

Research Article

Effects of endophytic fungi colonization on the growth and physiological responses of *Satureja khuzestanica*

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Abstract: Plant endophytes deployment at the field level affects the productivity of agricultural products. Savory *Satureja khuzestanica* Jamzad is a valuable medicinal plant native to Iran. In this study, in order to improve the quantitative and qualitative yield of the plant, the development of a suitable method for inoculation of native fungal endophytes to *S. khuzestanica* is surveyed. Three fungal endophytes (P.I.S.7, D.2.F.1 and D.1.S.1), isolated from savory plants, were inoculated on *S. khuzestanica* using four different methods (stem injection, foliar spray, soil drench and adding fungi to the soil in granular form) and their impact on plant's growth and its physiological response was analyzed. All inoculation methods, except adding granular form of fungi to the soil, resulted in endophytic colonization of the savory tissues. However, P.I.S.7 isolate was not established in these plants using any inoculated methods. Extent of fungal colonization showed a significant difference with the inoculation method, such that foliar spray had the highest colonization level, followed by stem injection and root drench. The highest rate of fungal colonization belonged to D.1.S.1. isolate. Spraying leaves and stem injection with endophytic fungi, especially in the case of D.1.S.1, significantly enhanced the morphological and physiological characteristics of host plants and the percentage of essential oil yields in these plants. All in all, using the D.1.S.1 isolate and foliar spray method led to savory plants' improved quantitative and qualitative product.

Keywords: essential oil, endophytic fungi, *Satureja khuzestanica*

Introduction

Endophytic fungi are endosymbiotic microorganisms living throughout the plant system without deleterious consequences and establish useful interactions with the host (Lareen *et al.*, 2016). Their interactions

contribute to beneficial effects on plants in terms of plant growth and disease management (Khare *et al.*, 2018). Therefore, these plant-associated microorganisms can be used as environment-friendly bio-fertilizers in the sustainable cultivation of important crops (Kalra *et al.*, 2010; Lobo *et al.*, 2019). Such

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applications of endophytes in the plant system establish beneficial effects in plants, such as improving nutrient uptake (phosphorus and nitrogen), increasing plant productivity, and preventing colonization by foreign parasitic organisms (Bamisile *et al.*, 2018). For example, colonization of *Colletotrichum tofieldiae* significantly improves phosphorus uptake and its transport in the host plant, *Arabidopsis thaliana*, which increases fertility and enhances plant growth under phosphate-deficient conditions (Hiruma *et al.*, 2016). There are also numerous examples of other roles of endophytes in host plants, including the production of plant hormones and the protection of plants against pathogens (such as Waqas *et al.*, 2012; Rabha *et al.*, 2014). In addition, under stress conditions, endophytes induce the ability of plants to tolerate heavy metals, drought, and salinity stress and help plants mitigate stress by exhibiting ACC deaminase activity and subsequent siderophore formation (Kong *et al.*, 2015; Ullah *et al.*, 2015). In addition to the aforementioned beneficial effects, endophytes play a crucial role in the production of important pharmaceutical compounds such as taxol (Kumaran *et al.*, 2010), vincristine (Kuriakose *et al.*, 2016), hypericin (Kusari *et al.*, 2008) and podophyllotoxin (Eyberger *et al.*, 2006). It has also been shown that endophytic fungi, in addition to their *in vivo* production, can significantly increase the production of secondary metabolites in plants (*in planta*) after application as bioinoculants and successful colonization of the host plant (Wani *et al.*, 2017). It has been reported that the inoculation of *Bacillus megaterium*, *Glomus intraradices*, and *Trichoderma harzianum* ThU and their simultaneous inoculation significantly increases the bacoside content in *Bacopa monnieri* (Gupta *et al.*, 2015); whereas, inoculation of chitinolytic microbes such as *Chitinophilus* sp. MTN22 and *Streptomyces* sp. MTN14, alone and when co-inoculated, significantly increase the biosynthetic pathway genes of bacoside A and modulates the systemic defense mechanism of *Bacopa monnieri* against

Meloidogyne incognita nematode (Gupta *et al.*, 2017). Similarly, previous studies on the increase of secondary metabolites in plants by inoculation with endophytic fungi have confirmed that inoculation of endophytes increases vindoline content in *Catharanthus roseus* (Pandey *et al.*, 2016a) and benzyloquinoline alkaloids in *Papaver somniferum* (Pandey *et al.*, 2016b). Another study reported that using bio-inoculants such as *Glomus mosseae* and *Glomus fasciculatum* significantly enhanced root yield and forskolin content in *Coleus forskohlii* (Singh *et al.*, 2009).

Satureja khuzestanica, known by the common Persian name “Marzeh Khuzestani”, is a plant of the family Lamiaceae that has various medicinal properties and grows in the south and southwest of Iran. Hadian *et al.* (2011) stated that 89.5-95% of carvacrol monoterpene could be found in essential oils extracted from *S. khuzestanica* populations. According to current evidence, the high purity of carvacrol in the essential oil is a unique feature that has not been reported in any other plant. Therefore, in recent years, many studies have been conducted on the traditional uses and biological characteristics of *S. khuzestanica*. These studies have revealed a wide range of pharmacological activities, including antiviral, antimicrobial, antifungal, antidiabetic, and antioxidant effects of *S. khuzestanica* (reviewed in Khaledi and Meskini, 2020). However, it should be noted that the yield of essential oil in this plant is not very high (Saadat *et al.*, 2004; Haeri *et al.*, 2006). Cultivation of a medicinal plant is cost-effective when not only producing suitable biomass but also reaching the desired level of the quantity and quality of its products, such as essential oils, are considered. One of the effective ways to increase the yield of medicinal plants and plant production efficiency is the application of endophytic fungi as biological regulators of plant growth. Green approaches like applying crop-specific endophytes in appropriate inoculation methods could be economical during the sustainable cultivation of medicinal plants.

Therefore, efforts were made to develop an appropriate inoculation method to transfer three functional fungal endophytes to “Marzeh Khuzestani”, and evaluate the effects of these fungi on the greenhouse cultivation of *S. khuzestanica*. Understanding the vegetative, physiological and biochemical performance of *S. khuzestanica* in response to inoculation with endophytic fungi is the first and most significant step in using these growth-promoting microorganisms for increasing the efficiency of host essential oil.

Materials and Methods

The experiment was conducted in the greenhouse at the Research Institute of Forests and Rangelands of Iran from February to May 2021. The temperature inside the sunlight greenhouse was set at 25 ± 2 °C.

Experimental designs and treatments

The experiment was set up as a completely randomized design with treatments consisting of four different fungal inoculation methods (including 1- injection of spores of endophytic fungi into the stem, 2- spray of spores on foliage, 3- addition of fungi to the soil in granular form and 4- addition of fungi to the soil by soaking with spores, and the control) using three endophytic fungal isolates that were isolated from savory plants in our previous study (Alipour, 2020). The characteristics of these isolates are presented in Table 1. The percentage of colonization of each endophyte for each inoculation method was examined in ten replications. Their biological effects were evaluated by assessing the morphological and physiological traits of *S. khuzestanica* plants in five repetitions.

Table 1 Characteristics of endophytic isolates included in this study.

Region of isolation	Host	Source	Code of isolates	Fungal species
Lorestan-Pol Dokhtar	<i>Satureja khuzestanica</i>	Stem	P.I.S.7	<i>Thielavia basicola</i>
North Khorasan-Darkesh	<i>Satureja mutica</i>	Flower	D.2.F.1	<i>Xenodidymella applanata</i>
North Khorasan-Darkesh	<i>Satureja mutica</i>	Stem	D.1.S.1	<i>Chaetosphaeronema achilleae</i>

Sterilization of greenhouse, seeds and soil

The pots used in the test and the greenhouse platforms were disinfected with 1% sodium hypochlorite. Initially, to ensure the germination ability of *S. khuzestanica* seeds, the germination percentage of seeds was evaluated, and the potency was estimated to be 85%.

S. khuzestanica seeds were washed under running water for 10 minutes and disinfected by immersion in 1% sodium hypochlorite for 2 minutes and 70% ethanol for 1 minute. The substrate was autoclaved twice for two consecutive days at 121 °C and at a pressure of 1 atmosphere for 60 minutes. The potting mix for the seedling tray was at the ratio of 1: 1: 2 peat: perlite: coco peat, and for pots was at the ratio of 1: 1 field soil: sand.

After disinfecting the seeds, they were planted in seedling trays, watered regularly, and weeded. Finally, the healthy seedlings

were transferred to the pot at the stage of 10 to 20 leaves.

Preparation of endophytic inoculum

Inoculum preparation of endophytic fungi on solid wheat medium was performed according to the method of Mastan *et al.* (2019) with some changes.

Initially, the wheat bran was boiled in water for 30 minutes, and then 100 grams of it was added in special bags, autoclaved, and cooled to room temperature. After cooling, five circular agar plugs (5mm in diameter) containing pure endophytic fungi P.I.S.7, D.2.F.1, and D.1.S.1 were inoculated into sterilized wheat bran. The inoculated bags were incubated at 30 °C until fungi covered the entire medium. After ten days, the bags were shaken to prevent clumping of the contents due to fungal growth and for proper aeration. Also, by shaking the bags, the inoculum is spread

evenly on the substrate, and uniform spores are obtained at harvest time.

To collect the produced spores, the spores were washed from each medium with 100 ml of sterile distilled water and 0.1% Tween 80 and vigorous hand-shaking for one min. A Neubauer hemocytometer estimated the spore concentration of stock suspension, and Sterile distilled water was used to adjust the stock concentration to a final concentration of 1×10^8 conidia ml^{-1} .

Spore viability was assessed just after harvest and before inoculating plants by spreading 100 μl of 1×10^8 conidia ml^{-1} on potato dextrose agar and incubating at 28 °C for 24 hours. In three replications (three separate Petri dishes), the spores were examined using a stereoscope to estimate percent germination.

A spore was considered germinated when a visible germ tube longer than half the diameter of the spore was observed. Spore germination of more than 90% was considered acceptable for use in experiments. The suspension stock was stored in darkness at 4 °C until use.

***S. khuzestanica* inoculation**

The plants were saturated with sterile distilled water 24 hours before inoculation. Four different methods were used for inoculation.

For inoculation by stem injection, a hole was made in the primary shoot using a sterile Hamilton syringe needle to facilitate injection of 1 ml of spore suspension (Posada *et al.*, 2007).

For inoculation by foliar spray, each plant was sprayed separately from the others to prevent accidental inoculation of other treatments. A manual spray was used to apply 100 ml of inoculum (10^8 spore ml^{-1}) with 0.1% Tween 80 on the foliage of savory plants. After spraying, the plants were covered with a plastic bag for 24 hours to maintain a high humidity level to facilitate fungal germination and plant colonization before being returned to the experimental blocks of the greenhouse (Parsa *et al.*, 2013). The tops of the plastic pots were covered with aluminum foil to avoid spore runoff into the soil.

To perform inoculation by fungal granular inoculum, 10 g of wheat bran colonized by each

endophytic fungi were added to the soil next to the crown. For inoculation by soil drench, 100 ml of inoculum with 0.1% Tween 80 was applied to the soil surface around the crown. In this method, the plants were covered with a plastic bag for 24 h to facilitate the germination of the fungus and inoculation of the plant in high humidity. Control plants were treated using sterile water with Tween 80%.

Assessment of endophytic colonization on *S. khuzestanica*

Sampling of savory plants to evaluate endophytic colonization was performed ten weeks after inoculation. At the time of sampling, ten plants (replicates) of each inoculation method (four types) for each fungus (three species) and control plants were harvested.

To determine the colonization of inoculated fungal isolates in each of inoculation methods, the procedure outlined by Greenfield *et al.* (2016) was followed.

Seedlings were divided into different sections (leaves and stems) using a sterile scalpel. The different plant parts were then aseptically surface-sterilized and cut under a laminar air flow hood into 5×5 mm pieces for leaves and 10 mm long pieces for stem. From each replicate, 50 randomly selected leaf and stem subsamples were placed 4 cm apart on potato dextrose agar plates. The Plates were sealed with parafilm and incubated in the dark at 28 ± 1 °C for 10 days, after which the presence of endophytes was determined.

The colonization of the different plant parts was recorded by counting the number of pieces of the different plant parts that showed the presence of inoculated fungal growth/mycelia according to Koch's postulates. The plates were inspected for fungal growth (every 2-3 days) for four weeks. Fungal isolates were identified morphologically using slides prepared from the mother plates. If fungal growth was detected and verified, subsamples containing the fungal colony were counted, and the corresponding subsamples were discarded. The success rate of fungal endophyte colonization (%) of host plant parts was calculated as follows:

$$\text{Colonization (\%)} = \left(\frac{\text{Number of pieces exhibiting fungal growth}}{\text{Total number of pieces plated out}} \right) \times 100$$

To assess sterilization method success, 100 µl of the last rinse water was also plated on PDA and incubated at 28 °C for 10 days. No fungal growth on the medium used for the imprint confirmed that sterilization was successful.

Evaluation of morphological and physiological characteristics of the inoculated plants

14 weeks after inoculation, five seedlings from each treatment and control plant were subjected to morphological and physiological evaluations. The morphological parameters were evaluated, plant height, leaf number, area, and shoot dry weight. The dry weight of shoots was measured after drying the tissue at 50 °C for 72 hours (Greenfield *et al.*, 2016). Leaf area was measured using a Leaf Area Meter. Chlorophyll content, photosynthesis, transpiration and stomatal conductance were evaluated among the physiological parameters. Chlorophyll Content Meter was used to measure leaf chlorophyll content. LCA-4 assessed photosynthesis, transpiration, and stomatal conduction, ACD between 10 am, and 11 am.

The percentage of essential oil was measured with the Clevenger method. Based on the method recommended by the European Pharmacopeia, after complete drying, dry aerial parts of *S. Khuzestanica* were subjected to hydro distillation for 3 h, using a clevenger-type apparatus. Then obtained essential oil was dried upon anhydrous sodium sulfate and according to the dry weight of the plant, the percentage of essential oil and its yield were determined.

Statistical analysis

Statistical analyses were performed by SPSS 20.0. GLM analysis was used to test significant differences between treatments, and general means (per isolate and inoculation method) were compared using the Duncan's test. A normality test (Kolmogoroff-Smirnoff) showed that the log-transformed data sets were normally distributed, and their variances were spread homogeneously.

Results

Assessment of endophytic colonization on *Satureja khuzestanica* plants

It was found that inoculation methods, including foliar spray, stem injection and soil drench resulted in establishment of fungal isolates D.2.F.1 and D.1.S.1 as an endophyte in *S. khuzestanica* tissues. While, none of the fungal isolates were established when fungi were added to the soil in the form of granules. Also, it was found that P.I.S.7 isolate was not established in these plants using any of the four inoculation methods.

Fungal colonization levels differed significantly with inoculation methods ($F = 11.56$; $df = 2, 15$; $P < 0.001$) and kind of isolates ($F = 20.635$; $df = 2, 15$; $P < 0.001$). Foliar spray caused the highest colonization of 44.85% followed by stem injection (41.14%) and soil drench inoculation (5.5%). The highest colonization rate belonged to D.1.S.1 (47.03%) and D.2.F.1 (44%). In soil drench inoculation, D.1.S.1 could not colonize the leaves of savory plants. The highest leaf colonization rate was recorded for D.2.F.1 and D.1.S.1 using the foliar spray method, while the stem injection led to a higher rate of colonization in the stems. No fungi were recovered from the tissues of control plants (Fig. 1).

Evaluation of morphological and physiological characteristics of the inoculated plants

The establishment of endophytic fungi had a significant effect on height, biomass, number of leaves and area, while the type of inoculation method only affected plant height and dry weight. Plants inoculated with endophytic fungi had significantly more leaves than controls in both types of fungi. Moreover, the type of inoculation had no significant effect on leaf size and number of leaves; but the leaf area increased in plants inoculated with endophytic fungi compared to control. (Table 2). The height of plants reached to 19.4, 17.5 and 15.7 cm in foliar spray, spore injection to the stem and soil drenching with spores, respectively (Table 3). Also, the shoot dry weight was 8.2, 7.3 and 6.05 g by the foliar spray, the stem injection and adding spores to the soil (soil drench), respectively.

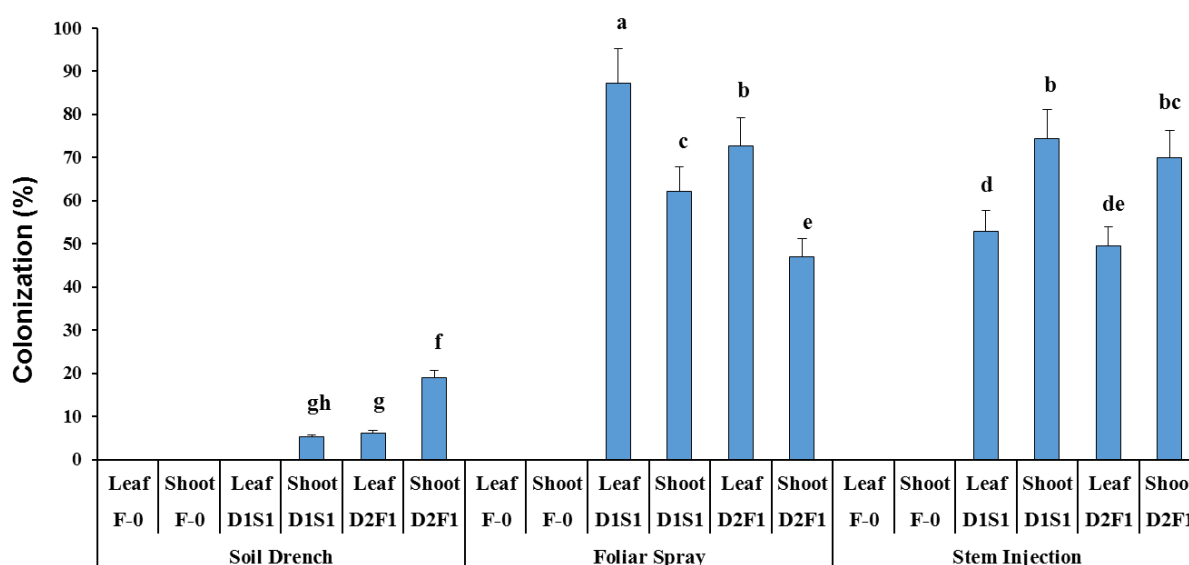


Figure 1 Colonization percentage of plant part pieces with endophytic fungi isolates (D.2.F.1 and D.1.S.1) and control (without fungus: F-0) recovered from leaves and stems following foliar spray, stem injection, or soil drench. Different letters above columns indicate statistical difference among treatments ($p < 0.05$). Treatments with zero percentage indicate failure in colonizing.

Although the dry shoot weight of plants treated with D.1.S.1 or D.2.F.1 endophytic fungi did not differ significantly but was significantly higher than control plants. So that the average dry weight of shoots in plants treated with D.1.S.1 and D.2.F.1 and control was 8.8 g, 8.6 g, and 4.1 g, respectively (Table 3).

Overall, the spore spraying method followed by the spore injection method to the stem showed the highest means of morphological traits, including plant height, shoot dry weight, number of leaves and leaf area.

Analysis showed that the establishment of endophytic fungi, as well as the type of

inoculation method had a significant effect on photosynthesis, transpiration, stomatal conductance and chlorophyll of host plants (Table 4). So that the rate of photosynthesis, transpiration, stomatal conductance and chlorophyll in endophytic fungi treatments was significantly higher than plants without endophytes (Table 5). Rate of photosynthesis and transpiration in plants inoculated by soil drenching was lower than in other treatments (Table 5). Also, stomatal conductance and chlorophyll content was significantly higher in the plants with spore foliar spraying treatment (Table 5).

Table 2 Results of analysis of variance for growth rate and morphological characteristics of *Satureja khuzestanica* in different treatments.

Sources of variation	df	Mean of squares			
		Plant height (cm)	Shoot dry weight (g)	Leaf number	Leaf area (cm ²)
Inoculation method	2	31.64*	10.43*	611.11 ^{ns}	0.031 ^{ns}
Fungal isolate	2	141.25**	62.607**	3530.33*	0.306**
Interaction effects	4	10.04 ^{ns}	5.53 ^{ns}	185.61 ^{ns}	0.003 ^{ns}
Error	18	8.07	2.69	192.15	0.013

* Significance level $p < 0.05$

** Significance level $p < 0.01$,

ns: non significant.

Table 3 Effects of different inoculation methods and endophytic fungal isolates on morphological characteristics of *Satureja khuzestanica*.

Inoculation method	Fungal isolate	Plant height (cm)	Shoot dry weight (g)	Leaf number	Leaf area (cm ²)
Soil drench	F-0	12.50 ± 2.16c	4.44 ± 1.50d	83 ± 16.4bc	0.45 ± 0.06b
	D.1.S.1	15.82 ± 3.25bc	6.01 ± 1.21cd	129 ± 17.2ab	0.57 ± 0.10a
	D.2.F.1	18.60 ± 2.21ab	7.71 ± 1.80bc	159 ± 23.6ab	0.59 ± 0.08a
Foliar spray	F-0	13.26 ± 2.31c	4.19 ± 1.58d	91 ± 13.4bc	0.47 ± 0.04b
	D.1.S.1	23.16 ± 6.21a	10.87 ± 3.64a	209 ± 22.0a	0.64 ± 0.08a
	D.2.F.1	21.83 ± 4.17a	9.53 ± 3.42ab	186 ± 31.2ab	0.61 ± 0.07a
Stem injection	F-0	13.14 ± 1.92c	3.81 ± 0.90d	69 ± 16.4c	0.43 ± 0.08b
	D.1.S.1	20.67 ± 7.10ab	9.40 ± 2.33ab	155 ± 25.2ab	0.6 ± 0.10a
	D.2.F.1	18.89 ± 1.54ab	8.74 ± 2.19bc	179 ± 22.4ab	0.56 ± 0.06a

Different letters within each column indicate statistical differences among treatments ($p < 0.05$)

Table 4 Results of analysis of variance for physiological characteristics of *Satureja khuzestanica* in different treatments.

Sources of variation	df	Mean of squares			
		Photosynthesis ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	Transpiration ($\text{mmol m}^{-2}\text{s}^{-1}$)	Stomatalconductance ($\text{mmol m}^{-2}\text{s}^{-1}$)	Chlorophyll ($\mu\text{g cm}^{-2}$)
Inoculation method	2	4.048**	0.061**	0.001*	3.383**
Fungal isolate	2	14.301**	0.261**	0.012**	8.894**
Interaction effects	4	1.036*	0.024**	0.001*	0.900**
Error	18	0.277	0.005	0.000	0.081

* Significance level $p < 0.05$

** Significance level $p < 0.01$

Table 5 Effects of different inoculation methods and endophytic fungal isolates on physiological characteristics of *Satureja khuzestanica*.

Inoculation method	Fungal isolate	Photosynthesis ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	Transpiration ($\text{mmol m}^{-2}\text{s}^{-1}$)	Stomatal conductance ($\text{mmol m}^{-2}\text{s}^{-1}$)	Chlorophyll ($\mu\text{g cm}^{-2}$)
Soil drench	F-0	5.63 ± 0.68c	0.53 ± 0.04d	0.09 ± 0.03d	5.41 ± 0.39d
	D.1.S.1	6.80 ± 0.80b	0.63 ± 0.09cd	0.12 ± 0.05c	6.04 ± 0.82c
	D.2.F.1	6.98 ± 1.32b	0.69 ± 0.08b	0.14 ± 0.02bc	6.1 ± 0.74c
Foliar spray	F-0	5.87 ± 0.33c	0.51 ± 0.07d	0.08 ± 0.02d	5.50 ± 0.14d
	D.1.S.1	8.90 ± 1.05a	0.93 ± 0.12a	0.17 ± 0.03a	7.91 ± 0.48a
	D.2.F.1	8.65 ± 0.63a	0.89 ± 0.11a	0.16 ± 0.03ab	7.75 ± 0.77a
Stem injection	F-0	5.92 ± 0.94c	0.50 ± 0.09d	0.09 ± 0.04d	5.24 ± 0.85d
	D.1.S.1	8.76 ± 0.93a	0.86 ± 0.07a	0.15 ± 0.07ab	7.67 ± 0.54a
	D.2.F.1	7.47 ± 0.60b	0.87 ± 0.10a	0.15 ± 0.05ab	7.11 ± 0.27b

Different letters within each column indicate statistical difference among treatments ($p < 0.05$).

Investigation of the potential of endophytic fungi in greenhouse conditions showed that the highest averages of physiological traits, including photosynthesis, transpiration, stomatal conductance, and chlorophyll content in host plants were obtained by foliar spore spraying method followed by injection of spores into the stem.

Both the types of fungus ($F = 9.40$; $df = 2$, 24; $P = 0.007$) and the methods of inoculation

($F = 6.25$; $df = 2$, 24; $P = 0.04$) had significant effects on the percentage of essential oil. However, D.1.S.1 was more effective than D.2.F.2. in improving the quality of savory plants (Fig. 2). Also, foliar spraying (with the highest essential oil (3.60%)) was a preferred method for endophytes inoculation into savory plants over spore injection (3.09% essential oil) (Fig. 2).

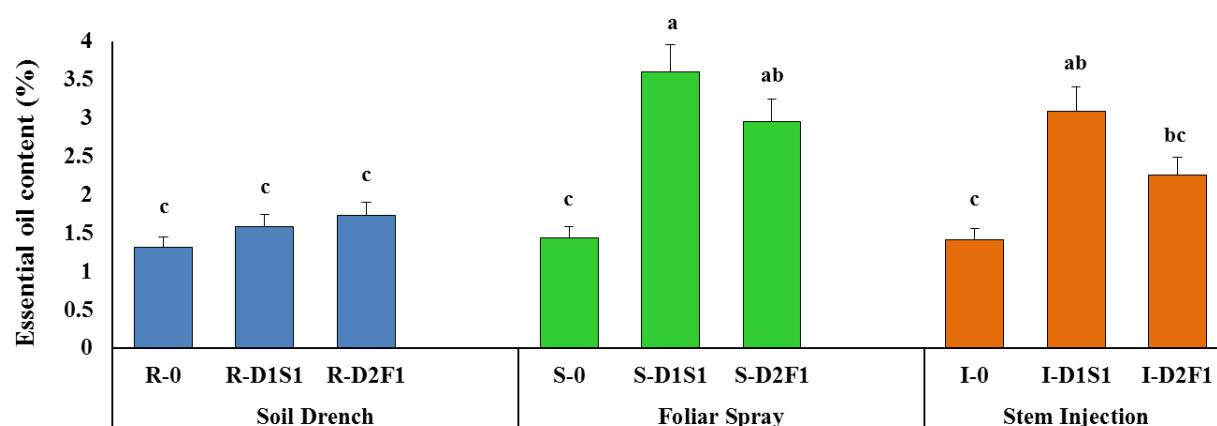


Figure 2 Effects of D.1.S.1 and D.2.F.1 endophyte fungal isolates on percentage essential oil yield of *Satureja khuzestanica* compared to control plants in different inoculation methods.

Discussion

The present study demonstrated for the first time establishment and colonization of two endophytic fungal isolates (D.1.S.1 and D.2.F.1) in stems and leaves of savory plants. Our results comply with Behie *et al.* (2015) and Kasambala Donga *et al.* (2018), who stated that many endophytic fungi such as *Metarhizium* spp., most other endophytic fungi do not display preferential tissue colonization. However, endophytic fungi may display different levels of colonization within different tissues of their host plants due to many factors such as plant tissue type, plant genotype, microbial taxon and strain type (Hardoim *et al.*, 2015).

In addition, our results showed that fungal recovery was significantly higher in plants inoculated via foliar sprays and stems injections than soil drenching, which has been noticed in some other studies also (e.g. Quesada-Moraga *et al.*, 2007; Guesmi Jouini *et al.*, 2014; Russo *et al.*, 2015; Jaber and Enkerli, 2017). Although, soil drenching was a more effective method for introducing endophyte fungal isolate than foliar sprays in coffee plants that can be due to the poor route of leaf entry of endophyte (Posada *et al.*, 2007).

Recovery of inoculated endophytic fungi from leaves and stems following foliar sprays, stem injections, and soil drenches can indicate

their ascending movement within the plant. Previously, such systemic spread of endophytes within the plant has been reported to occur in several crops such as *Vicia faba* and *Phaseolus vulgaris* (Akutse *et al.*, 2013) and coffee (Posada *et al.*, 2005). Yan *et al.* (2015) reported that fungal endophytes displayed limited systematic growth within host plants; the inoculated fungal endophyte remained localized in the plant part that had received the initial fungal treatment which seems to be the case for savory plants. However, in maize, mycelial growth in xylem vessels was the main mechanism of the fungus applied to the leaves that colonized the stem (Cherry *et al.*, 2004).

Alipour (2020), isolated 87 fungal endophytes from *Satureja mutica* and *S. khuzestanica*, among which only three fungal endophytes (including P.I.S.7, D.2.F.1 and D.1.S.1) showed the highest potential in controlling plant pathogenic fungi. He also developed a suitable inoculation method for the delivery of functional fungal endophytes as potential biostimulants for *S. khuzestanica* cultivation.

According to previous studies, the successful colonization of functional endophytes can significantly improve host plant growth (Das *et al.*, 2014), essential oil yield and secondary metabolites enhancement (Yan *et al.*, 2015). Application of fungal endophytes can improve

plant fitness, yield and productivity. So far, with several modes of practical inoculation, significant colonization of fungal endophytes in various important crops, including *Opium poppies* by *Beauveria bassiana* (Quesada-Moraga et al., 2006), wheat by *Metarhizium brunneum* (Jaber, 2018), rice by *Piriformospora indica* (Mohd et al., 2017), Soybean by *Cladosporium sphaerospermum* (Hamayun et al., 2009) and *Ocimum basilicum* by *Serendipita indica* (Sabra et al., 2018) has been noticed.

Inoculation method, inoculum concentration and host plant characteristics are important factors in evaluation the effect of fungal endophytes as plant growth stimulants (Jaber and Enkerli, 2017). In this study, inoculation methods showed different effects on *S. khuzestanica*, so that foliar spray methods had the highest colonization level followed by stem injection and root drench, but adding granular form of fungi to the soil was not effective.

Tamreihao et al. (2016) showed that application of plant growth promoting *Streptomyces corchorusii* through foliar spray significantly enhanced the shoot length, weight of shoot and root, total grain yield and weight of grains in rice plants. Whereas, Harris (2000) stated that adding solid substrate formulations of *Rhizoctonia* spp. in the pot mixture significantly was effective in suppression of damping-off diseases in *Capsicum annuum* as well as plant growth promotion. In our study, by evaluating the potential of endophytic fungi in improving the morphological traits of *Satureja khuzestanica* plants in the greenhouse, it was determined that spore spraying of the leaves and then spore injection method to the stem did result in enhanced plant growth and morphological traits including plant height, shoot dry weight, number of leaves and leaf area (number of sett roots). Also, the highest averages of physiological traits in host plants (including photosynthesis, transpiration, stomatal conductance and chlorophyll content) were obtained by spore spraying method and then injection of spores into the stem.

On the other hand, plants that had received stem injection were significantly shorter than

plants in foliar spray treatments. It can be related to the wounding of the stem caused by stem injection, which could have affected plant health (Doccola and Wild, 2012). In this regard, more in-depth studies need to be conducted to elucidate the mechanism responsible for increasing plant growth. However, endophytes are known to affect primary productivity of host plants via increasing photosynthetic rate and chlorophyll content (Khan et al., 2012). Colonization of *Dactylis glomerata* by fungal endophyte, *Epichloë typhina* results in improved host photosynthetic efficiency by increasing photosynthetic pigments in its host orchard grass (Rozpądek et al., 2015). Similarly, field trials of *C. forskohlii* with fungal endophyte formulations improved chlorophyll content significantly as an indication of higher photosynthetic rate (Mastan et al., 2019).

In our investigations, two types of endophyte isolates were evaluated for in-plant enhancement of essential oil yield as bio-stimulants under pot conditions. Inoculation of D.1.S.1 endophyte, especially with spraying method, had the highest essential oil yield. Endophytes can act as both producers and bio stimulants of secondary metabolites. The *Talaromyces radicus* endophyte isolated from *Catharanthus roseus* produces vincristine and vinblastine in the fermentation medium (Palem et al., 2015). While, colonization of *C. roseus* by endophytic fungi *Curvularia* sp. and *Choanephora infundibulifera* increased the content of vindoline in host as a biological stimulant (Pandey et al., 2016a).

Conclusions

D.1.S.1 endophyte fungal isolate, which in previous studies exhibited significant biocontrol potential against important fungal pathogens, was also positive for further growth and physiological traits of *S. khuzestanica*.

This isolate could significantly enhance the growth and essential oil yield of savory plants under pot conditions. Foliar spraying of fungal spores also significantly increased savory plants' quantitative and qualitative yield and their

economic productivity under pot conditions. The isolate could be promising for development as biofertilizers for major crops, especially savory plants, using the foliar spraying method to facilitate its use in crop plantations. Therefore, this strategy is worth conducting additional studies (technical and economic) for commercialization.

In addition to the use of this fungus in field conditions, future studies will focus on determining the effects of endophytism on savory pests, interaction with other endophytes of the host plant, and clarifying the mechanisms responsible for increasing plant growth.

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تأثیر کلونیزاسیون قارچ‌های اندوفیت بر رشد و فیزیولوژی مرزه خوزستانی *Satureja khuzestanica* Jamzad

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چکیده: به‌کارگیری اندوفیت‌های گیاهی در سطح مزرعه بر بهره‌وری محصولات کشاورزی تأثیر می‌گذارد. مرزه *Satureja khuzestanica* بومی مناطق جنوبی ایران است و یک گیاه دارویی ارزشمند متعلق به خانواده Lamiaceae می‌باشد که عمدتاً برای اهداف دارویی و پزشکی در سراسر جهان کشت می‌شود. در این مطالعه، به‌منظور بهبود عملکرد کمی و کیفی گیاه مرزه خوزستانی، توسعه روشی مناسب برای تلقیح اندوفیت‌های قارچی بومی به *S. khuzestanica* بررسی شده است. سه اندوفیت قارچی (D.2.F.1، PIS7 و D.1.S.1)، جدا شده از گیاهان مرزه، با استفاده از چهار روش مختلف (تزریق ساقه، محلول‌پاشی شاخه و برگ، خیساندن خاک و افزودن قارچ به خاک به‌صورت گرانول) به مرزه خوزستانی تلقیح شده و تأثیر آن بر رشد گیاه و پاسخ فیزیولوژیکی آن مورد بررسی گرفت. همه روش‌های تلقیح، به‌جز افزودن شکل گرانولی قارچ‌ها به خاک، منجر به کلونیزاسیون بافت‌های مرزه شد. جدایه P.I.S.7 با استفاده از هر کدام از روش‌های مایه‌زنی در این گیاهان مستقر نشد. میزان کلونیزاسیون قارچی تفاوت معنی‌داری در میان روش‌های مختلف تلقیح نشان داد، به‌طوری‌که محلول‌پاشی منتج به بالاترین سطح کلونیزاسیون قارچی شد و پس از آن تزریق ساقه و خیساندن خاک قرار داشت. بیش‌ترین میزان کلونیزاسیون قارچی متعلق به جدایه D.1.S.1 بود. محلول‌پاشی شاخه و برگ‌ها و تزریق ساقه با قارچ‌های اندوفیت، به‌ویژه در مورد D.1.S.1 ویژگی‌های مورفولوژیکی و فیزیولوژیکی گیاهان میزبان و هم‌چنین درصد عملکرد اسانس این گیاهان را به‌میزان قابل‌توجهی افزایش داد. درمجموع، استفاده از جدایه D.1.S.1 و روش پاشش برگی منجر به افزایش کمی و کیفی گیاهان مرزه می‌شود.

واژگان کلیدی: اسانس، اندوفیت قارچی، *Satureja khuzestanica*