Effects of mycorrhizal inoculation on growth and some quality parameters of *Matthiola incana* (L.) cultivation under salt stress

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Abstract

Aim: This research was carried out to investigate the effect of mycorrhizal inoculation on growth and some quality parameters of *Matthiola incana* cultivation under salt stress condition.

Methodology : The experiment was carried out in a greenhouse in Mugla-Turkey between 2016 and 2017. There were three replications with two factorial randomized plot design. Plant were given two treatments endomycorrhizal inoculation of *Glomus intraradices* in the root zone of plants without NaCl and 100 mM NaCl. The number of shoot, shoot length, shoot thickness, root length, wet and dry weights of root, leaf number on the pedicel, length and thickness of pedicel, length of spike, wet and dry weights of pedicel were estimated. The percentage of mycorrhizal infection, total N, P, K, Ca, Na, Mg, Fe, Cu, Zn and Mn contents in the roots were also determined.

Results : In all parameters, salt application had greatly diminished the performance of plants. However, mycorrhizal inoculation after salt application had effected the shoot and root growth as well as quality parameters of flower.

Interpretation: The results showed that mycorrhizal application to *M. incana* positively effected the plant growth and some quality parameters by increasing tolerance towards salt stress.

Keywords: Floriculture, *Glomus intraradices*, *Matthiola incana*, Mycorrhizal inoculation, Salt stress

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Introduction

Parallel to increase in global temperature, the increase in soil salinity around the world is restricting the use of such areas for cultivation. This stresses the fact to look for an alternative application to overcome soil degradation effects arising from salinity (Vibhuti *et al.*, 2015; Shahi *et al.*, 2015; Awasthi *et al.*, 2016). One way to overcome such problem is increased tolerance to salinity stress. Application of mycorrhizal fungus to the growth medium or roots, commonly known as biological fertiliser, leads to increased plant growth in degraded soils (Al Karaki, 2000; Cekic and Yilmaz, 2011). Mycorrhiza inoculation is reported to increase the uptake of water and nutrients, leading to growth enhancement, increased tolerance against stress factors and increased the productivity (Kaya *et al.*, 2009; Miao Xie *et al.*, 2017). Several studies carried out in this direction during the last decade have reported that mycorrhiza can overcome the negative effects of salinity as a bioregulator under abiotic stress factor (Rabie and Almadini, 2005; Kumbar *et al.*, 2017). Salt stress increases the problem of nutrient limitation but arbuscular mycorrhizal fungus increases the total root area with length of external mycelium enhancing the reabsorption capacity and provides higher accumulation of soluble sugars in plant roots under saline conditions (Jakobsen *et al.*, 1992; Feng *et al.*, 2002). *Glomus intra radices* an arbuscular mycorrhizal fungus (AMF) is a type of mycorrhiza widely preferred at industrial level, because of its adaptation to several soil types, showing colonisation with numerous hosts, early proration and preserving living conditions for longer periods. This research was carried out to investigate the effect of mycorrhizal inoculation on growth and some quality parameters of *M. incana* under salt stress condition.

Materials and Methods

Matthiola incana (L.) is an ornamental plant that belongs to Brassicaceae family. This species has different varieties which produce single and double flowers. The flowers have pleasant smell and are short-living perennial species widely distributed in

the Mediterranean region (Shanan, 2015). The study was undertaken during the year 2016-2017 in a greenhouse following two factorial random plot design in triplicate, using 9 plants per replica. *M. incana* 'Iron Apricot' cultivar was selected as plant material. In order to get a product without any deformation in quality on economical terms, the salt and mycorrhiza were applied together as two factorial design, in order to see if better growth performance could be obtained. Treatment was given as non saline (S0) and 100 mM NaCl (S1) added with irrigation water. Glomylgel (*Glomus intraradices*, Mycovitro) was used as source of mycorrhiza. Mycorrhizal application included AMF0 concentration as control without mycorrhiza and AMF1 concentration as 100 g da⁻¹ on the lines recommended for industrial scale. The soil was sterilized at 100°C for 180 min. AMF inoculum was added to pot soil with irrigation water at planting time. The plants were sown in 26 l pots and salt treatment was started on 22.11.2016 followed by once a week application. 15 days after sowing, root zone of plants were inoculated with mycorrhiza after dissolving in water with the suggested concentration (Table 1).

Once a week Hoagland solution (pH 5.5-6.5) was supplied as nutrients to plants (Brohi *et al.*, 1994). Salinity level was determined as per to Richards (1954), pH with Beckman pH meter, organic matter was calculated following the method of Reuterberg ve Kremkus (Black, 1965), total nitrogen by Bremner and Shaw's modified macro Kjeldal method (Bremner, 1965) and sodium according to Kacar (1972) (Table 2).

In this research after salt and mycorrhiza application, number of leaves on shoot, shoot length and thickness, root length, wet and dry weights of root, leaf number on the pedicel, length and thickness of pedicel, length of spike, wet and dry weights of pedicel was recorded. In roots, mycorrhizal infection percentage was determined without staining using "Grid Line Intersection" method (McGonigle *et al.*, 1990). Macro and micro elements were analysed. Nitrogen was determined by Kjeldahl method, Phosphorus by Vanadomolybdo phosphoric yellow

Table 1 : Production schedule of the study

Applications	Date of application
Seed sowing	09.09.2016
Transplantation	13.10.2016
Planting	15.11.2016
First salt application	22.11.2016
Mycorrhiza application	30.11.2017
First harvest	16.02.2017
End of the experiment	01.04.2017

Table 2 : Physical and chemical characteristics of soil used

Texture	pH (1:2.5)	Salt (%)	CaCO ₃ (%)	Organic matter (%)	Total N (%)	Na (ppm)
Sandy-loam	7.88	0.02	11.40	4.00	0.19	86.00

colour method with the help of Eppendorf Calorimeter; potassium, sodium, and calcium were determined with Flame Photometer; whereas magnesium, iron, copper, zinc and manganese by Atomic Absorption Spectrophotometer (Kacar and Inal, 2008). The results obtained were statistically analyzed SPSS (11.0) statistical package programme.

Results and Discussion

The results related to mycorrhizal inoculation in the roots are presented in Table 3. Mycorrhizal colonisation was observed under both saline as well as non-saline conditions without mycorrhizal application, depicting that soils possess mycorrhizal colonies under natural conditions. Mycorrhizal colonization in the roots under non-saline conditions was 65.67% whereas under saline conditions it was 36%. Mycorrhiza colonization of *Citrus karna* and *Poncirus trifoliata* x *Citrus sinensis* under salt stress decreased from 66.8 % and 62.4% to 31.3% and 39.7% as compared to non-saline salt stress (Murkute *et al.*, 2006). These results corroborates with our findings. In our study, the colonisation ratio in the roots decreased by 65.67% and 36% after mycorrhizal application under non-saline and saline conditions.

The role of mycorrhizal application on the vegetative growth of plant is presented in Table 3. Salt application significantly decreased the number of shoot and shoot length whereas other parameters showed negative relation. Salt stress decreased the length of root, shoot and stem, leaf number, dry and fresh weight in addition to leaf area (Koksai *et al.*, 2014). Application of salt to *Matthiola incana* decreased the plant growth parameters by approximately 8 to 20%. As compared to many cut flowers, the decrease due to negative effects of salt can be accepted on the trade scale in the case of *M. incana*. Heuer and Ravina (2004) and Grieve *et al.* (2006) in this study reported that although increased levels of salt can negatively effect the growth and development of *M. incana*, but this effect does not change the market productivity. These findings corroborates with our results.

On the other hand, mycorrhizal application positively affected all growth parameters at $p < 0.01$. AMF application generally increases quality of ornamental plants (Puschel *et al.*, 2014). According to Sohn *et al.* (2003), AMF inoculation in *Chrysanthemum morifolium* leads to increase in plant height, leaf area, dry and wet weight of stem, shoot, and root. Meir *et al.* (2010) also reported similar results and data of this study is also in confirmation with the above studies.

Table 3 : Mycorrhizal inoculation and the effect of mycorrhizal application on plant growth

Treatments	Mycorrhizal inoculation (%)		Leaf number of shoot (per plant ⁻¹)		
S	36.94 ^a		36.33 ^a		
S1	18.67 ^b		33.17 ^b		
LSD _{0.05}	3.72*		2.72*		
AMF0	4.77 ^b		32.17 ^b		
AMF1	50.84 ^a		37.33 ^a		
LSD _{0.05}	3.72**		2.72**		
S0*AMF0	8.20 ^c		34.00		
S0*AMF1	65.67 ^a		38.67		
S1*AMF0	1.33 ^d		30.33		
S1*AMF1	36.00 ^b		36.00		
LSD _{0.05}	5.47**		ns		

Treatments	Shoot length (cm)	Shoot thickness (mm)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
S	27.15 ^a	4.53 ^a	19.83 ^a	11.04 ^a	1.74 ^a
S1	23.57 ^b	4.19 ^b	17.17 ^b	9.32 ^b	1.40 ^b
LSD _{0.05}	3.12*	0.22**	1.71**	0.86**	0.10**
AMF0	22.33 ^b	4.06 ^b	15.94 ^b	9.09 ^b	1.36 ^b
AMF1	28.39 ^a	4.67 ^a	21.05 ^a	11.28 ^a	1.79 ^a
LSD _{0.05}	3.12**	0.22**	1.71**	0.86**	0.10**
S0*AMF0	24.17	4.26	17.50	10.02	1.53
S0*AMF1	30.14	4.80	22.16	12.07	1.95
S1*AMF0	20.50	3.85	14.39	8.16	1.19
S1*AMF1	26.64	4.53	19.94	10.49	1.62
LSD _{0.05}	ns	ns	ns	ns	ns

S0: Non saline; S1: 100 mM NaCl application; AMF0: Without mycorrhiza (Control); AMF1: Application of 100 g da⁻¹ *Glomus intraradices*; ns: nonsignificant; Within each column, values followed by same letters are not significantly different as per LSD test; ** Significant at $p < 0.01$; * Significant at $p < 0.05$ level

Mycorrhiza is known to increase the nutrient uptake and plant growth in many studies (Shokri and Maadi, 2009; Asrar and Elhindi, 2011). It is also clear that the advancement of nutrient uptake and plant growth increase salt resistance due to improvement of root and soil conditions. In this context, our results are consistent with the previous studies.

In case of salt-mycorrhiza interactions, mycorrhizal application under saline condition (S1*AMF1), as compared to control treatment under saline condition (S1*AMF0), revealed that there was an increase in the leaf number of shoot by 18.69%, shoot length by 29.95%, shoot thickness by 17.66%, root length by 38.56%, root fresh weight 28.55% and root dry weight by 36.13%. As compared to control (S0*AMF0), mycorrhizal application under non-saline condition (S0*AMF1) led to increase in leaf number of shoot (13.73%), shoot length (24.70%), shoot thickness (12.67%), root length (26.62%), root fresh weight (20.45%) and root dry weight (27.45%). As compared to non-saline conditions, application of mycorrhiza under saline conditions led to positive impact on all vegetative growth parameters. Therefore, a decrease in the growth values due to salt application, with mycorrhizal application as compared to the mycorrhizal application under non-saline conditions makes it more important. According to the control (S0*AMF0), mycorrhiza application under saline condition (S1*AMF1) had lead to increase in leaf number (5.88%), shoot length (10.21%), shoot thickness (6.33%), root length (13.94%), root dry weight (4.69%), root weight (5.88%) and increase the rates of plant growth were also observed in mycorrhiza condition despite salt application. A decrease in plant growth due to salinity conditions was reversed by mycorrhizal application with positive effects in different parameters in different ratios. Some studies have shown that AMF inoculation develops salt resistance through advanced

osmoregulation (Feng *et al.*, 2002). Sonmez *et al.* (2013) have reported that a decrease in plant height and fresh weight due to salt application, mycorrhizal application under salt conditions increased plant dry and fresh weight to a great extent. Geren *et al.*, (2011) reported that with *Lathyrus ochrus*, increase in salt concentration, root dry and fresh weight are negatively affected whereas mycorrhizal application produced a positive impact on the plant physiological behaviour. In addition, several studies have reported that mycorrhizal application positively affect plant growth due to increased nutrient uptake (Shokri and Maadi, 2009).

Table 4 presents data on the parameters related to flower quality. The effect of salt and mycorrhiza separately on leaf number of pedicel, pedicel length and thickness, spike length, dry and fresh weight of pedicel resulted in a difference significant at $p < 0.01$. Under the following treatments, interaction of salt and mycorrhiza, mycorrhiza application under salt stress (S1*AMF1), in comparison to the situation without mycorrhiza under salt stress (S1*AMF0) increase the number of leaf pedicel (21.09%), pedicel length (15.03%), pedicel thickness (31.36%), spike length (26.53%), fresh weight of pedicel (14.46%) and dry weight of pedicel (47.07%). Similarly, in the case of mycorrhiza application without salt (S0*AMF1), when compared to the control (S0*AMF), significant increase in number of leaf pedicel (12.74%), pedicel length (17.79%), pedicel thickness (30.92%), spike length (26.46%), fresh weight of pedicel (15.41%) and dry weight of pedicel (47.98%) was noted.

Application of mycorrhiza under salt stress condition, when compared with control, showed increase in leaf number on the pedicel by 4.02%, pedicel length by 7.98%, pedicel thickness by 9.50%, spike length by 8.82%, fresh weight of pedicel by

Table 4 : Effect of mycorrhizal application on flower quality

Treatments	Leaf number on the pedicel (per plant ⁻¹)	Pedicel length (cm)	Pedicel thickness (mm)	Spike length (cm)	Pedicel fresh weight (g)	Pedicel dry weight (g)
S	52.83 ^a	59.17 ^a	16.17 ^a	10.27 ^a	121.11 ^a	24.63 ^a
S1	47.17 ^b	54.83 ^b	13.50 ^b	8.83 ^b	115.13 ^b	21.33 ^b
LSD _{0.05}	1.88**	2.18**	1.49**	0.57**	4.60**	2.42**
AMF0	46.17 ^b	52.67 ^b	12.83 ^b	8.43 ^b	109.90 ^b	18.56 ^b
AMF1	53.83 ^a	61.33 ^a	16.83 ^a	10.67 ^a	126.33 ^a	27.40 ^a
LSD _{0.05}	1.88**	2.18**	1.49**	0.57**	4.60**	2.42**
S0*AMF0	49.67	54.33	14.00	9.07	112.44	19.86
S0*AMF1	56.00	64.00	18.33	11.47	129.77	29.39
S1*AMF0	42.67	51.00	11.67	7.80	107.36	17.27
S1*AMF1	51.67	58.67	15.33	9.87	122.89	25.40
LSD _{0.05}	ns	ns	ns	ns	ns	ns

S0: Non saline; S1: 100 mM NaCl application; AMF0: Without mycorrhiza (Control); AMF1: Application of 100 g da⁻¹ *Glomus intraradices*; ns: nonsignificant; Within each column, values followed by same letters are not significantly different as per LSD test; ** Significant at $p < 0.01$; * Significant at $p < 0.05$ level.

9.29% and dry weight of pedicel by 27.89%, respectively. In parallel with plant growth, quality of flowers was also affected in the same condition. All the growth parameters decreased under salt treatment but with mycorrhizal inoculation all the studied growth parameters increased. The results obtained in 0.674% salt condition by Akat and Ozzambak (2013) showed that *Limonium sinuatum* 'Compindi White' flower peduncle thickness showed 10.61 % loss and *L. sinuatum* 'Compindi Deep Blue' showed 19.64 % loss. Similarly, significant negative effects of salinity on flower quality and growth has been reported (Carter and Grieve, 2006; Akat and Ozzambak, 2014; Akat and Akat-Saracoglu, 2017). Salt application *Gerbera jamesonii* (Akat, 2008). *Hyacinthus orientalis* (Koksal et al., 2014) produced negative effect on pedicel length and thickness. Bessemer and Koide (1999) reported that mycorrhiza produce a positive effect on the flowering of *Narcissus papyroceus* and *Antirrhinum majus* by increasing the number of flowers and their vase life. According to Sohn et al. (2003), AMF inoculation in *Chrysanthemum* sp. lead to important difference in the pedicel thickness and length as well as fresh weight of pedicel, thus effecting the above growth parameters and flowering time in a positive way. In the case of *Eustoma grandiflorum*, *G. intradices* application increased the number of flowers and length of pedicel (Meir et al., 2010). It has also been helpful on the market level for cultivating *Lisianthus* spp.

and its use in other ornamental plants has been stressed with the fact that they may show similar potential. Similarly, mycorrhizal application in *Tagetes erecta* produced positive effects on all parameters related to flower (Asrar and Elhindi, 2011). Moreover, application of AMF increased the number of flowers and their quality (Long et al., 2010; Deljou et al., 2014). The results obtained during present study clearly show that application of mycorrhiza under non-saline condition increased flower development and quality. Mycorrhizal involvement under salt application clearly shows that with regard to different parameters related to flower quality, negative impact is overrun in different ratios. In floriculture, AMF application has shown positive effects on flower quality, flower growth and flowering time (Miao Xie et al., 2017).

The perusal of data showed that salt application had decreased nitrogen, calcium, zinc and manganese contents by 1%; phosphorus potassium and magnesium level by 5%, however, sodium level increased after salt application. Mycorrhizal application in roots had increased the levels of nitrogen, calcium, zinc and manganese at 1% level, and for potassium at 5% level (Table 5). The large hyphal system of AMF discovered more soil area and enhanced the absorption surface of roots, thus contributing towards an increase in the elemental

Table 5 : Effect of mycorrhizal application on the nutrient contents in roots

Treatments	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (%)
S	1.78 ^a	0.38 ^a	2.64 ^a	0.48 ^a	0.44 ^a	0.07 ^b
S1	1.56 ^b	0.35 ^b	2.47 ^b	0.32 ^b	0.31 ^b	0.45 ^a
LSD _{0.05}	0.045**	0.021*	0.129*	0.038**	0.091*	0.021**
AMF0	1.61 ^b	0.36	2.47 ^b	0.37 ^b	0.34	0.26
AMF1	1.72 ^a	0.37	2.64 ^a	0.43 ^a	0.41	0.25
LSD _{0.05}	0.045**	ns	0.129*	0.038**	ns	ns
S0*AMF0	1.74	0.38	2.61	0.45	0.39	0.07
S0*AMF1	1.82	0.39	2.67	0.51	0.49	0.07
S1*AMF0	1.49	0.35	2.34	0.29	0.29	0.46
S1*AMF1	1.63	0.36	2.61	0.35	0.34	0.44
LSD _{0.05}	ns	ns	ns	ns	ns	ns

Treatments	Fe (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)
S	377.20	16.42 ^a	38.28 ^a	40.27 ^a
S1	378.63	13.10 ^b	31.52 ^b	34.20 ^b
LSD _{0.05}	ns	0.783**	2.249**	2.875**
AMF0	325.42	14.40	32.50 ^b	34.87 ^b
AMF1	430.41	15.12	37.30 ^a	39.60 ^a
LSD _{0.05}	ns	ns	2.249**	2.875**
S0*AMF0	378.65	16.50	36.03	38.45
S0*AMF1	375.75	16.35	40.54	42.10
S1*AMF0	272.20	12.30	28.98	31.30
S1*AMF1	485.07	13.90	34.06	37.10
LSD _{0.05}	ns	ns	ns	ns

S0: Non saline; S1: 100 mM NaCl application; AMF0: Without mycorrhiza (Control); AMF1: Application of 100 g da⁻¹ *Glomus intradices*; ns: nonsignificant; Within each column, values followed by same letters are not significantly different as per LSD test; ** Significant at p<0.01; * Significant at p<0.05 level

concentration in AMF plants (Ruiz-Lozano and Azcon, 2000). Similarly, Abd El Aziz *et al.* (2011) reported that with increase in salt concentration, uptake of nitrogen, phosphorus and potassium and content decreased in *M. incana*. Shanan (2015) reported that in *M. incana*, stress created due to salt application increased sodium and chloride ions as well as proline content. Plants under salt stress showed increase in the level of sodium ion followed by a decrease in the potassium ion (Turkan and Demiral, 2008). Under saline conditions Na^+/K^+ ratio is abnormally high and makes enzymes more ineffective by inhibiting protein synthesis. This destroyed protein metabolism as compared to control can be explained by reduction in the nitrogen uptake. According to Soliman *et al.* (2012), decrease in the NPK and microelements is related to increase in the percentage of sodium and chloride. Na salts increase the pH of soil and uptake of P is limited. In addition, under stress conditions there is decrease in the mineral element contents as compared to the control is because, in order to reduce the solute potential in cytosol the balance created by the synthesis of soluble components like glycine, betaine, proline, sorbitol, mannitol, pinitol, and saccharose gives answer against salt stress. As it is well known, mycorrhizal application increases the nutrient content in plants. According to Sieverding (1991) and Ortas (2012), mycorrhizal application supports our findings that refinement of root cells increases the uptake of most minerals, except phosphorus. There is no significant difference between salt and mycorrhiza interactions. Under salt conditions non-mycorrhizal application (S1*AMF0), in comparison to mycorrhizal application (S1*AMF1) increased all plant nutrients, except Na (Table 5).

M. incana 'Iron Apricot' variety tolerant to medium salt level was negatively affected under saline conditions in connection to all parameters related to plant growth and flower quality leading to economic loss. In view of this, negative effects imposed by salt application were slightly overcome by mycorrhizal application by increasing tolerance to salt stress. In particular, the growth of *M. incana* was dense under green house as well as under open conditions with mycorrhizal application. The effects on growth and flower quality were on positive side, by increasing tolerance to salt stress it can add to the economy. This study has fully enlightened that mycorrhiza can be used as a positive strategy to increase tolerance to salt stress, which should be applied on other ornamental plants as well. Under non-saline conditions mycorrhizal application can increase the quality of flowers and plant growth, and at the same time can prove useful for the producers using fertilisers so as to bring down their monetary input.

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