Fatty Acid Composition and Eco-Agronomical Traits of Lallemantia Species Modulated Upon Exposed To Arbuscular Mycorrhizal Fungi and Nano Iron Chelate Fertilizers Under Water Deficit Conditions

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Research article

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Posted Date: January 14th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-143811/v1

Fatty Acid Composition And Eco-Agronomical Traits Of *Lallemantia* Species Modulated Upon Exposed To Arbuscular Mycorrhizal Fungi And Nano Iron Chelate Fertilizers Under Water Deficit Conditions

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Abstract

Background: Application nano iron chelate and AMF fertilizer can increase plants' tolerance against water deficit stress. The main objectives of the current study were to investigate the effect of arbuscular mycorrhizal fungi (AMF) and nano iron chelate fertilizer under drought stress on grain yield, leaf chlorophyll contents, root colonization, oil percentage, and fatty acids profile of *Lallemantia* species. The experiment was carried out as a factorial based on a complete randomized block design consisting of three factors of irrigation levels of 90 (I_{90}), 60 (I_{60}), and 30% (I_{30}) depletion of available soil water (ASW)), fertilizer levels of control (no fertilizer), AMF inoculation, and nano iron chelate, and plant species of *Lallemantia* (*L. iberica* and *L. royleana*) at the Research Farm of College of Agriculture, Shahed University, Tehran, Iran, in 2018/2019.

Results: The results showed that increasing water deficit stress significantly decreased the above traits while applying nano iron and AMF fertilizers significantly increased them across water treatments. AMF fertilizer inoculation significantly improved both species yield. Higher root colonization by AMF inoculation enhanced seed oil and fatty acids (palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid, and Eicosenoic acid). In contrast, applying nano iron chelate by increasing chlorophyll content in any irrigation regime could enhance seed oil and some fatty acids such as palmitoleic acid.

Conclusions: Water deficit stress and application of fertilizers had different effects on both species. *L. iberica,* compared to *L. royleana,* had the most tolerance to water deficit stress and the highest dependence on AMF inoculation. Overall, these results demonstrated that the application of AMF could improve major features of *Lallemantia* species under deficit irrigation conditions, especially at the I_{60} irrigation level.

Keywords: *Lallemantia iberica*, *Lallemantia royleana*, Root colonization, Oil content, Fatty acid

Background

Plants belonging to the genus *Lallemantia sp.* are known because of their economic features. These plants can be either served as food, industrial crop, or medicinal plant. The genus *Lallemantia sp*. (Balangu) belongs to the Lamiaceae family and comprises five species. Of these, *L. iberica* (Dragon head) and *L. royleana* (Lady's mantle) are mainly noticeable because of their high concentration of oilseed (approximately 30-45%) [1, 2, 3, 4]. The oil of *L. iberica* seeds is known as Iberian oil and is rich in ω-3 polyunsaturated fatty acids (PUFAs, approximately 67–74%). It has been shown that PUFAs in plants are tightly associated with cell structure and membrane fluidity, especially under stress conditions [5]. The PUFAs, such as linolenic acid (C18:3) and linoleic acid (C18:2), play crucial roles in maintaining plant cell membrane and seed storage lipids [6, 7]. A strong genotype effect mainly determines the composition of fatty acids in seeds, available water resources, and soil nutrients influence them concurrently [7].

The most critical production input is water that significantly affects plant growth and crop production [8, 9]; however, water deficit reduces drought stress [10]. Drought stress mainly leads to decreased plant growth [11] and photosynthetic production [12, 13], nutritional disturbance, restriction of water absorption, and damage to the plasma membrane [14]. Grain yield of *L. iberica* and *L. royleana* plants decrease in severe drought stress due to the reduction of photosynthetic materials [14]. Previous research has shown that water deficits cause a reduction in grain yield, oil percentage, and PUFA composition, including that of linolenic acid, linoleic acid, palmitic acid, and oleic acid in purslane [15]. Likewise, similar alterations have been shown in *Oenothera biennis* L. [16], sunflower, safflower, sesame [7], and purslane [17]. These findings indicate that the biosynthesis pathways of fatty acids and the percentage of oil content are involved in plant responses to drought stress [18]. One way to enhance crop performance, growth improvement, and plant tolerance is to use fertilizers.

A balanced nutrient concentration in the soil is another essential element for plant growth regulation [19]. A balanced nutrient concentration can be supplied by applying chemical and biofertilizers, critical for the sustainability of soil fertility, photosynthetic reactions, assimilate transportation, photosynthesis, oil synthesis, and final performance [19, 20, 21]. The application of biofertilizers such as arbuscular mycorrhizal fungi (AMF) not only keeps the environment healthy [8] but also is essential for the sustainability of soil fertility yield performance in the agricultural section or under drought stress [19]. The AMF establishes a symbiotic relationship with the host plant roots to absorb carbon. Carbon enables the fungi to grow and complete their life cycle [22]. In return, AMF helps the host plants absorb mineral nutrients (phosphorus) [23]. Improvement of nutrients such as P in soil and increasing root colonization via AMF can maintain optimal growth and water relations and, in contrast, enhance host plant resistance under drought stress [18]. Root colonization by mycorrhizal fungus is an alternative strategy to enhance plants' drought tolerance and increase water balance in drought conditions [12, 24]. It has been reported that adding AMF inoculum in soil not only enhances root colonization and water use efficiency but also has an influence on increasing photosynthetic pigment and fatty acid synthesis under drought stress [12, 18, 25]. One study has suggested that AMF inoculation can result in the highest oil percentage and oleic acid in flax (*Linum usitatissimum* L) [21] and purslane (*Portulaca oleracea*) [8]. Generally, in plant cell metabolism-enhancing, the biosynthesis of beneficial host phytochemicals, stimulation of photosynthesis, and nutrient acquisition can be changed by AMF symbiosis [26].

The third most limiting nutrient for the growth of plants is iron (Fe) [27] that plays a vital role in plant growth and food production [28] and, as a cofactor for approximately 140 enzymes, can improve photosynthesis and assimilates transportation to sinks and eventually amends grain yield and oil content under drought stress [20, 29, 30]. The nano iron chelates fertilizers application is highly efficient due to excessive chemical fertilizers and the resulting contamination of groundwater and soil salinization [31]. Applying nano iron chelate over other Fe fertilizers is a significant step to reach sustainable agriculture due to improving plant mineral nutrition and decreasing conventional fertilizer consumption [32, 33]. Nano iron, by decreasing Fe availability in the rhizosphere, stimulates operation of the proton pump in plants and by activating plasma membrane H+-ATPase to secretion protons leads to acidification of the rhizosphere of plant roots [32], eventually improves the solubility and dispersion of insoluble nutrients in the soil [29]. An in-depth review of the literature suggests that the interaction between available soil water and different fertilizers on grain and oil yield in *Lallemantia* species is still limited. Thus, the current study's goal was to evaluate AMF and nano iron fertilizer's effects on grain yield and fatty acids composition of *L. iberica* and *L. royleana* under deficit irrigation.

Results

We observed significant effects of treatments on grain yield, chlorophyll contents, root colonization, seed oil percentage, and fatty acid composition (Table 1).

Table 1 Analysis of variance for plant characteristics of *Lallemantia* species affected by irrigation regime and fertilizer treatments

Source	Df	Grain yield	Root colonization	Oil percentage	Chlorophyll a	Chlorophyll b
Block	2	496.71n.s	0.512n.s	0.34n.s	1.355n.s	1.331n.s
I	2	661945.48**	451.23**	54.96**	76.802**	22.811**
Error a	4	728.81	3.97	0.61	1.79n.s	0.347n.s
S	1	1043577.26**	1843.65**	1387.91**	113.381**	51.406**
F	2	2232472.64**	11438.49**	179.12**	152.135**	37.706**
I×S	2	35953.30**	5.37n.s	11.84**	33.827**	2.04*
I×F	4	378359.14**	187.69**	1.33**	39.58**	8.75**
S×F	2	31929.97n.s	263.23**	35.73**	76.799**	7.372**
I×S×F	4	9118.49**	13.39**	8.32**	39.302**	6.954**
Error b	30	1272.02	87.53	0.284	1.06	0.561
CV (%)		3.14	4.69	2.24	5.85	14.91

ns: non-significant. ** Significant at $P < 0.01$, * Significant at $P < 0.05$.

Table 2 Analysis of variance for fatty acids composition in *Lallemantia* species

Source	Df	EA	AA	LNA	LA	OA	SA	POA	PA
Block	2	800.48n.s	0.0001n.s	.248n.s	0.310n.s	0.61**	0.003**	0.0002*	0.671**
I	2	0.10*	0.009**	81.78**	7.99**	19.73**	0.43**	0.009**	13.63**
Error a	4	60.62	0.00003	0.428	0.016	0.03	0.0001	0.00003	0.089
S	1	0.41**	0.029**	311.20**	67.89**	47.07**	4.42**	0.040**	0.205n.s
F	2	1.73**	0.023**	109.04**	19.33**	33.81**	0.458**	0.011**	27.83**
I×S	2	0.02*	0.0003*	22.30**	1.31**	1.87**	0.002n.s	0.0002**	2.65**
I×F	4	0.016*	0.001**	20.04**	2.26**	0.271*	0.018**	0.0003**	1.77**
S×F	2	0.31**	0.001**	7.88*	2.67**	2.31**	0.0003n.s	0.0001**	0.771**
I×S×F	4	0.032**	0.0004**	2.86**	0.735**	0.35*	0.037**	0.0002**	1.49**
Error bb	30	0.004	0.6×10^{-4}	0.343	0.048	0.094	0.0030	0.1×10^{-5}	0.107
CV (%)		7.3	9.30	1.09	1.73	2.23	2.66	1.58	4.14

ns: non-signifcant. ** Signifcant at P < 0.01, * Signifcant at P < 0.05.

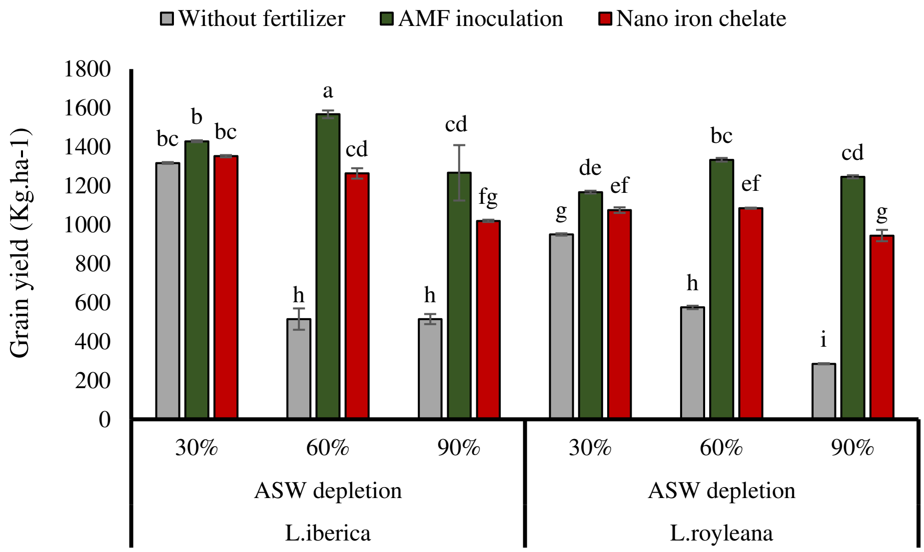


Fig 1. The effect of AMF and nano iron chelate application on grain yield of *L. iberica* and *L. royleana* species under different water treatments. The same letters in each column show non-significant differences at $P \leq 0.05$, analyzed by Tukey's honest test.

Grain yield

By increasing the severity of drought, grain yield reduced in both species, and the most increasing grain yield was observed when both species were grown under I_{60} condition. Application of AMF and nano iron chelate fertilizers significantly influenced grain yield of both species compared to that of the without fertilizer. AMF fertilizer significantly increased the grain yield of both species across all irrigation treatments. The amount of grain yield of *L. iberica* was higher than that of *L. royleana* across all irrigation regimes and fertilizer treatments. With AMF biofertilizer application, the highest grain yield for *L. iberica* was obtained at I_{60} and I_{90} irrigation treatments, while for *L. royleana* species, the highest grain yield was obtained from I_{30} to I_{90} level of irrigation treatment. Under different irrigation levels, the lowest grain yield (Fig. 1) in both species were observed in control (without fertilizer application).

Table 3 The oil percentage and leaf chlorophyll contents in *L. iberica* and *L. royleana* species inoculated with nano iron chelate and AMF under irrigation wate

Treatments: Irrigation regime (%)	Treatments: Species	Treatments: Fertilizer	Oil percentage (%)	Chlorophyll- a (µg/g FW)	Chlorophyll- b (µg/g FW)
I_{30}	S1	F1	27.05±0.37f	16.3±0.07f.h	5.18±0.32c.e
		F2	32.6±0.21bc	12.02±0.69i	4.41±0.2c.g
		F3	33.3±0.15b	20.07±0.18c.e	4.41±0.1c.g
	S2	F1	18.28±0.26ij	14.52±0.29g.i	3.07±0.24e.g
		F2	19.92±0.22h	13.11±0.96hi	4.32±0.15c.g
		F3	19.26±0.21hi	15.85±0.65f.h	2.24±0.04g
I_{60}	S1	F1	30.09±0.21d	12.18±0.04i	3.29±0.54e.g
		F2	32.82±0.06bc	27.52±0.24a	11.5±0.1a
		F3	34.81±0.03a	24.47±0.21ab	6.28±0.72bc
	S2	F1	19.2±0.08hi	14.22±0.28g.i	4.61±0.11c.g
		F2	21.4±0.22g	13.03±0.54hi	6.53±0.18bc
		F3	19.71±0.02h	18.78±0.89d.f	4.89±0.55c.f
I_{90}	S1	F1	28.78±0.53e	13.3±0.79hi	4.33±0.26c.g
		F2	31.75±0.67c	22.76±0.79bc	8.69±0.26b
		F3	33.19±0.37b	23.21±0.98bc	5.95±0.77cd
	S2	F1	17.91±0.22j	18.06±0.76d.f	3.5±1.08d.g
		F2	19.85±0.17h	17.09±0.98e.g	4.64±0.11c.g
		F3	18.8±0.17hij	21.08±0.15b.d	2.67±0.45fg

The same letters in each column show non-significant differences at $P \leq 0.05$, analyzed by Tukey's honest test.

Leaf chlorophyll contents

Increasing available soil water depletion caused a reduction and enhancement in leaf chlorophyll-a in L. *iberica* and L. *royleana*, respectively. Fertilizers application were effective in improving chlorophyll content across all irrigation treatments. In *L. royleana* species from I_{30} to I_{90} level of irrigation treatments, the highest and lowest chlorophyll-a content was related to plants treated with nano iron chelate and AMF fertilizer.

In contrast, in *L. iberica* species at I_{30} and I_{90} levels of irrigation regimes, the highest chlorophyll-a content was obtained when grown under nano iron chelate. At I_{30} irrigation level treatment, the highest chlorophyll content was observed in plants inoculated by AMF fertilizer. In *Lallemantia* species, chlorophyll-b content was reduced in irrigation regimes from I_{30} to I_{90} level.

Across all irrigation treatments, the highest chlorophyll-b content in *Lallemantia* species was obtained in plants inoculated by AMF. Simultaneously, the lowest chlorophyll-b content was observed in *L. iberica* and *L. royleana* at control treatment (neither biofertilizer nor nano iron chelate fertilizer). Higher chlorophyll-a and chlorophyll-b content were observed in *L. iberica* leaves, compared to *L. royleana* across all irrigation regimes and fertilizer treatments (Table 3).

Root colonization

A significant reduction was observed in the root colonization of *Lallemantia* species underwater deficiency stress, especially under I_{90} conditions. On average, both species under the I_{60} irrigation regime condition had more root colonization than that with I_{30} and I_{90} irrigation regime. No difference between plants treated by nano iron chelate and control was found under different irrigation regimes, and more differences in root colonization were found in plants inoculated by

AMF. Colonization in both species of *Lallemantia* roots improved by AMF inoculation in any irrigation regime treatments; however, *L. royleana* roots had the highest colonization (Fig. 2).

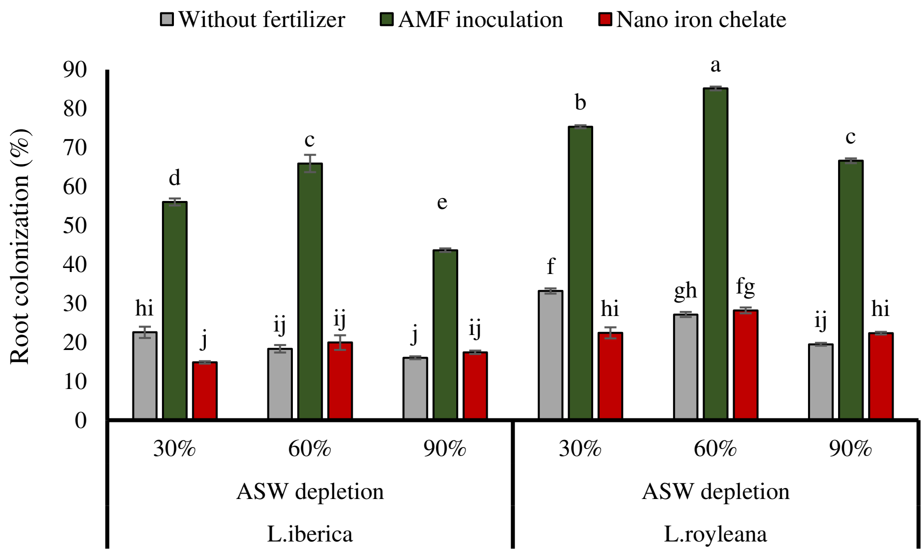


Fig 2. The effect of AMF and nano iron chelate application on root colonization of *L. iberica* and *L. royleana* species under different water treatments. The same letters in each column show non-significant differences at $P \leq 0.05$, analyzed by Tukey's honest test.

Oil percentage

As the availability of irrigation water reduced, the oil percentage of *Lallemantia* species followed a decreasing trend. Fertilizer application (especially AMF) significantly increased oil percentage. In all irrigation regimes (I_{30} and I_{90} levels) in both *Lallemantia* species, the highest and lowest oil percentage of seeds obtained in plants inoculated by AMF fertilizer and control (without fertilizer), respectively. Across all irrigation regimes and fertilizer treatments, *L. iberica* performed significantly higher seed oil percentage compared to *L. royleana* (Table 3).

Fatty acids composition

In both *Lallemantia* species by increasing water deficit, the amount of EA, AA, LNA, LA, OA and SA faty acid significantly reduced, while PA and AA increased. Using of AMF and nano

Table 4 The composition of fatty acids in *L. iberica* and *L. royleana* species inoculated with nano iron chelate and AMF under irrigation water treatments

Treatments			Fatty acid components (%)							
Irrigation regime (%)	**Species**	**Fertilizer**	**EA**	**AA**	**LNA**	**LA**	**OA**	**SA**	**POA**	**PA**
I_{30}	S1	F1	0.68±0.02f.h	0.02±0.00i	54.07±0.62h	13.07±0.09fg	11.77±0.11g	1.52±0.00m	0.02±0.00n	5.45±0.16i
		F2	1.52±0.04b	0.05±0.00g	56.94±0.54fg	14.29±0.25c	14.39±0.22d	1.84±0.02j	0.03±0.00l	8.47±0.14d.f
		F3	0.70±0.03f.h	0.04±0.00h	54.78±0.34h	14.94±0.03b	13.29±0.18e	1.58±0.00lm	0.06±0.00j	8.37±0.59ef
	S2	F1	0.73±0.03f.g	0.04±0.00h	61.75±0.35c	11.48±0.11i	9.85±0.23h	2.12±0.01fg	0.06±0.00j	4.97±0.17i
		F2	0.97±0.06de	0.11±0.00cd	62.84±0.08b	10.53±0.08 kl	13.42±0.08e	2.31±0.01b.d	0.08±0.00g	7.81±0.10gh
		F3	0.62±0.02h	0.11±0.01cd	58.11±0.01e	11.95±0.14i	12.55±0.04f	2.20±0.00e.f	0.11±0.00e	7.5±0.02h
I_{60}	S1	F1	0.89±0.01e	0.03±0.00hi	54.03±0.34h	12.59±0.10h	14.48±0.06d	1.63±0.01lm	0.05±0.00k	6.67±0.20i
		F2	1.69±0.05a	0.09±0.00c	59.54±0.17d	14.95±0.07b	16.64±0.36a	1.91±0.0ij	0.07±0.00h	8.92±0.2c.e
		F3	0.69±0.03g.h	0.06±0.00g	57.19±0.44e.g	15.36±0.05a	15.63±0.32b	1.67±0.00kl	0.10±0.00f	6.70±0.09i
	S2	F1	0.78±0.03f	0.06±0.00g	59.52±0.05d	10.56±0.12kl	11.34±0.07g	2.21±0.01d.f	0.12±0.00d	6.57±0.12i
		F2	1.05±0.04d	0.13±0.00b	68.12±0.43a	12.95±0.13gh	14.56±0.10d	2.40±0.02b	0.13±0.00b	9.1±0.05bc
		F3	0.75±0.02fg	0.10±0.00h	62.58±0.17bc	13.43±0.01ef	13.34±0.04e	2.27±0.01c.e	0.15±0.00a	8.83±0.03c.e
I_{90}	S1	F1	0.71±0.07f.h	0.04±0.00h	50.90±0.11i	10.85±0.00jk	14.54±0.18d	1.74±0.01k	0.02±0.01m	8.53±0.34d.f
		F2	1.25±0.08c	0.12±0.00c	56.76±0.42fg	13.82±0.38de	15.75±0.42b	2.07±0.07gh	0.05±0.00k	9.63±0.22b
		F3	0.68±0.04f.h	0.08±0.00f	56.63±0.56g	14.07±0.14cd	15.70±0.16b	1.99±0.07hi	0.07±0.00i	9.01±0.08cd
	S2	F1	0.65±0.03gh	0.08±0.01f	53.91±0.20h	10.36±0.09l	11.66±0.20g	2.33±0.03bc	0.06±0.01j	7.31±0.05h
		F2	1.01±0.07d	0.20±0.01a	59.45±0.04d	11.07±0.08i	15.12±0.08c	2.84±0.03a	0.12±0.01c	10.32±0.04a
		F3	0.68±0.03f.h	0.12±0.01bc	57.76±0.12ef	11.69±0.12i	13.54±0.05e	2.39±0.01b	0.13±0.00b	8.17±0.28fg

The same letters in each column show non-significant differences at $P \leq 0.05$, analyzed by Tukey's honest test.

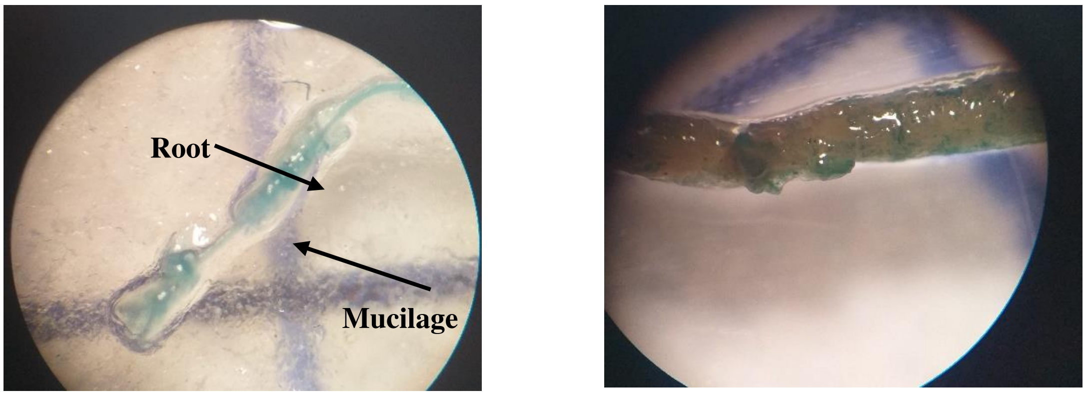
-iron chelate lead to a significant enhancement in the content of fatty acids compared to that control plants. The amount of POA and LA by using of nano iron chelate and fatty acids of EA , AA, LNA, OA, SA, PA with inoculation AMF significantly increased during drought stress. The highest value of AA, LNA and POA was observed in *L. royleana* species. Additionally, the EA, LA, OA, SA content in *L. iberica* species was significantly higher than *L. royleana* (Table 4).

Discussion

Drought stress is one of the environmental stress that influences growth and crop production; however, plants can create beneficial associations that reduce drought stress effects [18]. Increasing rates in grain yield for both species of *Lallemantia* under the I_{60} irrigation regime; in contrast, increasing severity of water deficit (I_{90}) caused a considerable reduction in grain yield. This may be due to the reduction of water and nutrient absorption [34]. However, AMF inoculation noticeably enhanced grain yield in both species. In agreement with *Portulaca oleracea* L. [8] and *Dracocephalum moldavica* [35], our results showed that AMF inoculation during drought stress improved grain yield. The AMF assisted in the uptake of microelements from the soil, and subsequently, metabolite production and enzyme activity could change positively [35, 36] and facilitated assimilate transition to grain [19], and these alterations could improve grain yield during drought stress [18]. The higher grain yield was observed in *L. iberica* across all irrigation regimes and fertilizer treatments.

Based on our results, by increasing water deficit in both species of *Lallemantia sp.,* chlorophyll-a and chlorophyll-b content at flowering stages decreased; however, it increased in response to AMF and nano iron chelate fertilizer. Water shortage leads to stomata closure and decreasing CO_2 diffusion into leaves, resulting in a reduction of chlorophyll levels and photosynthesis [11, 37]. Our results indicated that the rise of yield in any irrigation regime in *Lallemantia* species treated by nano iron chelate and AMF increased chlorophyll content. Application of nano iron chelate fertilizer improved chlorophyll-a content in both species of *Lallemantia* in any irrigation regime. Iron is a micronutrient that prevented water deficiency stress in photosynthesis by improving chlorophyll pigments' synthesis and enhancing the soil's water holding capacity [38]. In any irrigation regime, AMF inoculation improved and increased chlorophyll-b content in both species of *Lallemantia*. The AMF inoculation by developing the host plants' root system could keep the stomata open to facilitate the exchange of gases for photosynthesis [39]. Higher chlorophyll-a and chlorophyll-b content in *L. iberica* leaves indicated their better water deficit tolerance compared to *L. royleana*. The results are in agreement with the results of the research reported on *Lallemantia* species [3].

Percentage of root colonization of AMF inoculation in both species of *Lallemantia* significantly enhanced from I_{30} to I_{60} level of irrigation regime. The presence of AMF modified drought stress adverse effects on *Lallemantia* species, which is in agreement with the results reported on *Lavandula officinalis* and *Rosmarinus officinalis* [18]. Increasing root colonization in the I_{60} level of irrigation regime is probably due to the carbon supplies for growth and spore germination of fungus in the host plant roots [34]. As the water deficiency increased to (I_{90}) irrigation regime, root colonization in both species of *Lallemantia* reduced indicating that AMF had a lower symbiosis relationship with roots of *Lallemantia* species under severe drought stress conditions. Organisms such as AMF in soil often react adversely to drought stresses; soil moisture above field capacity favor their spore germination [40]. Increasing colonization with AMF inoculation under the I_{60} level of irrigation regime well explains improving grain yield and harvest index and also improved water use efficiency and leaf chlorophyll content. The current study results showed that the colonization rate of *L. royleana* was more than *L. iberica*. Also, we observed a layer of mucilage around *L. royleana* roots (Fig. 3), which is not reposted so far. Mucilage around the root tends to facilitate aggregation, providing carbon for microorganisms in soil and facilitated water and elements (like N, P, and K) absorption [41].



A **B**

Fig 3. A) *L. royleana* and **B)** *L. iberica* root covered with mucilage and no mucilage, respectively

Oil percentage of both species of *Lallemantia* significantly increased upon exposure to moderate water deficits I_{60}. It seems that the I_{60} water deficit could induce a specific defense mechanism in seeds of groundnuts through fatty acid synthesis as osmotic regulator metabolites [26]. The oil percentage was higher in *L. royleana* species treated by nano iron chelate than *L. iberica* across all irrigation regimes compared to other fertilizer treatments.

It has been shown that the application of nano iron chelate improved the oil percentage of seeds [30]. Iron as a micronutrient had a positive effect on photosynthetic products, especially carbohydrate [42]. Export of carbohydrates from photosynthesizing leaves to seeds of *L. royleana* may be caused to increasing oil percentage because carbohydrates are precursors of fatty acid biosynthesis pathways [38].

The AMF inoculation in *L. iberica* species improved and increased oil percentage across all irrigation regimes. The present research findings are consistent with results, which found drought stress-induced reduction of unsaturated fatty acids, whereas mycorrhizal treatment increased the unsaturated fatty acid concentration in drought-stressed *Lavandula officinalis* and *Rosmarinus officinalis* seeds [18]. The extension of mycorrhizal hyphae may cause this ability, thereby increasing water and microelements (*e.g.*, phosphorus) absorption from soil [12].

Water treatments influenced all identified fatty acids. The I_{90} treatment could significantly reduce the percentage of unsaturated fatty acids, such as POA, OA, LA, LNA, and EA. However, it increased the concentration of saturated fatty acids, such as PA, SA, and AA. The increase in fatty acid components under moderate water shortage improved both Lallemania species tolerance against water deficits. Moderate water shortage could probably cause an increase in the relevant mechanisms involved in assimilating remobilizing of fatty acids. The present research findings are consistent with results reported that severe water deficits caused an enhancement in saturated fatty acids (PA, SA, and AA) and decreased unsaturated fatty acids, including POA, OA, LA, LNA, and EA [40]. The main components of membrane lipids are saturated and unsaturated fatty acids, which induce cell membranes' integrity and functions. Any change that increases saturated fatty acids and decreases unsaturated fatty acids, such as water deficits, causes a decline in the cell membrane lipid fluidity [40]. Across all irrigation regimes, the POA, PA, SA, LA, and AA percentages were higher in *L. royleana* than in *L. iberica*. However, the higher OA, LNA, and EA percentage in *L. iberica* than *L. royleana,* maybe because of different genetic backgrounds. The results showed that AMF inoculation compared to that of the control, led to increased percentages of PA, SA, OA, LNA, AA, and EA in the harvested grain of *Lallemantia* species when exposed to water deficits. These results correspond to the results obtained by other researchers on these oilseed crops [7, 19, 21, 26]. It has been shown that under water deficit conditions, the application of AMF can enhance water balance and expand mycorrhizal hyphae in soil, which increases the uptake of mineral nutrients, such as phosphorus from the soil and releases it into the root cortex cells [43]. The phosphorus absorption from soil may supply the necessary ATP and NADPH for the fatty acid synthesis pathway [44]. The current study results showed that POA and LA percentages were considerably increased by nano iron chelate fertilizer under water deficit. These findings are in line with studies that reported the application of nano iron chelate could change the concentration of saturated and unsaturated fatty acids under water deficit [30]. Iron is a micronutrient and leads to the protection of treated plants under water deficit, which is related to the improvement of photosynthesis [39] and carbon absorption [30]. Increased carbohydrates serve as a precursor of the fatty acid biosynthesis pathways [30, 41, 45].

Conclusion

In conclusion, we found that increasing water deficit stress reduced both species' growth; however, the I_{60} level of irrigation regime could be considered optimal irrigation compared to other irrigation regime treatments. Moderate water deficit (I_{60}) caused a significant increase in grain yield, leaf chlorophyll contents, root colonization, oil percentage, and unsaturated fatty acids. These traits were higher in plants inoculated with AMF and nano iron chelate across all irrigation regimes compared to control (no fertilizer). These results were related to the ability of AMF to increase root colonization, leaf chlorophyll-b content, and accumulated unsaturated fatty acids composition in both species under different irrigation regimes. In contrast, nano iron chelate mostly influenced and improved leaf chlorophyll content. However, inoculation with AMF fertilizer had more effect on reducing water consumption and reduced the impact of drought stress. Almost negligible differences among both species were found for most traits in any irrigation regime and fertilizer treatments; however yield, leaf chlorophyll contents, oil percentage features of *L. iberica* seeds were better than that of *L. royeana* seeds, which demonstrated that *L. iberica* was more tolerant to water deficit condition. Overall, these results explained oil production ability and importance of *Lallemantia* species as an oilseed crop. Deficit irrigation and inoculation with AMF can increase quantitative and qualitative grain oil traits, particularly oil and fatty acid components of *Lallemantia* species. Therefore, this study provided further information on how to produce *Lallemantia* species under deficit irrigation regimes by applying beneficial fertilizers, such as AMF.

Methods

Study location specifications

The field trial was conducted at Research Farm of the College of Agriculture, Shahed University, Tehran, Iran, during the cropping seasons of 2018/2019. The site is located in the northern latitude of 35^{o} and 34' and the eastern longitude of 51^{o} and 8' and is 1190 m above the sea level. The soil's physical and chemical properties are determined in a combined soil sample from 0 to 30 cm depth. The soil had a Field capacity (FC) 20.86%; Permanent wilting point (PWP) 10.81%; Electrical conductivity (?) ; pH 7.09; Nitrogen (N) 0.11%; Iron (Fe) 12 $mg.kg^{-1}$; Phosphorus (P) 8.52 $mg.kg^{-1}$; potassium (K) 346 $mg.kg^{-1}$. The texture of soil comprised 20% clay, 50% silt, and 30% sand. Figure 4 represents total rainfall and average monthly air temperature during the growing season.

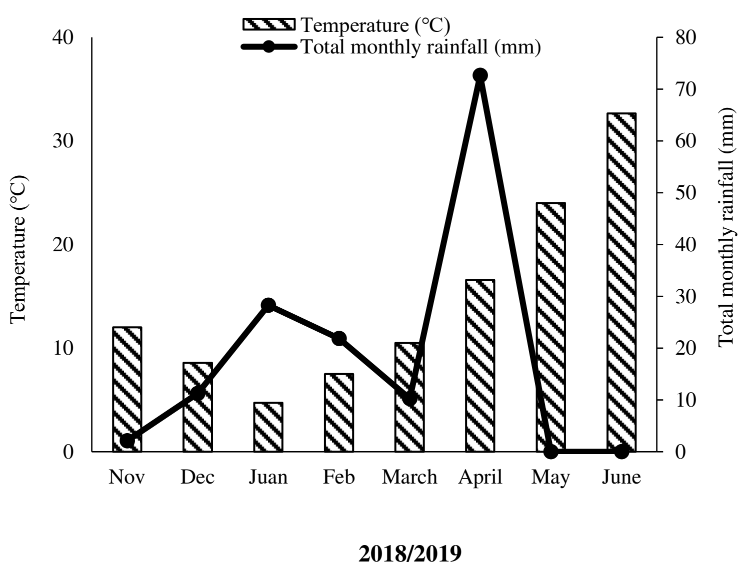


Fig 4. Total rainfall and average monthly air temperature for the 2018/2019 growing seasons

Plant materials and experimental design

Lallemantia iberica and *Lallemantia royleana* seeds were kindly provided by the Agricultural Research Center of Urmia and Pakan Bazr Company, Iran, respectively. A split-factorial layout based on randomized complete block design (RCBD) was employed to study three factors: Irrigation regimes (available resource of water (ASW); Fertilizer treatments: bio-fertilizers (AMF), and nono iron chelate; and *Lallemantia* species: *L. royleana,* and *L.iberica* using three replications. Main plots were assigned to the three-level of irrigation, including 30% (I_{30}; without stress), 60% ($I_{60;}$ mild stress), and 90% (I_{90}; severe stress) depletion of ASW. Subplots were allocated to the factorial combination of fertilizers and species. Fertilizer treatments comprised of without fertilizer (F_1), combined arbuscular mycorrhizal fungi (AMF) of three species, including *Funneliformis mosseae, Claroideoglomus etunicatum, and Rhizophagus intraradices* (F_2) and nano iron chelate (F_3). Two species of *Lallemantia* were *L. iberica* (S_1) and *L. royleana (*S_2). After seed-bed prepration, 54 experimental plots (2×2 m^2) with 1 m interval between plots and 2 m interval between blocks were assigned to all treatments. Seeds were planted manually on 14th November, 2018 in a depth of 1-2 cm at a row spacing of 50 cm and plant spacing of 5 cm on the rows. AMF fertilizer was obtained from the Soil-Biology Laboratory of Soil and Water Research Institute, Tehran, Iran. To apply AMF fertilizer, 20 g of mycorrhizal fungi (20 spores/g inoculums) was thoroughly mixed with each row's soil at a depth of 2 cm. The size of nano iron particles was 40 nm and contained 9% water-soluble iron chelate. Nano iron chelate fertilizer was applied as a solution in irrigation water after sowing at a rate of 5 $kg.ha^{-1}$. By manual weeding, weed control of experimental plots was performed twice 20 and 45 days after planting, respectively. During soil preparation and the growing season, herbicides, pesticides, and chemical fungicides were not used

Regulated deficit irrigation

A mount field capacity (FC) and permanent wilting point (PWP) were measured using a pressure plate, and plots were irrigated based on the allocated water. The Maximum percentage of allowable depletion of available soil water in the 0-30 cm soil depth was calculated using equation MAD (%) $= 100 \times \frac{Fc-\theta}{Fc-PWP}$ [46]. Where, MAD, FC, θ, and PWP represent maximum allowable depletion, soil volumetric moisture at field capacity, soil volumetric moisture, and soil volumetric moisture at the permanent wilting point, respectively. The volume of allocated water was measured using equation $In = \frac{(Fc-\theta)\times D\times A}{100}$ [3]. Where, In, FC, θ, D, and A represent the volume of allocated water, field capacity, soil moisture, the effective rooting depth, and plot surface area (4 m^2), respectively. Before applying irrigation treatments, the amount of water required for irrigation of *Lallemantia* species was calculated for 30% depletion of ASW (without stress or normal irrigation) by monitoring changes in soil water gravimetrically. According to this method, 24 h after irrigation, samples from an experimental plot were taken from the root development depth (0-30 cm), and the samples were immediately weighed and then dried in an oven at 105 °C for 24 h. Indeed, the percentage of soil water content [47] was registered according to sampling taken daily from an experimental plot. According to that measured amount (30% depletion of ASW), the water used in each level of drought stress (60 and 90% depletion of ASW) was calculated. The irrigation volume was controlled using the meter was installed at the beginning of the irrigation system (Fig. 5). Measured values with the help of a volumetric flow of water were performed at intervals of once every five days and separately for each drought stress level. The irrigation treatments were applied at the onset of plants established at the 8-12 leaf stage (on 1 February 2019).

Measurements

Agronomical traits

At the end of the growing season (on 13 June 2019), 20 plants from each plot's inner area (2×1 m^2) were selected to remove marginal effects.

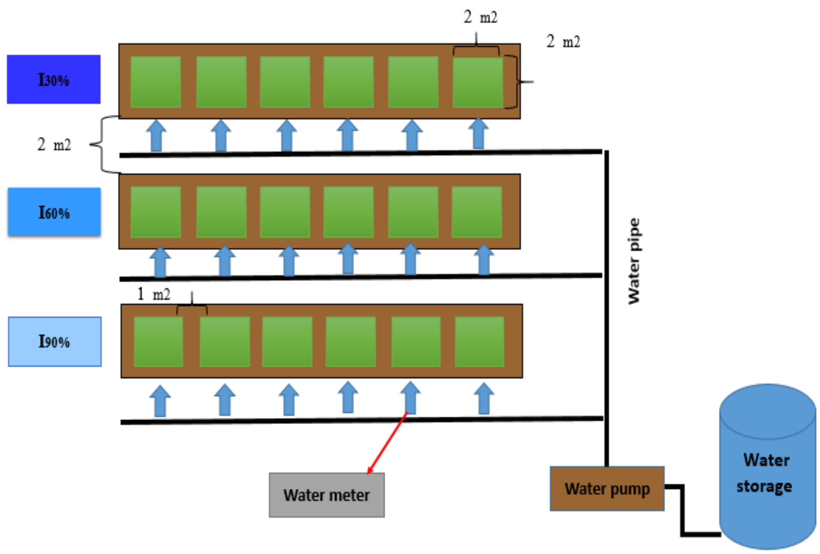


Fig 5. Schematic figure of the irrigation in the farm

Leaf Chlorophyll contents

Leaf chlorophyll (Chl) at flowering stage (on 11 April 2019) were measured on leaves (in 3 plants from each treatment randomly collected) sampled. After cutting, the plant leaves were placed in plastic bags, immediately transported to the laboratory. For measuring chlorophyll contents, leave samples were extracted in 80 % acetone, then the extracts material was centrifuged at 6000 rpm for 15 min and recorded at 645 and 663 nm [48].

Determination of arbuscular mycorrhizal colonization

The percentage of AMF root colonization was estimated in 5 plants from each treatment randomly collected after the seed maturity stage (on 30 May 2019). The roots were cleaned in 100 g/l KOH in a 90 °C water bath for 30 min, rinsed and cooled down with distilled water for 3 min, stained with 50 ml/l Ink blue 90 °C for 5 min, and washed with tap water for 3 min [49].

Oil extraction

Oil percentage of *L. iberica* (with 5% moisture content) and *L. royleana* (with 7% moisture content*)* seeds was measured using the standard Soxhlet method with hexane solution (ACS grade, Reag. PhEur; obtained from Merck Chemical Co., Germany), The solvent (150 mL) was poured in a Soxhelt apparatus, and then 10 g seeds of each treatment were added. The solvent was boiled and evaporated. This evaporation condensation process continued for 10 h, and after solvent removal, oil was extracted from brown seeds [50].

Determination of fatty acid composition

Briefly, the composition of the Fatty acids of Lallemantia seeds was determined according to the methylation (transformation of fixed oil into fatty acid) and gas chromatography (GC) [51]. Generally, in a 5-ml screw-top test tube, 0.10 g of the oil sample was weighed. Afterward, 3 ml of heptane and 500 ml of 2 N methanolic potassium hydroxide solutions were added and shaken at 10000 rpm for 15 s. Finally, one µL of the FAME sample was injected into the gas chromatograph using a microliter syringe. The fatty acid methyl esters were analyzed in an Agilent 7890A GC (Agilent Technologies, Inc. 2010) equipped with flame ionization detector (FID), using a BPX capillary (part number:054980) column (50 m × 0.22 mm internal diameter, 0.2 µm film, nitrogen was the carrier gas with a head pressure at 60 psi, Agilent Technologies, Inc. 2010). The initial column temperature was set at 165 °C and maintained for 10 min, then programmed to increase from 165 °C to 200 °C at 1.5 °C/min. Injector and detector temperatures were adjusted to 250 °C and 280 °C, respectively.

Statistical analysis

Data were submitted to statistical analysis using SAS software version 9.3; the mean values were compared using Tukey's honest significance test at 5% probability level.

Abbreviations

AMF: Arbuscular mycorrhizal fungi, I: Irrigation regime; S: Species, F: Fertilizer; AA: Arachidic acid, SA: Stearic acid, POA: Palmitoleic acid, PA: Palmitic acid, LNA: Linolenic acid, LA: Linoleic acid, OA: Oleic acid, EA: Eicosenoic acid. I_{30}: 30%, I_{60}: 60%, I_{90}: 90% depletion of available soil water, S_1: *Lallemantia iberica*, S_2: *Lallemantia .roylean*, F_1: without fertilizer, F_2: AMF, F_3: iron nano chelate, Df: Degree freedom.

Authors' contributions

AP, SMF, and ARR designed the concept study. AP carried and executed the experimental work. RAR, AP, SMF, and ARR jointly wrote the manuscript. All the authors have approved the manuscript and agree with the submission. The authors declare no conflicts of interest.

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Acknowledgment

We kindly acknowledge the Shahed University of Tehran for their support of this research.

Conflict of interest

This manuscript has not been published or presented elsewhere in part or entirety and is not considered by another journal. All the authors have approved the manuscript and agree with the submission. The authors declare no conflicts of interest.

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Acknowledgment

We kindly acknowledge the Shahed University of Tehran for their support of this research.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

Funding

This research project was supported by Shahed University, Tehran, Iran.

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Figures

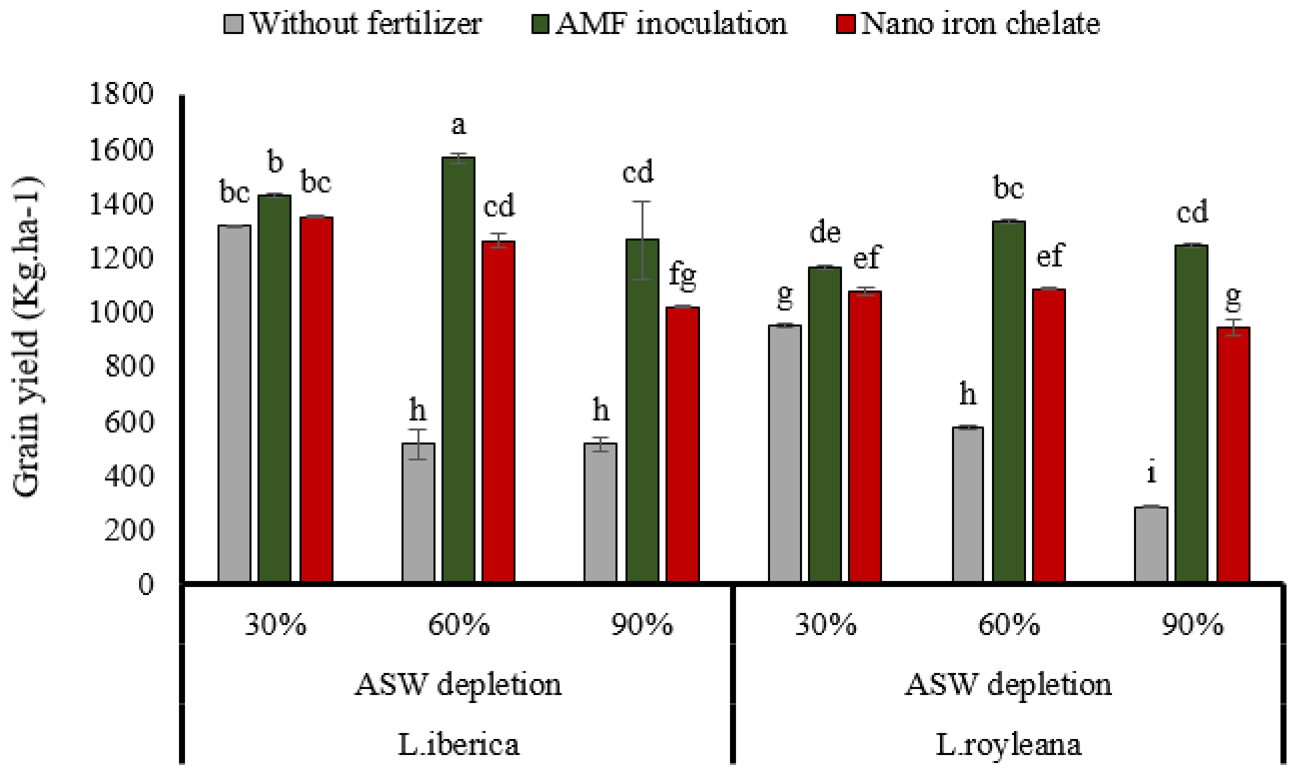

Figure 1

The effect of AMF and nano iron chelate application on grain yield of L. iberica and L. royleana species under different water treatments. The same letters in each column show non-significant differences at P ≤ 0.05, analyzed by Tukey's honest test.

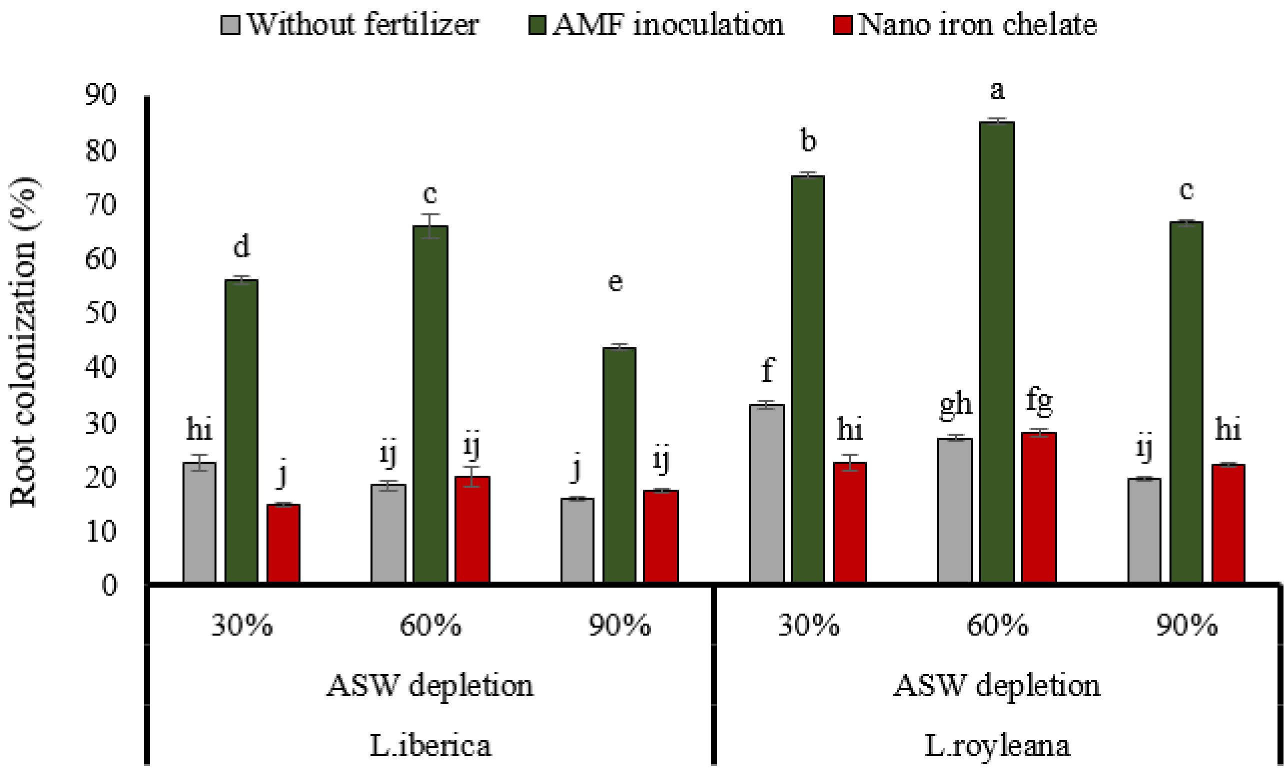

Figure 2

The effect of AMF and nano iron chelate application on root colonization of L. iberica and L. royleana species under different water treatments. The same letters in each column show non-significant differences at $P \leq 0.05$, analyzed by Tukey's honest test.

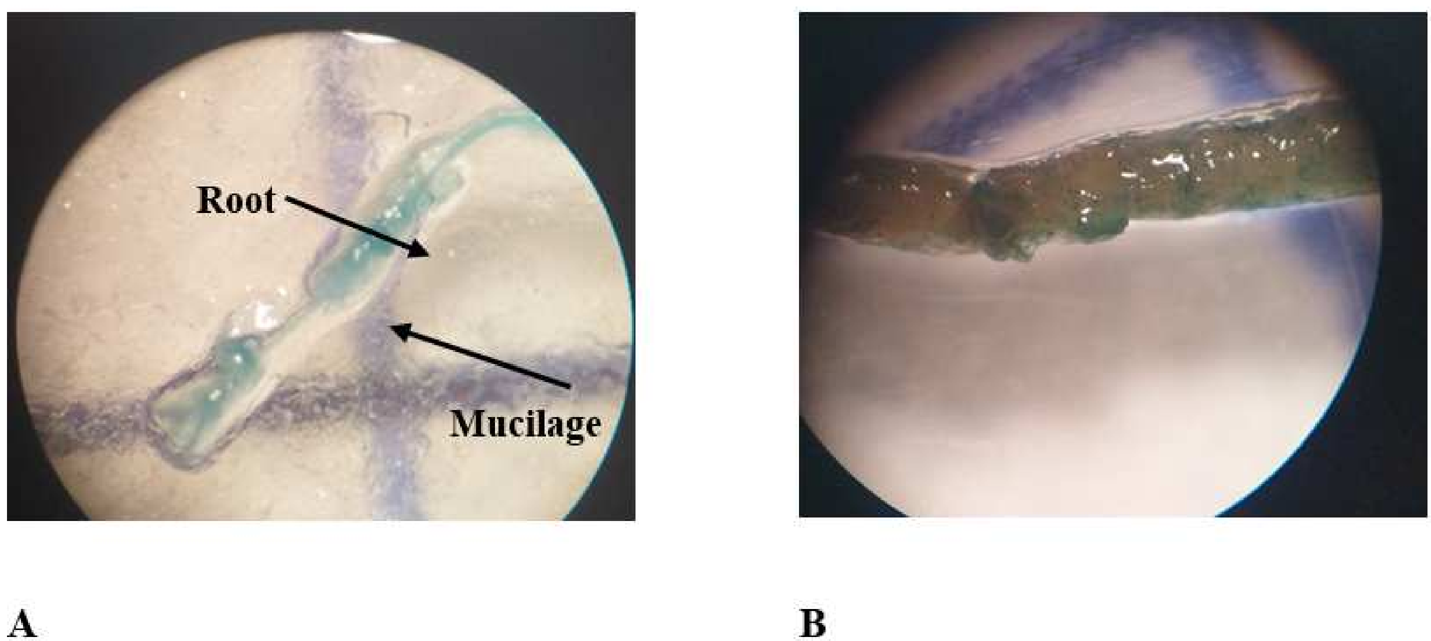

Figure 3

A) L. royleana and B) L. iberica root covered with mucilage and no mucilage, respectively

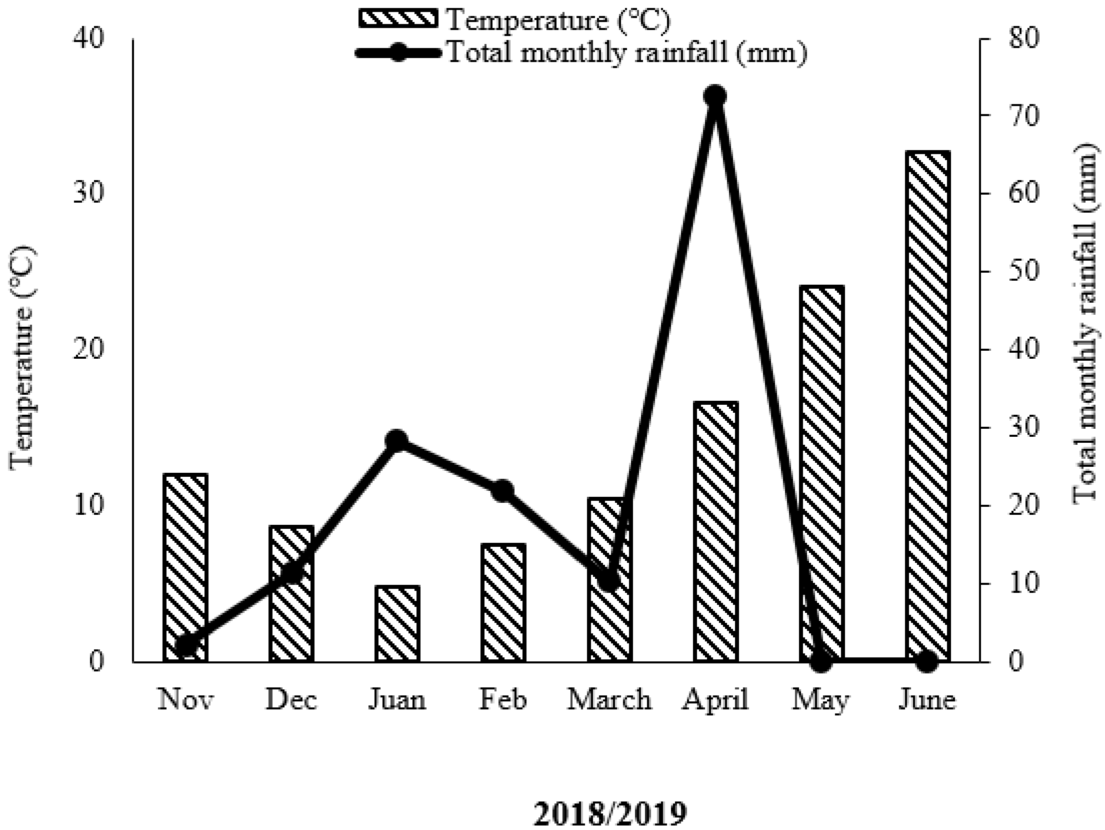

Figure 4

Total rainfall and average monthly air temperature for the 2018/2019 growing seasons

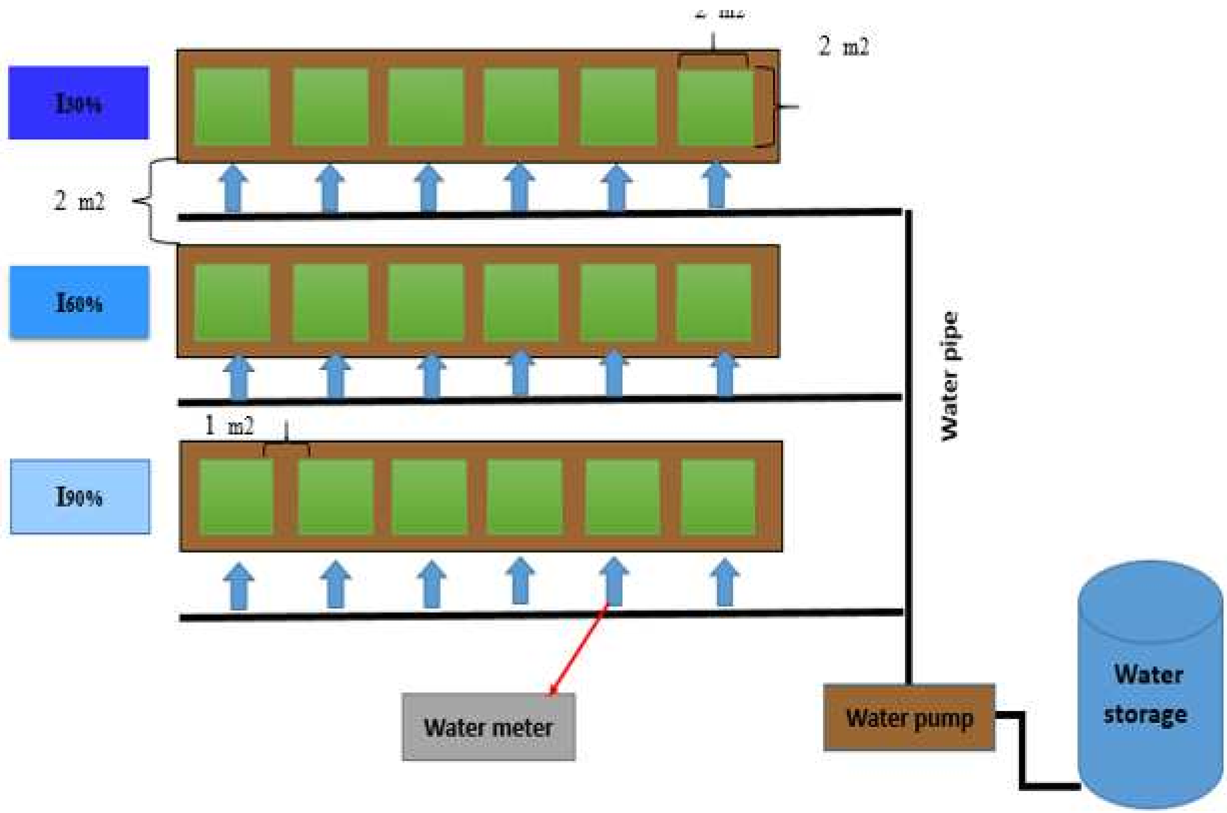

Figure 5

Schematic figure of the irrigation in the farm