

Nitrogen application and irrigation with purified urban wastewater effect on NPK accumulation in Fenugreek (*Trigonella foenum L.*)

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Abstract: This experiment was factorial based on completely randomized block design in three replications. The treatments were nitrogen (0.0, 3.0 and 6.0% Urea solution) and purified domestic wastewater (0.0, 50.0 and 100.0 mg/l) that developed in 27 standard 40 kg plastic pots on 28 days old plants. The results show significant differences ($p < 0.01$) between all of the measured traits in different levels of nitrogen foliar application and urban wastewater and their interactions too. The stem biomass of Fenugreek was 158.55 gram per plant and flower biomass by 483.66 gram per plant were highest when irrigated by 100.0 mg/l⁻¹ purified domestic wastewater without Nitrogen foliar application plants compare to control consequently. The most essential oil percentage was obtained by 100 mg/l⁻¹ purified domestic wastewater plus 6% nitrogen application about 0.53%. Thus, the nutrients containing in urban wastewater such as nitrogen, phosphorus and potassium can be a good alternative to chemical fertilizers in Fenugreek. After irrigation of Fenugreek by wastewater we observe changes in essential oil quantity by increased from 44.00% to 53.00%. We realize that wastewater has a beneficial effect in increasing the biomass yield of essential oil.

Key words: *Biomass; Essential oil; Foliar application; Urban wastewater; Urea*

1. Introduction

The search for new water resources in Iran is vital. The use of urban wastewater has been implemented and used in Mediterranean countries (Massoud et al., 2003). The use of Purified Urban wastewater for agricultural usage continues to expand due to the benefits it offers such as a solution to irrigation water insufficiency, the reduction of fertilizers needed due to the nutrients contained in this type of water; protection of the environment and regeneration of Wetlands (Papadopoulos, 1995) and dry land condition (Segura et al., 2004). However, inadequate handling of fertilization and irrigation with these types of water could supply the crop with higher quantities of nutrients and produce excessive accumulations within the plant and soil, negatively affecting the yield and production quality (Mohamed et al., 2003).

The main problems caused by the use of urban wastewater regarding to the presence of biological and chemical contaminants. These could harm the agricultural environment, as well as the health of farmers and consumers by build-up of chemical contaminants and pathogens in the soil (Khan et al., 2008). Previous investigational results specify that no major limitation for the use of the wastewaters as an irrigation source in plant nursery was found (Lubello et al., 2004). In plant production programs, the use of low-input farming systems has found a considerable position in order to invent modern

methods to management of resource utilization, and achieve the goals of sustainable agriculture, So that, management of production systems plays a more important role (Yadav et al., 2002). Foliar application is one of these strategies that previous studies showed that using urea as foliar application on green organs of the plant, causing the further production quantity of essential oil of fennel compared to the direct use in the soil. The constituents of the essence of the plant were also influenced by fertilization method. Foliar application, also, preserve the health of the environment, particularly soil resources (Khan et al., 1992).

Fenugreek (*Trigonella foenum L.*) is an angiosperm plant from Fabaceae plant family. Fenugreek is native of Iran and later transferred to other areas of the world (Dini, 2006; Mozafarian, 1995). The main chemical constituents of fenugreek are saponins, alkaloids and mucilage fibers (50 %). Fenugreek has a wide range of therapeutic effects. For example, studies are demonstrating that fenugreek seeds are using to reduce blood sugar levels in type II diabetic patients (with insulin independent). It is also use to reduce the cholesterol and triglyceride levels, treating gastritis, headache, flatulence, osteoporosis, cancer, anemia and pulmonary infections (Sharma & Raghram., 2000). Fenugreek seeds are used as spices in food preparations to improve or impart flavor and are good sources of protein, fat, minerals, and dietary fiber. The main chemical constituents of Fenugreek are proteins rich in lysine and tryptophan, flavonoids

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(e.g. quercetin, Trigonelline, saponins, and phytic acid), and polyphenols (Kochhar *et al.*, 2006).

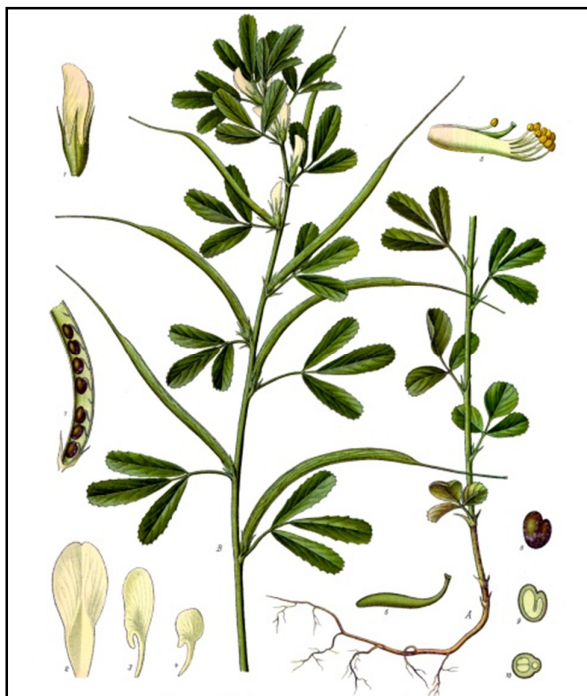


Fig. 1: Fenugreek (*Trigonella foenum L.*) Immage by Thomas Schoepke

2. Material and method

The present study was complete at normal nursery greenhouse (27° 38' N and 40° 21' W with an altitude of 1417 m) in 2012. This experiment was conducted in factorial experiment based on completely randomized block design in three replications. Experimental treatments were nitrogen foliar application in three levels of N1= 0.0, N2=3.0 and N3=6.0% Urea solution. Purified domestic wastewater also were in three levels (W1=0.0, W2=50.0 and W3=100.0 mg/l). The plant material was Fenugreek medicine plant (*Trigonella foenum L.* cv Persian) that sown in sand for 28 days then transplants planting in pots with analyzed soil 5 plants each. For these purpose, 27 polyethylene plastic containers (60*100cm) were used. To remove drainage water from containers, perforate pipes were considered in the bottom of the containers.

Table 1: Chemical and physical soil properties

Parameters	Physical	Chemical
Sand %	58.72	-
Silt%	21.38	-
Clay%	19.90	-
pH	-	7.90
N (ppm)	-	0.004
P (ppm)	-	38.00
K (ppm)	-	210.00
Total Organic C	0.88%	-

Thus, a hole with a diameter of 4.0 cm was placed at the bottom of the containers. Then flexible pipes were attached to the bottom of containers as

drainage. To setup all containers, they were filled by analyzed agricultural soil (Table 1). Four days after soil filling in containers and first watering the planting of transplants were started.

To prevent of soil water the surface of containers covered by 1.0 cm sand. Weekly Irrigation treatments regards to purified domestic wastewater in three levels of 0.0, 50.0 and 100.0 mg⁻¹ were done (Table 2). Nitrogen foliar applications were performed in three and eight leaf growth stages and beginning of stem elongation and flowering stages. To evaluation of biomass amount of stem, flower and whole plant yield in the physiological maturity stage were determined. Mineral accumulation of N, P, and K in Fenugreek tissues and Essential oil were measurement (Cadho and Rajender, 1995).

Statistical analysis of data was carried out using SPSS software. Mean separation was performed using Duncan's Multiple Range test at 0.05 probability level after ANOVA.

3. Results

3.1. Biomass yield

The results of this study showed the nitrogen foliar application were significant ($p < 0.01$) on every plant part and whole plant biomass. Irrigation with urban wastewaters had the same effects on leaf biomass.

Thus, the interaction effects between treatments had significant effects on each plant part and also whole plant biomass too (Table 3). The total biomass production of Fenugreek is shown in Table 4.

The maximum assimilation of Fenugreek leaves biomass was 223.30 gram per plants when sprayed by 6.0% urea solution foliar application (N3). The production of Fenugreek stem and flower parts biomass were highest when irrigated by 100.0 mg⁻¹ purified domestic wastewater and non-Nitrogen foliar application by 158.55 and 483.66 gram per plants (N1W3) continuously (Table 4).

The maximum Fenugreek whole plant biomass was obtained by 775.89 gram per plants in non-nitrogen application treatment level (N1) and 436.22 gram per plants when irrigated by Purified domestic wastewater 100.0 mg⁻¹ (W3).

Thus, irrigating Fenugreek by 100.0 mg⁻¹ Purified domestic wastewater were highest records in stem, flowers and whole plant biomass comparing to control. Furthermore, the correlation index between Fenugreek leaf, stem and flowers were significant and positive to each other (Table 5). The same results also were reported by others (Kochhar *et al.*, 2006 and Khan *et al.*, 1992).

The results showed that there were significant differences between Nitrogen, different levels of urban wastewater and also between their interactions for nitrogen (N) phosphorus (P) and potassium (K) accumulation sharply (Table 3).

Table 2: Domestic waste water characteristics

Parameters	Unit	Concentration
COD	mg ^l ⁻¹	232
BOD ₅	mg ^l ⁻¹	150
EC	Dsm ⁻¹	1.83
PH	-	7.90
N	mg ^l ⁻¹	32.11
P	mg ^l ⁻¹	3.41
K	mg ^l ⁻¹	12.18
Total Organic C	mg ^l ⁻¹	150.47
Ni	mg ^l ⁻¹	1.83
Cd	mg ^l ⁻¹	0.04
SAR	-	1.01
Na	meq ^l ⁻¹	13.82
Ca	meq ^l ⁻¹	3.51
Mg	meq ^l ⁻¹	2.71
Sulfate	meq ^l ⁻¹	3.42
Bicarbonate	meq ^l ⁻¹	11.18
Cl	meq ^l ⁻¹	8.20
Fe	mg ^l ⁻¹	1.51
Pb	mg ^l ⁻¹	0.00
Ni	mg ^l ⁻¹	0.20
Cr	mg ^l ⁻¹	0.00
Sn	mg ^l ⁻¹	0.002
Co	mg ^l ⁻¹	0.00
Coliform bacteria faecal	No/100 ml	1.2*10 ¹⁸
Parasite eggs	No/ 1 Liter	0.1 <

3.2. Mineral accumulation

The most N accumulation in leaf was obtained in N3W3 by 6.07 ppm and the smallest amounts were 5.39 ppm observed in N1W1 and N2W1 without statistical difference (table 4). Application of Nitrogen and urban wastewater increased the N accumulation in the fenugreek shoots (Figure 3). Former studies have indicated that due to having different forms of nitrogen in urban wastewater, specific organic compounds of this element can have an important role in plant nitrogen supply (Ayers & Westcot., 1985).

The results showed that there were significant differences between nitrogen levels, different levels of urban wastewater and also between their interactions for phosphorus accumulation (P%) in the fenugreek shoots (Table 3). The maximum phosphorus accumulation in the Fenugreek shoots was observed in the N1W3 treatment by 0.35 % and the smallest amount 0.15% was observed in N1W2 and N2W1 without statistical difference (Table 4). Therefore, using urban wastewater solely could increase the phosphorus accumulation in the fenugreek shoots (Fig. 1).

Table 3: Analysis of variance for some characteristics

SOV	df	Mean square							
		Biomass yield			Mineral accumulation			Essential oil %	
		Leaf	Stem	Flower	Whole plant	N	P	K	oil %
Replication	2	0.002ns	4.778ns	0.007*	50.704ns	0.001ns	0.001ns	0.010*	0.001*
Foliar-N	2	0.039**	27.444**	3.580**	1156.491**	0.124**	0.005**	2.168**	0.001**
Wastewater	2	0.003ns	0.333**	3.830**	101.926*	0.452**	0.026**	5.443**	0.014**
N*W	4	0.001ns	4.250**	0.022**	6.481ns	0.007**	0.005**	1.897**	0.001**
Error	16	0.001	1.403	0.001	27.120	0.001	0.005	0.002	0.005
C.V %		5.129	7.666	6.38	13.103	3.770	5.405	6.790	3.370

ns, * and **: non-significant, significant at 5% and 1% levels of probability, respectively.

As a next result, the significant difference ($p < 0.01$) was observed between nitrogen levels, different levels of urban wastewater and also between their interactions for potassium accumulation percentage (Table 3). The most potassium accumulation percentage was observed in N3W3 and N1W3 treatments and the lowest was observed in the control treatment (Table 4).

The potassium accumulation percentage was approximately equal in the N2W1 and N2W2 treatments. Thus, application or not use of urban wastewater (50 mg/l) in the 0.03 % nitrogen had no effect on the potassium accumulation percentage in fenugreek shoots (Figure 1). The same results were reported by Kalavrouzotis and colleagues in 2008 as well. In addition, the correlation index between nitrogen (N) phosphorus (P) and potassium (K) were significant and positive to each other too (Table 5).

3.3. Essential oils percentage

In this study, significant difference was observed between nitrogen levels, different levels of urban wastewater and also between their interactions for

essence percentage (Table 3). Increasing in the nitrogen levels and urban wastewater, increase the essence percentage in the Fenugreek. As shown in the table 4 the greatest essence percentage was obtained in the N3W3 when application 0.06 % urea plus 100 mg/l urban wastewater in plots. The average of essence percentage was 0.53 % and the smallest amount was 0.44 % that observed in N1W2 (100 mg^l⁻¹ urban wastewater without nitrogen application) and control. This indicates that regarding to application do nitrogen, non-use of urban wastewater caused reduction in production of essence (Figure 1). Additionally, the correlation indexes between essence percentages were significant and positive to all other measured treats in this report significantly (Table 5).

4. Discussion

The results indicated positive effect of the nitrogen application and urban wastewater at third level were common in most important Fenugreek characteristics.

Table 4: Mean comparison of determined characteristics for some characteristics

Treatments	Means of treats				Mineral accumulation (%)			Essential oil (%)
	Biomass yield (g/plant)				N	P	K	
	Leaf	Stem	Flower	Whole plant				
Foliar-N								
N1	219.20b	144.47b	412.22a	775.89a	5.59b	0.24c	3.87a	0.47c
N2	218.73c	140.58c	342.88c	702.19b	5.61b	0.20a	2.94c	0.48b
N3	223.30a	148.04a	386.33b	757.67b	5.80a	0.21b	3.67b	0.49a
Wastewater								
W1	218.10a	137.39c	341.00c	696.49c	5.44c	0.17c	2.84c	0.44c
W2	220.18a	142.84b	364.22b	727.24b	5.68b	0.21b	3.29b	0.47b
W3	222.94a	152.86a	436.22a	812.02a	5.88a	0.27a	4.36a	0.52a
Interaction								
N1W1	217.08a	135.11d	356.66c	708.85a	5.39c	0.17c	2.76e	0.44c
N1W2	219.30a	139.75c	396.33b	755.38a	5.59b	0.21b	3.13d	0.45b
N1W3	221.23a	158.55a	483.66a	863.44a	5.79ab	0.35a	5.72a	0.51ab
N2W1	218.46a	136.46d	331.00d	685.92a	5.39c	0.15c	2.85d	0.45b
N2W2	218.53a	139.18c	346.00c	703.71a	5.65b	0.21b	2.81d	0.47b
N2W3	219.20a	146.09b	351.66c	716.95a	5.79ab	0.23b	3.17d	0.51ab
N3W1	218.76a	140.62bc	335.33d	694.71a	5.52b	0.18c	2.92d	0.45bc
N3W2	222.73a	149.59b	350.33c	722.65a	5.81ab	0.21b	3.93b	0.49ab
N3W3	228.40a	153.93ab	473.33ab	855.66a	6.07a	0.24b	4.18b	0.53a

Nitrogen solution were define by N1= 0.0, N2=3.0 and N3=6.0% levels and Purified domestic wastewater were at three levels of W1=0.0, W2=50.0 and W3=100.0 mg/l.

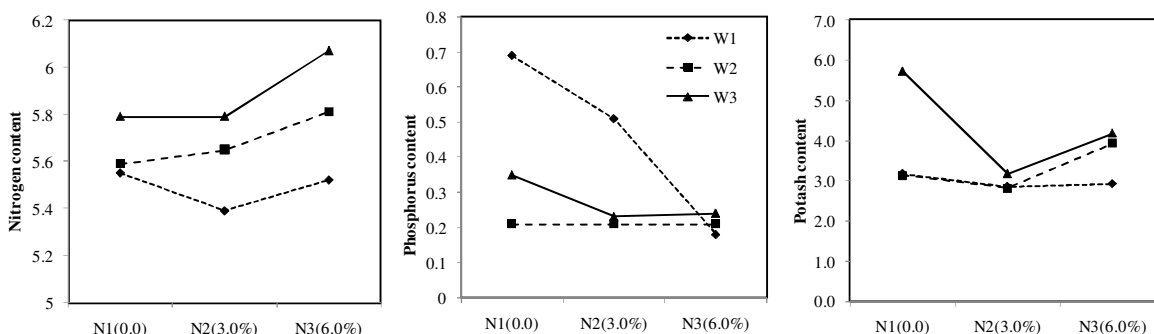


Fig. 2: Effects of Nitrogen and domestic wastewater on NPK accumulation in Fenugreek. There were W and N those define by N1= 0.0, N2=3.0 and N3=6.0% levels and Purified domestic wastewater and W1=0.0, W2=50.0 and W3=100.0 mg/l.

Table 5: Correlation coefficients for experimental characters

Treats	Biomass yield			Mineral accumulation			Essential oil %
	Leaf	Stem	Flower	N	P	K	
	I	II	III	IV	V	VI	
I	1.000						
II	0.735**	1.000					
III	0.629**	0.734**	1.000				
IV	0.871**	0.840**	0.615**	1.000			
V	0.378 ^{ns}	0.856**	0.716**	0.600**	1.000		
VI	0.560**	0.925**	0.777**	0.615**	0.855**	1.000	
VII	0.733**	0.856**	0.513*	0.906**	0.697**	0.697**	1.000

ns, * and **: Correlation is no-significant, significant at the 0.05 and 0.01 level

This suggests that the nutrients containing in urban wastewater such as nitrogen, phosphorus and potassium can be a good alternative to chemical fertilizers and will also save on costs. So, it looks that Nitrogen foliar application is required in this situation. The gradual release of N in urban wastewater can reduce loss of nitrogen in different forms and this makes it more important using treated urban wastewater in agriculture.

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