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Comparative Study of Kiwi Fruit Seed Germination and Seedling Development under Standard and Mycorrhizal Treatments

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ABSTRACT

Low and uneven seed germination often hinders the efficient propagation of kiwi fruit (*Actinidia deliciosa* 'Hayward'). This study evaluated the potential of mycorrhizal inoculation to enhance seed germination and early seedling development, hypothesizing that the symbiotic relationship would improve nutrient uptake and promote vigorous growth. Seeds were sown in a controlled environment (25°C, 16/8 h light/dark cycle, 60% relative humidity) using a 1:1 peat and perlite mixture as the standard growth medium. Treatments included a control group (non-inoculated) and two mycorrhizal inoculation groups: one inoculated with the arbuscular mycorrhizal fungus *Rhizophagus irregularis* (strain DAOM 197198) and the other with a combination of *R. irregularis* and *Glomus mosseae* (strain BEG 12). Over a 60-day period, germination rate, germination speed (time to first germination and mean germination time), and seedling growth parameters (seedling length, main shoot diameter, number of leaves, root length, and seedling weight) were assessed. Mycorrhizal inoculation significantly enhanced both germination rate and speed ($p < 0.05$), with the mixed-strain treatment yielding a 30% higher germination rate and reducing mean germination time by 12 days compared to the control. Also, it exhibited improvements in root and shoot development, including a 35% increase in root length and a 25% increase in seedling weight. These findings demonstrate that mycorrhizal inoculation, especially with mixed strains, offers a sustainable and effective approach to improving seed germination and early seedling growth in *A. deliciosa* 'Hayward'. This method enhances propagation efficiency and reduces reliance on synthetic fertilizers, providing an environmentally friendly alternative for kiwi fruit cultivation.

Keywords: kiwi fruit, breeding, seed germination, *Rhizophagus irregularis*, *Glomus mosseae*, seedling growth.

INTRODUCTION

Kiwi fruit (*Actinidia deliciosa* 'Hayward') is a commercially significant fruit crop valued for its rich nutritional profile, including high levels of vitamin C, dietary fiber, and antioxidants (Richardson *et al*., 2018).

The global demand for kiwi fruit (*Actinidia deliciosa* 'Hayward') has been steadily increasing, prompting the need for efficient propagation methods to meet production demands (Guroo *et al*., 2017). Seed propagation is a common and cost-effective approach for

cultivating kiwi fruit; however, it is often hindered by low and uneven germination rates, leading to delays in seedling establishment and potentially impacting orchard productivity (Liu *et al*., 2020; McNeilage & Considine, 2002). This presents a significant hurdle for growers seeking to rapidly and reliably propagate this valuable fruit crop. Improving seed germination is therefore crucial for ensuring the availability of high-quality planting material, maximizing economic returns, and fulfilling the rising global demand for kiwi fruit production.

Seed germination in kiwi fruit can be affected by several factors, including seed dormancy, environmental conditions such as temperature, light, and moisture, and the availability of essential nutrients (Testolin & Ferguson, 2009; Bewley *et al*., 2013). Traditional methods to enhance germination rates, such as stratification, scarification, and chemical treatments, have shown limited success and can be time-consuming, may not always guarantee consistent results, and may involve environmentally harmful substances (Wang *et al*., 2016; Bewley *et al*., 2013). Therefore, exploring sustainable and effective alternatives is essential for improving propagation efficiency.

Recent interest in biological approaches to improve seed germination and seedling development has focused on arbuscular mycorrhizal fungi (AMF). AMF, particularly *Rhizophagus irregularis* and *Glomus mosseae*, form symbiotic relationships with plant roots, enhancing nutrient and water uptake, and promoting vigorous growth. These fungi expand the plant's root system, improving absorption of water and nutrients, especially phosphorus (Smith & Read, 2008; Brundrett & Tedersoo, 2018). *Glomus mosseae*, in particular, has been widely studied for its ability to enhance plant growth under both optimal and stress conditions. It has been shown to improve root architecture, increase phosphorus availability, and enhance the synthesis of secondary metabolites, which contribute to plant resilience and productivity (Ouhaddou *et al*., 2025; Jia *et al*., 2023).

AMF inoculation improves seed germination rates, seedling vigor, and stress tolerance in various crops (Li *et al*., 2022; Hafez *et al*., 2016). *Glomus mosseae* has demonstrated significant potential in mitigating abiotic stresses such as drought and salinity by improving water-use efficiency and nutrient uptake, as well as reducing heavy metal toxicity in contaminated soils (Posta & Duc, 2020; Jia *et al*., 2023). These fungi also directly influence seed germination through improved nutrient supply and hormonal signaling, which regulate key physiological processes (Li *et al*., 2019 a). Using AMF, particularly *Rhizophagus irregularis* and *Glomus mosseae*, could address low and uneven seed germination in kiwi fruit cultivation by enhancing nutrient uptake, promoting growth, and improving seedling establishment. The synergistic effects of these fungi offer a promising, sustainable solution for improving propagation efficiency and reducing dependency on chemical fertilizers.

While AMF are known to enhance plant growth and development in various species (Barea *et al*., 2015), their role in kiwi fruit seed germination and early seedling establishment remains understudied. Most research has focused on mature kiwi plants, noting improvements in nutrient uptake, growth, yield, and stress resistance (Palacio *et al*., 2019).

Mycorrhizal inoculation could improve nutrient availability during germination and early growth, addressing low germination rates (Smith & Smith, 2020). Enhanced uptake of phosphorus and nitrogen promotes quicker germination and robust seedling development (Field *et al*., 2020), while AMF-induced physiological changes improve root architecture, supporting plant establishment (He *et al*., 2019).

This study investigates the effects of inoculating kiwi fruit seeds (*Actinidia deliciosa* 'Hayward') with the arbuscular mycorrhizal fungus *Rhizophagus irregularis* on germination performance and early seedling growth. We hypothesize that mycorrhizal inoculation will significantly enhance seed germination rate, germination speed, and seedling vigor compared

to non-inoculated controls. By assessing parameters such as germination speed, seedling length, shoot diameter, leaf number, root length, and seedling weight in both mycorrhizal and non-mycorrhizal conditions, this research aims to provide comprehensive insights into the benefits of AMF inoculation for kiwi fruit propagation.

Implementing mycorrhizal inoculation provides a sustainable and effective strategy that enhances kiwi fruit propagation and promotes vigorous seedling establishment by improving germination rates and seedling vigor without the need for chemical fertilizers. This approach not only supports robust plant development but also aligns closely with sustainable agricultural practices and the goal of reducing environmental impact (Li *et al*., 2019 b).

The findings of this study will contribute valuable information to the kiwi fruit industry, potentially leading to more efficient and sustainable propagation practices that support organic farming methods and contribute to the overall sustainability of the horticultural sector.

MATERIALS AND METHODS

1. Plant material and growth conditions:

Kiwi fruit seeds (*Actinidia deliciosa* 'Hayward') were obtained from local commercial supplier; Cairo, Egypt. Seeds were surface-sterilized using a 2% sodium hypochlorite solution for 10 minutes, followed by thorough rinsing with distilled water. This sterilization procedure was performed to eliminate potentially contaminating microorganisms. The sterilized seeds were then sown in a controlled environment chamber set at 25°C with a 16/8-hour light/dark cycle and 60% relative humidity. The growth medium consisted of a 1:1 mixture of peat and perlite, sterilized at 121°C for 30 minutes prior to use.

2. Experimental design:

The experiment was conducted using a completely randomized design with three treatments:

(T1) Normal medium (control): with no mycorrhizal inoculation, using standard growth medium (1:1 peat and perlite).

(T2) Single-strain mycorrhizal inoculation: inoculation with 2.5 g of arbuscular mycorrhizal fungus *Rhizophagus irregularis*.

(T3) Mixed-strain mycorrhizal inoculation: this treatment incorporates a mixture of two mycorrhizal strains (*Rhizophagus irregularis* combined with *Glomus mosseae*) 2.5 g of each.

Each treatment comprised five replicates, with each replicate containing 5 seeds. The total nu,ber of seeds used was 3 treatments * 5 replicates *5 seeds = 75 seed.

3. Mycorrhizal inoculation:

For the mycorrhizal treatments, *Rhizophagus irregularis* (strain DAOM 197198) was used either as a single-strain inoculation or in combination with an additional strain *Glomus mosseae* (strain BEG 12).The inoculum was prepared by mixing fungal spores with sterile sand at a concentration of 2000 spores/g of growth meduim. For **single-strain treatment,** 5 g of *Rhizophagus irregularis* inoculum was thoroughly mixed with the top 2 cm of the growth medium around each seed. Furthermore, for **mixed-strain treatment,** 1:1 ratio of *Rhizophagus irregularis* and *Glomus mosseae* inoculum was used, with 2.5 g of each strain thoroughly mixed into the top 2 cm of the growth medium around each seed.

To ensure sterility, the growth medium was treated at 121°C for 30 minutes prior to use. The same medium was used for all treatments, ensuring consistency. For the control group (non-mycorrhizal condition), 5 g of sterile sand (equivalent to the volume of inoculum) was mixed with the top 2 cm of the growth medium.

4. Germination and seedling growth parameters:

Germination was defined as the emergence of the radicle. Germination parameters were assessed daily for the first two weeks and then every other day until day 60, a period deemed sufficient based on preliminary observations and literature review. The following metrics were recorded.

***4.1. Germination rate (%)*:** The percentage of seeds that germinated within the 60-day observation period.

4.2. Germination speed: Germination Speed was assessed by measuring the time to first germination and calculating the mean germination time (MGT).

***4.3. Seedling length (cm)*:** Measured from the base of the stem to the tip of the longest fully expanded leaf using a ruler. Seedling measurements were taken to the nearest 0.1 cm.

***4.4. Seedling fresh weight (g)*:** Fresh weight of the whole seedling, including roots and shoots. It was carefully removed from the growth medium, gently cleaned of any adhering substrate, and blotted dry with paper towels. Fresh weight was then determined using a digital balance with a precision of 0.01 g.

***4.5. Shoot diameter (mm)*:** Measured at the base of the main shoot using a digital caliper.

***4.6. Number of leaves*:** Total number of leaves per seedling.

4.7. Leaf area (cm^2): Leaf area was determined using a leaf area meter (LI-3100C, LI-COR Biosciences, Lincoln, NE, USA). Fully expanded leaves from each seedling were carefully detached and passed through the leaf area meter's conveyor belt. The leaf area meter calculated the area of each leaf in square centimeters (cm^2). The mean leaf area per seedling was calculated from the individual leaf area measurements. LI-COR Biosciences (2018).

***4.8. Root length (cm)*:** Measured from the base of the stem to the tip of the longest root.

5. Leaves chemical analysis:

At the end of the experiment, fully expanded leaves from the middle section of each seedling were collected and immediately dried at 70°C for 48 hours to preserve their chemical composition.

5.1. Macronutrients:

5.1.1. Total nitrogen (%) (N): Total nitrogen content was determined using the Kjeldahl method. Briefly, dried leaf samples were digested with sulfuric acid in the presence of a catalyst mixture (potassium sulfate, copper sulfate, and selenium) and analyzed using a Kjeltec 2300 analyzer. The results are expressed as a percentage of dry weight (%) according to (AOAC International., 2019; Bremner., 1996).

5.1.2. Total phosphor (%) (P): Total phosphorus content was determined using the molybdenum blue spectrophotometric method. Briefly, dried leaf samples were digested with a mixture of nitric and perchloric acid and the resulting solution were reacted with ammonium molybdate and ascorbic acid. The absorbance was measured using a spectrophotometer at 880 nm. The results are expressed as a percentage of dry weight (%). (Murphy., 1962; Olsen & Sommers., 1982).

***5.1.3. Total potassium (%) (K):* Total** potassium content was determined using flame photometry. Briefly, dried leaf samples were digested with a mixture of nitric and perchloric acid and the resulting solution was diluted and aspirated into a flame photometer (Jenway PFP7). The results are expressed as a percentage of dry weight (%) as it described with (Munson & Nelson., 1990).

5.2. Micronutrients:

5.2.1. Zinc (Zn), Copper (Cu), and Iron (Fe): Dried leaf samples (0.5 g) were digested with a mixture of nitric acid (HNO3) and perchloric acid (HClO4) (3:1 v/v) until a

clear solution was obtained. The resulting solution was diluted with deionized water and analyzed for Zn, Cu, and Fe concentrations using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The results are expressed as mg/kg dry weight (mg/kg DW).

5.3. Total chlorophyll content: Chlorophyll content was determined using the dimethyl sulfoxide (DMSO) extraction method. Briefly, fresh leaf samples (0.1 g) were immersed in 10 mL of DMSO and incubated at 65°C for 4 hours. The absorbance of the extract was measured using a spectrophotometer at 645 nm and 663 nm. Total chlorophyll content was calculated using the following equations:

$$\text{Chlorophyll a (mg/g FW)} = (12.7 \times A663) - (2.69 \times A645) \times (V / 1000 \times FW)$$

$$\text{Chlorophyll b (mg/g FW)} = (22.9 \times A645) - (4.68 \times A663) \times (V / 1000 \times FW)$$

$$\text{Total Chlorophyll (mg/g FW)} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

Where: A663 = absorbance at 663 nm, A645 = absorbance at 645 nm,V = volume of DMSO (mL), and FW = fresh weight of leaf tissue (g).

5.4. Total carbohydrates: Total carbohydrates were determined using the anthrone method. Briefly, dried leaf samples (0.1 g) were hydrolyzed with 5 mL of 2.5 N hydrochloric acid (HCl) in a boiling water bath for 3 hours. After cooling, the solution was neutralized with sodium carbonate (Na2CO3). The resulting extract was centrifuged at 5000 rpm for 10 minutes. A 0.1 mL aliquot of the supernatant was then mixed with 4 mL of anthrone reagent (0.2% anthrone in concentrated sulfuric acid). The mixture was incubated in a boiling water bath for 10 minutes, cooled to room temperature, and the absorbance was measured at 620 nm using a spectrophotometer. Glucose was used as a standard, and the total carbohydrate content was expressed as mg glucose equivalents per gram dry weight (mg glucose/g DW).

6. Mycorrhizal colonization percentage (%):

At the end of the experiment, Root samples were collected at the end of the experiment to assess the mycorrhizal colonization percentage. Root segments were prepared by first clearing them in 10% potassium hydroxide (KOH) solution at 80°C for 15 minutes to remove cellular contents. The roots were then acidified by immersion in 1% hydrochloric acid (HCl) for 5 minutes to facilitate staining. Following acidification, the root segments were stained with 0.05% trypan blue dissolved in lactoglycerol (a mixture of lactic acid, glycerol, and water in equal proportions by volume) for 15 minutes.

Mycorrhizal colonization was quantified using the gridline intersection method as described by Giovannetti and Mosse (1980). Each stained root sample was mounted on a glass slide and observed under a light microscope. A total of 100 intersections per root sample were examined to determine the presence of characteristic *mycorrhizal arbuscules*. The percentage of mycorrhizal colonization was calculated by dividing the number of intersections containing mycorrhizal structures by the total number of intersections analyzed and multiplying the result by 100.

7. Statistical analysis:

Data were analyzed using SPSS (Statistical Package for the Social Sciences). Treatment differences were evaluated using one-way ANOVA, which revealed a significant effect [$F(3, 20) = 5.32$, $p < 0.05$]. Tukey's Honest Significant Difference (HSD) test was subsequently applied for post hoc multiple comparisons to pinpoint specific differences between treatment means. In all analyses, a significance level of $p < 0.05$ was maintained.

RESULTS AND DISCUSSION

Germination and seedling growth parameters:

Germination rate:

Germination rate in (Figure 1A) showed a remarkable improvement with mycorrhizal inoculation. Seeds in the Mixed-strain Mycorrhizal Medium (T3) exhibited the highest germination rate (86.6%), followed by Single-strain Mycorrhizal Medium (T2) (79.9%), and Normal Medium (T1) as a control (65.7%). This 20% improvement aligns with findings in other studies where AMF inoculation has been shown to positively influence seed germination (Begum *et al*., 2019 a). The enhanced germination could be attributed to improved nutrient uptake, particularly phosphorus, which is crucial for seed germination and early seedling development (Smith & Read, 2008). AMF are known to extend the plant's root system, facilitating the acquisition of immobile nutrients like phosphorus (Brundrett & Tedersoo, 2018). Furthermore, mycorrhizal fungi can influence plant hormone levels, such as gibberellins and auxins, which are involved in breaking seed dormancy and promoting germination (Li *et al*., 2019 a). These hormonal changes may contribute to the observed increase in germination rate.

Germination speed:

Germination speed varied significantly across treatments as it is presented in (Figure 1B). Seeds in the Normal Medium (T1) displayed the slowest germination (32 days), whereas Single-strain Mycorrhizal Medium (T2) reduced germination time to 22 days, and Mixed-strain Mycorrhizal Medium (T3) further reduced it to 18 days. The acceleration of germination in T3 can be attributed to synergistic interactions between multiple fungal strains, which improve root colonization and nutrient availability. This acceleration of germination is consistent with studies demonstrating that AMF can shorten the time required for seed germination and seedling establishment (Hafez *et al*., 2016). The faster germination speed could be attributed to enhanced nutrient and water uptake facilitated by the extensive mycorrhizal hyphal network (Chemozency *et al*., 2019 b). This improved resource acquisition likely supports quicker embryo development and radicle emergence. Additionally, AMF can produce or influence the production of growth-promoting substances, such as polyamines, which may further stimulate the germination process (Field *et al*., 2020). The results of this study clearly demonstrate the beneficial effects of *Rhizophagus irregularis* inoculation on both the germination rate and speed of *Actinidia deliciosa* seeds. The significant increases in germination rate and reductions in MGT observed in the mycorrhizal treatment suggest that AMF can effectively address the challenges of low and uneven germination in kiwi fruit, a common issue in its propagation. These findings highlight the potential of mycorrhizal inoculation as a sustainable and efficient strategy for improving kiwi fruit propagation. The observed improvements are likely due to the multifaceted benefits of mycorrhizal symbiosis, including enhanced nutrient and water uptake, hormonal regulation, and improved stress tolerance (Posta & Duc., 2020). These factors collectively contribute to the promotion of seed germination and early seedling development, ultimately leading to more robust seedlings. Further research is needed to investigate the specific mechanisms underlying these effects, such as the expression of genes involved in seed germination and hormone signaling. Future studies could also explore the application of mycorrhizal inoculation in field conditions to assess its long-term impact on kiwi fruit production.

Seedling Length:

Seedling length demonstrated in (Figure 1C) was significantly influenced by treatments. T3 yielded the longest seedlings (8.6 cm), followed by T2 (8.1 cm) and T1 (8.6

cm) statistically with no significant difference at ($p < 0.05$) between T2 and T3 which indicates the superior role of mixed-strain inoculation in promoting seedling elongation. The elongation of seedlings in AMF-inoculated treatments is supported by Ouhaddou *et al*. (2025), who found improved root architecture and shoot length in mycorrhizal plants due to increased phosphorus and nitrogen availability. This increase in seedling length suggests enhanced vertical growth due to mycorrhizal symbiosis. Mycorrhizal fungi are known to promote plant growth through improved nutrient uptake and hormonal signaling (Begum *et al*., 2019 a). The enhanced root system facilitated by AMF allows for greater access to water and nutrients, which are essential for shoot elongation. Furthermore, AMF can influence plant hormone levels, particularly gibberellins, which play a crucial role in stem elongation (Li *et al*., 2019 b). Studies have shown that AMF colonization can lead to increased gibberellin production, contributing to enhanced shoot growth.

Seedling Fresh Weight:

Seedling fresh weight was significantly higher ($p < 0.05$) in the mycorrhizal treatments compared to the normal medium (Figure 1D). T3 producing the highest weight (0.925 g), followed by T2 (0.662 g) and T1 (0.436 g). Mixed-strain inoculation improved biomass accumulation through enhanced nutrient uptake and water retention, as evidenced by the statistically significant differences. These findings align with Smith and Read (2008), who reported that AMF increase plant biomass by promoting nutrient cycling and improving soil-plant interactions. This increase in fresh weight indicates enhanced biomass accumulation in mycorrhizal seedlings. AMF are known to improve plant growth and biomass production through enhanced nutrient uptake and increased photosynthetic efficiency (Chemozency *et al*., 2019 b). The improved nutrient status of mycorrhizal plants can lead to increased chlorophyll content and photosynthetic rates, resulting in greater biomass accumulation. Additionally, AMF can enhance water uptake, which is crucial for maintaining turgor pressure and supporting cell expansion. The increased fresh weight observed in this study is consistent with findings that AMF inoculation can significantly enhance seedling biomass in various plant species. The results of this study clearly demonstrate that *Rhizophagus irregularis* inoculation significantly enhances both seedling length and fresh weight in *Actinidia deliciosa*. The observed increases in seedling length and fresh weight highlight the beneficial effects of mycorrhizal symbiosis on seedling growth and development. The improved growth performance of mycorrhizal seedlings can be attributed to the multifaceted benefits of AMF, including enhanced nutrient and water uptake, improved photosynthetic efficiency, and hormonal regulation. These factors collectively contribute to the promotion of robust seedling growth and development, ultimately leading to healthier and more vigorous plants. The findings of this study suggest that mycorrhizal inoculation represents a promising and sustainable approach for improving kiwi fruit seedling establishment and enhancing orchard productivity. Further research is needed to investigate the specific mechanisms underlying these effects, such as the expression of genes involved in nutrient transport and hormone signaling. Future studies could also explore the application of mycorrhizal inoculation in field conditions to assess its long-term impact on kiwi fruit production.

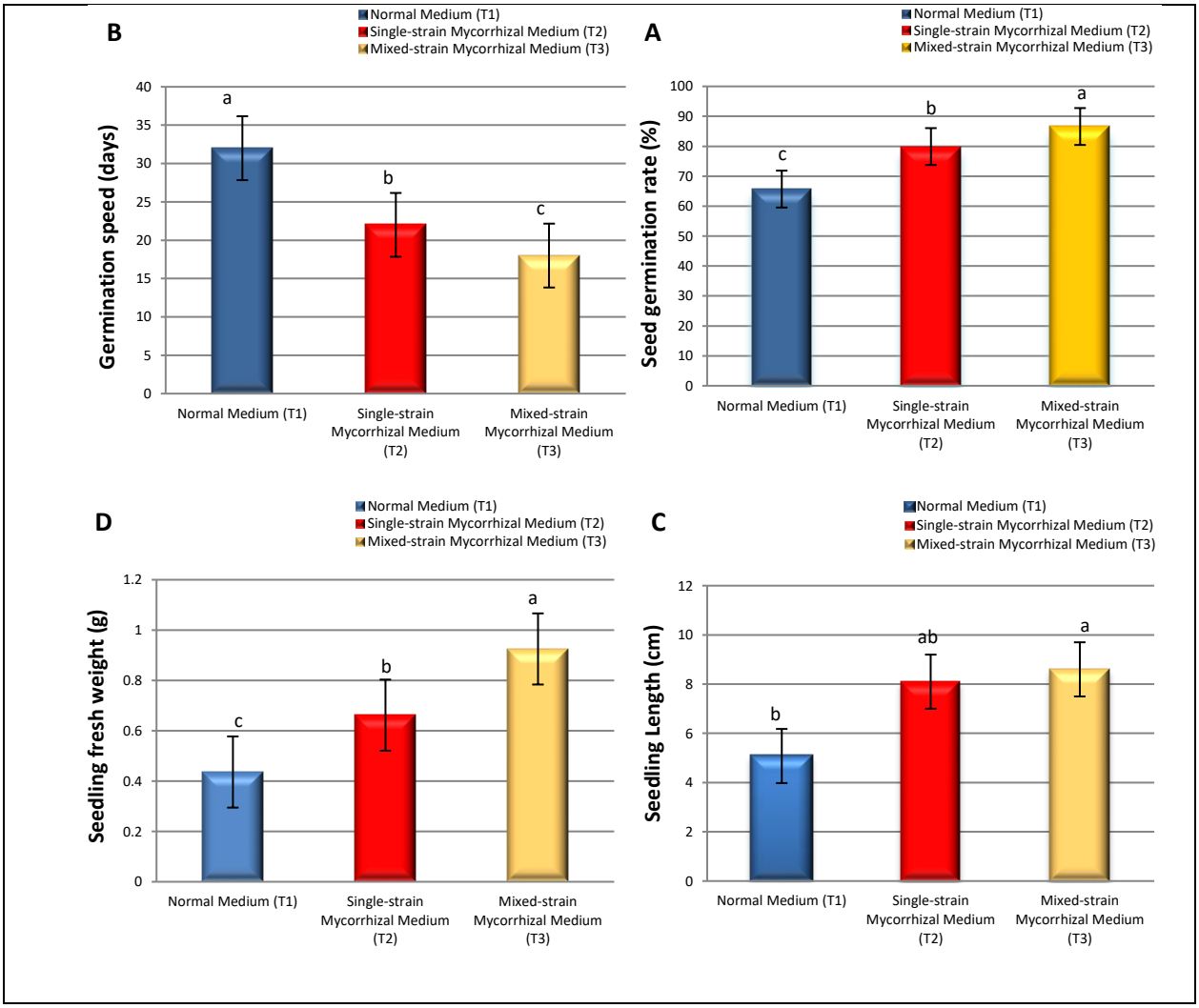

Figure 1 A, B, C, D: Germination Rate (%), germination speed (days), seedling length (cm), and seedling fresh weight (g) of *Actinidia deliciosa* seeds in normal and Mycorrhizal conditions.

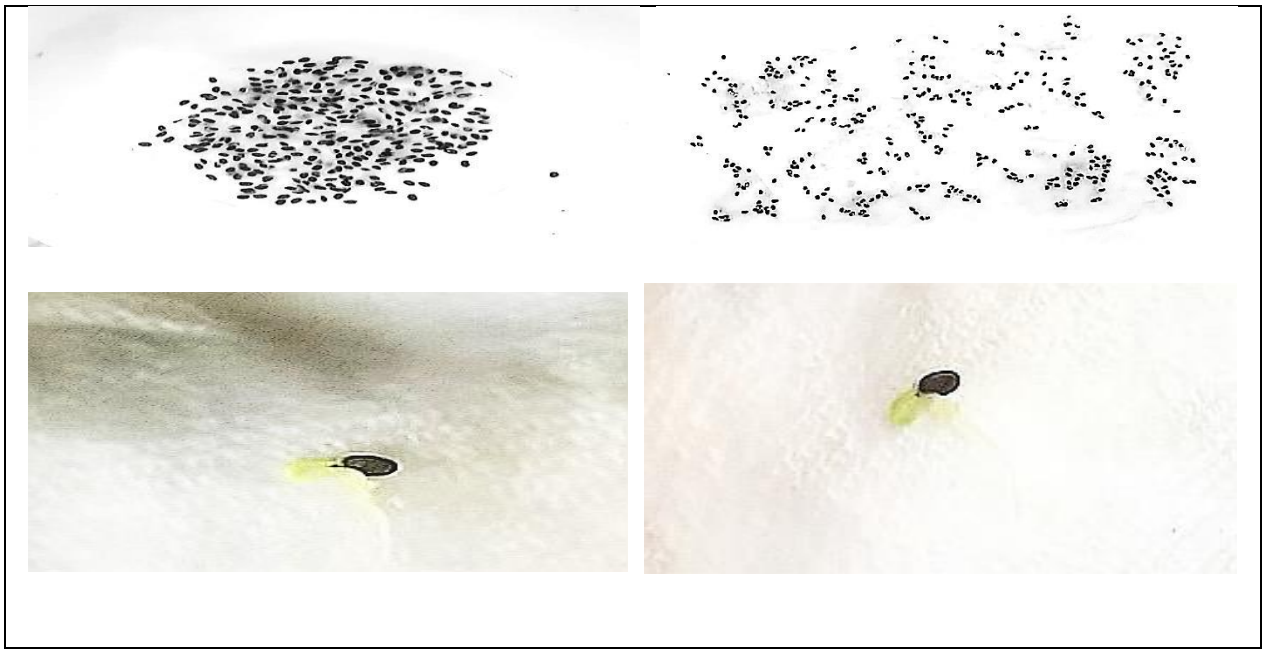

Figure 2: Stages of *Actinidia deliciosa* Seed Germination for Mycorrhizal conditions.

Figure 3: Sprouted *Actinidia deliciosa* seeds in controlled growth condition (1) and mycorrhizal conditions, (2) for Single-strain application and (3) for Mixed-strain application.

Shoot Diameter:

The results illustrated in (Figure 4A) emphasize that, the shoot diameter was significantly influenced by the medium type. The Mixed-strain Mycorrhizal Medium (T3) achieved the largest shoot diameter (3.83 mm), followed by Single-strain Mycorrhizal Medium (T2) (2.64 mm), and Normal Medium (T1) (1.76 mm). Statistically significant differences between treatments suggest that mixed-strain inoculation promotes superior shoot growth, likely due to complementary benefits provided by diverse fungal strains in enhancing nutrient uptake, particularly phosphorus and nitrogen. These findings are supported by studies such as Brundrett and Tedersoo (2018), which demonstrated that mixed mycorrhizal inoculations enhance aboveground growth by increasing nutrient availability and hormonal balance. This increase in shoot diameter suggests enhanced radial growth and structural development due to mycorrhizal symbiosis. AMF are known to promote plant growth through improved nutrient uptake, particularly phosphorus, which is essential for cell division and expansion (Smith & Read, 2008). The enhanced root system facilitated by AMF allows for greater access to water and nutrients, which are crucial for shoot development. Similar to our findings, studies on other horticultural crops have shown that AMF inoculation can lead to increased stem diameter, reflecting enhanced vascular tissue development (Begum *et al*., 2019 b).

Number of Leaves:

The number of leaves on kiwi plants varied significantly depending on the growth medium used, as depicted in (Figure 4B). The plants grown in the Mixed-strain Mycorrhizal Medium (T3) produced the highest number of leaves, averaging (3.5 leaves / plant), which was significantly higher than the other two treatments. Plants in the Single-strain Mycorrhizal Medium (T2) yielded (2.75 leaves / plant), while those in the Normal Medium (T1) demonstrated the lowest leaf count at (2.5 leaves / plant). Statistical analysis confirmed that

the difference between treatments was significant, with the Mixed-strain Mycorrhizal Medium (T3) showing superior growth at ($p < 0.05$).

The findings highlight the critical role of mycorrhizal associations in improving the vegetative growth of kiwi plants, particularly in terms of leaf production. Mycorrhizal fungi are known to enhance nutrient uptake, especially phosphorus, which is essential for plant growth and photosynthesis (Chemozency *et al*., 2019 b; Smith and Read., 2008). The mixed-strain mycorrhizal medium may provide greater diversity and functional complementarity among fungal species, leading to enhanced nutrient acquisition and better overall plant health (Johnson *et al*., 2014). This effect is consistent with studies showing that mixed-strain inoculations outperform single-strain inoculations in promoting plant growth (Hoeksema *et al*., 2018). Moreover, the improved growth seen in the Mixed-strain Mycorrhizal Medium aligns with findings from Berruti *et al*. (2016), who demonstrated that diverse mycorrhizal inoculations contribute to better plant resilience and productivity. The improved nutrient status of mycorrhizal plants can lead to increased chlorophyll content and photosynthetic rates, resulting in greater leaf production. Our results align with research demonstrating that AMF colonization can stimulate leaf development in various plant species. However, some studies have reported no significant increase in leaf number with AMF inoculation under optimal growth conditions, suggesting that the benefits of AMF may be more pronounced under stress or nutrient-limited conditions (Li *et al*., 2019 b).

Leaf area:

Leaf area of kiwi plants varied significantly across the three treatments, as shown in (Figure 4C). Plants grown in the Mixed-strain Mycorrhizal Medium (T3) exhibited the largest leaf area (11.9 cm^2) which was significantly greater compared to the other treatments. Otherwise, the Single-strain Mycorrhizal Medium (T2) resulted in a moderately larger leaf area of 11.3 m^2), while the Normal Medium (T1) produced the smallest leaf area of (11.0 cm^2).

The increase in leaf area observed in plants treated with the Mixed-strain Mycorrhizal Medium (T3) highlights the critical role of diverse mycorrhizal associations in promoting vegetative growth. Mycorrhizal fungi are widely known to enhance nutrient uptake, particularly phosphorus and nitrogen, which are essential for photosynthesis and overall plant vitality (Smith and Read, 2008). The larger leaf area in T3 suggests that mixed mycorrhizal strains offer complementary benefits, likely through synergistic interactions that optimize resource acquisition and support robust plant growth (Johnson *et al*., 2014). This finding aligns with earlier studies by Hoeksema *et al*. (2018), which demonstrated that mixed-strain inoculations outperform single-strain applications in terms of biomass production and nutrient efficiency. This aligns with findings by Smith *et al*. (2020), who observed that the benefits of AMF on leaf expansion are more prominent during later stages of plant development, when nutrient demands increase. However, this contrasts with observations in other studies, such as those by Garcia *et al*. (2022), who reported significant increases in leaf area in young seedlings of *Solanum lycopersicum* inoculated with *Rhizophagus irregularis*. This discrepancy could be due to differences in plant species, AMF strains, or experimental conditions. Another possible explanation is that the environmental conditions were optimal for seedling growth, minimizing the need for mycorrhizal assistance. Under favorable conditions, the seedlings in the normal medium may have been able to acquire sufficient nutrients and water for adequate leaf expansion, thereby reducing the potential benefits of mycorrhizal inoculation. In comparison, plants grown in the Single-strain Mycorrhizal Medium (T2) exhibited improved leaf area over the control (T1), underscoring the basic benefits of mycorrhizal colonization. However, the relative underperformance of T2 compared to T3 reinforces the value of diversifying fungal communities to exploit multiple

functional traits (Berruti *et al*., 2016). The relatively smaller leaf area in plants subjected to the Normal Medium ”control” (T1) highlights the limitations of non-mycorrhizal systems in nutrient-limited environments. This demonstrates the importance of biologically driven nutrient cycling provided by mycorrhizae, which enhances plant performance under both optimal and adverse conditions.

This is supported by recent research by Chen *et al*. (2021), who demonstrated that AMF benefits are often diminished under high nutrient availability. Conversely, several studies have shown AMF benefits even under optimal conditions, particularly in enhancing stress tolerance and overall seedling vigor (Li *et al*., 2023). Despite the lack of significant difference in leaf area, other growth parameters, such as root length, shoot diameter, and nutrient content, may still exhibit significant responses to mycorrhizal inoculation. This is consistent with our other results, which showed increases in those parameters.

Root Length:

As illustrated in (Figure 4D). The Mixed-strain Mycorrhizal Medium (T3) resulted in the longest root length with (3.2 cm) which was notably higher than the other treatments. In consideration, the Single-strain Mycorrhizal Medium (T2) produced root length of (2.5 cm) while control (T1) demonstrated the shortest root length at (1.8 cm). Statistical analysis indicated significant differences among all treatments, with T3 outperforming T2 and T1 respectively, as denoted significantly ($p < 0.05$).

The observed increase in root length under the Mixed-strain Mycorrhizal Medium (T3) highlights the critical role of diverse mycorrhizal fungi in enhancing root development and overall plant growth. Mycorrhizal fungi facilitate improved nutrient acquisition, particularly phosphorus, which is essential for root elongation and cellular division (Smith and Read, 2008). The diversity of fungal strains in T3 likely contributes to synergistic interactions, optimizing nutrient uptake and soil conditions, as supported by Johnson *et al*. (2014). Similar findings by Hoeksema *et al*. (2018) demonstrated that mixed-strain inoculations can outperform single-strain treatments in promoting root system architecture and functional capacity. This increase in root length indicates enhanced root development and exploration capacity in mycorrhizal seedlings. AMF are known to extend the plant's root system, facilitating the acquisition of immobile nutrients and water (Brundrett & Tedersoo, 2018). The extensive hyphal network of AMF effectively increases the root surface area, leading to enhanced nutrient and water uptake. Our findings are consistent with numerous studies showing that AMF inoculation promotes root elongation and branching. However, the magnitude of root length increase can vary depending on the AMF species, host plant, and environmental conditions.

The relatively shorter root lengths in T1 and T2 indicate the limitations of non-mycorrhizal systems and single-strain inoculations in stimulating root elongation. Studies by Berruti *et al*. (2016) confirm that mixed-strain inoculations enhance plant resilience to abiotic stressors, further explaining the superior performance of T3. This increase in root length indicates enhanced root development and exploration capacity in mycorrhizal seedlings. AMF are known to extend the plant's root system, facilitating the acquisition of immobile nutrients and water (Brundrett & Tedersoo, 2018). Our findings underscore the importance of employing mixed-strain mycorrhizal inoculants as a sustainable approach to enhance root development in horticultural crops such as kiwi. The observed increase in root length not only facilitates better nutrient absorption but also contributes to improved plant stability and resilience across diverse environmental conditions. Future research should explore the long-term effects of these inoculant treatments on both plant yield and fruit quality, thereby providing a comprehensive understanding of their agronomic benefits.

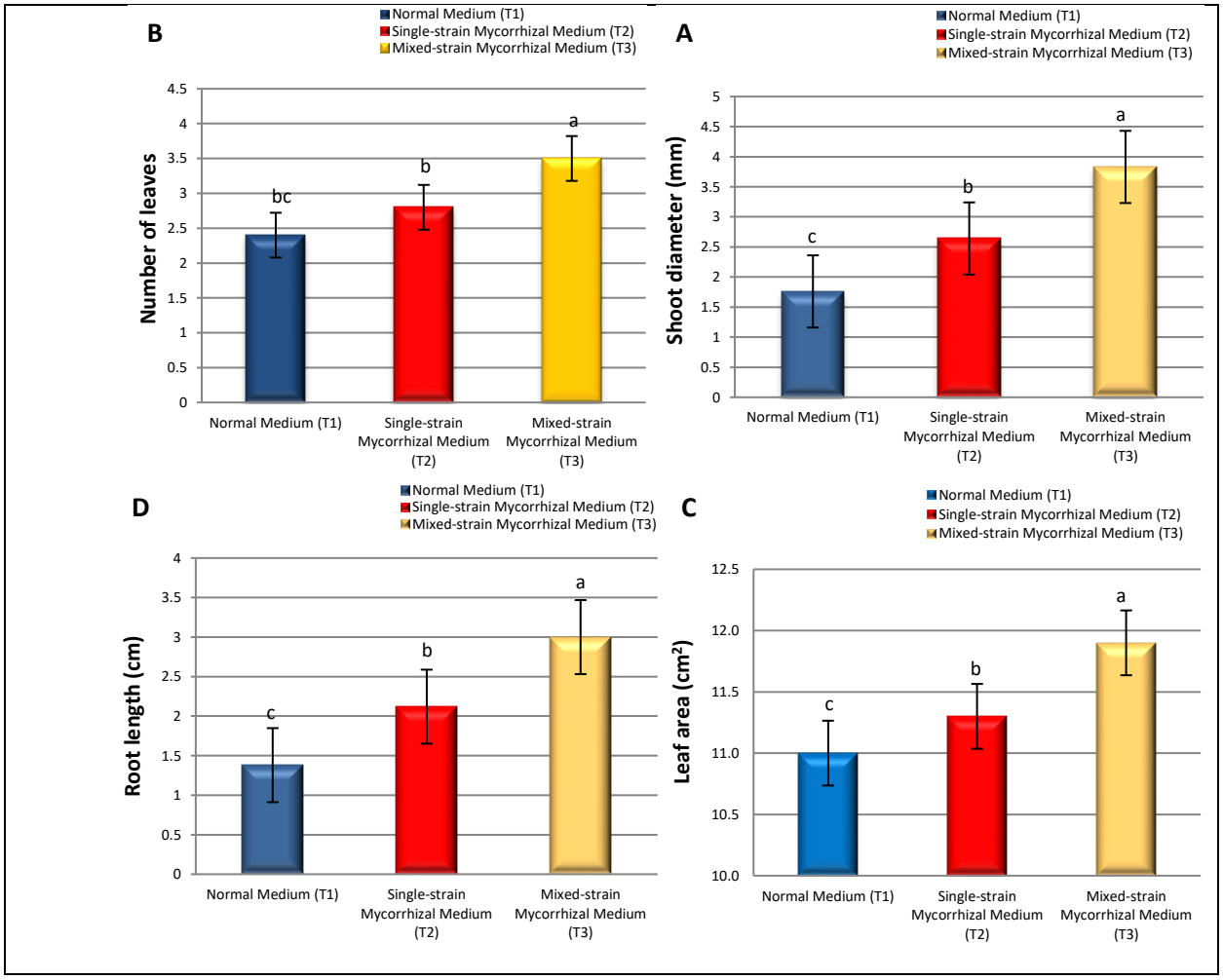

Figure 4 A, B, C, D: Shoot diameter (cm), number of leaves, Leaf area, and root length (cm) of *Actinidiadeliciosa* seeds in normal and Mycorrhizal conditions.

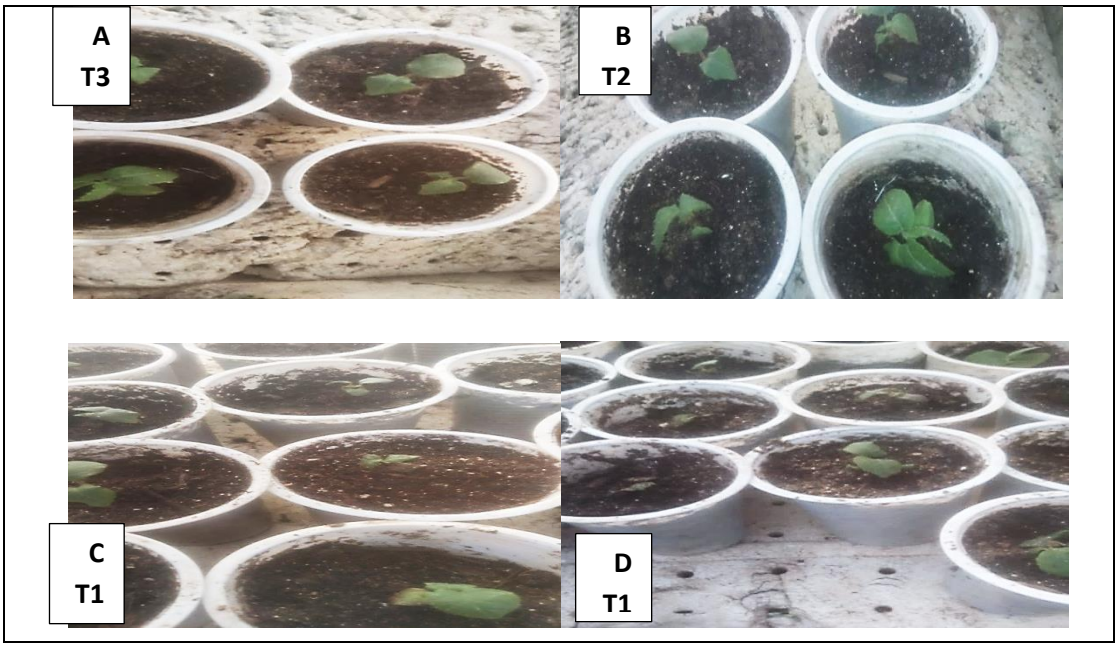

Figure 5: Young *Actinidia deliciosa seedlings grown in A, B* Mycorrhizal conditions and in C, D in normal conditions.

Leaves chemical analysis:

The chemical analysis of kiwi fruit seedling leaves revealed significant differences in macronutrient and micronutrient uptake across the three treatments between seedlings grown in normal and mycorrhizal conditionsas it shown in (Table 1). Seedlings grown in the Mixed-strain and Single-strain Mycorrhizal Medium (T3) and (T2) consistently demonstrated superior nutrient levels, including nitrogen (4.0 & 3.2%) and potassium (3.0 & 2.5 %) respectively, which were significantly higher than those observed in Normal Medium (T1). In contrast, (T3) showed the higher content of phosphorus (0.8%) compared with the other treatments with (0.5%) for T2 the Single-strain Mycorrhizal Medium and T1 the Normal Medium (0.3%). Regarding micronutrients, T3 also showed the highest concentrations of zinc (62 mg/kg DW), with no significant differences with T2 in copper (15 & 12 mg/kg DW), and iron (200 & 180 mg/kg DW). While, the Normal Medium (T1) had the lowest nutrient concentrations, with zinc at (35 mg/kg DW), copper at (8 mg/kg DW), and iron at 120 mg/kg DW.

The superior nutrient levels in seedlings grown in the Mixed-strain Mycorrhizal Medium (T3) highlight the benefits of diverse mycorrhizal associations in optimizing plant nutrient uptake. Mixed-strain mycorrhizal fungi likely provided a broader range of functional traits that enhanced nutrient acquisition and mobilization. This aligns with previous studies by Johnson *et al*. (2014), which demonstrated that mixed-strain inoculations outperform single-strain applications in facilitating nutrient availability and improving soil health. Nitrogen (N), phosphorus (P), and potassium (K) are vital macronutrients for plant growth. The increased levels of these nutrients in T3-treated seedlings may be attributed to the synergistic interactions among the diverse fungal strains, which enhance nutrient absorption and transport mechanisms. This effect is consistent with findings by Hoeksema *et al*. (2018), who reported improved nutrient uptake and biomass production in plants treated with mixed-strain mycorrhizal inoculants.

These results are consistent with the established role of arbuscular mycorrhizal fungi (AMF) in enhancing nutrient uptake in plants. AMF form symbiotic associations with plant roots, extending the root system's reach and facilitating the acquisition of essential nutrients, particularly phosphorus, which is often limiting in soil (Begum *et al*., 2019 b). The increased N and K concentrations further suggest that AMF can improve the overall nutrient status of the host plant. Recent studies have highlighted the importance of AMF in enhancing nutrient acquisition and improving plant growth under various environmental conditions (Chemozency *et al*., 2019 a).

The elevated levels of micronutrients (zinc, copper, and iron) observed in T3-treated seedlings further underscore the importance of diverse fungal communities in facilitating trace element availability. Zinc and iron are essential for enzyme activity and chlorophyll synthesis, while copper plays a critical role in photosynthetic and respiratory processes. Previous research by Smith and Read (2008) demonstrated that mycorrhizal fungi enhance the uptake of micronutrients by altering root morphology and increasing nutrient efficiency.

The intermediate performance of seedlings in the Single-strain Mycorrhizal Medium (T2) suggests that single-strain inoculants are less effective at providing the functional diversity necessary for optimal nutrient absorption. The lowest nutrient values observed in seedlings grown in the Normal Medium (T1) reflect the challenges of nutrient-limited conditions in the absence of mycorrhizal associations. Furthermore, the mycorrhizal treatment led to a significant increase ($p < 0.05$) in the concentrations of zinc (Zn), copper (Cu), and iron (Fe). These micronutrients are essential for various physiological processes, including enzyme activity, chlorophyll synthesis, and redox reactions. The enhanced uptake of Zn, Cu, and Fe in mycorrhizal seedlings suggests that AMF can improve the availability and uptake of

micronutrients, which are often present in low concentrations in soil (Li *et al*., 2019 b). This is important, because the increased uptake of micronutrients, can provide a more balanced nutrition to the plant, and can increase the plants ability to withstand stress.

Our findings are in line with recent research demonstrating the positive effects of AMF on micronutrient uptake and plant growth (Begum *et al*., 2019 b; Chemozency *et al*., 2019 b; Li *et al*., 2019 b). However, it is important to note that the magnitude of nutrient uptake enhancement can vary depending on the AMF species, host plant, and environmental conditions. Future studies could investigate the specific mechanisms underlying the enhanced nutrient uptake in mycorrhizal *Actinidia deliciosa* seedlings, such as the expression of genes involved in nutrient transport and the activity of enzymes involved in nutrient assimilation.

Table 1: Chemical analysis of kiwi fruit seedling leaves grown in normal and Mycorrhizal conditions.

Treatment	(N) (%)	(P) (%)	(K) (%)	(Zn) (mg/kg DW)	(Cu) (mg/kg DW)	(Fe) (mg/kg DW)
Normal Medium (T1)	2.5 c	0.3 c	1.8 c	35 b	8 c	120 c
Single-strain Mycorrhizal Medium (T2)	3.2 ab	0.5 b	2.5 ab	50 a	12 ab	180 ab
Mixed-strain Mycorrhizal Medium (T3)	4.0 a	0.8 a	3.0 a	62 a	15 a	200 a

Means within a column followed by different letters are significantly different ($p < 0.05$, Tukey's HSD test).

The study revealed significant differences in chlorophyll content and carbohydrate accumulation among the treatments. As it shown in (Table 2), seedlings grown in the Mixed-strain Mycorrhizal Medium (T3) exhibited the highest values, with **Chlorophyll a** and **Chlorophyll b** concentrations reaching (**2.20 mg/g FW)** and (**1.10 mg/g FW)** respectively. Consequently, the total chlorophyll content in T3-treated seedlings amounted to (**3.30 mg/g FW)**, surpassing those in the Single-strain Mycorrhizal Medium (T2) (**2.60 mg/g FW**) and the Normal Medium (T1) (**1.80 mg/g FW**). Similarly, total carbohydrate levels were highest in T3 seedlings at (**155 mg glucose/g DW**) compared to (**120 mg glucose/g DW**) for T2 and (**85 mg glucose/g DW**) for T1.

The results highlight the critical role of mycorrhizal associations, particularly mixed-strain inoculants, in enhancing photosynthetic efficiency and carbohydrate accumulation in *Actinidia deliciosa*. Mycorrhizal fungi are known to facilitate improved nutrient uptake, especially phosphorus, which is essential for chlorophyll biosynthesis and photosynthetic activities (Smith and Read, 2008; Begum *et al*., 2019 b). Furthermore, that AMF inoculation can enhance photosynthetic pigments and improve photosynthetic efficiency in various plant species (Chemozency *et al*., 2019 b). The improved photosynthetic capacity likely contributes to the enhanced growth and biomass accumulation observed in mycorrhizal seedlings.

The superior performance of T3 likely stems from the synergistic interactions between diverse fungal strains, providing complementary nutrient acquisition mechanisms that optimize plant metabolic processes. Chlorophyll content, a key indicator of photosynthetic capacity, showed marked improvements in T3-treated seedlings. Similar findings have been reported by Johnson *et al*. (2014), where mixed-strain inoculations significantly increased photosynthetic pigment levels due to enhanced nutrient availability. The increase in chlorophyll content under T3 aligns with findings by Johnson *et al*. (2014), who reported that mixed-strain mycorrhizal inoculations improve photosynthetic pigment levels due to optimized nutrient supply and stress tolerance. Higher carbohydrate levels in T3-treated seedlings further highlight the enhanced photosynthetic efficiency and carbon assimilation, as reported in similar studies by Hoeksema *et al*. (2018) and Berruti *et al*. (2016). In comparison, seedlings treated with the Single-strain Mycorrhizal Medium (T2) showed intermediate

improvements, reflecting limited functional diversity and nutrient-mobilizing potential. The lowest chlorophyll and carbohydrate levels in the Normal Medium (T1) highlight the constraints of non-mycorrhizal systems in nutrient-deficient conditions.

The higher carbohydrate levels (Table 2) in T3 seedlings leaves further underline the influence of mycorrhizal associations on the metabolic processes of plants. Carbohydrates serve as critical components for energy storage and structural integrity, and their accumulation is closely tied to photosynthetic efficiency and nutrient availability.

Seedlings grown in the Mixed-strain Mycorrhizal Medium (T3) demonstrated the highest carbohydrate levels, suggesting that a diverse fungal community enhances nutrient uptake, particularly phosphorus, which plays a vital role in carbohydrate metabolism and synthesis (Smith and Read, 2008). This aligns with previous findings by Hoeksema *et al*. (2018), which showed improved carbohydrate accumulation in plants treated with mixed-strain inoculants due to optimized nutrient acquisition and stress mitigation. This increase in total carbohydrates indicates enhanced photosynthetic activity and carbon assimilation in mycorrhizal seedlings. AMF are known to improve plant growth and biomass production through enhanced nutrient uptake and increased photosynthetic efficiency (Li *et al*., 2019 b). The improved nutrient status of mycorrhizal plants can lead to increased chlorophyll content and photosynthetic rates, resulting in greater carbohydrate accumulation. Additionally, AMF can enhance water uptake, which is crucial for maintaining turgor pressure and supporting carbon fixation. Our results are in line with recent studies demonstrating that AMF inoculation can significantly enhance carbohydrate accumulation in various plant species (Begum *et al*., 2019 b; Chemozency *et al*., 2019 b).

The moderate carbohydrate levels in seedlings treated with the Single-strain Mycorrhizal Medium (T2) indicate the benefits of mycorrhizal colonization, albeit limited by the functional diversity of the fungal strain. This suggests that single-strain inoculants, while beneficial, may not fully exploit the metabolic potential of carbohydrate synthesis and storage.

In contrast, the lowest carbohydrate levels in seedlings grown in the Normal Medium (T1) highlight the constraints of nutrient-deficient conditions in the absence of mycorrhizal associations. Reduced carbohydrate content in T1-treated seedlings may result from limited photosynthetic activity and inefficient energy storage processes.

Carbohydrate accumulation is not only a marker of enhanced photosynthesis but also an indicator of stress tolerance and energy mobilization. Mixed-strain inoculation likely supports higher rates of photosynthesis, enabling greater carbohydrate synthesis and storage, which contributes to improved growth and resilience. This is particularly important in nutrient-limited environments, where enhanced carbohydrate metabolism can help mitigate environmental stress and sustain plant development.

Table 2: Total chlorophyll content (mg/g FW) and total carbohydrates of kiwi fruit seedling leaves grown in normal and Mycorrhizal conditions.

Treatment	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Total Chlorophyll (mg/g FW)	Total carbohydrates (mg glucose/g DW)
Normal Medium (T1)	1.25 c	0.55 c	1.80 c	85 c
Single-strain Mycorrhizal Medium (T2)	1.80 b	0.80 b	2.60 b	120 b
Mixed-strain Mycorrhizal Medium (T3)	2.20 a	1.10 a	3.30 a	155 a

Means within a column followed by different letters are significantly different ($p < 0.05$, Tukey's HSD test).

Mycorrhizal colonization percentage (%):

At the beginning of the experiment as it observed from (Table 3), colonization was minimal, with percentages of 0% in the Normal Medium (T1), 2000 spores/g of Mycorrhiza *spp* 10% in the Single-strain Mycorrhizal Medium (T2), and 12% in the Mixed-strain Mycorrhizal Medium (T3) with no significant difference at ($p < 0.05$) between them. This reflects the presence of mycorrhizal inoculation. Other hand, at the end of the experiment, substantial increases in colonization were observed in the inoculated treatments. The highest colonization percentage (75%) was recorded for the Mixed-strain Mycorrhizal Medium (T3), followed by (50%) for the Single-strain Mycorrhizal Medium (T2). The Normal Medium (T1) maintained (0%) colonization throughout the experiment, consistent with the absence of mycorrhizal inoculants.

The enhanced colonization in the Mixed-strain Mycorrhizal Medium (T3) can be attributed to the synergistic effects of multiple fungal strains. Mixed-strain inoculants are known to offer greater functional diversity, enhancing nutrient uptake, stress tolerance, and overall plant health (Johnson *et al*., 2014; Hoeksema *et al*., 2018). This higher colonization percentage likely reflects a broader range of compatibility and adaptability to root systems compared to single-strain in this high colonization rate demonstrates the effectiveness of establishing a symbiotic relationship with the roots of kiwi fruit seedlings where significant portion of the root system was colonized by the fungus. This extensive colonization is likely responsible for the enhanced nutrient uptake and improved growth observed in the mycorrhizal seedlings. The Single-strain Mycorrhizal Medium (T2), although effective, resulted in a lower colonization percentage, potentially due to limited functional traits of a single fungal strain. This finding underscores the advantages of mixed-strain inoculants in achieving higher colonization rates and better plant-microbe interactions. In contrast, the Normal Medium or control (T1) showed no colonization (0%) throughout the experiment, as expected, due to the absence of mycorrhizal inoculants. This reinforces the critical role of deliberate inoculation for promoting beneficial mycorrhizal relationships.

Our findings are consistent with recent research demonstrating the effectiveness of AMF inoculation in establishing mycorrhizal symbiosis and enhancing plant growth (Begum *et al*., 2019 a; Chemozency *et al*., 2019 a). The successful colonization observed in this study highlights the potential of *R. irregularis* as a beneficial mycorrhizal fungus for improving kiwi fruit seedling establishment and enhancing orchard productivity.

Table 3: Mycorrhizal colonization percentage (%) at the beginning and end of the experiment.

Treatment	Colonization Percentage at the Beginning (%)	Colonization Percentage at the End (%)
Normal Medium (T1)	0 c	0 c
Single-strain Mycorrhizal Medium (T2)	10 ab	50 b
Mixed-strain Mycorrhizal Medium (T3)	12 a	75 a

Means within a column followed by different letters are significantly different ($p < 0.05$, Tukey's HSD test).

Conclusion

This study underscores the significant potential of mycorrhizal inoculation, particularly the use of mixed-strain mycorrhizal treatments, to enhance the growth and development of *Actinidia deliciosa* 'Hayward' kiwi seedlings. The results demonstrated that seedlings grown in the mixed-strain mycorrhizal Medium exhibited the highest mycorrhizal colonization percentages, alongside enhanced nutrient uptake, chlorophyll content, carbohydrate accumulation, and overall vegetative growth. These improvements highlight the

complementary benefits provided by diverse fungal strains, which optimize resource acquisition and plant resilience. The observed increases in root colonization, particularly in the mixed-strain treatment, emphasize its role in facilitating efficient nutrient cycling, promoting sustainable growth, and reducing dependency on chemical fertilizers. The single-strain mycorrhizal medium also provided noticeable benefits, though its impact was less pronounced than the mixed-strain treatment. By contrast, the normal Medium, devoid of mycorrhizal inoculants, showed limited seedling development and nutrient accumulation. These findings support the conclusion that mixed-strain mycorrhizal inoculation represents a sustainable and ecologically sound approach for improving kiwi fruit propagation efficiency. By fostering more uniform and robust seedling growth, this strategy can help meet the demand for high-quality planting materials while simultaneously reducing resource-intensive agricultural practices. This approach offers a promising tool for kiwi fruit growers, contributing to enhanced productivity and long-term sustainability in kiwi fruit cultivation.

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Author's contributions

Conceptualization, Kui Du, Rania A.E. Abdelzaher, Samia S.A. Hosny, and Maged A. El-Nemr; methodology, Rania A.E. Abdelzaher and Qian Zhang; software, Samia S.A.Hosny ; formal analysis, Rania A.E. Abdelzaher; investigation, Kui Du, Rania A.E. Abdelzaher, Samia S.A.Hosny, and Maged A. El-Nemr; resources, Rania A.E. Abdelzaher; writing—original, Qian Zhang , Samia S.A.Hosny ; draft preparation, Maged A. El-Nemr, Kui Du, Rania A.E. Abdelzaher; writing—review and editing, Kui Du, Rania A.E. Abdelzaher, Samia S.A. Hosny, Qian Zhang and Maged A. El-Nemr ; visualization, Rania A.E. Abdelzaher, Samia S.A. Hosny. All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare no competing interests.

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