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Valorization of secondary treated wastewater in *Trigonella foenum-graecum* L. irrigation

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Abstract
Facing the water scarcity, several countries had recourse to increase the water supply for agricultural irrigation by reusing treated wastewater. This study aimed to assess the impact of secondary treated wastewater irrigation on agronomic characteristics, nutrient and metal trace elements rates and also on the health quality of *Trigonella foenum-graecum* (fenugreek) cultivated in open field and in plastic greenhouse, with and without addition of cattle manure as fertilizer. Irrigation control water was the groundwater of the region of Oued Souhil in Nabeul, northeast of Tunisia. Results showed that field cultivation was more productive. Irrigation water quality and fertilizer use did not significantly affect the vegetative and reproductive development of cultivated crops. A nutrient accumulation in the fenugreek aerial parts was observed. Plants cultivated in the greenhouse were richer in Na, K and P than those of the field. No significant bacterial contamination was recorded on fenugreek crops.

Keywords: Agronomic characteristics, Bacterial contamination, Fertilization with treated wastewater irrigation, Nutrients, Metal trace elements.

1. Introduction

Fresh water is a fundamental resource for human life and the natural environment. This resource is becoming increasingly scarce facing the growing population demands. In recent decades, climate change and human socioeconomic development changed the universal hydrological cycles, threatening human water security, health and biodiversity of aquatic environments [1, 2, 3]. The United Nations estimated that 1.8 billion people will live in countries or regions with water scarcity by 2025, and population growth added to climate change will continue to exert additional stress on fresh water resources with great intensity, even in developed countries [4]. Facing the growing problem of water shortage, many countries such as Tunisia, Spain, China and Jordan adopted adaptation policies with two options, those focused on improving supply and those aimed the demand management. Improving supply means increasing access to conventional water resources, reuse of unconventional water, transfers between basins, desalination and pollution control [5].

Reuse of treated wastewater (TWW) began in several countries such as Australia, France, Germany, India, the United Kingdom, the USA and Mexico since the late 19th century [6]. This practice became increasingly important in water resources management for environmental and economic reasons. It was mainly applied in agriculture, but increasingly, industrial, household and urban uses are being developed [7]. In Tunisia, a North African developing country with arid and semi-arid climate and low availability of fresh water resources, agriculture consumes near 80% of conventional water resources [8]. Reuse of treated wastewater in agriculture can relieve the exploitation of traditional resources which cannot meet the needs of intensive agriculture, and reduce the water deficit [9]. Since 1965, the developed areas for irrigation with treated wastewater in Tunisia were amplified from 1000 ha to 8415 ha in 2016 and this is equivalent to 2% of the total irrigated agricultural area [10]. In addition to increasing water supply for irrigation, agricultural reuse of treated wastewater can provide a significant amount of nutrients, particularly nitrogen, potassium and micro-nutriments which can improve soil fertility, stimulate plant growth and enhance agricultural production by reducing the needed amount of commercial fertilizer and increasing the economic benefits for farmers [11]. Moreover, this water may have adverse impacts on environment and public health because discharge and reuse of raw wastewater pose serious sanitary problems related to the presence of metal trace elements (MTE) and pathogenic microorganisms such as total and fecal coliforms and fecal streptococci present in these waters [12].

The amount of reused treated wastewater in Tunisia reached 25% of the total treated wastewater in 2016 and was used for irrigation of agricultural land, parks and golf courses. Crops allowed to be irrigated with treated wastewater are fodder crops, fruit trees (olives, citrus, pomegranate and peach), cereals and industrial crops [13]. In this context we studied the impact of irrigation with secondary treated wastewater on cultivated *Trigonella foenum-graecum* (fenugreek), a species which green matter is used as fodder and seeds are used as fodder and human food [14]. Fenugreek is widely grown in Asia and North Africa [15]. Treated wastewater irrigation impact on this species was studied mainly in Asian countries and such a study was not conducted in Tunisia before. Scientists proved that this crop had good yield when it was irrigated with treated wastewater and this was not the case when the irrigation water was heavily loaded with biological and chemical contaminants [16, 17, 18]. In this study, the impact of secondary treated wastewater irrigation was assessed through monitoring variations of morphological characteristics, nutrient composition, MTE content and bacterial quality of irrigated plants.

2. Materials and Methods

2.1 Site and protocol description

The study was conducted during October 2014 - June 2015 on an experimental station located in the region of Oued-Souhil (36°27'22''N 10°42'02''E) near Nabeul city, northeast of Tunisia (Figure 1). The altitude is 25 m above mean sea level. The region has a Mediterranean semi-arid climate characterized by rainy and fresh winter without frosts, mean annual precipitation of 391.6 mm (concentrated from September to December) and a mean annual temperature of 18.3°C [19]. The main irrigation water resource in the station is the groundwater (GW) of the Hammamet–Nabeul aquifer. At that level, the groundwater table is estimated to be about 10 m deep [19]. Moreover the station is equipped with a localized irrigation site with secondary treated wastewater (TWW) from the treatment plant SE4 (activated sludge). Once arrived at the station, the TWW is filtered, stored and decanted into two 500 m^3 basins. The soil of the experimental field is sandy loam, with an alkaline pH, a rather low electrical conductivity (0.4 to 0.9) and low organic matter content, ranging from 0.39% to 1.98% [20]. Fenugreek was cultivated in open field and plastic greenhouse. The plots were irrigated with TWW and GW with drip system after making a deep plowing. Half of crops were fertilized with cattle manure (20 ton/ha) (Figure 2). The physicochemical and bacteriological characteristics of irrigation waters are shown in Table 1.

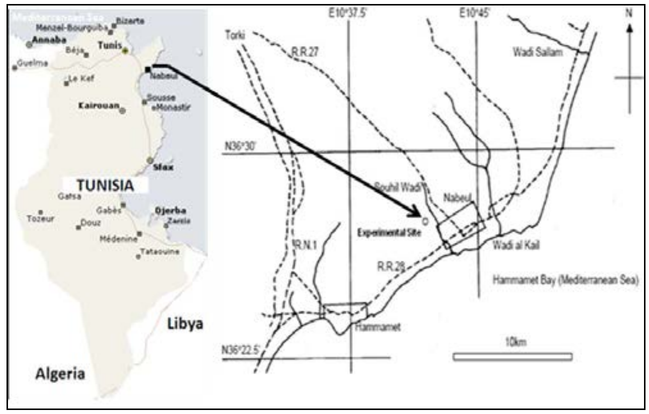

Fig. 1. Location map of Oued Souhil experimental site [19]

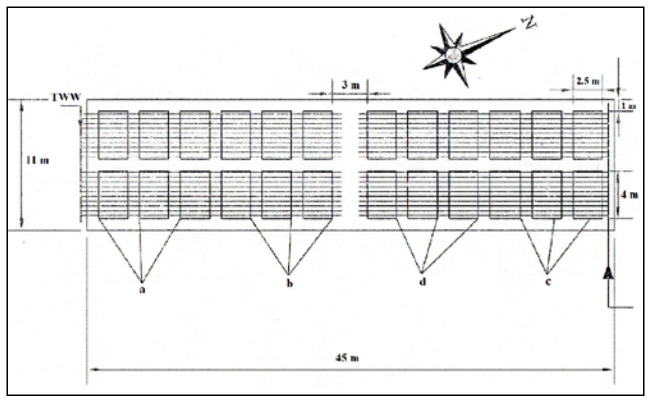

Fig. 2. Experimental setup of fenugreek culture (elaborated by Autocad 2008)

Notes. a: irrigation with TTW and fertilization with manure, b: irrigation with TWW and without fertilization, c: irrigation with GW and fertilization with manure, d: irrigation with GW and without fertilization.

2.2 Study of fenugreek parameters

2.2.1 Agronomic parameters

The fenugreek morphological development was studied at different stages (0 - 120 days) following standard methods. The measured parameters were leaves number, leaf area (cm²), stem height (cm), plant height (cm), branches number, number of plants in 1 m², fresh and dry weights of the aerial part (g), root fresh and dry weights (g), pods number, pods length (cm), seeds number per pod, pod weight in 1m² (g), weight of seeds harvested in 1m² (g) and harvest index [21].

Table 1. Comparative table of physicochemical and bacterial characteristics of irrigation water with the Tunisian standard for treated wastewater reuse in agriculture NT 106.03 [22].

Parameters	***GW***	***TWW***	***NT 106.03***
pH	7.20	7.54	6.5 - 8.5
Electrical Conductivity (mS/cm)	4.34	3.01	7
Sodium (mg/l)	547.56	636.18	NS
Total Suspended Solid (mg/l)	NS	24.44	30
Dry Residue (mg/l)	NS	1742.22	NS
BOD5 (mg/l)	NS	53.44	30
COD (mg/l)	NS	121.34	90
Magnesium (mg/l)	2.82	36.29	NS
Calcium (mg/l)	12.25	26.05	NS
Potassium (mg/l)	29.8	26.1	NS
Ammonium ion (mg/l)	3.26	37.0	NS
Chloride (mg/l)	1050	500	2000
Bicarbonate ion (mg/l)	504.9	1285.2	NS
Cadmium (mg/l)	0.02	0.01	0.01
Cobalt (mg/l)	0.03	0.02	0.1
Chromium (mg/l)	0.10	0.03	0.1
Copper (mg/l)	0.02	0.01	0.5
Iron (mg/l)	0.05	0.27	5
Manganese (mg/l)	0.01	0.01	0.5
Nickel (mg/l)	0.03	0.02	0.2
Lead (mg/l)	0.12	0.04	1
Zinc (mg/l)	0.04	3.6	5
Total coliforms (MPN/100 ml)	4.1 103	2.24 104	NS
Escherichia coli (MPN/100 ml)	3.3 103	7.74 103	NS
Fecal streptococci (MPN/100 ml)	4.8 103	1.07 104	NS

Note. NS: not stated.

2.2.2 Chemical parameters

The fenugreek aerial parts (leaves and stems) were dried at 70◦C for 48 h and dry weights determined. A quantity of 1g of plant powder was wet-digested with $HNO_3/HClO_4$ (1/1, V/V) and the concentration of P in aerial parts was determined on the digests with colorimetric standard method (Model AE-11, Erma Optical Works Ltd., Japan) [23]. The concentrations of Na and K were determined with flame spectrophotometry (Jenway PFP7, U.K.) [23] and the concentrations of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in aerial parts were dosed using flame atomic absorption spectrophotometry (PerkinElmer A Analyst 400, U.S.A.) according to Jedrzejczak and Szteke (1990) method [24]. All samples were analyzed in triplicate with reagent blanks.

2.2.3 Bacterial parameters

The aerial parts of *T. foenum-graecum* were prepared for bacteriological analysis using the method described in ISO 6887-1 standard. Bacterial counts were made by the MPN technique in liquid media [25].

2.2.4 Statistical analyses

The considered statistical model included three qualitative variables namely the type of irrigation water (GW or TWW), location of fenugreek culture (field or greenhouse) and use of fertilizer (without or with fertilizer) which were considered as factors explaining the variations of quantitative variables (agronomic, chemical and bacterial parameters). All tests were performed using version 3.3.2 of R software [26]. Normality of the quantitative data was assessed by the Shapiro-Wilks test and variability of quantitative parameters was evaluated through a three factors ANOVA. Variations of fenugreek harvest index were explained and predicted by a multiple linear regression analysis involving the rest of the studied parameters.

3. Results and discussion

3.1 Agronomic study

Variations in the studied vegetative parameters based on the location of fenugreek crop, irrigation water type and use or not of fertilizer were shown in Figure 3. The three-factor variance analysis revealed no significant variations in all studied vegetative parameters according to the irrigation water type and the use or not of fertilizer (ANOVA, $p > 0.05$) and significant variations depending on the location of the crop ($p < 0.05$) for the majority of considered parameters (except for branches number). The highest values for the parameters number of leaves, number of plants in 1 m^2, number of branches, fresh weights of root and aerial part and dry weight of aerial part were recorded in the field crop (Figure 3).

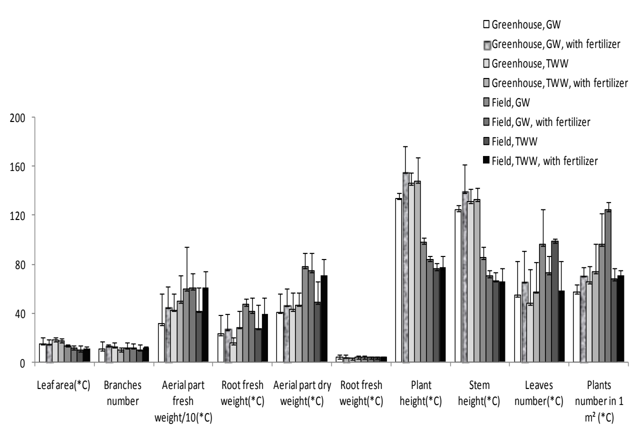

Fig. 3. Variations of *Trigonella foenum-graecum* vegetative parameters.
Note. *C: indicates significant variations according to the culture location factor ($p < 0.5$).

Regarding reproductive parameters, statistical analysis showed also no significant variations according to the irrigation water type and the use or not of fertilizer (ANOVA, $p > 0.05$) and significant variations depending on the location of the fenugreek crop ($p < 0.05$). The highest values of the considered reproductive parameters were detected in the field crop except for pods number (Figure 4). Consequently, *T. foenum-graecum* culture in the open field was more productive than the greenhouse as was the case for other leafy vegetable crops such as spinach (*Spinacia oleracea*), lettuce (*Lactuca sativa* var. crispa), celtuce (*Lactuca sativa* var. angustana), cabbage (*Brassica oleracea*), Chinese celery (*Apium graveolens*), rape (*Brassica campestris* var. capitata), etc. For these latter, the greenhouse was a way to ensure an out-of-season production higher to that obtained in the open field cultivation [27]. However, a decline in greenhouses production occurred due to acidification and accumulation of nutrient, salts and metal trace elements in soils [28]. In this study, the decrease in fenugreek production in the greenhouse could also be due to the fact that *T. foenum-graecum* species, preferring temperate climates [29], did not stand the greenhouse effect. In very sunny countries such as Tunisia (semi arid and arid climate), despite the natural or forced ventilation, the greenhouse effect in summer generates a considerable increase in the internal temperature of up 40 °C and a considerable decrease in the relative humidity of the air. Such conditions prevent greenhouse production for several months [30].

3.2 Chemical study

Plant growth requires a nutrient supply of nitrogen, potassium, phosphorus and trace elements.

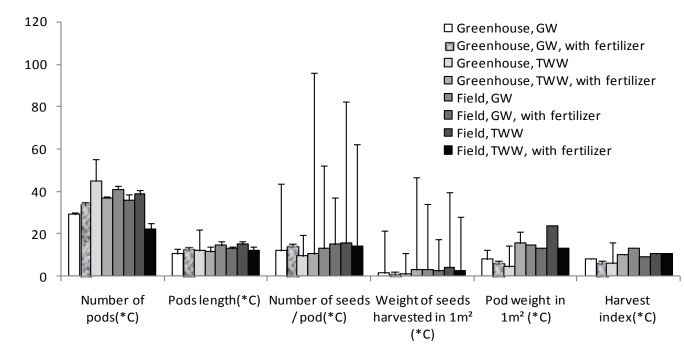

Fig. 4. Variations of *Trigonella foenum-graecum* reproductive parameters.
Note. *C: indicates significant variations according to the culture location factor ($p < 0.5$).

These elements are essential to plant growth and are found in significant quantities but in very different proportions compared to the growing needs in raw and TWW [31]. Thus irrigation by TWW could be an asset to fenugreek culture. Results showed that K, Na and P were accumulated in the fenugreek aerial parts and that the plants grown under the plastic greenhouse were richer in Na, K and P than those grown in the open field (Figure 5). This may be due to the fact that the soil under plastic greenhouses are often very enriched because of the excessive application of fertilizers in order to increase the yield of crops under greenhouses [32]. The statistical study revealed that K and Na variations were not significant according to the type of irrigation water and the use or not of fertilizer (ANOVA, $p > 0.05$) and significant depending on the location of the fenugreek crop. Moreover P content presented significant variations ($p < 0.05$) according to the irrigation water type and not significant ($p > 0.05$) depending on the use or not of fertilizer and the crop location.

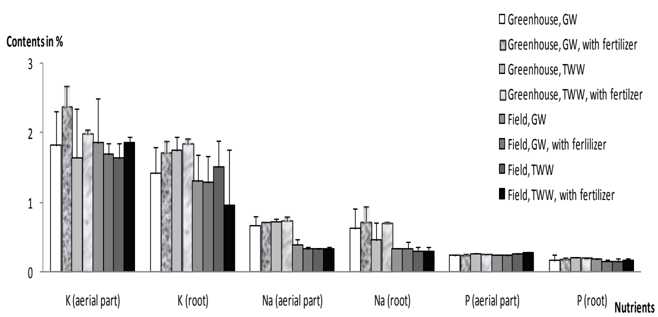

Fig. 5. Variations of *Trigonella foenum-graecum* nutrient contents.

Concerning the MTE, results showed that the Cr, Cu, Fe, Mn, and Zn were accumulated in the plants roots whileCo, Cd and Pb were accumulated in the fenugreek aerial parts (Figure 6). Statistical analysis showed that the contents of the aerial and root parts of Cu, Cr and Zn were significantly affected by the culture location (ANOVA, $p < 0.05$). Root Cd concentrations showed significant variations according culture location ($p < 0.05$) whereas the aerial parts concentrations in this metal did not present significant variations regarding this factor ($p > 0.05$). The variations in contents of aerial and root parts of Pb, Fe, Mn and Co were not significant on the basis of the culture location variation ($p > 0.05$). Based on the classification criterion type of irrigation water, only the content of the aerial parts in Co showed significant variation (ANOVA, $p < 0.05$), for the other MTE, whether in root or aerial parts of the plant, variations depending on the irrigation water type were not significant ($p > 0.05$). As for the use of fertilizer, it did not significantly influence variations of all studied MTE ($p > 0.05$).

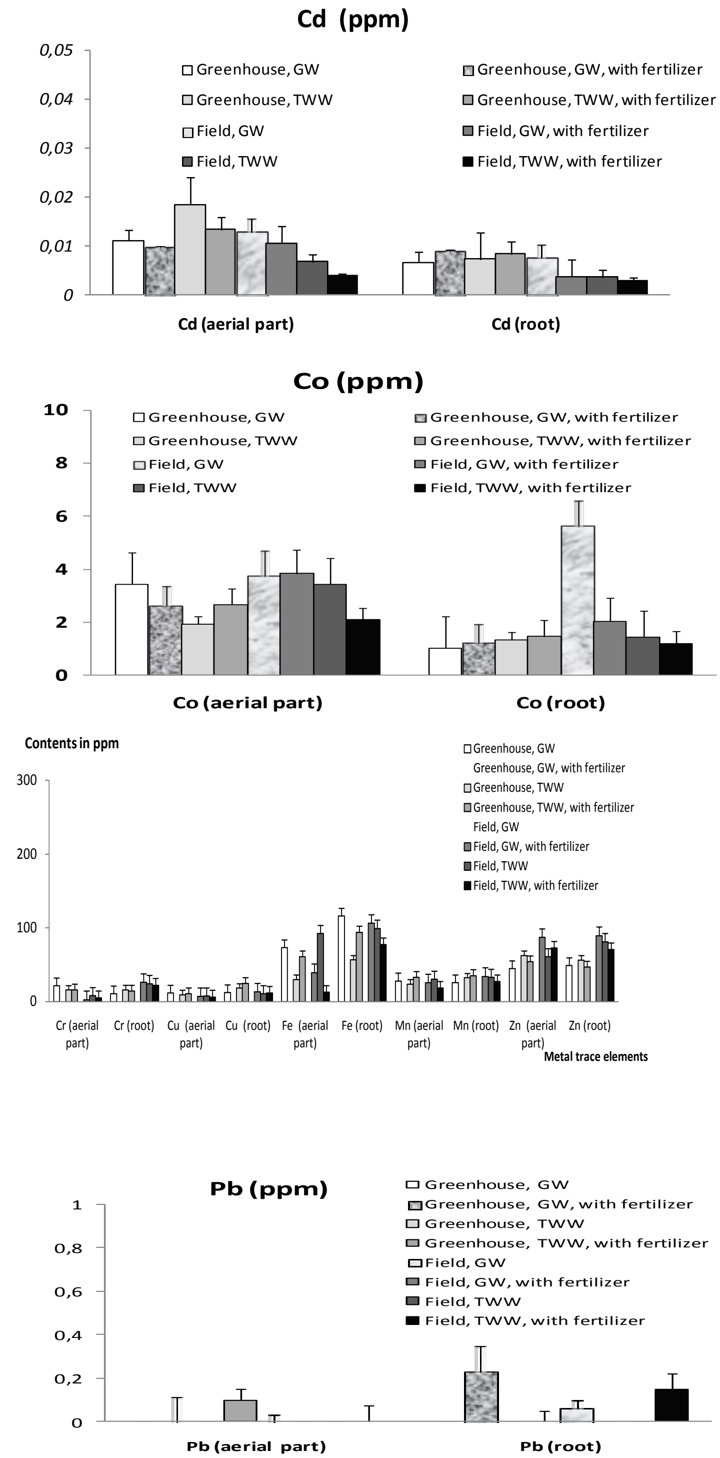

Fig. 6. Variations of *Trigonella foenum-graecum* metal trace elements contents.

As shown in Table 1, MTE concentrations of waters used in fenugreek irrigation were acceptable according to the Tunisian Standards NT 106.03 [22]. Irrigation with such waters prevents soil pollution and does not cause heavy metals accumulation until reaching the thresholds of phytotoxicity [33]. The comparison between the contents of nutrients and MTE of our study with the fenugreek contents mentioned in the literature showed that irrigation with TWW and GW of the culture region leads to the rising of concentrations of Na, Mn and Zn in aerial tissues [14, 29]. This can be justified by the results of other scientific works which confirmed that irrigation by TWW leads to an enrichment of cultivated plant tissues in nutrients and trace elements [34, 35] and also it could be due to the nature of the soil, the age of the irrigation with TWW and bad practice of fertilization [36].

3.3 Bacterial study

The bacteriological results showed that the TWW used for experimental plots irrigation had roughly the same quality as the GW (Figure 7). Indeed the numbers of total coliforms and fecal indicators (*E. coli* and fecal streptococci) showed no significant differences according to the origin of the water (ANOVA, $p > 0.05$). TWW at secondary level generally carry a larger quantity of fecal indicators than conventional water. For our study, the TWW coming from the treatment plant were kept for about a week in a storage basin built on the experimental site before being used in irrigation. Many previous studies showed that TWW storage constitutes an additional treatment which reduces microbial contamination to a level allowing them use in irrigation of all crops without restriction [37, 38, 39]. GW was in fact consistent with the quality of river water used in many countries without known harmful effects for crop irrigation [37]. Regarding the bacteriological quality of cultivated fenugreek, analysis results showed that the number of E. coli recorded on the various samples was low. This number is less than one bacterium per gram of plant material regardless of the origin of irrigation water which reflects good bacteriological quality of the cultivated *T. foenum-graecum*.

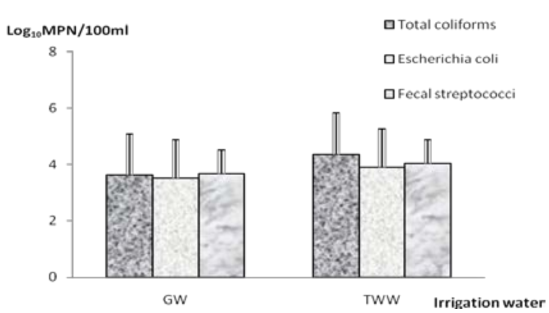

Fig. 7. Content of total coliforms, *Escherichia coli* and fecal streptococci in GW and TWW.

4. Conclusions

The imbalance between supply and demand of water resources is becoming more threatening in the North African countries. So the use of non-conventional water resources such as TWW becomes necessary and requires the mobilization and promotion of these marginal resources use such as additional resources. For this, the reuse of TWW must be increased in an economically advantageous manner, and especially do that by respecting the requirements for public and animal health protection and for environment preservation. In this study, TWW was used in the irrigation of *T. foenum-graecum* cultivated under greenhouse and in open field. The morphological characteristics, nutrient composition, MTE content and bacterial quality of TWW irrigated crops were compared to those of crops irrigated with GW. Morphologically, the location of culture had a significant impact on variations of the majority of studied vegetative parameters and all analyzed reproductive parameters. Fenugreek culture in the field was more productive than the greenhouse. The irrigation water type and fertilizer use did not significantly affect the vegetative and reproductive development of crops. Results of the chemical study showed a nutrient accumulation in the fenugreek aerial parts and that the crops grown under plastic greenhouse were richer in Na, K and P than the crops of the open field. The majority of MTE were accumulated in fenugreek roots. Bacteriological analysis led to the conclusion that the culture location, irrigation water type and the use of manure as fertilizer did not significantly affect the health quality of fenugreek crops.
Secondary treated wastewater could be considered as potential water for the irrigation of *Trigonella foenum-graecum*. Results suggested also that farmers could minimize the cost of the fenugreek culture and preserve the environment by avoiding the use of fertilizers and greenhouse.

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