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# *Lallelantia Iberica* and *Lallelantia Royleana*: The Effect of Mycorrhizal Fungal Inoculation on Growth and Mycorrhizal Dependency under Sterile and Non-sterile Soils

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## ABSTRACT

This study evaluated the effects of mycorrhizal species inoculation on the growth of *Lallelantia* species. Two pot experiments were performed to determine mycorrhizal species effects on growth, yield, mycorrhizal dependency, root colonization, and seed quality. Under sterile soil conditions, *Lallelantia iberica* and *Lallelantia royleana* were inoculated with *Funneliformis mosseae*, *Funneliformis caledonius*, *Rhizophagus intraradices*, *Claroideoglossum etunicatum*, *Claroideoglossum claroideum*, *Rhizophagus fasciculatus* and *Diversisporles epigaea*. Among mycorrhizal species, *Cl. etunicatum* (M<sub>1</sub>), *Fu. mosseae* (M<sub>2</sub>) and *R. intraradices* (M<sub>3</sub>) were most effective on root colonization on both species of *Lallelantia*. In the main experiment, for both plant species of *Lallelantia*, the growth, yield and yield components, seed quality, and mycorrhizal dependency (MD) parameters were studied under non-sterile and sterile soil conditions using three optimal mycorrhizal species (M<sub>1</sub>), (M<sub>2</sub>), (M<sub>3</sub>), and their mixture. Both plant species grown in non-sterile soil grew better than in sterile soil conditions. The integrated application of M<sub>1</sub> + M<sub>2</sub> + M<sub>3</sub> had a better effect on measured parameters of *Lallelantia* species. Inoculated and non-inoculated *L. iberica* showed higher growth and produced more yield, thousand grain weight, phosphorus, and oil content. The number of siliques in plant, number of seed in plant, mucilage, and MD of inoculated and non-inoculated *L. royleana* were higher than *L. iberica*. Generally, our result showed that both species of *Lallelantia* were mycorrhizal dependent, and soil sterilization led to killing of all indigenous mycorrhizal species, and as a result, it caused to decrease the growth of *Lallelantia* species.

## ARTICLE HISTORY

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## KEYWORDS

AM fungi species; grain yield; root colonization; oil content; seed phosphorus

## Introduction

The critical members of the plant microbiome are mycorrhizal fungi that have been promoted as biofertilizers for many years; yet their potential to improve the nutritional value of crops has mostly been overlooked (Hart and Forsythe 2012). However, mycorrhizal fungi application in soil provides mutual benefits for host plants and fungi as improved plant growth by acquiring soil nutrients by fungi, which are difficult for plants to uptake (Averill et al. 2019), and nutrient-efficient exchange such as N and P to the plant and sucrose and lipids to the fungi are mediated via specialized structures within the roots (Feng et al. 2020; Owen et al. 2015). Also, the non-nutritional benefits of mycorrhizal fungi to host plants are alleviation of environmental stressors such as drought as well as providing resistance to pathogens and herbivory (Koziol, Crews, and Bever 2019).

Nearly 90% of plant species can form mycorrhizal symbioses (Ortas and Bykova 2018): 79% of monocotyledons and 83% of dicotyledonous plants (Owen et al. 2015). There are seven main groups of mycorrhizas such as arbuscular mycorrhizas (AM), ecto- (EcM), ectendo-, arbutoid, ericoid, monotropoid and orchid mycorrhiza (Smith, Anderson, and Andrew Smith 2018), whereas the most widespread and ecologically important types of mycorrhiza are AM and EcM that are used commercially in agriculture and forestry, which have an important role in P uptake (Owen et al. 2015). AM belongs to the phylum Glomeromycota that is the most widely used in agriculture (Ziane et al. 2017); Notably, formerly *Glomus* such as *Funneliformis mosseae*, *Funneliformis caledonius*, *Rhizophagus intraradices*, *Claroideoglossum etunicatum*, *Claroideoglossum claroideum*, *Rhizophagus fasciculatus* (Krüger et al. 2012; Schüßler and Walker 2010), and also *Diversisporles epigaea* often named *Glomus versiforme* (Krüger et al. 2012; Schüßler et al. 2011).

A significant factor determining microbial community structure for plant growth and rhizosphere nutrient dynamics is the soil type such as sterile and non-sterile soil (Owen et al. 2015). In non-sterile soil, there are living organisms including mycorrhizal spores that have beneficial effects on plant growth (Ortas, Akpınar, and Demirbas 2016; Surendirakumar, Pandey, and Muthukumar 2019). It has been reported that sterilized soil reduced plant growth due to the elimination of viable mycorrhiza, while non-sterilized soil had positive effect on development and growth of plants (Ortas 2019). As the degree to which a plant is dependent on the mycorrhizal population to produce its maximum growth or yield at a given level of soil fertility is defined “mycorrhiza dependency” (Janos 1980). Mycorrhiza dependency is assessed by examining the effects of a range of soil fertilities (especially, that of available phosphorus) on mycorrhizal inoculated and non-mycorrhizal plants (Janos 2007). Ortas, Iqbal, and Cem Yücel (2019) tested that mycorrhizal “inoculation dependency” and nutrient uptake were significantly increased by five different AMF species used to inoculate green peppers, tomatoes, eggplants and bell peppers. More to the point, the symbiosis between AMF and plant considerably increased root colonization that had different effects on roots and shoots growth (Askari et al. 2019).

Inoculation of *Fu. mosseae* increased plant growth, yield and root colonization of *Lavandula officinalis*, *Rosmarinus officinalis* and *Thymus vulgaris* (Pirzad and Mohammadzadeh 2018). Inoculated plants exhibited changes in increasing seed quality as seed mucilage, oil content (Rahimzadeh and Pirzad 2019) and phosphorus concentration (Ortas and Bykova 2018).

As regards some plants showed positive and negative responses to a mycorrhizal inoculant, the importance of plants within the bio-inoculant design and testing is highlighted by the contradictory results obtained by different plant species, because mycorrhizal growth dependency of host species can have a bearing on the success of AM colonization (Owen et al. 2015). For this reason, in this research, two host of *Lallemantia*, including *L. ibeica* and *L. royleana*, were used to identify host plants’ potential in association with mycorrhizal growth dependency.

*Lallemantia iberica* (Dragon head) and *Lallemantia royleana* (Lady’s mantle) as an annual herb belonging to genus *Lallemantia* are the major medicinal and aromatic plants in the Lamiaceae family (Paravar et al. 2021b), and their seeds are widely used in cosmetic, food and pharmaceutical industries because of high mucilage and oil content (Abdolahi and Maleki Farahani 2019; Paravar et al. 2018). Although these plants are native to Asia, they are cultivated in some central and southern European countries (Paravar et al. 2021b; Abdolahi and Maleki Farahani 2019; Al-Snafi 2020). Nowadays, cultivation of *Lallemantia* plants are mainly noticeable because of their high oil seed (approximately 30–45%) and the high content of the valuable omega-3 fatty acid (Al-Snafi 2019a, 2019b; Zlatanov et al. 2012). This study was based on the hypothesis that under sterile and non-sterile conditions, mycorrhizal inoculation increases seedling growth, colonization and mycorrhizal inoculation dependency on *Lallemantia* species. The objective of the study was to evaluate and detect the mycorrhizal association on *Lallemantia* species.

**Table 1.** Description of mycorrhizal species treatments in main experiment.

Mycorrhizal species	Treatments	Code
Single	<i>Cl. etunicatum</i>	M <sub>1</sub>
	<i>Fu. mosseae</i>	M <sub>2</sub>
	<i>R. intraradices</i>	M <sub>3</sub>
Mixing	<i>Cl. Etunicatum, Fu. mosseae</i>	M <sub>1</sub> + M <sub>2</sub>
	<i>Cl. Etunicatum, R. intraradices</i>	M <sub>1</sub> + M <sub>3</sub>
	<i>Fu. Mosseae, R. intraradices</i>	M <sub>2</sub> + M <sub>3</sub>
	<i>Cl. Etunicatum, Fu. Mosseae, R. intraradices</i>	M <sub>1</sub> + M <sub>2</sub> + M <sub>3</sub>

**Table 2.** Physicochemical properties of non-sterile and sterile soil.

	Soil texture	Fe (ppm)	P (ppm)	K (ppm)	N (%)	Organic carbon (%)	Inorganic carbon (%)	pH
Non-sterile	Loamy	646	8.52	646	0.11	3.21	3.94	7.19
Sterile	Loamy	611	8.21	632	0.10	3.11	3.27	7.19

## Materials and methods

The seeds of *Lallemantia iberica* and *Lallemantia royleana* were provided by the Agricultural Research Center of Urmia and Pakan Bazr Company, Iran, respectively, and the mycorrhizal species were supplied from the Soil and Water Research Institute. The pre-experiment and main experiment were conducted as factorial based on RCBD in four replications in the greenhouse of Shahed University, Tehran, in November 2019. The seeds were sown in pots and were grown for 160 days in a controlled greenhouse with day–night temperatures of 25°C under a 16 h photoperiod, using cool white fluorescent lamps, at approximately 350  $\mu\text{mol m}^{-2} \text{s}^{-1}$  with relative humidity 70 to 80% at night and 80 to 85% during the day. Distilled water was added daily to maintain the moisture at 75% of field capacity after pots were weighed daily.

A pre-experiment was performed with seven mycorrhizal species (*Funneliformis mosseae*, *Funneliformis caledonius*, *Rhizophagus intraradices*, *Claroideoglossum etunicatum*, *Claroideoglossum claroideum*, *Rhizophagus fasciculatus* and *Diversisporles epigaea*) on plant species of *Lallemantia* (*L. iberica* and *L. royleana*). The plastic pots were filled with 5 kg of sterile soil, and 1000 spores of mycorrhizal species were applied per seedling (approximately 50 mm below the seedling root in pots). Five seeds of *L. iberica* and *L. royleana* were sown per pot and thinned to two seedlings after 2 week. The soil was sterilized at  $121 \pm 3^\circ\text{C}$  for 2 h in an autoclave (Ortas, Demirbas, and Akpinar 2018) for use as a growth medium and kept in laboratory condition for 2 weeks before being repacked into the pots. Non-inoculated pots were assigned as control. Transplanted plants into soil without an inoculum were assigned as control. In among treatments, three optimal fungal were selected based on root colonization, which are used for the main experimental.

The main experiment was performed to the investigation of mycorrhizal inoculation (single and mixed fungal of *Cl. etunicatum*, *Fu. mosseae* and *R. intraradices*) (Table 1) effects on growth, yield, seed quality (mucilage, phosphorus and oil content) and mycorrhizal dependency in plant species *Lallemantia* under sterile and non-sterile soils. Filled soil was applied as the non-sterile soil, and some properties of sterile and non-sterile soils were determined (Table 2).

## Traits measurement

The *Lallemantia* species were harvested 160 days after sowing, and the following length of root and shoot, shoot length, number of silique in plant, number of seed in plant and grain yield parameters were measured (Abdolahi and Maleki Farahani 2019; Omidi et al. 2018).

The percentage of AMF root colonization was estimated after the harvest stage. 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 at 90°C for 5 min and washed with tap water for 3 min (Giovannetti and Mosse 1980).

The phosphorus concentration of seeds was measured after ashing (450°C) and solubilizing in 139 ml/l hydrochloric acid and 217 ml/l nitric acid by spectrophotometric analysis using a spectrophotometer at 410 nm after the addition of vanadate molybdate (Liu et al. 2018).

To determine seed mucilage, 10 g seeds were dispersed in boiling water at 100°C for 30 min. At the end of the extraction period, the extract was left to cool down at room temperature. The extract was filtered through glass wool, and the filtrate volume was reduced by rotary evaporation. The mucilage was precipitated by adding ethanol to a final concentration of 80% (v/v). After 24 h at 5°C, the precipitate was removed by centrifugation (4500 g for 30 min at 5°C), homogenized in water and freeze-dried (Bhatty 1993).

Oil content in each treatment was measured using the standard Soxhelt 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 (Visavadiya, Soni, and Dalwadi 2009).

For each species based on the following formula, mycorrhizal dependency (MD) of AM fungal yield was measured ;  $MD(\%) = \frac{GY(+M) - GY(-M)}{GY(+M)} \times 100$ , where GY, +M and -M represent the grain yield, inoculated plants and non-inoculated plants, respectively (Ortas 2019).

### Statistical analysis

Data were submitted to statistical analysis using SAS software version 9.3 (SAS, 2011). The analysis of variance (ANOVA) was carried out at  $P < .05$ . Mean values were compared using Duncan's Multiple Range Test.

## Results

### The pre-experiment

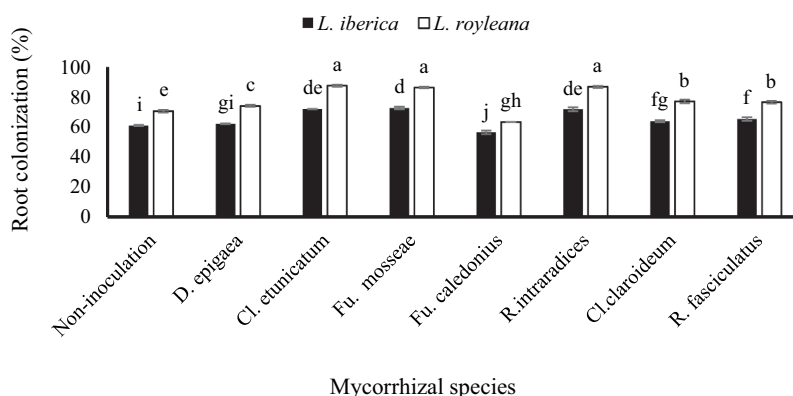
#### Root colonization

All mycorrhizal species used in the pre-experiment significantly increased the root colonization in both plant species of *Lallemantia* compared to non-inoculation plants. The present study indicated a 19% increase of root colonization for *L. royleana* exposed to *Cl. etunicatum*, *Fu. Mosseae* and *R. intraradices* than the non-inoculation plants, whereas for *L. iberica*, this increase was 16% under *Cl. etunicatum*, *Fu. Mosseae* and *R. intraradices* (Figure 1).

### The main experiment

#### Growth, yield and yield components

Compared to sterile soil, length of root and shoot, shoot length, number of silique in plant, number of seed in plant (Table 3), grain yield (Figure 2) and thousand grain weight (Figure 3) of both inoculated and non-inoculated plant species were higher under non-sterile soil. Single and mixed mycorrhizal species, especially  $M_1 + M_2 + M_3$  inoculated *Lallemantia* species, produced a higher yield and yield components, and growth were also higher under both type of soil conditions. The application of single and mixed mycorrhizal species to both type of soil significantly increased the growth, yield and yield components compared to the non-inoculated plants. The inoculated and non-inoculated plants of *L. royleana* had the most number of silique

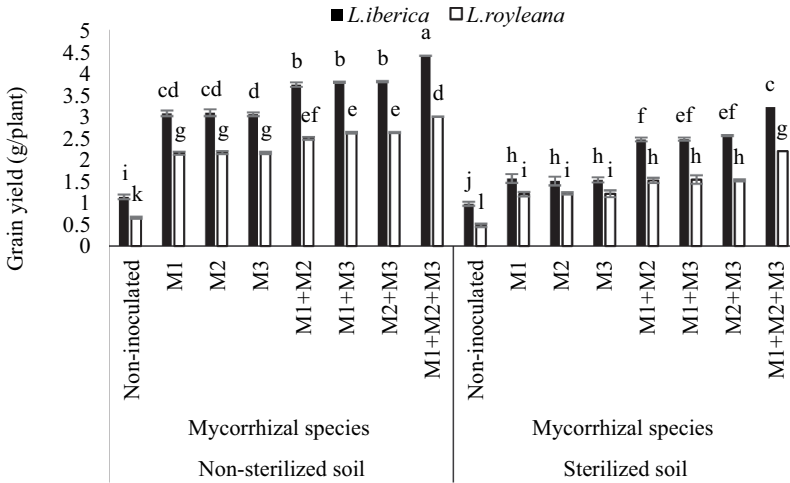


**Figure 1.** The effect of mycorrhizal species on root colonization of *Lallemantia* species. Means with similar letters in each column are not significantly different at 5% level according to Duncan test. Averages of three replicates are presented. Error bars show standard errors.

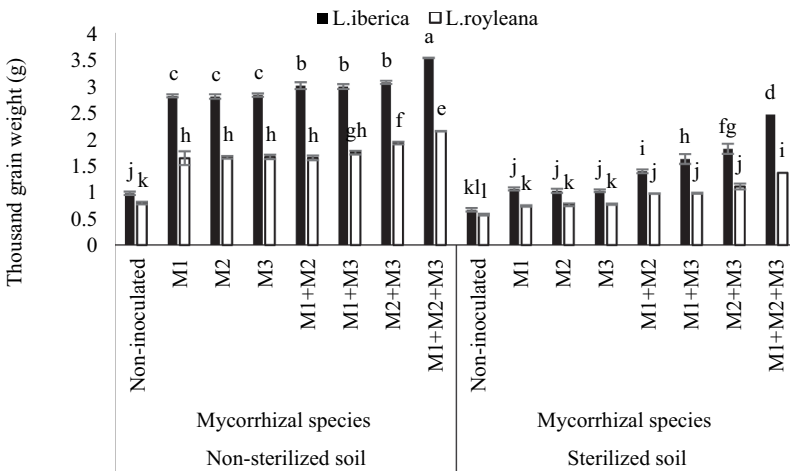
**Table 3.** The effect of mycorrhizal species and their interaction on different parameters of two *Lallemantia* species under sterile and non-sterile soil.

Treatments	Root length (cm)	Shoot length (cm)	Number of silique in plant	Number of seeds in plant
Mycorrhizal species	Plant species	Non-sterilized soil		
Non-inoculation		7.35 ± 0.15 g	42.83 ± 0.87k.m	432.2 ± 26.54 l
M <sub>1</sub>		10.28 ± 0.08 c.e	55.83 ± 0.29c	505.87 ± 3.18k
M <sub>2</sub>		10.26 ± 0.08 c.e	51 ± 0.88de	517.43 ± 29.87k
M <sub>3</sub>		10.23 ± 0.03 c.e	46.83 ± 0.87gh	579.8 ± 1k
M <sub>1</sub> + M <sub>2</sub>	<i>L. iberica</i>	10.69 ± 0.09 c	47.44 ± 0.23fg	594.43 ± 9.6j
M <sub>1</sub> + M <sub>3</sub>		10.71 ± 0.20 c	52.76 ± 0.49d	595.33 ± 34.72 j
M <sub>2</sub> + M <sub>3</sub>		10.54 ± 0.06 c	52.31 ± 0.83d	602.37 ± 33.56 j
M <sub>1</sub> + M <sub>2</sub> + M <sub>3</sub>		12.88 ± 0.11 a	64.27 ± 0.23a	775.27 ± 8.41e
Non-inoculation		5.43±0.11 j	37.94 ± 0.87o	476.53 ± 32.65 h
M <sub>1</sub>		9.69 ± 0.00 d.f	50.95 ± 0.3de	734.93 ± 52.23 f
M <sub>2</sub>		9.66 ± 0.03 ef	45.02 ± 0.3h.j	723.33 ± 76.6f
M <sub>3</sub>		9.66 ± 0.01 ef	42.44 ± 0.29 lm	757.47 ± 88.27 f
M <sub>1</sub> + M <sub>2</sub>	<i>L. royleana</i>	10.40 ± 0.03 c	44.98 ± 0.5 h.j	863.17 ± 119.26c
M <sub>1</sub> + M <sub>3</sub>		10.33 ± 0.05 cd	47.6 ± 0.66fg	861.37 ± 92.17c
M <sub>2</sub> + M <sub>3</sub>		10.75 ± 0.05 c	44.71 ± 0.73i.k	864.67 ± 43.32c
M <sub>1</sub> + M <sub>2</sub> + M <sub>3</sub>		11.75 ± 0.10 b	59.38 ± 0.23b	1151.33 ± 581.38a
Sterilized soil				
Non-inoculation		6.21 ± 0.05 i	35.4 ± 0.64p	584.43 ± 26.54 l
M <sub>1</sub>		7.76 ± 0.01 g	37.57 ± 0.61op	472.7 ± 3.18k
M <sub>2</sub>		7.66 ± 0.27 g	37.63 ± 0.87op	475.47 ± 29.87k
M <sub>3</sub>		7.20 ± 0.52 gh	38.3 ± 0.59o	495.07 ± 1k
M <sub>1</sub> + M <sub>2</sub>	<i>Li berica</i>	9.24 ± 0.43 f	42.3 ± 0.64 m	495.93 ± 9.6 j
M <sub>1</sub> + M <sub>3</sub>		9.63 ± 0.22 ef	41.07 ± 0.67mn	493.63 ± 34.72 j
M <sub>2</sub> + M <sub>3</sub>		9.55 ± 0.14 f	41.04 ± 0.22mn	493.77 ± 33.56 j
M <sub>1</sub> + M <sub>2</sub> + M <sub>3</sub>		10.83 ± 0.03 c	49.37 ± 0.46ef	386.5 ± 8.41e
Non-inoculation		4.47 ± 0.07 k	39.47 ± 0.49no	538.67 ± 64.47i
M <sub>1</sub>		6.59 ± 0.01 hi	47.2 ± 0.35 g	674.73 ± 6.56 g
M <sub>2</sub>		6.08 ± 0.16i i	44.33 ± 0.55 j.l	695.8 ± 46.13.00 g
M <sub>3</sub>		6.62 ± 0.31 hi	45.83 ± 1.26 g.j	676.17 ± 103.26 g
M <sub>1</sub> + M <sub>2</sub>	<i>L. royleana</i>	7.57 ± 0.27 g	47.6 ± 1.18fg	755.07 ± 125.65d
M <sub>1</sub> + M <sub>3</sub>		7.76 ± 0.22 g	47.27 ± 0.55 g	763.77 ± 100.53d
M <sub>2</sub> + M <sub>3</sub>		7.62 ± 0.20 g	46.73 ± 0.68 g.i	767.07 ± 136.79d
M <sub>1</sub> + M <sub>2</sub> + M <sub>3</sub>		9.38 ± 0.05 f	54.7 ± 1.25c	1074 ± 71.00b

Values were means of three replicates ± standard deviation.



**Figure 2.** The effect of mycorrhizal species on grain yield of *Lallemantia* species under sterilized and non-sterilized soil. Means with similar letters in each column are not significantly different at 5% level according to Duncan test. Averages of three replicates are presented. Error bars show standard errors. M<sub>1</sub>, *Cl. etunicatum*; M<sub>2</sub>, *Fu. mosseae*; M<sub>3</sub>, *R. intraradices*; M<sub>1</sub>+ M<sub>2</sub>, *Cl. Etunicatum*, *Fu. mosseae*; M<sub>1</sub>+ M<sub>3</sub>, *Cl. Etunicatum*, *R. intraradices*; M<sub>2</sub>+ M<sub>3</sub>, *Fu. Mosseae*, *R. intraradices*; M<sub>1</sub>+ M<sub>2</sub>+ M<sub>3</sub>, *Cl. Etunicatum*, *Fu. Mosseae*, *R. intraradices*.



**Figure 3.** The effect of mycorrhizal species on thousand grain weight of *Lallemantia* species under sterilized and non-sterilized soil. Means with similar letters in each column are not significantly different at 5% level according to Duncan test. Averages of three replicates are presented. Error bars show standard errors. M<sub>1</sub>, *Cl. etunicatum*; M<sub>2</sub>, *Fu. mosseae*; M<sub>3</sub>, *R. intraradices*; M<sub>1</sub>+ M<sub>2</sub>, *Cl. Etunicatum*, *Fu. mosseae*; M<sub>1</sub>+ M<sub>3</sub>, *Cl. Etunicatum*, *R. intraradices*; M<sub>2</sub>+ M<sub>3</sub>, *Fu. Mosseae*, *R. intraradices*; M<sub>1</sub>+ M<sub>2</sub>+ M<sub>3</sub>, *Cl. Etunicatum*, *Fu. Mosseae*, *R. intraradices*.

in plant and the number of seed in plant under sterile and non-sterile soil. In contrast, the highest length of root and shoot, grain yield and thousand grain weight were observed in inoculated and non-inoculated *L. iberica* under sterile and non-sterile soil.

**Seed quality**

The inoculated and non-inoculated plants of both *Lallemantia* species exhibited the highest mucilage content at non-sterile soil condition compared to sterile soil condition. The content of mucilage in the non-inoculated *Lallemantia* species was lower than that in the inoculated plants. The increasing seed

mucilage content of *Lallemantia* species inoculated by single mycorrhizal species and their mixture was observed, so that the result exhibited that the application of  $M_1 + M_2 + M_3$  to both types of soil was more effective compared to other treatments. The highest mucilage in seed *L. iberica* and *L. royleana* inoculated by  $M_1 + M_2 + M_3$  was 4.19% and 15.27% under non-sterilized soil, respectively, whereas in sterilized soil, that was 3.85% and 9.45% for *L. iberica* and *L. royleana*, respectively (Table 4).

Phosphorus content in inoculated and non-inoculated *Lallemantia* species were more significantly changed under non-sterilized than sterilized soil condition. Compared with non-inoculated *Lallemantia* species, inoculated plants produced the highest phosphorus content under both types of soil conditions. The application of single and mixed mycorrhizal species to both types of soil were effective in increasing seed phosphorus in both species of *Lallemantia*. The result showed that inoculation of  $M_1 + M_2 + M_3$  increased the phosphorus content than other treatments. Under non-sterile soil, the phosphorus content in *L. iberica* and *L. royleana* inoculated by  $M_1 + M_2 + M_3$  was 96.52% and 87.84%, respectively, whereas in sterile soil, that was 85.15% and 76.79% for *L. iberica* and *L. royleana*, respectively (Table 4).

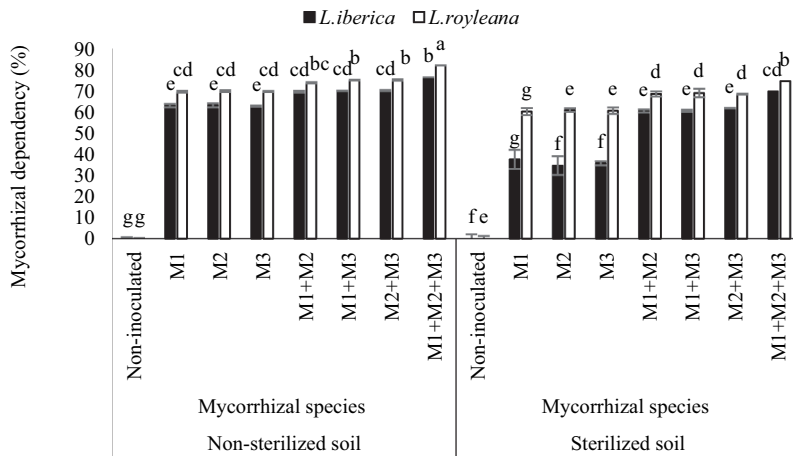
In this study, non-sterilized soil condition was observed to be highly effective on increasing oil content of *Lallemantia* species compared to sterilized soil. Mycorrhizal-inoculated *Lallemantia* species exhibited better oil content in both types of soil condition than non-inoculated plants. Inoculation by single and mixed mycorrhizal species, especially using  $M_1 + M_2 + M_3$ , had the most effect on increasing

**Table 4.** The effect mycorrhizal species and their interaction on different parameters of two *Lallemantia* species under sterile and non-sterile soil.

Treatments		Seed phosphorus (%)	Seed mucilage (%)	Oil content (%)
Mycorrhizal species	Plant species	Non-sterilized soil		
Non-inoculation		80.55 ± 0.34 e	1.94 ± 0.01 o	17.03 ± 0.74hij
$M_1$		92.33 ± 0.58 b	3.82 ± 0.01 k	21.1 ± 1.42e
$M_2$		91.27 ± 0.48 b	3.75 ± 0.05 k	20.07 ± 0.92efg
$M_3$		90.61 ± 0.23 b	3.73 ± 0.04 k	20.03 ± 1.19efg
$M_1 + M_2$	<i>L. iberica</i>	92.58 ± 0.01 b	4.07 ± 0.00 j	27.1 ± 0.89c
$M_1 + M_3$		92.56 ± 0.06 b	4.07 ± 0.01 j	26.7 ± 1.17 cd
$M_2 + M_3$		92.62 ± 0.04 b	4.11 ± 0.00 j	26.83 ± 0.91 cd
$M_1 + M_2 + M_3$		96.52 ± 0.53 a	4.19 ± 0.01 j	37.1 ± 0.32a
Non-inoculation		60.43 ± 0.06 jk	9.35 ± 0.07 e	14.45 ± 0.15 lm
$M_1$		74.39 ± 0.92 g	13.68 ± 0.11 d	16.47 ± 0.49ijkl
$M_2$		74.03 ± 0.81 g	13.65 ± 0.13 d	17.28 ± 0.42hi
$M_3$		73.01 ± 0.46 g	13.66 ± 0.16 d	16.77 ± 0.88hijk
$M_1 + M_2$	<i>L. royleana</i>	74.35 ± 0.01 g	14.68 ± 0.02 c	19.67 ± 0.58efg
$M_1 + M_3$		74.51 ± 0.03 g	14.87 ± 0.00 b	19.9 ± 0.66efg
$M_2 + M_3$		74.67 ± 0.02 g	14.72 ± 0.09 bc	20.13 ± 0.73efg
$M_1 + M_2 + M_3$		87.84 ± 0.84 c	15.26 ± 0.02 a	24.93 ± 0.33d
		Sterilized soil		
Non-inoculation		60.8 ± 0.65 jk	1.16 ± 0.02 p	16.73 ± 0.23 hijk
$M_1$		68.26 ± 0.51 h	2.36 ± 0.09 n	20 ± 0.26efg
$M_2$		67.43 ± 0.60 h	2.23 ± 0.09 n	19.93 ± 0.33efg
$M_3$		68.09 ± 0.29 h	2.22 ± 0.08 n	20.73 ± 0.33ef
$M_1 + M_2$	<i>L. iberica</i>	72.51 ± 0.73 g	3.16 ± 0.01 m	26.1 ± 0.35 cd
$M_1 + M_3$		73.71 ± 1.06 g	3.24 ± 0.02 lm	27.47 ± 0.58bc
$M_2 + M_3$		74.20 ± 1.84 g	3.35 ± 0.01 l	26.3 ± 0.62 cd
$M_1 + M_2 + M_3$		85.14 ± 0.95 d	3.85 ± 0.01 k	29.13 ± 0.26b
Non-inoculation		49.52 ± 0.58 n	6.71 ± 0.00 i	12.47 ± 1.23 m
$M_1$		55.08 ± 0.73 l	7.74 ± 0.03 h	14.83 ± 0.32kl
$M_2$		53.05 ± 0.69 m	7.72 ± 0.00 h	16.27 ± 0.12ijkl
$M_3$		55.94 ± 0.60 l	7.71 ± 0.04 h	15.03 ± 0.37jkl
$M_1 + M_2$	<i>L. royleana</i>	59.71 ± 0.65 k	8.47 ± 0.01 g	17.93 ± 0.33ghi
$M_1 + M_3$		62.37 ± 0.32 ij	8.61 ± 0.01 fg	18.7 ± 0.6 fgh
$M_2 + M_3$		63.20 ± 0.35 i	8.75 ± 0.01 f	17.9 ± 0.42ghi
$M_1 + M_2 + M_3$		76.78 ± 0.77 f	9.45 ± 0.01 e	21.27 ± 0.49e

Values were means of three replicates ± standard deviation





**Figure 4.** The effect of mycorrhizal species on micorrhizal dependency of *Lallemantia* species under sterilized and non-sterilized soil. Means with similar letters in each column are not significantly different at 5% level according to Duncan test. Averages of three replicates are presented. Error bars show standard errors. M<sub>1</sub>, *Cl. etunicatum*; M<sub>2</sub>, *Fu. mosseae*; M<sub>3</sub>, *R. intraradices*; M<sub>1</sub>+ M<sub>2</sub>, *Cl. Etunicatum*, *Fu. mosseae*; M<sub>1</sub>+ M<sub>3</sub>, *Cl. Etunicatum*, *R. intraradices*; M<sub>2</sub>+ M<sub>3</sub>, *Fu. Mosseae*, *R. intraradices*; M<sub>1</sub>+ M<sub>2</sub>+ M<sub>3</sub>, *Cl. Etunicatum*, *Fu. Mosseae*, *R. intraradices*.

oil content in both species of *Lallemantia* under both types of soil conditions. Compared to oil content of *L. royleana*, the highest oil content was observed in inoculated and non-inoculated *L. iberica* under sterilized and non-sterilized soil (Table 4).

### Mycorrhizal dependency (MD)

The MD in both *Lallemantia* species was more significantly affected by non-sterilized soil compared to sterilized soil. The MD of *Lallemantia* species inoculated was higher than non-inoculated plants under both types of soil conditions. In both species of *Lallemantia*, not only single use of mycorrhizal species significantly increased the MD in both types of soil conditions but also their mixture, especially M<sub>1</sub> + M<sub>2</sub>+ M<sub>3</sub> were highly effective. The highest MD of *L. iberica* (76.39%) and *L. royleana* (81.95%) was observed when inoculated by M<sub>1</sub> + M<sub>2</sub>+ M<sub>3</sub> and exposed to non-sterile soil conditions (Figure 4).

### Discussion

At pre-experiments, it seems that three mycorrhizal species such as *Cl. etunicatum*, *Fu. mosseae* and *R. intraradices* had more effect on increasing root colonization of *L. iberica* and *L. royleana*. This result is consistent with those reporting an enhanced colonization in the roots of flax (Rahimzadeh and Pirzad 2019) and tomato (Ghazanfar et al. 2015) plants by *Fu. Mosseae*, *R. intraradices* and *Cl. etunicatum* inoculation. The increasing root colonization were vital to AMF and host plant, since the high colonization provide more carbon from roots of the host plant in the soil, and in return, fungi the compensate the plant via enhanced nutrients (e.g. phosphorus); therefore, a symbiotic relationship was established between mycorrhizal fungi and plant roots (Wu, Kumar Srivastava, and Zou 2013).

Based on the pre-experiment results, the main experiment was conducted to determine the effect of soil sterility on symbiotic relationship between three optimal mycorrhizal *Lallemantia* species. According to this research, Ortas et al. (2016) has indicated that soil sterility was effective on mycorrhizal growth dependency of *Citrus aurantium* L. Our results showed that growth, yield and yield components in non-sterilized soil was higher compared to sterilized soil condition. The limitation of nutrients in sterilized soil than in non-sterilized soil can reduce colonization in roots of host plant and limit crop growth and yield in sterilized soil (Ortas and Yucel 2020). The inoculation of M<sub>1</sub> + M<sub>2</sub>+ M<sub>3</sub> mycorrhizal species led to increased growth, yield and yield components of

*Lallemantia* species under both types of soil conditions. Improvement in crop growth and yield by inoculation of mycorrhizal has been reported in *Lavandula officinalis*, *Rosmarinus officinalis* and *Thymus vulgaris* (Pirzad and Mohammadzadeh 2018). Indeed, application of mixed mycorrhizal compared to single mycorrhizal has good potential for increasing crop production in soil (Ortas, Iqbal, and Cem Yücel 2019). It has been reported that mixed mycorrhizal can accelerate revegetation soil and inoculation (Leung et al. 2013). Under both types of soil, the highest number of silique in plant and number of seed was demonstrated in inoculated and non-inoculated *L. royleana*; however, the most length of root and shoot, grain yield and thousand grain weight was obtained in inoculated and non-inoculated *L. iberica*. In this present study, it is observed that growth and yield of *L. iberica* and *L. royleana* were significantly affected by genotype. It is in agreement with the results of Abdolahi and Maleki Farahani (2019) and Omidi et al. (2018) reporting that genetic background influences both species of *Lallemantia* growth and yield.

The increased phosphorus, mucilage, and oil content in both *Lallemantia* species in the non-sterilized soil is probably related to the higher abundance of indigenous AMF in the non-sterilized soil compared to the sterilized soil (Ortas and Yucel 2020). According to the research, the highest phosphorus, mucilage, and oil content was observed in both species *Lallemantia* inoculated by  $M_1 + M_2 + M_3$ . Our results are consistent with reports on *Lavandula officinalis* (Pirzad and Mohammadzadeh 2018), *Dracocephalum moldavica* (Ghanbarzadeh et al. 2020) and *Linum usitatissimum* (Rahimzadeh and Pirzad 2019) that demonstrated that mycorrhizal inoculation played an effective role on increased seed quality. Indeed, high microbial abundance in soil promotes symbiosis with the host plant. This can increase root extension and improve photosynthesis through enhanced uptake of water and nutrients (Askari et al. 2019). It was indicated that the extension of roots provided more phosphorus and carbohydrates for the synthesis of oil and mucilage in seeds of *Lallemantia* species (Paravar et al. 2021a). Indeed, interaction between AM fungi and plant roots is bidirectional (Bücking, Liepold, and Ambilwade 2012). On one hand, AM fungi promote the carbon needed for germination of fungal spores and root colonization by increasing of the plant's photosynthesis activity (Ghorchiani, Etesami, and Ali Alikhani 2018). On the other hand, AM fungi improve nutrient uptake from soil via extending the root system (Ghanbarzadeh et al. 2020; Keymer et al. 2017). The highest content of phosphorus and seed oil in seeds of *L. iberica* compared with *L. royleana* due to the higher potential of *L. iberica* roots for taking up nutrients (Paravar et al. 2021a). Enhanced mucilage in *L. royleana* than *L. iberica* was probably due to the higher photosynthesis activity which increasing carbohydrates in seeds (Abdolahi and Maleki Farahani 2019).

The highest inoculation mycorrhizal dependency were observed when the inoculated and non-inoculated plants in both species grew under non-sterilized soil. According to these results, Ortas, Iqbal, and Cem Yücel (2019) reported that MD increased by mycorrhizal inoculation under non-sterilized soil compared to sterilized soil. Under sterilized soil, native mycorrhizal spores were eliminated; as a result, the plants had no strong inoculation dependency for better root colonization (Ortas, Demirbas, and Akpınar 2018) and secondary metabolites of seed (e.g., mucilage) (Rahimzadeh and Pirzad 2019). In this experiment, it is shown that application of mycorrhizal was more effective on MD on both plant species, but their integrated application, especially  $M_1 + M_2 + M_3$  treatment, was most effective on them. Our results are in agreement with Ortas (2019) reporting, who found inoculation plants by mycorrhizal species always better grew and exhibited higher MD than control plants. Under sterilized and non-sterilized soil, inoculation dependency was more for inoculated *L. royleana* compared to that of *L. iberica*. It can be concluded that *Lallemantia* species can influence the extent of mycorrhizal colonization and its potential benefits. The present research findings are consistent with the studies on soybean (Pawlowski et al. 2020) and onion (Taylor et al. 2015) that reported plant genotypic variation to AMF colonization or their benefits.

## Conclusions

In general, plants grown in non-sterilized soil show higher growth parameters, yield, yield components (thousand grain weight, silique in plant and number seed in plant), seed quality (phosphorus, mucilage and oil content) and MD than sterilized soil. It seems that in non-sterilized soil, because of the presence of native mycorrhizal spores, both *Lallelantia* species were able to grow better than in sterile soil. For both sterilized and non-sterilized soil, application of M<sub>1</sub> + M<sub>2</sub> + M<sub>3</sub> mycorrhizal species in both species of *Lallelantia* led to significantly increase growth, yield, yield components, seed quality and MD. Compared to non-inoculated plants, the inoculated plants exhibited better response in both non-sterilized soil and sterilized soil. Under sterilized soil conditions, the highest number of silique in plant and number of seed in plant, MD and seed mucilage were observed in *L. royleana*; however, the most length of root and shoot, yield and thousand grain weight, phosphorus and oil content were shown in *L. iberica*. Mycorrhizal growth dependency of *L. iberica* and *L. royleana* could have a bearing on the success of AM colonization; however, *L. royleana* plants were more dependent on mycorrhizal inoculation than *L. iberica*. Therefore, it seems that different genotypes of *L. iberica* and *L. royleana* influenced the extent of mycorrhizal colonization and its potential benefits.

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