were cultured in SMM and SPM to obtain disease-free plants. However, to facilitate the transportation of plants to aeroponic towers, rockwool was used as a growth medium. The use of rockwool for tissue culture has received little attention, with some experiments conducted in the late 90s. This experiment was designed to examine tissue culture in rockwool and address these issues. SUKSA-ARD et al. (1998) experiment involved tissue culturing papaya in rockwool to acclimatize the plants. The highest rooting rate was observed in starch media, followed by rockwool.

The tissue culturing of calypso strawberry plant cultivar was conducted in the SPM growth media without agar. Following the experimental aim, rockwool with liquid media was utilised instead of solid growth media.

4.2.2. Methods

This experiment was monitored for 4 weeks. Daily observations were recorded. Two square pieces of rock wool were placed in each Magenta vessel. The SPM growth media, prepared without agar, was transferred to the magenta vessels and then autoclaved. Fifteen sterilized magenta vessels containing rockwool were prepared for tissue culturing of the strawberry plants' cultivar Calypso. The Magenta vessels were kept and sealed for 2 weeks at room temperature before the tissue culturing process. None of the 15 Magenta vessels prepared containing Rockwool exhibited any contamination. The earlier tissue culturing procedures were adhered to, ensuring that contamination in the plants was prevented throughout the process, as shown in **Figure 52**.

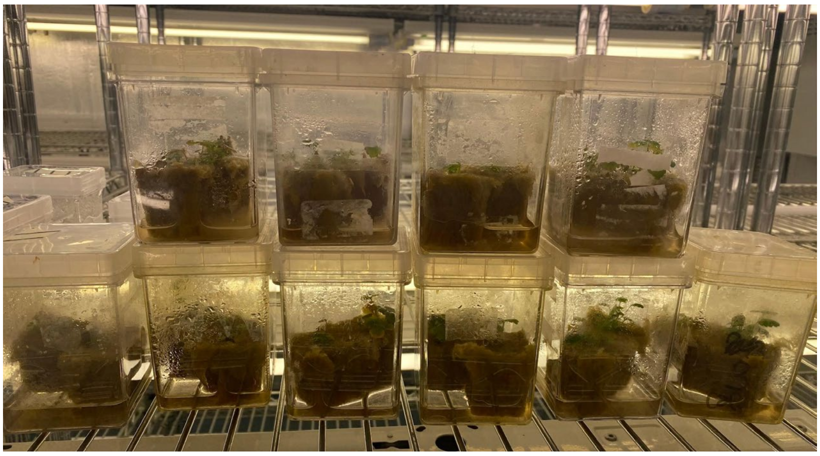

Figure 52. Tissue-cultured strawberry plants in SPM growth media in rock wool.

4.2.3. Results: Exploring Rockwool as a Substrate for Strawberry Tissue Culture

The contamination began in the first week, and it continued to increase over 4-5 weeks, as shown in **Figure 53**. The contamination was likely fungal; therefore, samples were analysed using PCR to identify the specific species responsible. QIAGEN kit was used for PCR, with the Plain Combi PPP Master Mix serving as a buffer during the procedure. All the species identified in the Magenta vessels were members of the fungal family, listed as follows.

Figure 53. Contamination on rock wool during tissue culturing.

Table 3. Fungal species identified in Contaminated Strawberry Plants within Magenta Vessels.

Bar code	Identified Species	Ribosomal (RNA) Primers
48733644	Cladosporium species	ITS1
48733651	*Tramates* species could be *versicolor* or *ochracea*	*ITS1*
48733668	*Phlebia* species could be *radiata* or *rufa*	*ITS1*
48733675	*Cladosporium* species; narrowed down probably to *Cladosporium cladosporioides,* but could be another species	*28S*
48733682	*Tramates* species; could be *versicolor* or *ochracea* or another one	*28S*
48733699	*Phlebia* species could be *radiata* or *rufa*	*28S*

Only four Magenta vessels containing eight uncontaminated strawberry plants were retained and transferred to a separate aeroponic tower after the acclimatisation stage. Although there was no visible contamination, all the plants died in two weeks, as shown in **Figure 54**.

Figure 54. Dead Tissue cultured strawberry plants cultivar Calypso in Aeroponic towers.

4.2.4. Discussion

This experiment aimed to observe the growth of strawberry plants in rockwool. However, the study did not yield significant results due to contamination found in the rockwool. Although some plants that showed no visible contamination were transported to towers, they died within two weeks, yielding no significant results. Tissue-cultured plants differ from other plants due to the specific environmental conditions found within a tissue-culture container (Capellades et al., 1989).

4.3. Experiment 4.3: Impact of Activated Charcoal on Tissue-Cultured Strawberry Plants

4.3.1. Introduction

In the previous experiment, contamination was found in rockwool; therefore, a new experiment was designed that included charcoal in solid media. Activated charcoal consists of carbon arranged in a quasi-graphitic structure with a small particle size. It is a porous, tasteless substance heated at a very high temperature that differs from elementary carbon due to the removal of non-carbon impurities and the oxidation of its surface (Mattson & Mark, 1971). Activated charcoal has demonstrated properties that help reduce contamination; activated charcoal is considered to have antimicrobial properties, making it effective in reducing contamination (Di Lonardo et al., 2013). Therefore, this experiment was designed to observe the impact of activated charcoal during tissue culturing.

4.3.2. Methods

This experiment was conducted for 4 weeks. In this experiment, eleven Magenta vessels were prepared with SMM growth media, protocols from experiment 4.1 were adhered to, with the slight modification being the addition of 5 g/L of activated charcoal in the growth media before autoclaving, and eleven Magenta vessels were prepared with SMM growth media that did not contain activated charcoal.

4.3.3. Results: Impact of Charcoal on Tissue-Cultured Strawberry Plants

Observations were recorded daily to monitor contamination. Out of eleven Magenta vessels with activated charcoal, there was contamination in only one Magenta vessel. In contrast, in Magenta vessels without charcoal, there was contamination in 4 Magenta vessels out of 11, as shown in **Figure 55**. The graph below, **Figure 56,** indicates the difference in the contamination levels with and without the addition of activated charcoal. A chi-square statistical analysis was performed where the F value was 1.075 ($p \leq 0.05$), which revealed a significant difference between the two conditions.

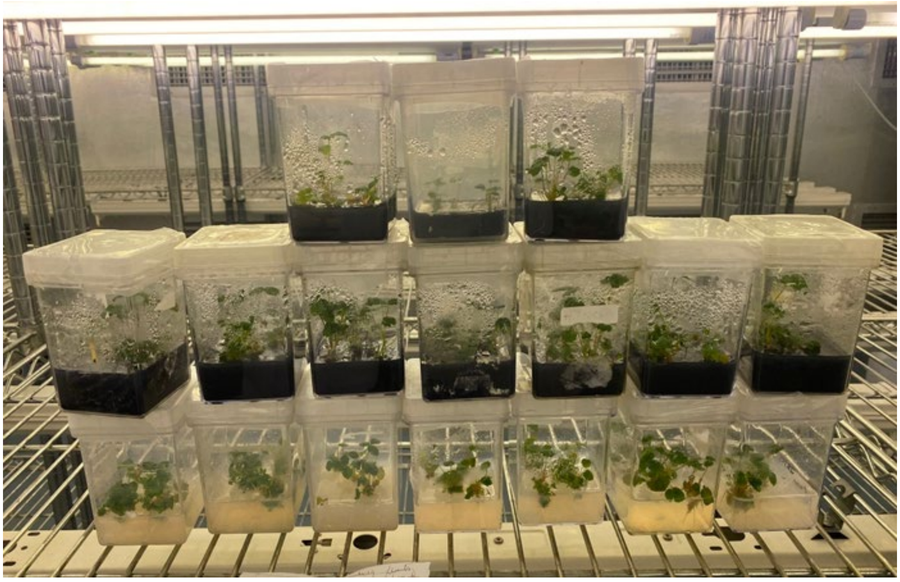

Figure 55. Uncontaminated Magenta vessels with and without the addition of charcoal.

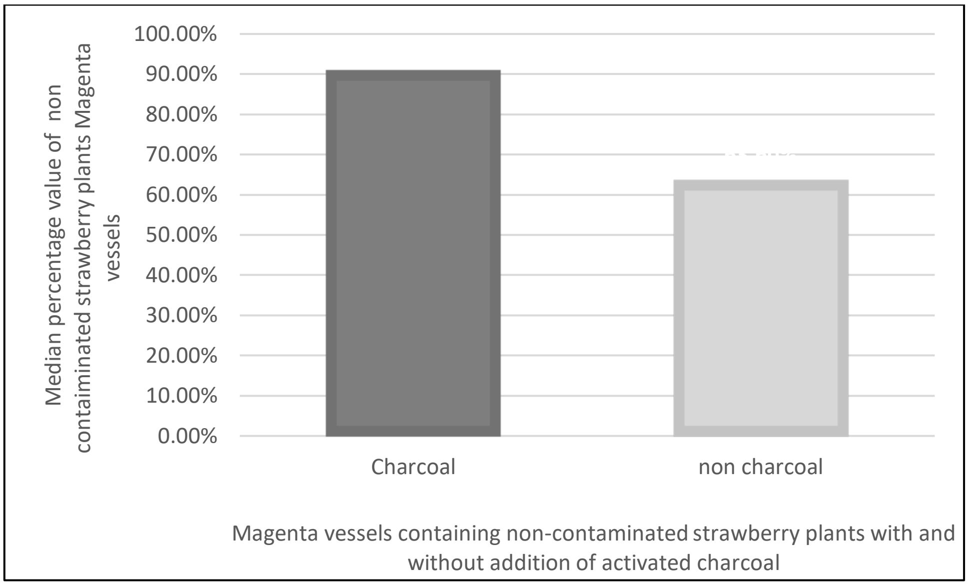

Figure 56. Comparison of Strawberry Plant Growth in Magenta Vessels with and without the addition of Activated Charcoal.

4.3.4. Discussion:

The study's results indicated that activated charcoal had a significant effect on reducing contamination in tissue culture. Activated charcoal reduces plant contamination by adsorbing organic chemicals and other pollutants, which keeps them from contaminating soil and water (Hilber et al., 2009).

Additionally, activated charcoal is known to enhance and adjust the pH levels, which can influence the growth of certain organisms. Activated charcoal can help retain nutrients, promote better plant growth, and reduce vulnerability to contaminants. Its addition to the media used for growing strawberries may aid in lowering contamination levels and enhancing overall plant development (Chaemsai et al., 2016).

4.4. Experiment 4.4 Exploring Rockwool as a Substrate for Strawberry Tissue Culture plants with the addition of charcoal

4.4.1. Introduction

The results of experiment 4.2 indicated that there was a lot of contamination in the tissue-cultured plants within Magenta vessels and no survival of tissue-cultured plants in the Rockwool within the aeroponic towers. Experiment 4.3 showed a significant reduction in contamination with the addition of activated charcoal. As a result, a new experiment was designed to incorporate activated charcoal to reduce contamination in the tissue-cultured plants within Magenta vessels.

4.4.2. Methods

For this experiment, SPM growth media was prepared without agar, incorporating 5(g/L) activated charcoal and rockwool substrate, and the rockwool was autoclaved twice to ensure proper sterilisation. The Magenta vessels were left at room temperature for 2 weeks. After 2 weeks, strawberry plants (from experiment 4.3 in SMM solid media) were transferred to autoclaved rockwool with activated charcoal.

4.4.3. Results: Exploring Rockwool as a Substrate for Strawberry Tissue Culture plants with the addition of charcoal

In total, fifteen Magenta vessels were prepared, of which seven were found to be contaminated. After completing the acclimatisation stage, sixteen plants that had been grown in the uncontaminated Magenta vessels were moved to the aeroponic towers.

After one week, the plants started to die gradually, with no visible signs of contamination; only two plants survived; plants that survived also demonstrated successful rooting, as shown in **Figures 57** and **58**.

Figure 57. Death of the plants in the tower.

Figure 58. Plants that survive in the tower.

4.4.4. Discussion

The experiment results indicated that rockwool can be used for tissue culture to grow and propagate plants, but the success ratio is very low. Only two plants survived in aeroponic towers in rockwool, suggesting that other environmental factors may be affecting their growth. This highlights the need for further experimentation in future work. Previously, the use of rockwool has been used by Suksa-Ard et al. (1998) to observe the acclimatisation of papaya. However, the rooting rate improved with higher concentrations of agar and gellan gum. There is a need to explore the potential of using rockwool in tissue culture. There is a need to observe and implement different weaning techniques to improve the success rates.

Chapter 5: General Discussion and Conclusion

5.1. Discussion

Strawberries are cultivated commercially in temperate and tropical zones across the globe, and their production has increased due to increasing consumer demands (James et al., 2022). However, strawberry growers face several issues, including increased labour costs, susceptibility to diseases, and pest attacks (Guthman & Jiménez-Soto, 2021). Most strawberries are propagated from runners rather than directly from seeds because of the low germination rate and other challenges (e.g. seed health) (Walter et al., 2005). The current experiments were designed to assess the effects of various growth media on strawberry seed germination, growth, and development, providing valuable insights into the use of substrates like rockwool and compost. As a preliminary work related to the experiments and to gain insight into the cultural practices surrounding strawberries, an interview with Bushby's Strawberry Grower 'David' was conducted

The interview focused on their cultivation methods, the types of strawberry cultivars they use, and their seasonal and cultural practices. The growers noted that most strawberry fields were planted with plants imported from Holland. They prefer strawberry cultivars Elsanta and Santa because of their best growth. Additionally, the grower cultivates strawberries over three months to harvest the fruit. Typically, biological control methods are employed to manage pathogens and pests. For the fertilization process, Fortafol is a biostimulant which is formulated as a water-soluble emulsion and contains humic and fulvic acids and plant extracts from Thymus and Mentha (Jackson et al., 2019). It is used for strength, while Agrolution Special serves as a nutrient supplement. Safe pesticides, such as Koppert Trianum P, are also utilized to combat soil-borne diseases. The commercial product Trianum-P is a registered plant protection product for the control of plant diseases in growing media. It contains the Trichoderma harzianum strain T22, which is arguably the most widely applied beneficial fungus in commercial horticulture (Harman et al., 2004a). These practices have helped the farmers enhance their yields.

The aeroponic tower system experiments were designed to observe the growth rates of strawberry seeds (cultivar Florian) and (cultivar Temptation) using different growth substrates. The study aimed to investigate the impact of different growth substrates (compost and Rockwool) on the germination of strawberry seeds (cultivars Temptation and Florian). The literature has demonstrated that rockwool is a widely used commercial horticultural substrate due to its ability to promote root development and provide a stable, effective environment for plant growth (Madhavi et al., 2021). The study findings align with Madhavi's work, which revealed that rockwool significantly influenced strawberry seed germination. Study monitored various growth parameters, such as runners, leaf size, and overall plant growth. Section 2.6.3 indicates that nutrient B was more effective in promoting plant growth compared to nutrient A in the aeroponic towers. An increase in the concentration of nutrient B led to a notable enhancement in plant growth, as evidenced by larger leaves and a greater number of runners in promoting plant growth compared to nutrient A in the aeroponic towers.

Another objective was to investigate the impact of the vertical aeroponic growing system on strawberry plant development. In the initial phase, aeroponic tower systems were set up, ensuring that strawberry plants received a fine mist of the nutrient solutions directly to their roots (Bai et al., 2024). This method was chosen for its potential to enhance plant growth by providing optimal hydration and nutrient delivery (Karablieh et al., 2024). The study was focused on observing the plant growth in aeroponic towers after transferring Florian strawberry plantlets to aeroponic towers.

Lettuce has a relatively short growth cycle, making it ideal for quick harvests in an aeroponic system. This study also focused on a series of experiments on lettuce seeds because of their rapid germination and leaf development. Lettuce seeds showed 100% germination in compost and rockwool. This suggests that various seed types yield different results Ameri et al., 2012). The aeroponic system's efficient oxygen delivery to the roots was also noted to enhance the overall growth rate of the lettuce plants (He, 2024). Referring to section 2.4.3, the ability to deliver nutrients and oxygen directly to the roots in a fine mist helped maximise plant health and yield. Rockwool and compost substrates are popular choices in horticulture and gardening, each with distinct advantages. Rockwool, an inert growing substrate made from spun molten rock, provides excellent aeration and water retention, making it ideal for aeroponic systems. However, it lacks organic nutrients and requires careful handling due to potential respiratory hazards. In contrast, compost is an organic growth media rich in nutrients and beneficial microorganisms, promoting healthy plant growth and improving soil structure. It is environmentally friendly but may require more frequent monitoring of moisture levels and nutrient balance compared to Rockwool (Bai et al., 2024).

The study also explored the impact of different light conditions, different temperature treatments, and chemical (ethephon) treatments on the germination of strawberry seed cultivars Temptation and Florian. The primary objective was to determine how light quality—specifically red, white, and dark environments—affects the germination rates and overall seedling development. The results indicated that there was no significant difference in strawberry seed germination cultivar Temptation when exposed to various light conditions. In contrast, Florian, the strawberry seeds cultivar, showed minimal germination across white and dark light treatments, with red light conditions resulting in no germination throughout the experiment. The seeds began to germinate when exposed to white light. This indicates that the strawberry seed cultivar Florian might have a different light sensitivity compared to the strawberry seed cultivar Temptation. Kettenring et al. (2005) demonstrated significant effects on seed germination of *Carex brevior* in white light.

The study also focused on the effect of pre-germination temperature on strawberry seeds (cultivars Temptation and Florian). The result showed no difference in the germination of the strawberry seeds cultivar Temptation and Florian when subjected to various temperature conditions. The findings align with existing literature on seed germination, which suggests that different plant species might have changing reactions to temperature. A few examinations have demonstrated that cold temperatures can restrain germination in specific species, while others have found that intensity can invigorate germination in others. Tang et al. (2020) have distinguished that seed germination of strawberries was ideal at the temperature of 20 to 27 ºC. Haj Sghaier et al.

(2022) have also observed the impact of temperature on seed germination (*Brassica napus* L.). The plants were studied under various temperature conditions (5°C, 10°C, 15°C, 20°C, 25°C, 30°C, and 35°C). The results showed that 20°C was the most favourable and optimal temperature for achieving the highest germination percentage.

The current study also examined the effects of ethephon treatment on strawberry seed cultivars Temptation and Florian before germination. The study indicates that the ethephon-treated seeds exhibited outcomes almost identical to those of the control group. **Figure 34** in section 3.2.3.2 indicates that ethephon speeds up the seed germination process. However, no statistically significant difference in germination rate was observed between the ethephon-treated seeds and the control group for both the cultivars Temptation and Florian by the end of the study. In contrast to the findings of this study, Zhang et al. (2018) reported significant effects of ethephon on seed germination and maize growth (*Zea mays* L.), which contradicts the results observed here. However, Watanabe et al. (2007) suggested that ethephon alone does not produce significant effects, as ethephon-treated rice seeds (*Oryza sativa* L.) showed no notable growth, a finding that aligns with the results of this study. The researchers also found that a combination of ethephon and Gibberellic acid led to a notable improvement in growth.

The study, which investigates strawberry seed germination under different light temperatures and chemical conditions, indicates that the germination rate of the strawberry seed cultivar Temptation was better than that of the strawberry seed cultivar Florian. The results indicate that there is a cultivar effect on the performance of crops in terms of germination and overall yield performances.

The study also addressed the tissue culture techniques using strawberry plantlets (Cultivar Calypso). Tissue culturing in rockwool was found difficult compared to agar gel media. Activated charcoal showed good results in terms of controlling contamination. The stock was saved and maintained in the agar gel media with and without activated charcoal. Activated charcoal has been widely used to reduce microbial contamination in the media. Its porous nature can assimilate and trap microbes, decreasing their presence in the media. The overall results demonstrated that the addition of activated charcoal significantly reduced contamination. In contrast, the tissue culture plants grown in rockwool did not survive in the aeroponic towers. Among the tissue-cultured plants grown in rockwool with the addition of activated charcoal, only two plants survived. This requires further experimentation in the future to fully understand and validate the results. Different weaning techniques can be used to improve the success rates.

5.2. Conclusion

In summary, the experiments in Chapter 2 demonstrated that aeroponic tower systems, when combined with the right nutrient solutions, can significantly enhance the growth and yield of strawberries. Throughout the study, the aeroponic tower systems proved highly effective in creating a controlled environment for plant growth. This setup allowed for precise monitoring and adjustment of conditions, leading to improved growth outcomes for strawberry plants. Despite the unknown composition of Nutrient

A and B, the results highlight the potential of aeroponics as a promising alternative to traditional farming methods, offering the possibility of year-round cultivation.

Future research could build on these findings by investigating the impact of different light spectra and exposure durations, aiming to gain a more comprehensive understanding of how these factors affect seed germination across various strawberry varieties. Additionally, the research could be extended to include other strawberry cultivars beyond ‘Florian’ and ‘Temptation’, allowing for a broader analysis of how light, temperature and chemical treatments affect seed germination across different varieties.

The use of rockwool for tissue culture technique requires attention, as the previous research is limited, and focused on the use of rockwool for the acclimatisation of Pappaya (Suksa-Ard et al., 1998). Therefore, it is necessary to explore the efficiency of rockwool in tissue culturing to promote growth. Skilled weaning is required to avoid shock to plantlets during transfer. If available, specific kits can be used to ease a protected transfer, prevent any shock, and encourage the transfer. The use of micropropagation techniques can help the plantlets to acclimatise to the environment and enhance growth and vigour.

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