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ORIGINAL RESEARCH

Treated wastewater as a partial nutrient source for Lily grown in a soilless system in presence of *Arbuscular Mycorrhizal* Fungi

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Abstract

Purpose The reuse of treated wastewater (TWW) in agriculture may affect the growth of plants. The goal of this study is to investigate the potential use of TWW in irrigating Lily in presence of Arbuscular Mycorrhizal Fungi (AMF) in a closed soilless system.

Method In the first experiment, plants were irrigated with a mixture of 3 part nutrient solution: 1 part treated wastewater (3NS:1TWW) supplemented with 50, 125, 160 or 200 mg L^{-1} Ca to determine the optimal Ca concentration supplement that would give the highest plant growth. In the second experiment, the plants were irrigated with a nutrient solution or a mixture of 3 part nutrient solution: 1part treated wastewater (3NS:1TWW) supplemented with 160 mg L^{-1} Ca in the presence or absence of AMF.

Results The results indicated that the plants receiving the higher concentrations of Ca (160 or 200 mg L^{-1}) showed better growth performance. The results showed that the plants receiving (3NS:1TWW) had a significantly higher dry weight of adventitious roots and fresh weight of the flower bud than those receiving nutrient solution. The flower bud was longer in presence of AMF. The highest shoot fresh weight was recorded for non-AMF plants receiving 3NS:1TWW, and the least shoot fresh weight.

Conclusion The most efficient solution is to supplement the 3NS:1TWW irrigation solution with 160 mg Ca L^{-1} to reduce the salinity effects of TWW. Moreover, it is recommended to inoculate the roots of the *Asiatic lily* plants with AMF to achieve the longest flower.

Keywords *Arbuscular Mycorrhizal* Fungi (AMF), Lilium, Wastewater, Hydroponics, Sustainability

Introduction

Water scarcity is a pressing issue worldwide (Rasheed et al. 2020) and specifically in many developing countries (Black et al. 2015). Jordan like many other countries is facing a severe water security situation due to scarce water resources and high population growth (Mkhinini et al. 2018; Atamaleki et al. 2019; Rasheed et al. 2020). To reduce the pressure on using freshwater in agriculture, treated wastewater (TWW) has been used as an alternative source of irrigation water (Vergine et al. 2016; Albalasmeh et al. 2020, 2022; Makhadmeh et al. 2021). Moreover, using TWW may compensate for partial fertilizer requirements for the plants (Makhadmeh et al. 2021). Another approach that has been used to conserve water in agriculture is the soilless culture. Soilless culture is not only efficient in using waster (70-90%) but also, reduce the

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amount of the chemical fertilizers needed for plant production as well as reducing the number of agro-chemicals used for pests and diseases control (Agrawal et al. 2020; Maya Waiba et al. 2020). Therefore, a synergistic way could be implemented to integrate the treated wastewater in soilless culture to reduce the freshwater use and get benefit from the high nutrient content in the TWW to decrease the fertilizers use for crop production (Cifuentes-Torres et al. 2021). Several studies investigated the potential use of TWW in soilless culture (Khan et al. 2011; Power and Jones 2016; Magwaza et al. 2020; Vera-Puerto et al. 2020; Al Hamedi et al. 2021; Lee et al. 2021) using different crops (lettuce; tomatoes; bell pepper; wheat; horticultural roses; Lilium) grown in different soilless systems. Arbuscular mycorrhizal fungi (AMF) play an important role in reducing the salinity effects resulting from the use of TWW and improving plant growth (Scagel et al. 2017). Therefore, the objective of this study was to investigate the potential use of treated wastewater in irrigating Lily in presence of Arbuscular Mycorrhizal Fungi (AMF) in a closed soilless system.

Materials and methods

Experimental setup

Sixteen growing beds were prepared using plastic baskets (60 cm length x 20 cm width x 25 cm depth) lined with 1000 µm polyethylene sheets. A drainage hole was made at the end of each bed and a screen was fitted at the hole to prevent the loss of the substrate particles. The beds were filled with two layers of zeolitic tuff; 3-8 mm tuff particles in the lower 5 cm and less than 3 mm tuff particles in the upper 15 cm of the bed. The beds were placed on benches at a 2% slope in the glasshouse. Pre-cooled *Asiatic lily* cv. *cavalese* bulbs (12-14 cm circumference) were obtained from Alisar Nursery for Production and Marketing of Cut Flowers (Baqa', Jordan). The bulbs were planted 14 cm deep in each bed with 15 x15 cm planting space between rows and plants. A metallic mesh was laid over each bed after planting to support the plant stem. The bulbs were irrigated with tap water for two weeks before applying the treatments. The experiment was conducted as a completely randomized design (CRD) with four replicates per treatment.

A 500 L tank containing the supply solution was assigned to each treatment. The supply solution was pumped to the plants via a polyethylene drip irrigation system with 6 drippers per bed (2 L h^{-1} per dripper). The plants were irrigated automatically with the supply solution three times daily (at 9.00 am, 12.00 pm and 15.00 pm) for 15 min duration. The drain solutions from the four beds of each treatment were collected daily in plastic containers, the volume of the drain was measured and then returned to the corresponding tank of the treatment to be circulated the next day. Electrical conductivity (EC) and pH of the drain and the supply solution were measured daily.

The plants were harvested when the first flower was fully colored and beginning to open (blooming). The plants were uprooted and washed with running tap water gently to remove tuff particles. Data were recorded for stem length (from the tip of the adventitious roots to the base of the peduncle), stem diameter (just above the adventitious root zone), leaf count and shoot (stem and leaves) fresh and dry (oven-dried at 70 °C for 48 h) weights. In addition, fresh and dry weights of adventitious roots (roots growing above the bulb) and basal roots (roots growing from the base of the bulb) and length of the longest adventitious root and basal root were recorded. Furthermore, data were collected on flower bud count and fresh weight and length of the longest flower bud.

Experimental 1: Effect of calcium concentrations on growth and flowering

A nutrient solution (NS) was prepared by dissolving 16.64 g NH_4NO_3, 91.34 g KNO_3, 46.69 g KH_2PO_4, 119.81 g $MgSO_4.7H_2O$, 3.17 g $FeSO_4.7H_2O$, 0.03 g $CuSO_4.4H_2O$,0.24g$ZnSO_4.7H_2O$,0.23 g $MnCl_2.4H_2O$, 0.23 g $(Na)_2MoO_2.2H_2O$ and 1.07 g H_3BO_3 in 375 L tap water. The NS solution was mixed with 125 L treated wastewater (TWW) to make up the supply solution (3NS:1TWW), the TWW used in the experiments was supplied from wastewater treatment plant located in Jordan University of Science and Technology (JUST) campus. Chemical analyses of tap water and treated wastewater used in the experiment are presented in Table 1. Chemical composition of the supply solution (3NS:1TWW) is presented in Table 2.

Table 1 Chemical analysis of tap water used to prepare the nutrient solution (NS) and of treated wastewater (TWW) used to prepare the supply solution (3 NS: 1 TWW) ratio

Nutrient	Unit	Tap water	Treated wastewater
EC	dS m^{-1}	0.48	1.13
pH		7.8	8.8
NO_3^-	mg L^{-1}	10	14
PO_4^{-3}		5.44	20.30
K		102	114
Ca		51	53
Mg		16.6	16.1
SO_4^{2-}		26	61
Na		81.1	500.0
Cl^-		23	285
SAR	-	2.51	12.40

EC: electrical conductivity; SAR: sodium adsorption ratio

In this experiment, the plants were irrigated with the supply solution (3NS:1TWW) containing different concentrations of Ca (50, 125, 160 or 200 mg L^{-1}). The lower concentration 50 mg Ca L^{-1} was the Ca concentration in tap water and treated wastewater (Table 1). The remaining treatments were supplied by a calcium source to reach the required concentration with considering the initial 50 mg Ca L^{-1} in tap water and treated (TWW).

Table 2 Chemical composition of the nutrient solution (NS) and the supply solution (3 NS: 1 TWW)

Nutrient	Unit	NS	3 NS: 1 TWW
NH_4^+	mg L^{-1}	10	7.5
NO_3^-		194	148.8
PO_4^{-3}		92	74.3
Ca		51	51.5
K		232	202.5
Mg		48	40.1
SO_4^{2-}		154	130.6
Na		81.2	185.9
Cl^-		23.4	88.8
Fe		1.70	1.28
Cu		0.02	0.02
Zn		0.23	0.17
Mo		0.24	0.18
Mn		0.27	0.20
B		0.56	0.42
SAR	-	1.95	4.7

SAR: sodium adsorption ratio

Experimental 2: Effect of *Arbuscular Mycorrhizal* fungus (AMF) on growth and flowering

The NS was prepared as mentioned in Experiment 1. The solution used for irrigation was the NS, and mixed solution of ratio 3NS:1TWW. Both solutions were supplemented by 160 mg Ca L^{-1} concentration (the optimal concentration in Experiment 1). *Glomus intraradices* (BioMyc, Germany) was used to inoculate the bulbs of the AMF before planting, 10 ml AMF granules were added to each planting hole near the basal root zone of the bulb. After planting the bulbs another 10 ml AMF granules were added above the bulb in the zone where adventitious roots emerge. Other bulbs were not inoculated with AMF. Each group of bulbs was irrigated with (NS) or (3NS:1TWW) prepared solution. The treatments were as follows: NS-AMF: Non-inoculated bulbs irrigated with NS; 3:1-AMF: Non-inoculated bulbs irrigated with 3NS:1TWW;

NS+AMF: Inoculated bulbs irrigated with NS and 3:1
+AMF: Inoculated bulbs irrigated with 3NS:1TWW.

Testing for AMF

The roots were examined for the presence of AMF using Trypan blue following the methodology of Phillips and Hayman (1970). In brief, one gram of each type of root (adventitious and basal roots) was cleaned with running tap water and was cut into pieces (1-2 cm). Then the root pieces were immersed in 15 ml of 10% KOH for 5-10 min, rinsed with distilled water, placed in 0.1 N HCl for 5 min. After this, roots were immersed in 0.01% Trypan blue stain. The roots were placed in Petri-dishes containing lactoglycerol solution for 5 min to de-stain them from excessive stain. Ten of the root pieces were then placed on a microscopic slide and examined under a light microscope (Nikon YS 100, Japan Optics, Japan) at 40 X and 10 X magnification. The areas stained with the blue stain in the intercellular spaces of the root tissue indicated AMF infection.

Plant tissue analysis

All leaves from four randomly chosen plants from the ten plants per replicate were dried and grounded using a micro plant grinding machine using 0.5 mm sieve hole diameter. One gram of the grounded leaves were placed in crucibles into a muffle furnace at 550 °C for 5 hrs. After which the ash was dissolved in 5 ml of 2 N hydrochloric acid (HCl) and mixed with a plastic rod, 15 minutes later volume made up to 50 mL with deionized water (DI) (Abbruzzini et al. 2014). The mixture was filtered through a filter paper to be analyzed for K, Ca, Mg, Na, Fe, Zn, Mn and Cu. All elements were analyzed using Unicam Atomic Absorption Spectrometer (SOLAAR M5, UK) fully equipped for flame and graphite furnace atomization. Nitrogen content was estimated using Kjeldahl digestion method (Jones and Case 1990).

Statistical analysis

In each experiment, data were subjected to analysis of variance (ANOVA) by the General Linear Models procedure using SAS (Statistical Analysis System, version 9.1, 2003). Mean comparison was performed using the Least Significant Difference (LSD) Method at $P \leq 0.05$.

Results and discussion

Effect of calcium concentrations on growth and flowering

Plant growth and flowering

The main characteristics of growth and flowering of *Asiatic lily* 'Cavalese' as affected by Ca concentrations are tabulated in Table 3. There were no significant effects of Ca concentration in the irrigation solution on shoot fresh weight, shoot dry weight and flower bud length (Table 3). Ca (160 and 200 mg L^{-1}) significantly affected the basal and adventitious root length, fresh and dry weight. However, no significant difference was observed in shoot weight between 50-200mg L^{-1} Ca. The 200 mg L^{-1} Ca treatment has a significantly higher flower bud fresh wt. (11.3 g) compared to other Ca concentrations whereas the 160 mg L^{-1} Ca treatment has a significantly higher flower bud number (3.7) compared to other Ca concentrations. These results confirmed the important role of calcium in the development of the root system and plant growth (Hepler 2005; Marschner 2012).Similarly, Choi et al. (2005) also reported that varying calcium concentrations did not affect significantly the number of flowers and flower length but a significant decrease in stem diameter was observed with increased Ca concentration (Choi et al. 2005).

Another study by Al-Ajlouni et al. (2017) reported a similar result where increasing Ca among other nutrients increased the root dry weight (Table 3).

Table 3 Growth and flowering of *Asiatic lily 'Cavalese'* as influenced by irrigation with a solution of ratio 3NS: 1 TWW containing different concentrations of Ca in a closed soilless system

Parameter	**Unit**	**Ca concentration (mg L^{-1})**			
		50	**125**	**160**	**200**
Basal root length	cm	7.7 b	7.5 b	11.3 a	14.4 a
Basal root fresh wt.	g	5.3 b	5.4 b	9.6 a	10.9 a
Basal root dry wt.	g	0.41 b	0.41 b	0.87 a	0.70 a
Adventitious root length	cm	5.1 b	5.0 b	11.0 a	13.1 a
Adventitious root fresh wt.	g	4.4 b	5.6 b	12.1 a	11.3 a
Adventitious root dry wt.	g	0.33 b	0.37 b	0.91 a	0.77 a
Shoot fresh wt.	g	72.4 a	76.4 a	77.5 a	76.8 a
Shoot dry wt.	g	10.06 a	10.21 a	11.66 a	11.61 a
Leaf number	-	47 c	49 bc	52 ab	56 a
Stem diameter	cm	0.93 a	0.94 a	0.68 b	0.65 b
Stem length	cm	73.9 a	72.6 a	68.6 b	70.6 ab
Flower bud length	cm	17.7 a	17.1 a	19.1 a	18.9 a
Flower bud fresh wt.	g	7.7 b	7.6 b	9.2 b	11.3 a
Flower bud number	-	3.2 b	3.2 b	3.7 a	3.4 b

Means within same row having different letters are significantly different according to LSD ($P \leq 0.05$). Values are means of 40 plants.

Moreover, Al-Rashdan (2012) reported that highest shoot fresh weight at Ca concentration of 150 ppm when plants were supplied with nutrient solution only whereas the highest shoot fresh and dry weight was recorded at 200 ppm Ca when plants irrigated by wastewater. These results could explain the role that Ca plays when the sodium (Na) ion is present in the TWW.

Plant tissue analysis

The minerals concentrations of *Asiatic lily 'Cavalese'* as affected by Ca concentrations are tabulated in Table 4. The results revealed that varying Ca concentrations affect significantly N, Fe and Zn only where there was no statistically significant difference was observed for other elements at different concentrations of Ca (Table 4). The highest content of N was 3.309 g Kg^{-1} dry weight in plants that received 125 mg Ca L^{-1}. Furthermore, the level of Fe (514.6 mg Kg^{-1} dry weight) was the highest in plants supplemented with 200 mg Ca L^{-1} but there is no significant difference between other treatments. The plants that received 50 or 125 mg Ca L^{-1} exhibited significantly higher levels of Zn (21.96 or 18.70 mg Kg^{-1} dry weight, respectively) compared to the other treatments.

These results could be explained by the well-adjusted (acidic) pH of the nutrient solution which makes all nutrients available to be absorbed and accumulated in the leaves (Choi et al. 2005; Marschner 2012). Moreover, Chang et al. (2012) found that Ca application to Oriental lily increased leaf contents of K and Fe. Similar results were obtained by Mohamed (2012) where applying Ca to maize grown under salt stress increased the uptake of K, P, N, Fe and Zn.

Table 4 Concentration of minerals in *Asiatic lily 'Cavalese'* leaves as influenced by irrigation with a solution of ratio 3NS: 1 TWW containing different concentrations of Ca in a closed soilless system

Parameter	Unit	Ca concentration (mg L^{-1})			
		50	**125**	**160**	**200**
N	g Kg^{-1}	3.246 ab	3.309 a	3.239 b	3.198 b
K		5.004 a	5.306 a	4.763 a	4.356 a
Ca		2.211 a	2.302 a	1.979 a	2.084 a
Mg		0.666 a	0.642 a	0.574 a	0.613 a
Na		0.843 a	0.748 a	0.670 a	0.921 a
Fe	mg Kg^{-1}	172.3 b	176.6 b	251.1 b	514.6 a
Zn		21.96 a	18.70 a	7.74 b	6.09 b
Mn		43.21 a	40.35 a	37.01 a	36.33 a
Cu		6.64 a	5.63 a	5.19 a	5.96 a

Means within same row having different letters are significantly different according to LSD ($P \leq 0.05$). Values are means of 4 replicates.

Effect of *Arbuscular Mycorrhizal* fungus (AMF) on growth and flowering

Plant growth and flowering

The main characteristics of growth and flowering of *Asiatic lily 'Cavalese'* as affected by Mycorrhiza (AMF) inoculation are tabulated in Table 5.

The results showed that there was no significant effect of the irrigation solution or inoculation with Arbuscular Mycorrhiza or their interaction on stem length, shoot dry weight, leaf number, adventitious root fresh weight or length, basal root fresh weight, dry weight and length or flower bud count and fresh weight (Table 5). The irrigation solution had significant effects on the dry weight of adventitious roots and the fresh weight of the flower bud.

The plants receiving 3NS:1TWW solution had higher adventitious root dry weight (0.64 g) and fresh weight of the flower bud (10.27 g) than those receiving only NS (average of 0.46 g and 8.85 g, respectively). Furthermore, inoculation had a significant effect only on the length of the flower bud, it reached 18.0 cm for inoculated plants compared with 17.0 cm for non-inoculated plants. Moreover, a significant interactive effect of the irrigation solution and inoculation with Arbuscular Mycorrhiza was detected on stem diameter and shoot fresh weight.

Similar to our results, Garmendia and Mangas (2012) concluded that the addition of mycorrhizal inoculum did not promote plant biomass of *Rosa cv. Grand Gala*. They explained it by the insufficient percentage of AMF colonization achieved by roots to influence the growth of the rose.

Moreover, Sohn et al. (2003) reported an increase in the height of flowers as results of AFM inoculation. Similarly, Maboko and Du Plooy (2013) concluded that AMF inoculation has limited effects on the yield and quality of tomatoes grown in a soilless growing medium.

Plant tissue analysis

The minerals concentrations of *Asiatic lily 'Cavalese'* as affected by AFM inculcation are tabulated in Table 6.

Table 5 Growth and flowering of *Asiatic lily 'Cavalese'* as influenced by inoculation with Mycorrhiza (AMF) and irrigation with NS or 3NS:1TWW ratio containing 160 mg Ca L^{-1} in soilless closed system

Parameter	**Unit**	**Irrigation solution**		**Inoculation**		**Significance**		
		NS	**3NS:1TWW**	**+ AMF**	**- AMF**	**Irrigation solution**	**Inoculation**	**Irrigation solution*Inoculation**
Basal root length	cm	11.0	10.2	10.5	10.7	NS	NS	NS
Basal root fresh wt.	g	6.9	7.06	7.62	6.33	NS	NS	NS
Basal root dry wt.	g	0.79	0.91	0.82	0.87	NS	NS	NS
Adventitious root length	cm	8.6	8.1	8.6	8.1	NS	NS	NS
Adventitious root fresh wt.	g	4.3	4.3	4.6	3.9	NS	NS	NS
Adventitious root dry wt.	g	0.46 b	0.64 a	0.57	0.53	0.03	NS	NS
Shoot fresh wt.	g	53.1	55.3	54.02	54.38	NS	NS	0.016
Shoot dry wt.	g	5.90	6.19	6.12	6.00	NS	NS	NS
Leaf number	-	61	62	62	62	NS	NS	NS
Stem diameter	cm	0.70	0.71	0.72	0.69	NS	NS	0.039
Stem length	cm	65.7	68.1	67.1	66.8	NS	NS	NS
Flower bud length	cm	17.6	17.6	18.0 a	17.0 b	NS	0.008	NS
Flower bud fresh wt.	g	8.85 b	10.27 a	9.82	9.30	0.014	NS	NS
Flower bud number	-	4.0	4.0	4.0	3.9	NS	NS	NS

Means within same row having different letters are significantly different according to LSD ($P \leq 0.05$). Values are means of 36 plants.

Tissue contents of Na, Mg, K and Cu were not affected by the AFM inculcation or irrigation solution. However, AFM inoculation and 3NS:1TWW irrigation solution were more effective in terms of Ca, Fe, Zn and Mn.

Tissue content of N was significantly higher in AFM inoculated plants and irrigated with NS (2.658 g Kg^{-1} dry weight) where the lowest in inoculated plants that were irrigated with 3NS:1TWW irrigation solution ratio (2.443 g Kg^{-1} dry weight). Levels of tissue Ca (3.456 g Kg^{-1} dry weight) and Fe (269.0 mg Kg^{-1} dry weight) were the highest in inoculated plants that were supplied with 3NS:1TWW solution but were not different among the other treatments. Furthermore, the highest concentration of Zn (19.21 and 23.88 mg Kg^{-1} dry weight) was measured in non-inoculated plants receiving NS and inoculated plants receiving 3NS:1TWW solution.

These results could be explained by the frequent irrigation with a nutrient (NS or 3NS:1TWW) in soilless systems which contain all nutrients required by plants (Dere et al. 2019; Cifuentes-Torres et al. 2021). Many studies observed increment in plant growth after AMF inoculation through enrichment of nutrient uptake (Michałojć et al. 2015; Chen et al. 2017; Dere et al. 2019).

Table 6 Concentration of minerals in *Asiatic lily 'Cavalese'* leaves as influenced by inoculation with Mycorrhiza (AMF) and irrigation with NS or 3NS:1TWW ratio containing 160 mg Ca L^{-1} in closed soilless system

Parameter	Unit	Treatments			
		NS+AMF	**NS-AMF**	**3:1+AMF**	**3:1-AMF**
N		2.658 a	2.510 bc	2.443 c	2.547 b
K		5.710 a	5.576 a	6.259 a	6.394 a
Ca	g Kg^{-1}	2.714 b	2.528 b	3.456 a	2.835 b
Mg		0.521 a	0.520 a	0.620 a	0.531 a
Na		0.573 a	0.515 a	0.508 a	0.693 a
Fe		183.8 b	182.2 b	269.0 a	185.0 b
Zn	mg Kg^{-1}	14.08 b	23.88 a	19.21 a	8.56 c
Mn		46.96 b	45.83 b	50.99 a	52.18 a
Cu		6.52 a	7.23 a	5.24 a	4.85 a

Means within same row having different letters are significantly different according to LSD ($P \leq 0.05$). Values are means of 4 replicates.

Conclusion

Water resources are limited especially in arid and semi-arid areas (Zone B, Köppen Geiger classification). Therefore, treated wastewater has been used to reduce freshwater consumption in the agriculture sector. The use of wastewater as a replacement for fresh water in a closed soilless system is recommended to save both water and fertilizer. Results of this study indicated a positive effect for adding Ca and inoculation with Arbuscular mycorrhizal fungi on reducing deleterious effect of TWW on *Asiatic lily cv. Cavalese* in a closed soilless culture and irrigated with a mixture of 3 NS: 1 TWW. Treated wastewater had the potential as irrigation water for the production of *Asiatic lily*, when mixed with potable water. It was concluded that the TWW and AMF inoculation could be used in soilless culture to reduce chemical fertilization.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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