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Under drought stress conditions, the presence of arbuscular mycorrhizal fungi (AMF) enhances the growth of biomass, improves mineral content, and increases antioxidant activity in tomato plants.

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Abstract

Arbuscular mycorrhizal fungi (AMF) are symbiotically associated with crops. They increase biomass production, nutritional elements, and antioxidant activities in food and vegetable crops grown in soil under stress conditions. The present study focused on the effects of AMF (*Acaulospora morrowiae*, *Paraglomus occultum*, *Funneliformis mosseae*, *Rhizophagus clarus*, and *Rhizophagus intraradices*) on biomass growth and yield, contents of chlorophyll and carotenoids, activities of catalase (CAT) and ascorbate peroxidase (APX), and contents of hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and minerals (Na, K, Ca, Mg, and Fe) in Unnayan, LT896, and Minto super tomato (*Solanum lycopersicum* L.) varieties grown in soil under drought stress (<10% moisture). The results showed that root length and shoot mass in plants treated with *R. clarus* and *P. occultum* were significantly higher than those of the control (non-AMF) in Minto super tomato. Compared to the control, the shoot's dry weight and yield were enhanced by 28% and 20% with AMF-treated tomatoes. The CAT activity in *P. occultum*-treated plants was statistically higher than that of the control in Unnayan tomatoes. H_2O_2 content was detected higher in the control than *R. clarus*-treated LT896 tomatoes. In plants treated with *A. morrowiae* and *R. clarus*, APX activity was significantly higher than that of the control in the Unnayan tomatoes. CAT and APX activity increased by 42% and 66% in AMF-treated leaves of tomatoes compared to non-AMF. Treatment with AMF reduced the content of MDA and H_2O_2 (ROS) in the leaves of tomato plants by 50% and 2% compared to the control, respectively. Potassium (K), calcium (Ca), magnesium (Mg), and iron (Fe) of tomato fruits increased by 2%, 13%, 24%, and 37% with AMF treatment compared to the control. These results suggested that biomass growth, yield, photosynthetic pigments, antioxidant enzyme activity, and mineral contents could be enhanced by AMF in food crops grown under drought stress. It is concluded that AMF might be used for the development of AMF-enriched biofertilizers that will improve the nutritional quality of food crops grown under stress conditions.

1. Introduction

Arbuscular mycorrhizal fungi are beneficial microbes forming a symbiotic relationship with food crops [1]. AMF could increase the nutrient and water uptake capacity through a hyphal network in the roots of the host plant [2, 3]. In contrast, plants serve as carbohydrates and organic nutrient sources for the AMF [4, 5]. The prominent role of AMF in boosting the uptake of various nutrients, especially phosphate, is already well recorded [3, 6]. This natural symbiosis is highly effective in increasing nutrients within the plant organs [7]. The concentration of nutrients in the colonization of AMF with host plants can be

increased by double compared to its non-mycorrhizal counterparts [8]. For instance, the concentration of nitrogen is increased in AMF-colonized leguminous species *Melilotus alba* [9].

AMF increase soil organic carbon and microbial activity through the interaction with the rhizosphere, which finally enhances the nutrient availability in soils. AMF also boost the photosynthetic contents and its following effect on biomass growth of plants [10]. Consequently, these ubiquitous fungi have various constructive effects on plant biomass growth, soil quality, and defense mechanisms against stresses [11, 12]. There are abundant records of AMF aiding in the vigorous vegetative growth of different plants. AMF increase plants' biomass of leaves, roots, and shoots [13, 14]. A majority of food crops such as onion, leeks, garlic, carrot, lettuce, cucumber, lentils, rice, mung beans, peas, tomato, and pepper form symbiotic associations with AMF [15, 16]. Recently, we showed that crop productivity increased significantly in AMF-colonized mung bean crops through the formation of photosynthetic products [12]. In addition to this, an excellent positive correlation between AMF colonization and biomass production in carrots and parsley was reported by Regvar et al. [17], suggesting that mycorrhizae can enhance biomass growth in food crops [18, 19].

Around 180 million tons of fresh tomato fruits are produced worldwide from 5 million ha of land [20]. Tomatoes can be consumed as both fresh salad and processed food. Daily consumption of tomato products may provide a superb combination of healthy substances such as minerals, vitamins, flavonoids, and antioxidant compounds like lycopene, beta-carotene, and lutein [21, 22]. Among the antioxidant compounds, lycopene and carotenoids contribute to reddening and enhancing the nutritional quality of tomato fruits [22]. Lycopene works as a scavenger of toxic substances such as reactive oxygen species (ROS) in food crops grown under stress conditions [23]. Consequently, antioxidants also work as potential inhibitors of different diseases like cancer, heart diseases, and macular degeneration [24]. The important incidence of AMF on P uptake was confirmed with generally double the concentration in mycorrhizal olive plants as compared to non-mycorrhizal controls, irrespective of genotype and inoculation. An evidence of higher crop yield and water use efficiency by AMF-colonized tomato genotypes has been reported [25], where elevated levels of N and P concentration, higher photosynthetic rate, and finally increased amount of fruit yield in AM tomato genotype are found. For example, AMF-inoculated tomato plants in arsenic-contaminated soil have been shown to increase nutrient uptake and biomass production, resulting in higher growth parameters [25]. Inoculation of AMF increased P and K concentration in tomato plants under abiotic stress [26].

Numerous research has investigated that AMF reduce oxidative stress in plants grown under abiotic stress [16, 27–29]. This mechanism also enhances superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), and glutathione reductase (GR) [12, 18, 30] in food crops grown in stressed conditions [14, 25, 31–34]. In stress conditions, carotenoids, anthocyanins, chlorophyll, and phenolics were higher in AMF-colonized lettuce leaves [13]. Furthermore, significant enhancement of SOD, CAT, POD, and APX was recorded in AMF plants under stress in food crops [26, 34]. Thus, inoculation of AMF with host plants has the greatest impact on antioxidant activity compared to other symbionts [35, 36].

Photosynthesis reduces due to the dropping of CO_2 in crops grown under drought stress [37]. Reactive oxygen species (ROS) is one of the major indicators of drought stress [38]. However, ROS reduces through different mechanisms in food crops grown under drought stress [38]. The key mechanism to mitigate the adverse impacts of drought stress is increasing antioxidant activities [39].

Tomato is highly sensitive to drought stress. The biomass production and photosynthetic pigments reduce in tomato plants under drought stress. Moreover, drought also reduces the enzymatic APX, CAT, DHAR (dehydroascorbate reductase), GST (glutathione S-transferase), GR (glutathione reductase), MDHAR

(monodehydroascorbate reductase), POD (peroxidase), and SOD (superoxide dismutase) and non-enzymatic antioxidant AsA (ascorbate), DHA (dehydroascorbic acid), GSH (glutathione), and GSSG (oxidized glutathione) activities and increases oxidative damage, H_2O_2 (hydrogen peroxide), MDA (malondialdehyde), and $O_2^{\cdot-}$ (superoxide ion) [40]. Arbuscular mycorrhizal fungi (AMF) are considered one of the most disseminated fungi throughout the world [41]. The symbiotic association between the host plant and AMF substantially improved the resistance to drought stress [42]. Drought stress is regulated in plants by AMF through diverse metabolic pathways [43]. AMF increase water uptake in the host plant, progress water use efficiency and gas change ability [43], alter the morphology of roots [44], adjust hormone levels [45], and decrease the generation of ROS [39] and thus decline the impacts of drought stress. Additionally, AMF also generate glomalin-related soil protein (GRSP), which works as a cement that encourages the creation of water-stable aggregates by extraradical hyphae, thus enhancing the water-holding capacity [46]. Additionally, under drought stress, AMF regulate antioxidant activities, osmolytes, and photosynthetic pigments [47].

The Unnayan, LT896, and Minto super tomatoes are hybrid day-length sensitive varieties. The yield is comparatively higher than other varieties. The influence of AMF species (*Acaulospora morrowiae*, *Paraglomus occultum*, *Funneliformis mosseae*, *Rhizophagus clarus*, and *Rhizophagus intraradices*) on biomass growth, photosynthetic pigments, and antioxidant activity in Unnayan, LT896, and Minto super tomato varieties remains unknown under drought stress. Therefore, we studied the effects of AMF on biomass growth, yield, chlorophyll and carotenoids, catalase (CAT), hydrogen peroxide (H_2O_2), malondialdehyde (MDA), ascorbate peroxidase (APX), and minerals (Na, K, Ca, Mg, and Fe) in tomato varieties grown in soil under drought stress. It is hypothesized that AMF will increase biomass growth, antioxidant activity, and nutritional quality in tomatoes and other food crops grown in soil under drought stress.

2. Materials and Methods

2.1. Soil Sampling

The soil sample was taken from a farmer's land in Gazipur of Bangladesh. The geographical position of Gazipur District is between 23°53′ and 24°20′ north latitude and between 90°09′ and 90°42′ east longitude. The collected soil was silty loam. The experiment was conducted in the net house during winter (November to February) of the Department of Environmental Science at Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU). The absence of mycorrhizal spores was confirmed in the soil using a stereomicroscope by following the wet sieving and decanting method [48]. The average temperature was 22°C during the experiment.

2.2. Chemical Analysis

The collected soil samples were brought into the laboratory for chemical analysis before fertilization [49]. Soil samples were dried with sunlight. Then, the samples were crushed and sieved with 250 μ size sieve and kept in Ziploc bags with proper tagging. The percentages of total nitrogen (0.086%), phosphorus (9.6 $mg \cdot kg^{-1}$), exchangeable potassium (0.28 meq^{-100g}), and pH (7.34) were detected by the Kjeldahl method [50], by the Olsen method [51], by the ammonium acetate extraction method [50], and by the glass electrode pH meter [50] in soils, respectively (Table 1).

Table 1
Quality control (QC) for the determination of chemical properties, photosynthetic pigments, antioxidants, and minerals.

2.3. Tomato Seedlings, Pots, and Fertilizers

Seeds of tomato (*Solanum lycopersicum* L.) varieties (Unnayan, LT896, and Minto super) were collected from the research and development wing of Lal Teer Institute in Bangladesh. These varieties were selected based on their growing season and height. These tomatoes are grown in winter (November to February) in Bangladesh. They need 18 to 22°C temperature. Seeds of three tomato varieties were spread into the soil in a concrete structured seedbed separately for the growing of seedlings during November 2020. Three tomato seedlings of each variety were transferred into the soil in each pot at week 3. Urea, triple superphosphate (TSP), and muriate of potash (MOP) were applied as nutrients. Plastic-made pots and pesticides were purchased from a local shop in Bangladesh. The sizes of the pots were 25/25 cm. The volume of the pot with soil was 8 kg. Urea (550 kg·ha^{-1}), triple superphosphate (TSP) (250 kg·ha^{-1}), and muriate of potash (MOP) (250 kg·ha^{-1}) were applied according to the BARC recommendations to the soil in each pot.

2.4. Mycorrhizal Fungi

Arbuscular mycorrhizal fungi (AMF) species (CL149: *Acaulospora morrowiae*, CL699: *Paraglomus occultum*, CA201: *Funneliformis mosseae*, BR143A: *Rhizophagus clarus*, and WV116: *Rhizophagus intraradices*) was cultured with *Sorghum* separately in a concrete structured seedbed for multiplication as a source of AMF in the Department of Environmental Science at BSMRAU. AMF species were collected from the International Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM). AMF species were collected separately from the inoculated plants of *Sorghum*. Hyphae, vesicles, and spores were included in the AMF inoculum (Figure 1). Mycorrhizal spores in the soil and vesicle, hyphae, and arbuscules in the root samples were observed before using as treatment in pot soils by following the wet sieving and decanting method [48]. The spores in the watch glass were observed under a stereomicroscope (Figure 1). The number of spores was expressed as the total number of spores in 100 g of soil. Each sample was observed around 100 intersections of roots under the compound microscope (Zeiss Primo Star, Carl Zeiss Ltd., Germany). Mycorrhizal colonization was recorded based on the presence of hyphae, vesicles, or arbuscule in a root segment (Figure 1). The colonization of AMF in roots was calculated using the following formula: % root colonization = total number of positive segments/total number of segments studied × 100 [52]. In *Sorghum*, the average percentages of root colonization and spores in 100 g soil of AMF species were 40 and 50, respectively, before using as treatment. In tomatoes, the average percentages of root colonization and spores of AMF species were 45 and 60, respectively, after using them as treatments (Figure 1).

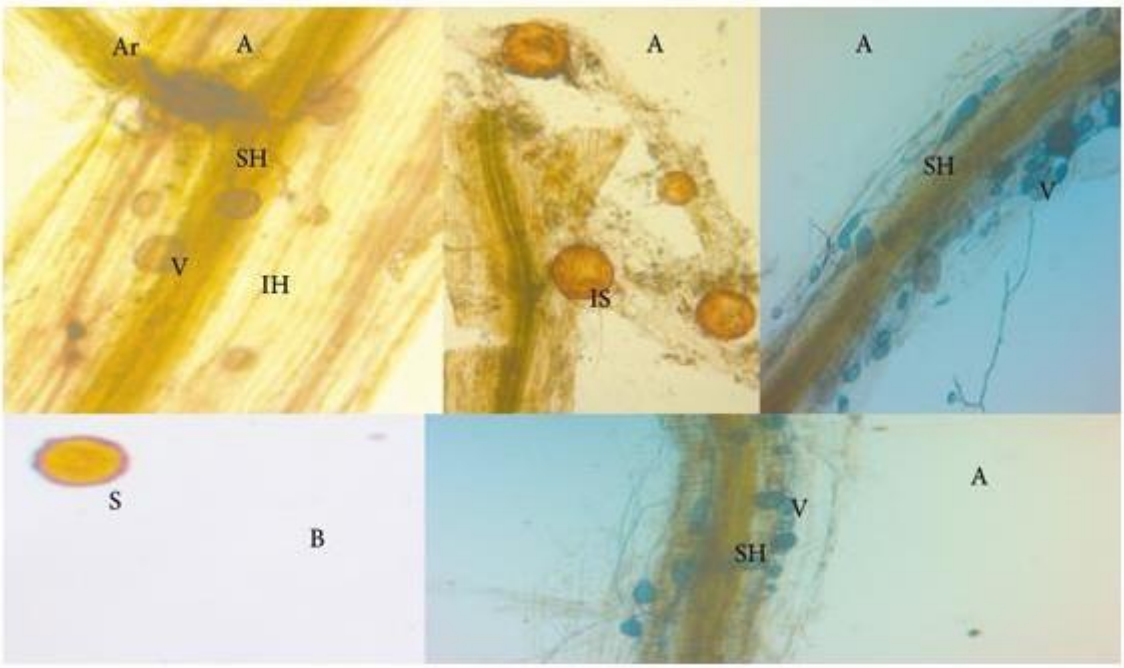


Figure 1
Morphology of AMF spore before being used as a treatment in tomato plants under drought stress. (A) Colonization structure of AMF within the roots, subtending hyphae (SH), intact spore (IS), vesicle (V), intraradical hyphae (IH), arbuscules (Ar), and spore (S). (B) Morphology of AMF spore in soil sample.

2.5. Treatments

Three tomato varieties (Unnayan, LT896, and Minto super) and six treatments comprised of T_1 = *A. morrowiae*, T_2 = *P. occultum*, T_3 = *F. mosseae*, T_4 = *R. clarus*, T_5 = *R. intraradices*, and T_6 = control (non-AMF). Five replications of each treatment were used, and the total number of pots was 90 in this experiment. Soil moisture was less than 10%. A soil moisture meter was used for maintaining expected moisture content in soil samples. Fifty gram AMF soil kg^{-1} pot soil was used in this experiment (5% of total biomass).

2.6. Biomass Growth

Average shoot length, number of leaves, length of leaves, the width of leaves, and branches of leaves were noted using a measuring tape (cm) during week 9 of each treated tomato plant. The root and shoot's average dry and fresh weight were measured separately using an electrical balance after harvesting each treated tomato plant at week 18. The yield was recorded during the collection of each tomato variety grown in pot soil (Figures 2 and 3).

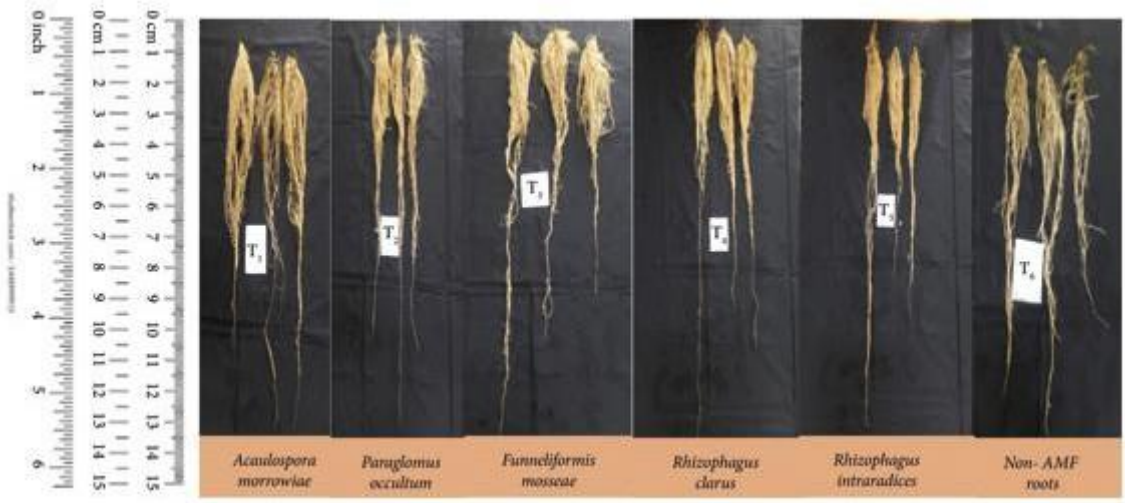
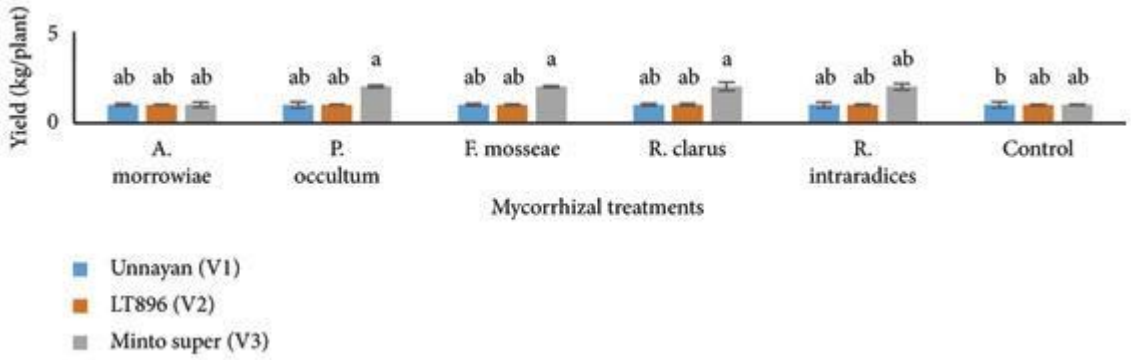
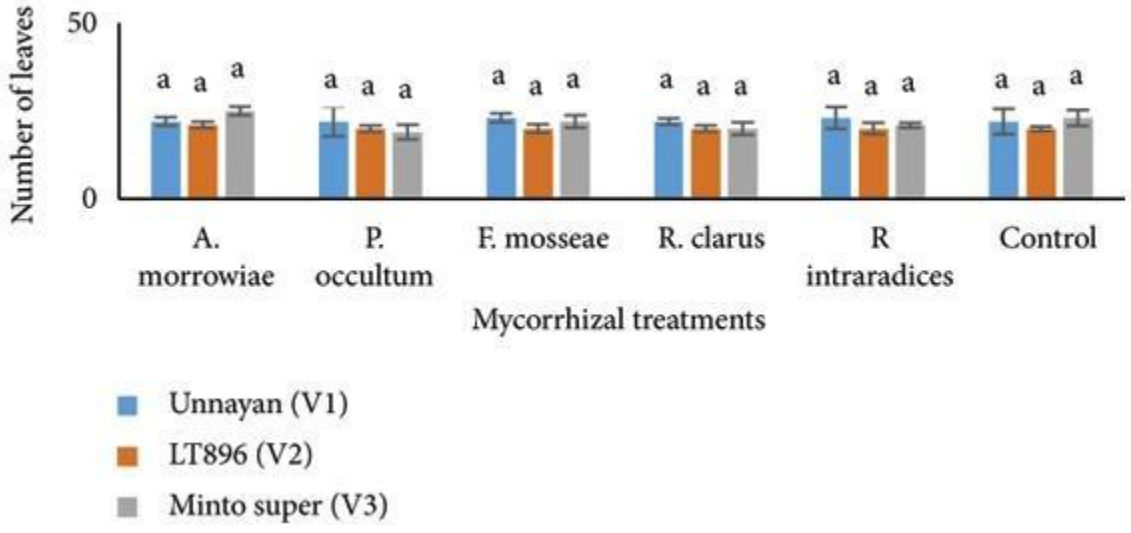


Figure 2
Mycorrhizal and non-mycorrhizal treated roots of tomato plants under drought stress.



(k)



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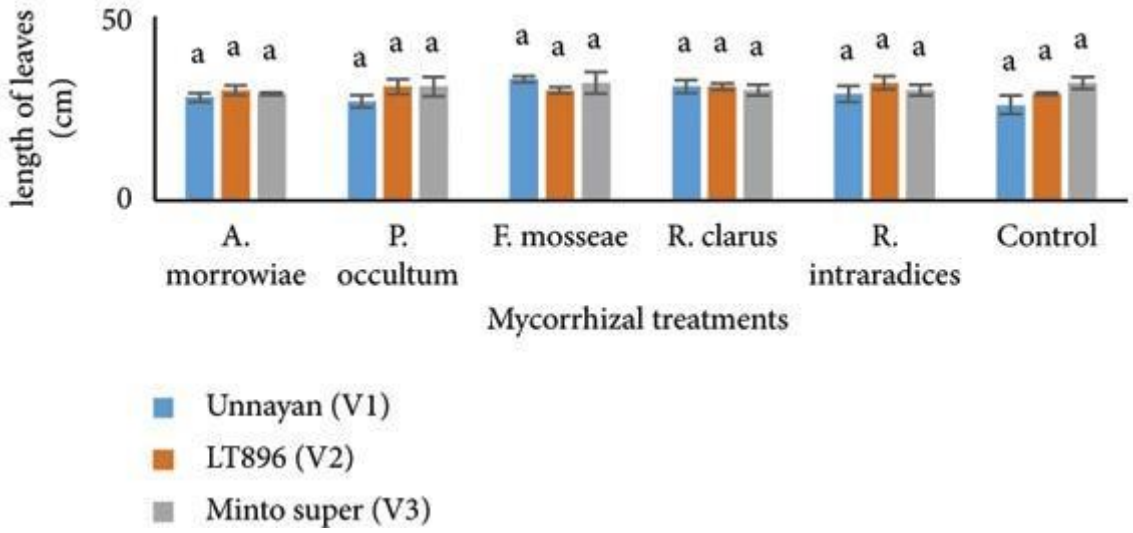
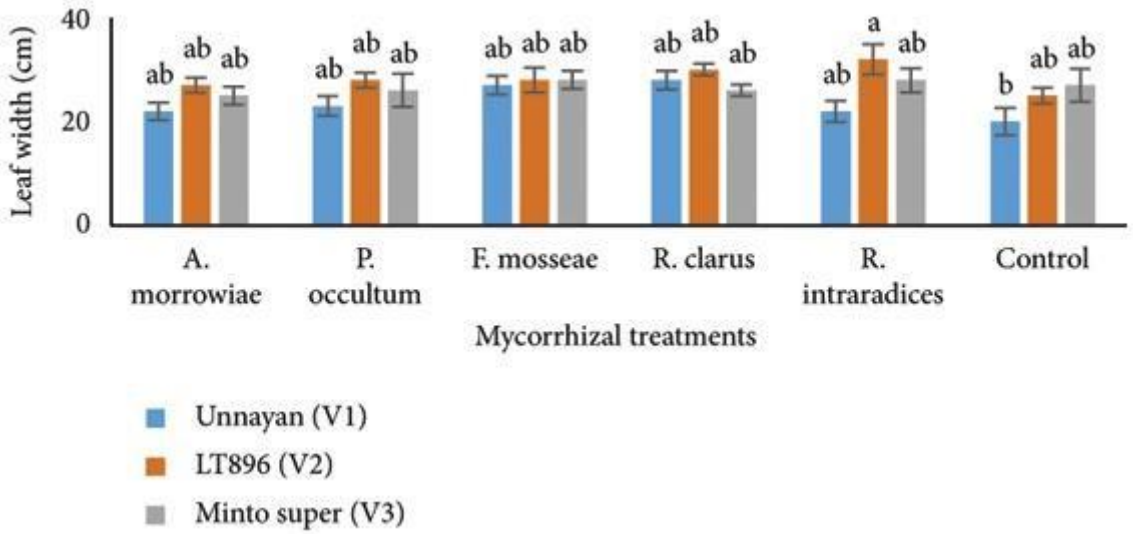
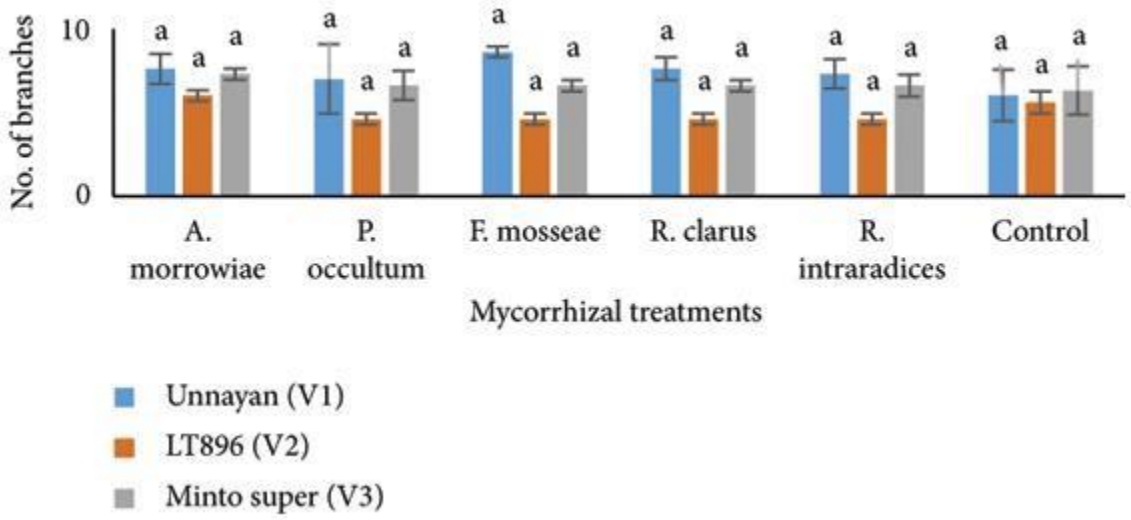

length of leaves (cm)
50
0
a a a
a a a
a a a
a a a
a a a
a a a
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

(b)


Leaf width (cm)
40
20
0
ab ab ab
ab ab ab
ab ab ab
ab ab ab
ab a ab
b ab ab
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

(c)


No. of branches
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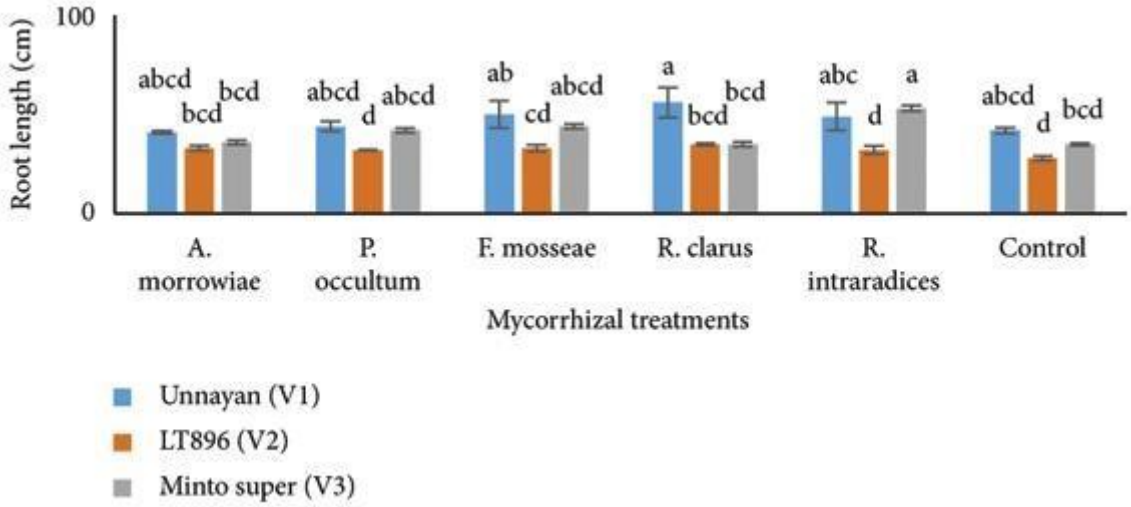
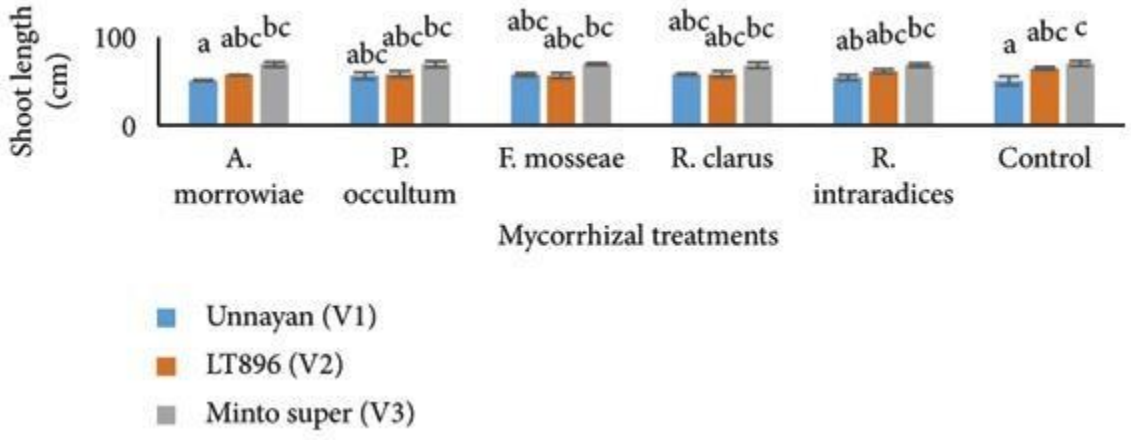
0
a a a
a a a
a a a
a a a
a a a
a a a
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

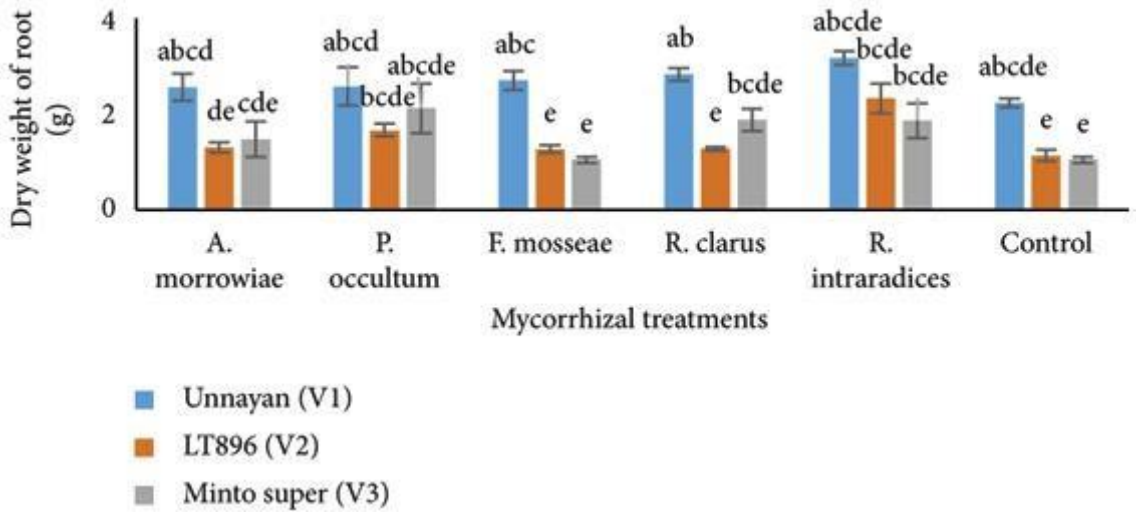
(d)


Root length (cm)
100
0
abcd bcd bcd
abcd d abcd
ab cd abcd
a bcd bcd
abc d a
abcd d bcd
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

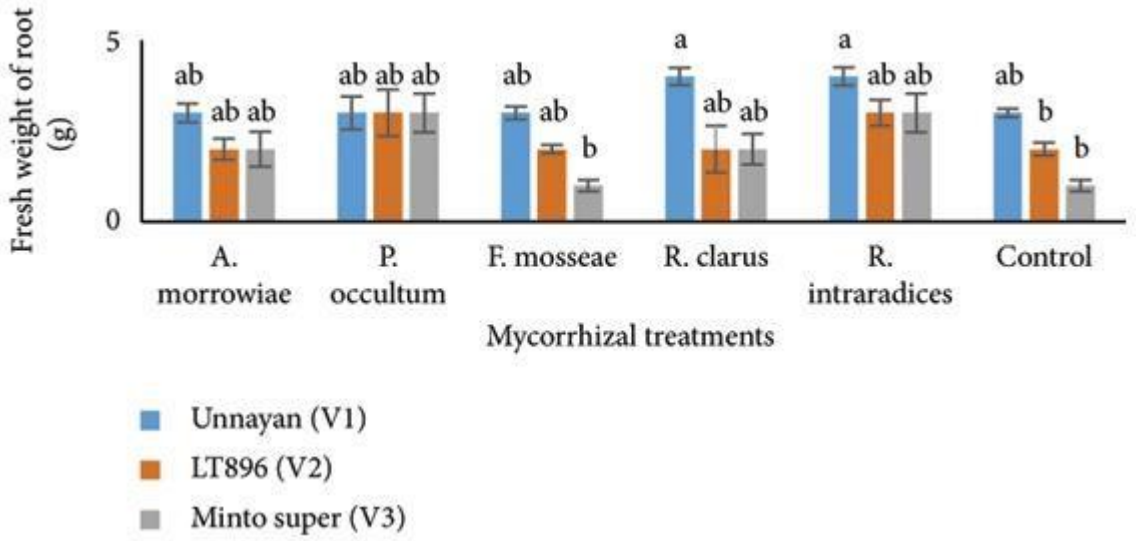
(e)


Shoot length (cm)
100
0
a abc bc
abc abc bc
abc abc bc
abc abc bc
ab abc bc
a abc c
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

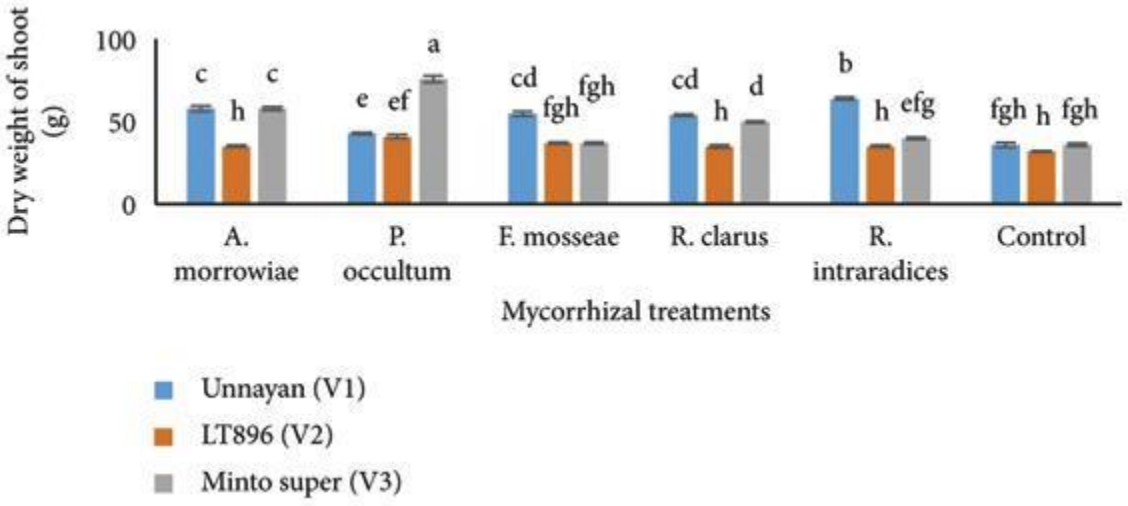
(f)


Dry weight of root (g)
4
2
0
abcd
de
cde
abcd
bcde
abcde
abc
e
e
ab
e
bcde
abcde
bcde
bcde
abcde
e
e
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

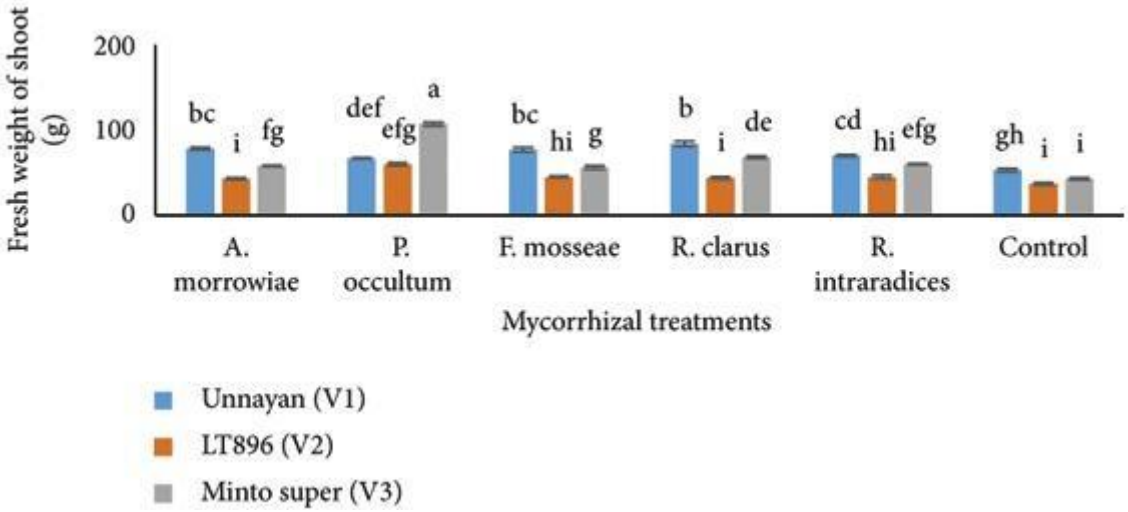
(g)


Fresh weight of root (g)
5
0
ab
ab ab
ab ab ab
ab
ab
b
a
ab ab
a
ab ab
ab
b
b
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

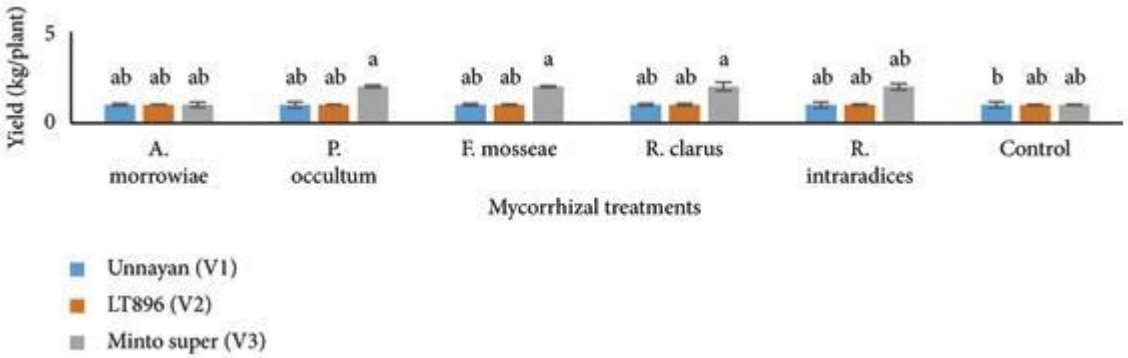
(h)


Dry weight of shoot (g)
100
50
0
c h c
e ef a
cd fgh fgh
cd h d
b h efg
fgh h fgh
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

(i)


Fresh weight of shoot (g)
200
100
0
bc i fg
def efg a
bc hi g
b i de
cd hi efg
gh i i
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

(j)


Yield (kg/plant)
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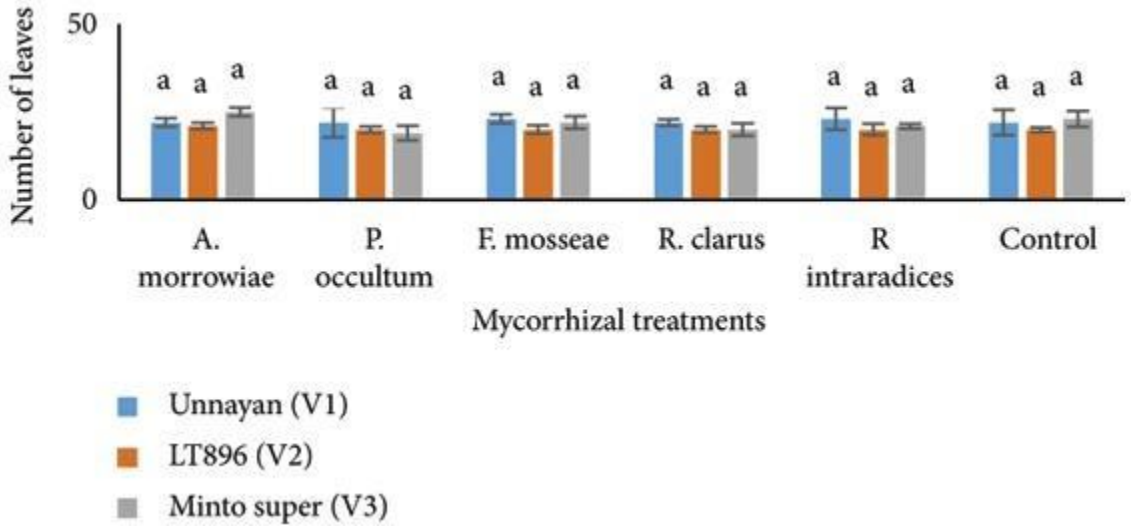
0
ab ab ab
ab ab a
ab ab a
ab ab a
ab ab ab
b ab ab
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

(k)


Number of leaves
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a a a
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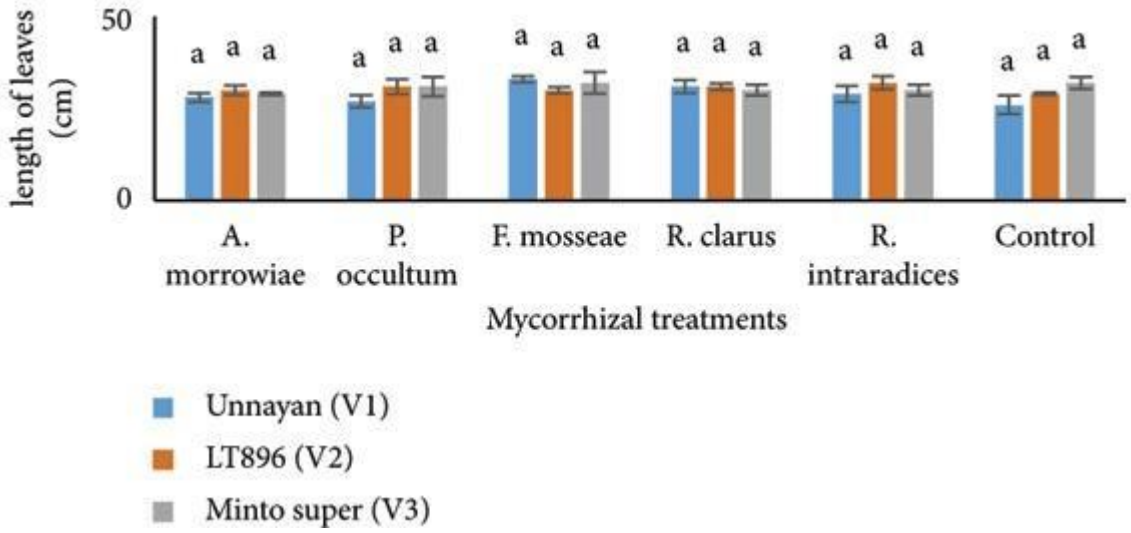
a a a
a a a
A. morrowiae
P. occultum
F. mosseae
R. clarus
R intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

(a)


length of leaves (cm)
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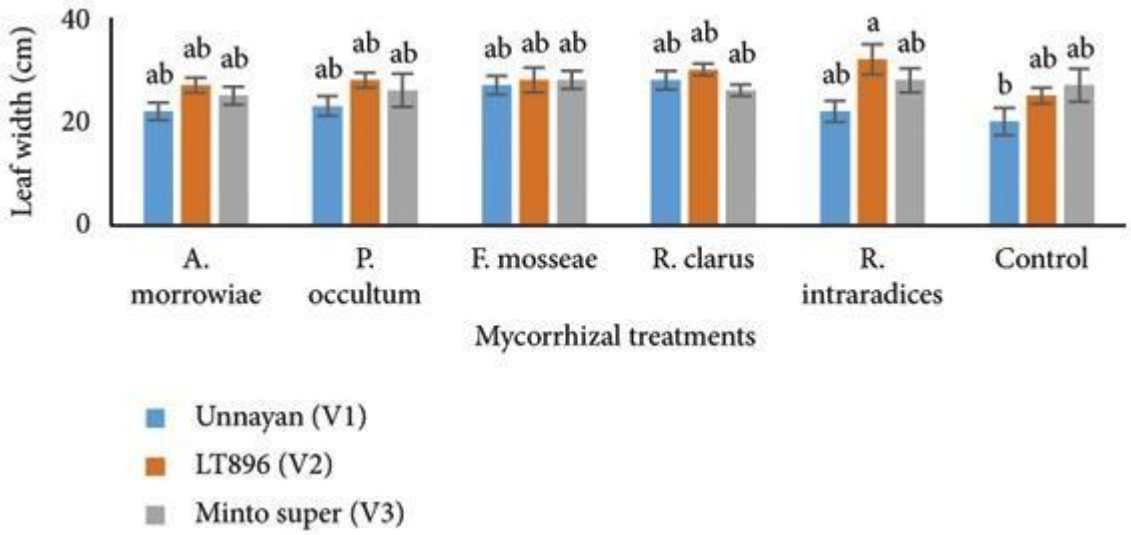
a a a
a a a
a a a
a a a
a a a
a a a
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

(b)


Leaf width (cm)
40
20
0
ab ab ab
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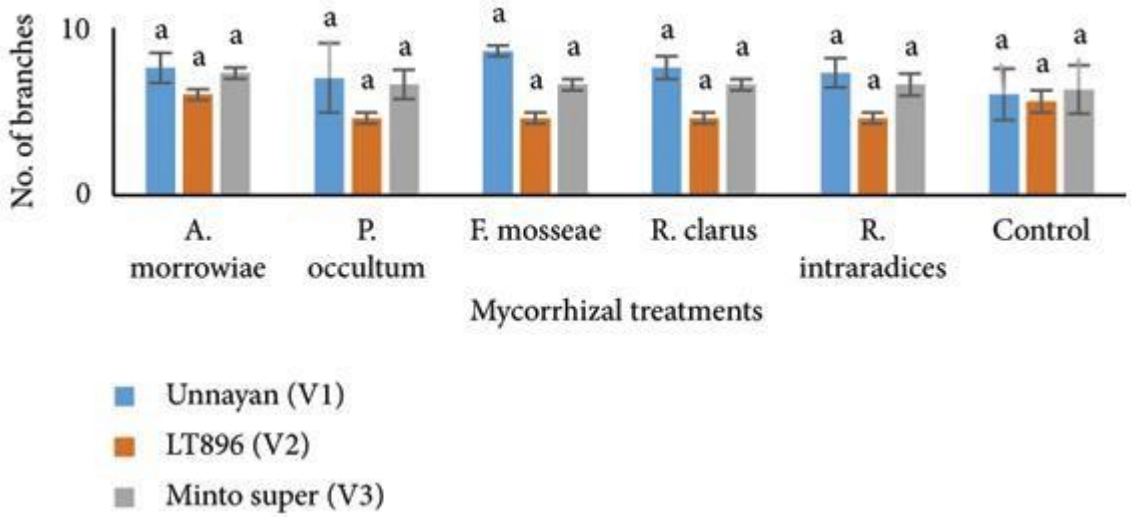
ab ab ab
ab ab ab
ab a ab
b ab ab
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

(c)


No. of branches
10
0
a a a
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a a a
A. morrowiae
P. occultum
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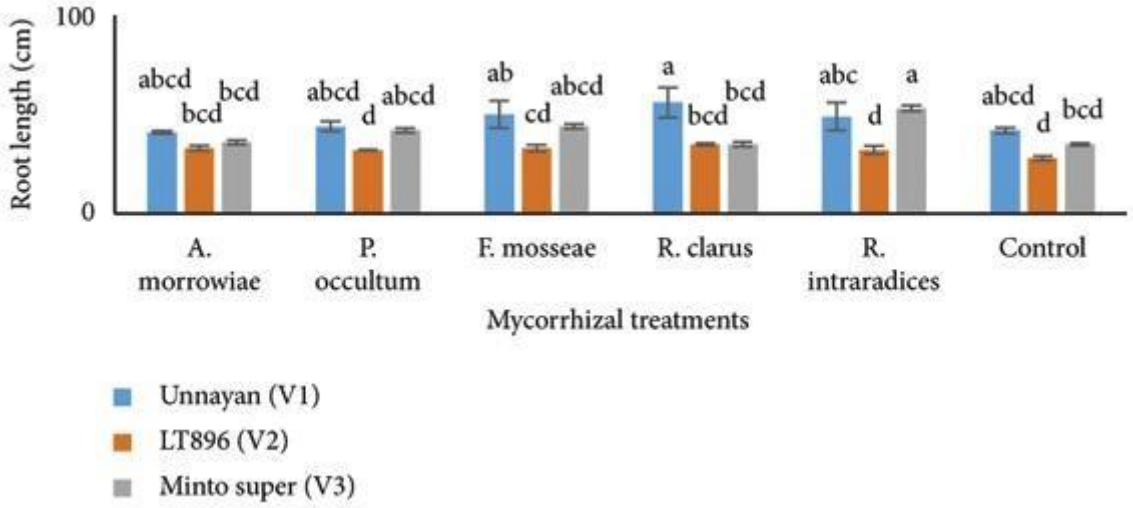
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

(d)


Root length (cm)
100
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abcd bcd bcd
abcd d abcd
ab cd abcd
a bcd bcd
abc d a
abcd d bcd
A. morrowiae
P. occultum
F. mosseae
R. clarus
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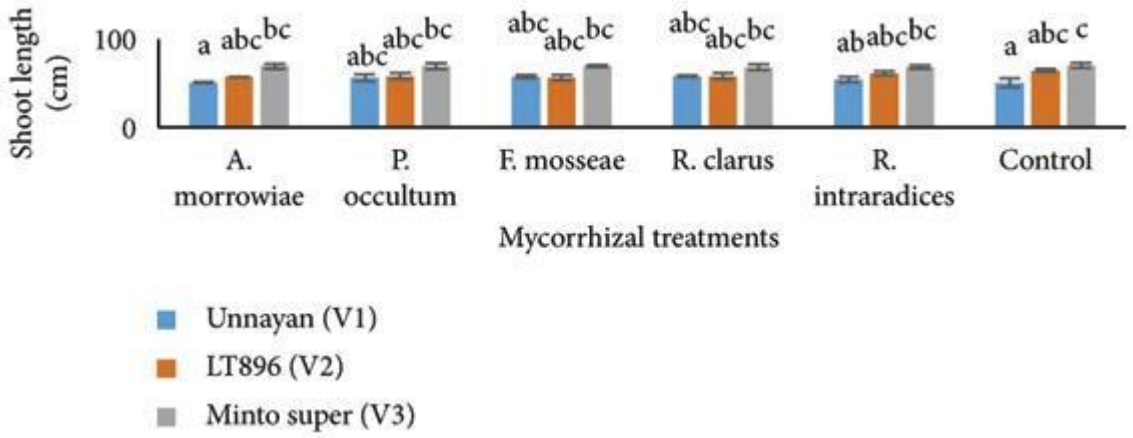
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

(e)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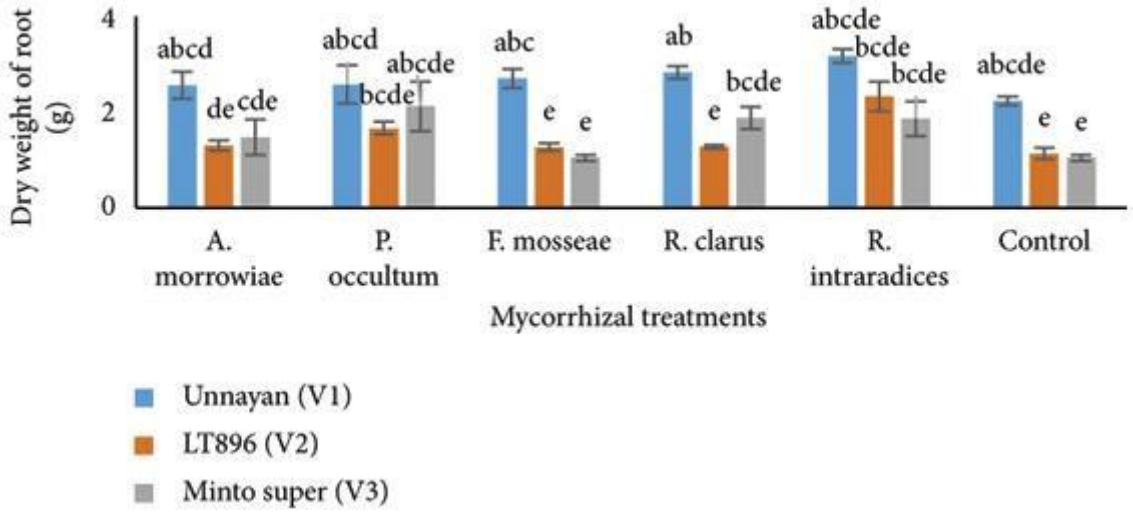
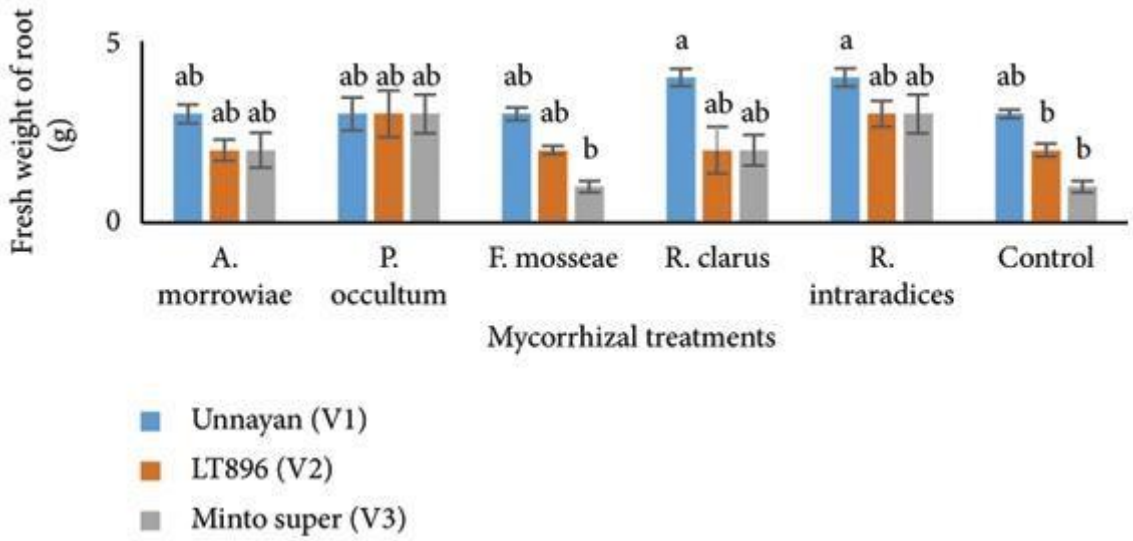
0
a abc bc
abc abc bc
abc abc bc
abc abc bc
ab abc bc
a abc c
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

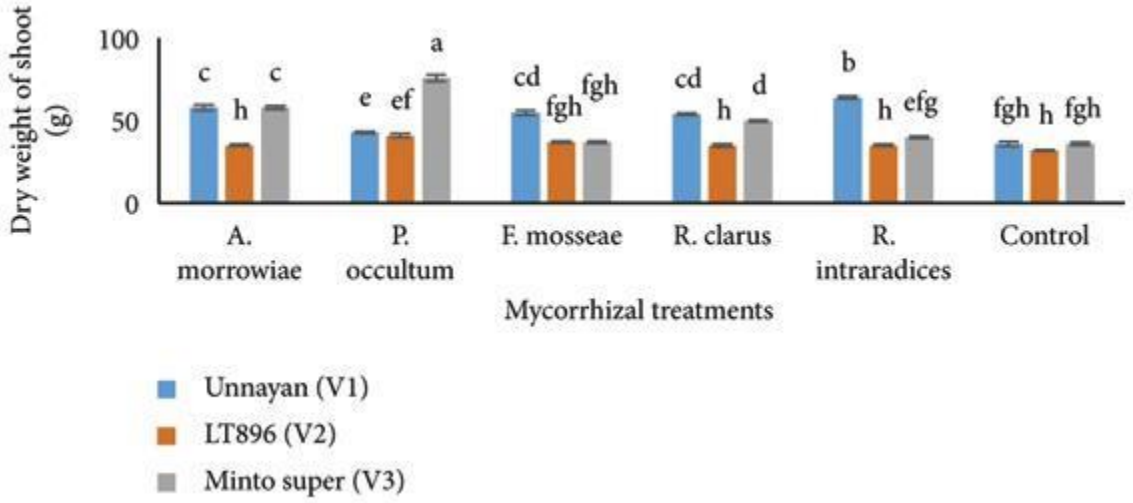
(f)


Dry weight of root (g)
4
2
0
abcd de cde
abcd bcde abcde
abc e e
ab e bcde
abcde bcde bcde
abcde e e
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

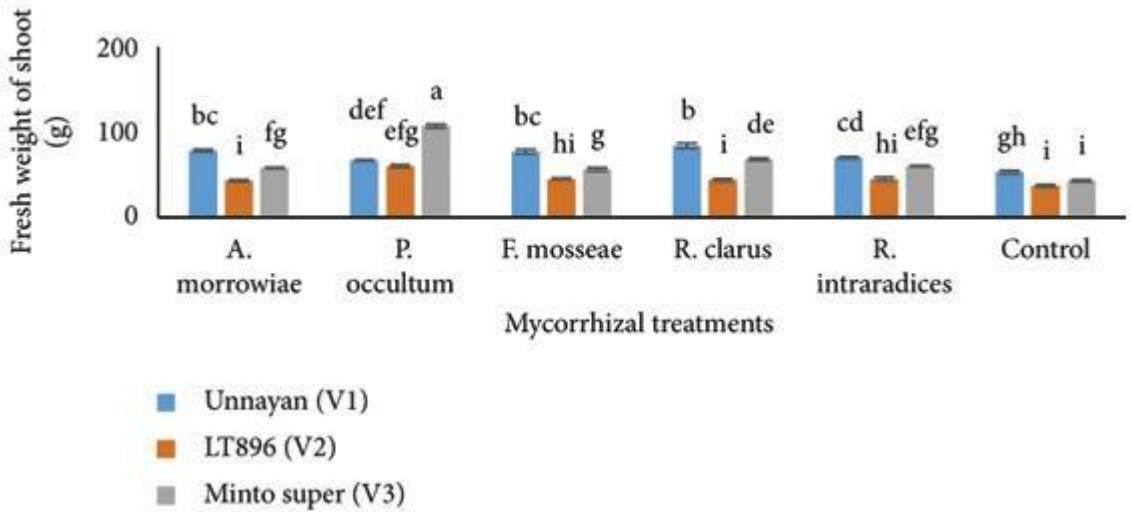
(g)


Fresh weight of root (g)
5
0
ab
ab ab
ab ab ab
ab
ab
b
a
ab ab
a
ab ab
ab
b
b
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

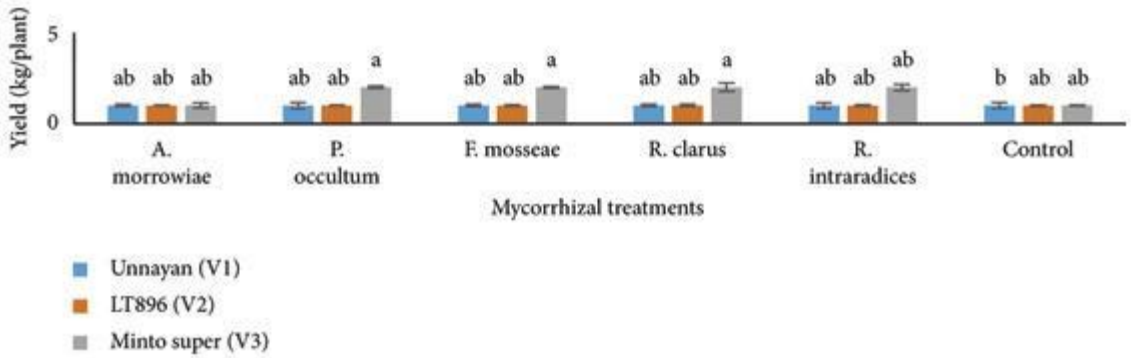
(h)


Dry weight of shoot (g)
100
50
0
c
h
c
e
ef
a
cd
fgh
fgh
cd
h
d
b
h
efg
fgh
h
fgh
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

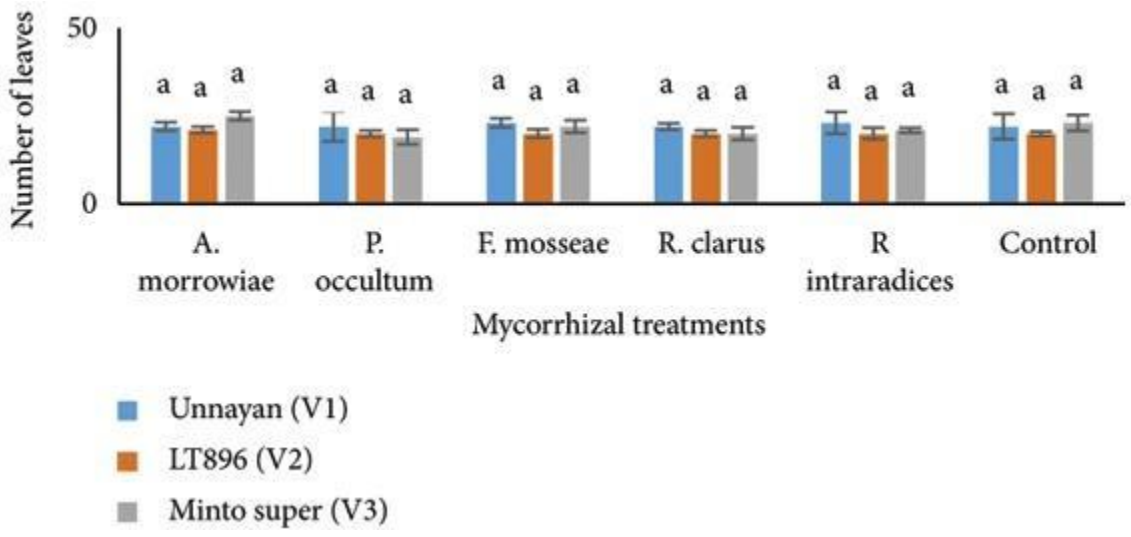
(i)


Fresh weight of shoot (g)
200
100
0
bc i fg
def efg a
bc hi g
b i de
cd hi efg
gh i i
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

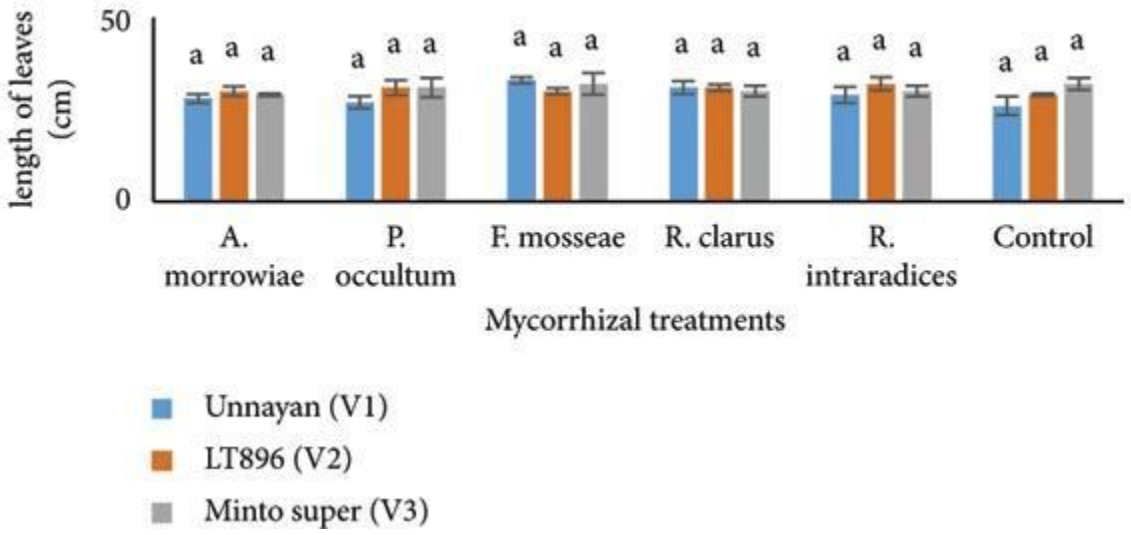
(j)


Yield (kg/plant)
5
0
ab ab ab
ab ab a
ab ab a
ab ab a
ab ab ab
b ab ab
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

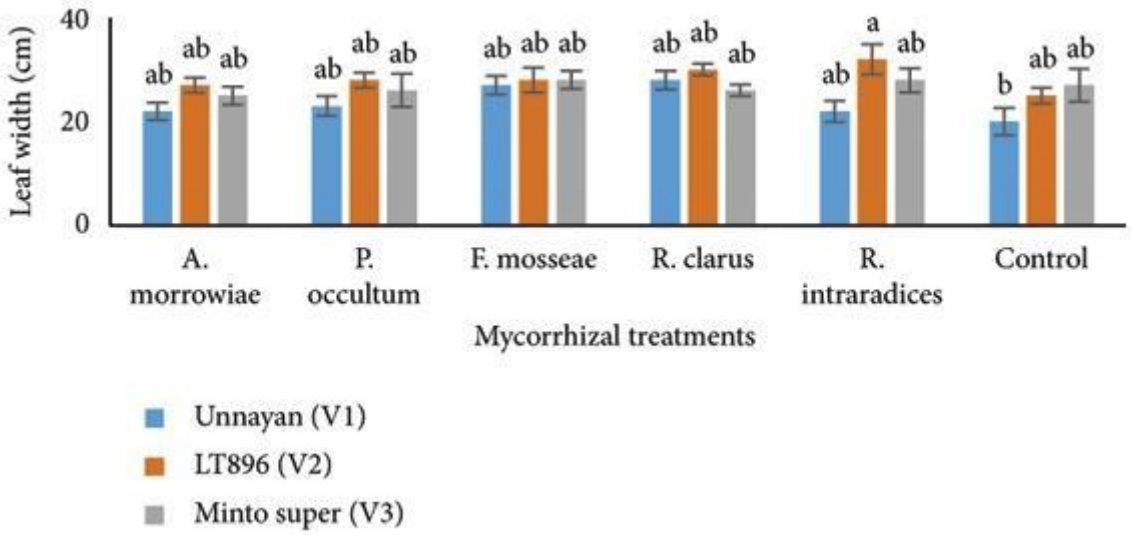
(k)

- 
Number of leaves
50
0
a a a
a a a
a a a
a a a
a a a
a a a
A. morrowiae
P. occultum
F. mosseae
R. clarus
R intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

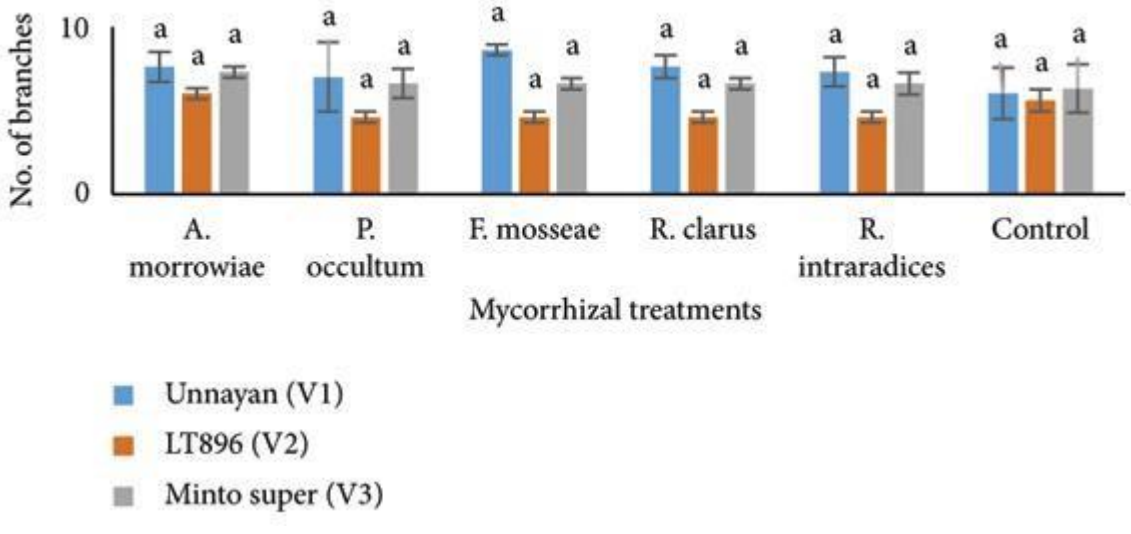
(a)


length of leaves (cm)
50
0
a a a
a a a
a a a
a a a
a a a
a a a
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

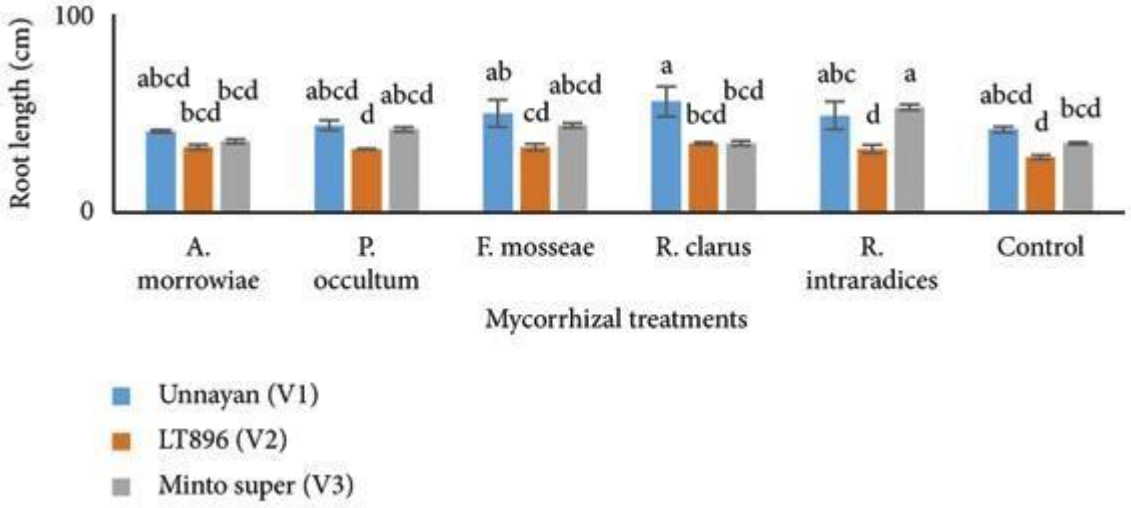
(b)


Leaf width (cm)
40
20
0
ab ab ab
ab ab ab
ab ab ab
ab ab ab
ab a ab
b ab ab
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

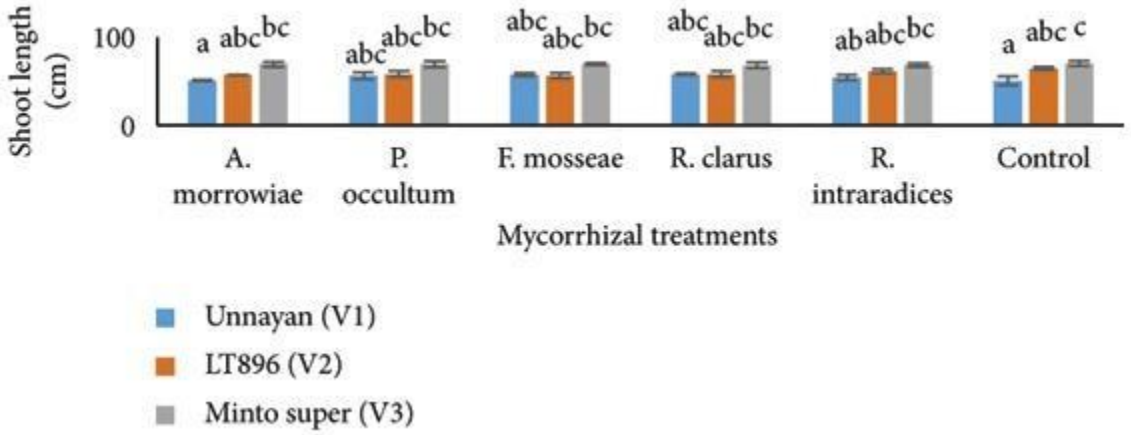
(c)


No. of branches
10
0
a a a
a a a
a a a
a a a
a a a
a a a
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

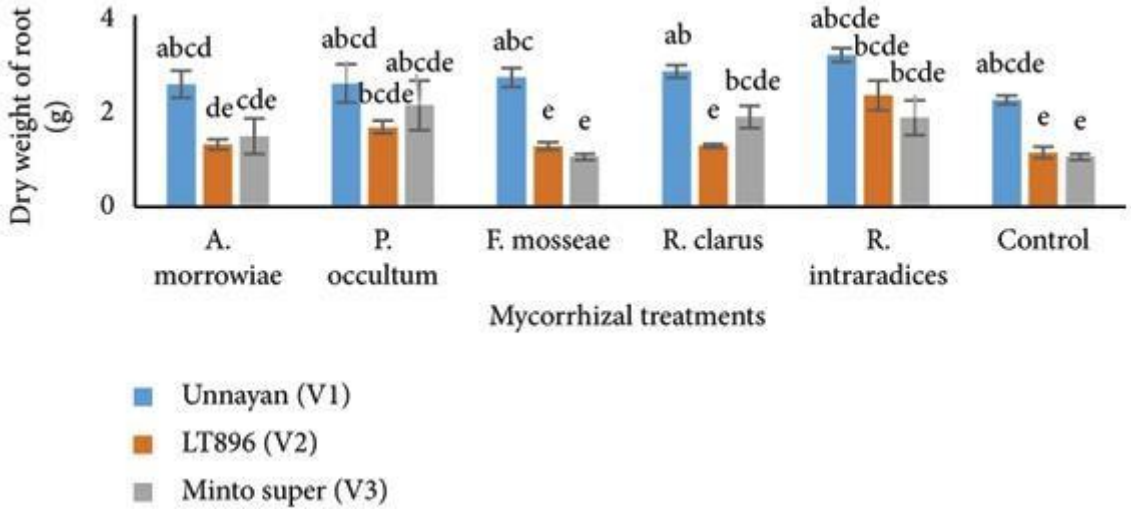
(d)


Root length (cm)
100
0
abcd bcd bcd
abcd d abcd
ab cd abcd
a bcd bcd
abc d a
abcd d bcd
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

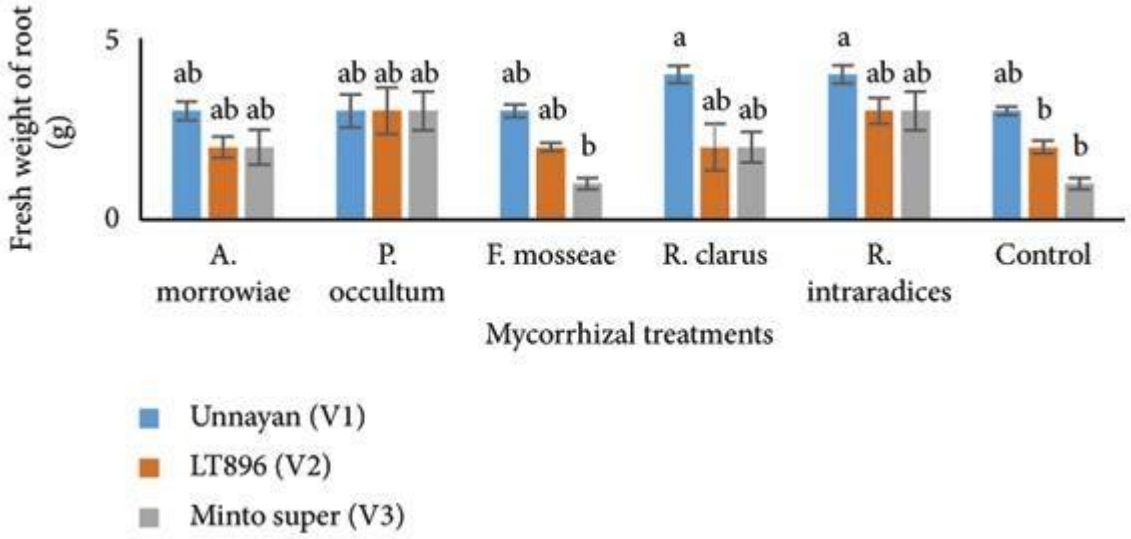
(e)


Shoot length (cm)
100
0
a abc bc
abc abc bc
abc abc bc
abc abc bc
ab abc bc
a abc c
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

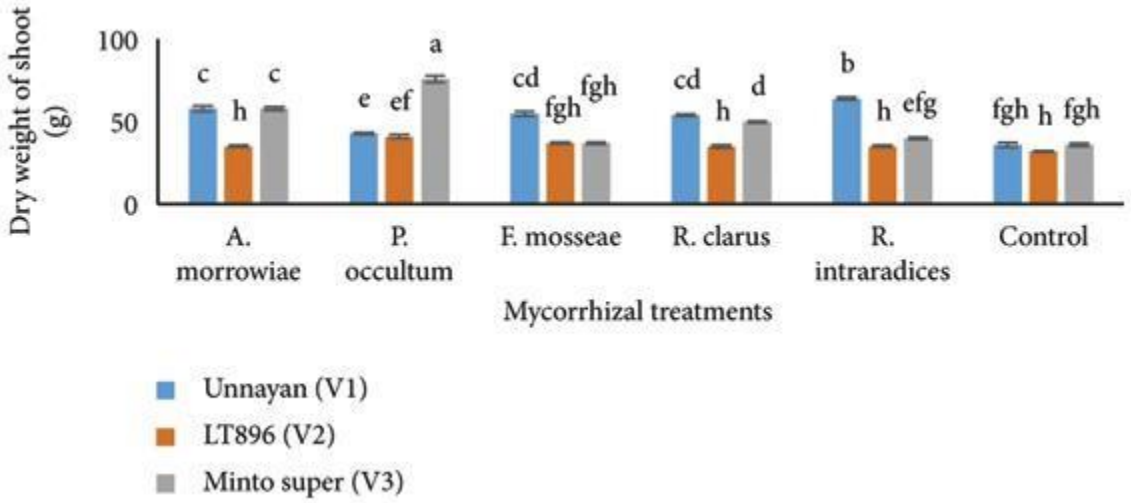
(f)


Dry weight of root (g)
4
2
0
abcd de cde
abcd bcde abcde
abc e e
ab e bcde
abcde bcde bcde
abcde e e
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

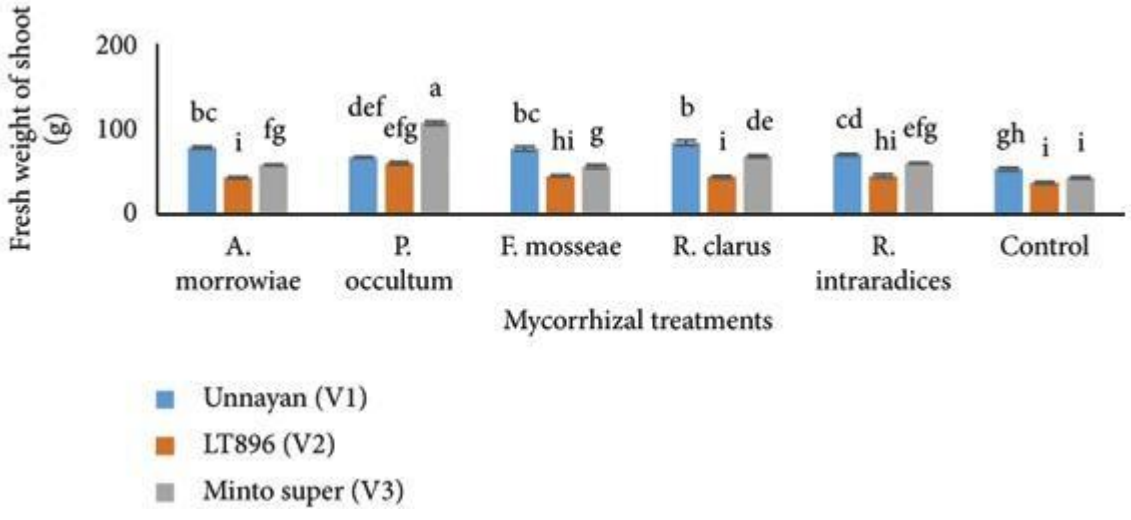
(g)


Fresh weight of root (g)
5
0
ab
ab ab
ab ab ab
ab
ab
b
a
ab ab
a
ab ab
ab
b
b
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

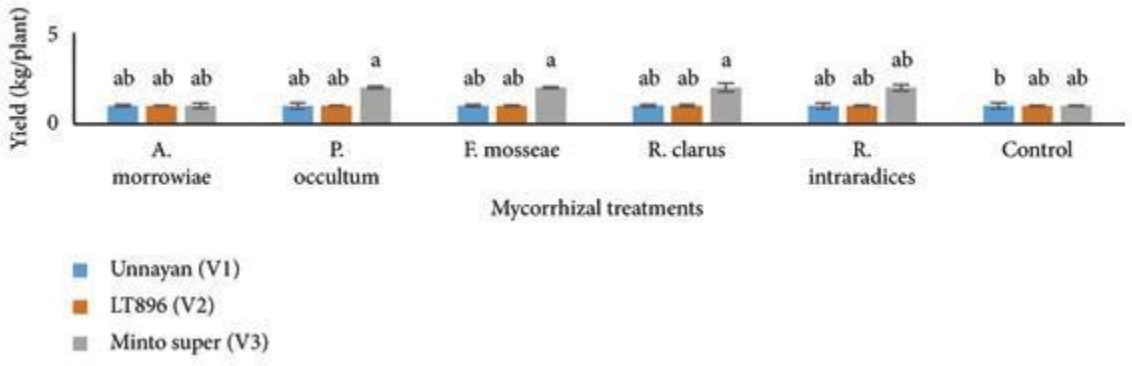
(h)


Dry weight of shoot (g)
100
50
0
c
h
c
e
ef
a
cd
fgh
fgh
cd
h
d
b
h
efg
fgh
h
fgh
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

(i)


Fresh weight of shoot (g)
200
100
0
bc
i
fg
def
efg
a
bc
hi
g
b
i
de
cd
hi
efg
gh
i
i
A. morrowiae
P. occultum
F. mosseae
R. clarus
R. intraradices
Control
Mycorrhizal treatments
Unnayan (V1)
LT896 (V2)
Minto super (V3)

(j)



(k)

Figure 3
Effect of AMF on (a) number of leaves, (b) length of leaves, (c) leaf width, (d) number of branches, (e) root length, (f) shoot length, (g) dry weight of root, (h) fresh weight of root, (i) dry weight of shoot, (j) fresh weight of shoot, and (k) yield of Unnayan, LT896, and Minto super tomatoes (mean ± SEM) grown in soil under drought condition. Means denoted by different letters indicate a difference at 0.05% level of significance. Three varieties, 5 replications, and 5 treatments with one control were used in this experiment.

2.7. Collection of Leaves and Fruits for the Analysis of Photosynthetic Pigments, Antioxidants, and Minerals

Tomato leaves were taken after inoculation with AMF on week 9 for biochemical analysis. Tomato fruits were collected on week 18 for the analysis of minerals. All treated samples were kept in Ziploc bags with proper labeling for chemical analysis. Then, samples with iceboxes containing liquid nitrogen were carried into the laboratory.

2.8. Chlorophyll and Carotenoids

The chlorophyll content of tomato leaves was detected following the method described by Arnon [53]. Chlorophyll contents were calculated using the following formula:(i)Chlorophyll a $(mg \cdot g^{-1} \cdot FW) = (0.0127 \times A_{663}) - (0.00269 \times A_{645})$.(ii)Chlorophyll b $(mg \cdot g^{-1} \cdot FW) = (0.0229 \times A_{645}) - (0.00468 \times A_{663})$.(iii)Total chlorophylls $(mg \cdot g^{-1} \cdot FW) = (0.0202 \times A_{645}) + (0.00802 \times A_{663})$.(iv)The carotenoids were determined as per the formula of $(mg \cdot g^{-1} \cdot FW) = (1000 \times A_{470} - 2.270 \times \text{Chl. a} - 81.4 \times \text{chl. b})/227$ [14].

2.9. Malondialdehyde (MDA)

The malondialdehyde content of tomato leaves was determined by the method of Heath and Packer [54]. The absorbance of the colored supernatant was taken at 532 nm. The MDA content was estimated using an extinction coefficient (Є) of 155 $mM \cdot cm^{-1}$.

The following formula was used for the calculation of MDA content = $3 \times 5 \times (\Delta A/Є) \times 2\ \mu m \cdot min^{-1} \cdot g^{-1} \cdot FW$.

2.10. Catalase (CAT)

Catalase (CAT) activity in tomato leaves was estimated by the method of Aebi [55]. The activity was calculated by using an extinction coefficient of 6.93×10^{-3} mM·cm^{-1}. The activity of CAT was determined by the following formula:

2.11. Ascorbate Peroxidase (APX) Activity

The APX was calculated using an extinction coefficient of 2.8 mM·cm^{-1}. The activity of ascorbate peroxidase (APX) was determined by the following formula: unit activity (units min^{-1}·g^{-1}·FW) = change in absorbance/min. × total volume (ml)/extinction coefficient × volume of samples (ml). APX activity (mM·min^{-1}·mg^{-1}·P) = unit activity (units min^{-1}·g^{-1}·FW)/protein mg·g^{-1}. Ascorbate peroxidase (APX) was estimated by the method of Nakano and Asada [56].

2.12. Hydrogen Peroxide (H_2O_2)

According to Loreto and Velikova [57], hydrogen peroxide (H_2O_2) content was determined in tomato leaves. Tomato leaves of 0.5 g were homogenized in 3 mL of 1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 rpm and 4°C for 10 min. Subsequently, 0.75 mL of the supernatant was added to 0.75 mL of 10 mM K-phosphate buffer (pH 7.0), and 1.5 mL of 1 M KI. H_2O_2 concentration of the supernatant was evaluated by comparing its absorbance at 390 nm to a standard calibration curve. The concentration of H_2O_2 was calculated from a standard curve plotted in the range from 100 to 1000 μ mol·mL^{-1}. H_2O_2 concentration was expressed as μ mol·g^{-1} FW.

2.13. Digestion and Detection of Minerals

Tomato fruits were digested individually using a hot plate [58]. For the fruit samples, 0.2 g fresh samples was placed into a clean beaker and 8 mL concentrated HNO_3 was mixed with it. The beaker was put into the hot plate for 2 hours at 90°C temperature on the following day. Usually, heating stopped when a white dense fume of HNO_3 was released into the air. Then, the samples were cooled and diluted to 25 ml with deionized water and filtered through Whatman No. 42 filter paper. Lastly, samples were kept in polyethylene bottles. All glassware was washed with 2% HNO_3 followed by rinsing with deionized water and drying. Minerals in tomato fruits such as Ca, Fe, K, Mg, and Na were analyzed by flow injection hydride generation atomic absorption spectrophotometry (FI-HG-AAS, Perkin Elmer A Analyst 400, USA) [59].

Discussion

The symbiotic relationship of arbuscular mycorrhizal fungi (AMF) with host plants is persistent for a prolonged period ensuring nutrient uptake into the host plant. Thus, AMF enhance biomass growth as well as enable the plant to resist environmental stresses. In this study, five species of AMF (*A. morrowiae*, *P. occultum*, *F. mosseae*, *R. clarus*, and *R. intraradices*) were used to treat three popular tomato varieties under drought stress. AMF are well known for their ability to increase biomass growth by colonizing the majority of food crops [61]. Here, we found that AMF increased shoot length, dry weight of root and shoots, leaves, and yield of tomato varieties (Figures 3 and 4). The mechanism of biomass growth is directly connected with the extended hyphal network of AMF in the root zone. The hyphal networks in non-AMF plants are much thinner than the roots of host plants [62, 63]. Thus,

nutrients are readily available for the host plants when colonized with AMF [5, 64–66]. AMF can also increase water use efficiency and improve the quality of fruits [63]. A similar growth effect of AMF is also found in many food crops such as *S. lycopersicum* L. [64], *Sorghum bicolor* (L.) [65], *Withania somnifera* (L.) [66], *Cucurbita maxima* [67], *Piper longum* L. [68], *Phaseolus vulgaris* L., *Pisum sativum*, *Lens culinaris*, and *Vigna radiata* [16, 19, 27].

Arbuscular mycorrhizal fungi (AMF) can prompt the buildup of carotenoids, phenolics, and anthocyanins in the leaves of different food crops. In most cases, AMF increased the contents of carotenoids, chlorophylls, and tocopherols in green and red leaf lettuce. These molecules are also important to enhance the minerals in edible vegetables [69]. For instance, chlorophyll contents are enhanced by 31–35% in AMF-treated pea crops [12]. Similarly, chlorophyll contents were higher compared to control in mung beans grown in AMF soils [14, 70]. In this study, both chlorophyll and carotenoids were found higher in AMF-treated tomato varieties under drought stress (Table 2 and Figure 4).

AMF enhance antioxidant enzyme activity in food crops. Literature showed that SOD, CAT, and APX increased in AMF (*R. irregularis*)-treated *Elaeagnus angustifolia* L. plants [71]. In peas, soil amendment with AMF increased CAT and POD activity by 24 to 46%. AMF also significantly improved the proline content in plants [42]. Similarly, CAT activity was found higher than that of the control in *P. occultum*-treated Unnayan tomatoes. In *A. morrowiae* and *R. clarus*-treated plants, APX activity was also found higher than that in the control (Table 2). Thus, soil amendment with AMF significantly improved antioxidant enzyme activity in food crops [12].

AMF produce glomalin, which is also known as glomalin-related soil protein (GRSP), which works as a glue that promotes the formation of water-stable aggregates by physical entanglement of extraradical hyphae, thus improving the water-holding capacity of the soil and stabilizing the structure of the soil [46]. Additionally, AMF regulate antioxidant activities, osmolyte accumulation, and gene expression and maintain plant water status and photosynthetic performance under drought stress [47]. As a result, AMF reduce drought stress in food crops through the stimulation of metabolites [72]. In this aspect, increased metabolites decrease the osmotic potential in the AMF-inoculated plants that improve the photosynthetic pigments [12]. AMF plants also alter drought-induced oxidative stress by scavenging ROS through antioxidant activities [73]. However, the activity of SOD, CAT, APX, and glutathione reductase (GR) increases through AMF inoculation in crops grown under drought stress [74]. The SOD is the principal defender for the reduction of oxidative stress. Several researchers also reported that AMF enhance APX, CAT, SOD, and GR activities, which verified to improve biomass growth in food crops grown under drought stress [73].

In this study, the increased antioxidant potential in AMF-treated tomato plants reduces ROS. For example, H_2O_2 content was detected higher in control than AMF (*R. clarus*)-treated LT896 tomato variety under stress. However, MDA was also found higher in the control than in the AMF-treated tomatoes under drought stress in this experiment (Table 2). An excessive amount of ROS such as hydroxyl radicals ($^{\cdot}OH$), superoxide radicals (O_2^{-}), singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2) creates oxidative stress and damages membrane lipids, proteins, and nucleic acids and even causes the death of cells [75, 76]. In this situation, antioxidants enable the removal of ROS [77–79]. Consequently, oxidative stress is reduced due to the minimization of ROS accumulation in plants under stress conditions [80, 81].

AMF increase mineral contents in tomato fruits [82, 83]. In this experiment, Na, Ca, Mg, K, and Fe contents under *A. morrowiae*, *P. occultum*, *R. clarus*, *R. intraradices*, and *F. mosseae* treatments were found higher than those of the control (Table 2 and Figure 4). The fungal structure of AMF-like arbuscules can assist in the exchange of inorganic minerals and compounds [84, 85]. Consequently, an association of AMF with host plants increases the nutritional value of fruits [82, 86, 87]. Although the

effect of AMF on the edible portions of plants is less clear, AMF can increase micronutrients and macronutrients in plants. For instance, AMF (*Rhizophagus irregularis* and *Funneliformis mosseae*) improve the quality of fruits, particularly minerals, vitamins, and flavor compounds (sugars, titratable acids, and volatile compounds) in fruits [88]. It was found that AMF inoculation increased the nutritional quality of tomato fruits. Likewise, antioxidant capacity, carotenoids, and volatile compounds were significantly higher in AMF plants compared with non-AMF under stress conditions. AMF might be used in other food crops grown in stress condition that will enhance nutritional quality and antioxidant activities [89]. Taken together, these results show that AMF represent a promising resource for improving both sustainable food production and human nutritional needs for future demand throughout the world.

5. Conclusions

AMF increased biomass growth, yield, and nutritional quality in tomato cultivars (Unnayan, LT896, and Minto super) grown in soil under drought stress. The shoot's dry weight and yield were enhanced by 28% and 20% on AMF-treated tomatoes, respectively, compared to the control under drought stress. AMF also increased the catalase (CAT) and ascorbate peroxidase (APX) activity as well as reduced MDA and hydrogen peroxide (H_2O_2) content. CAT and APX activity increased by 42% and 66% in AMF-treated tomatoes compared to non-AMF. In contrast, AMF treatment decreased MDA and H_2O_2 (ROS) in tomatoes by 50% and 2% compared to the control, respectively. K, Ca, Mg, and Fe of tomato fruits increased by 2%, 13%, 24%, and 37% with AMF treatments. Although AMF enhanced the yield and yield-contributing attributes of tomato varieties in pot trials, further field studies are suggested in multiple sites to verify these results. These results suggested that biomass growth, yield, photosynthetic pigments, antioxidant enzyme activity, and mineral contents could be enhanced by AMF in food crops grown under drought stress. It is concluded that AMF might be used for the development of AMF-enriched biofertilizers that will reduce fertilizer demand and improve the nutritional quality of food crops grown under stress conditions.

Data Availability

All data used to support the findings of the study are available within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Mohammad Zahangeer Alam was responsible for original draft preparation, funding acquisition, review, and editing. Tasrina Rabia Choudhury was responsible for formal analysis. MAU Mridha was responsible for review and investigation.

Acknowledgments

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Supplementary Materials

Supplementary Table: ANOVA-two way. *(Supplementary Materials)*

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