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Effect of Human Urine Fertilization and Foliar Spray with *Spirulina Platensis* Extract On Growth, Yield and Quality of Lettuce Plants in Pots

M. A. M. Abd-Elmotalib*, A. S. Abu-Zaid*, and L. A. A. Badr*
* Horticulture Dept., Fac. of Agric., Benha Univ., Egypt.
* Soils, Water Dept., Fac. of Agric., Benha Univ., Egypt.
** Central Administration for Soils, Water & Environment Ministry of Agriculture and Land Reclamation, Dokki, Giza , Egypt.

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Abstract

Two pot experiments were carried out during the two successive winter seasons of 2020-2021 and 2021-2022 at the Agricultural Research Center, Giza Governorate, Egypt, to study the effect of use of un-traditional nitrogen sources such as human urine and spraying with some growth stimulants such as ***Spirulina Platensis*** foliar extract to improve the vegetative growth, yield and its components as well as chemical composition traits of lettuce plants **(*Lactuca sativa, L.*).** This experiment included 15 treatments which were the combination of five human urine fertilization treatments and three foliar spray treatments by ***Spirulina Platensis*** extract. Human urine fertilization treatments were added beside plants six times during the growing season, the first dose after 21 days from transplanting and the second dose after 10 days from the first dose, then every 10 days by intervals. The foliar spray by ***Spirulina Platensis*** extract treatments were added four times during the growing season, the first dose after 21 days from transplanting and every 15 days by intervals. A split plot design with four replicates was adopted in this experiment where the mineral fertilizer and human urine treatments were located in the main plots and the foliar spray by ***Spirulina Platensis*** treatments were distributed randomly in the sub plots. The obtained results showed that lettuce plants were treated with 3.3 g ammonium nitrate plus (0.3 ml of human was diluted with 600 ml of water) per pot (50% menral nitrogen + 50% human urine-N); added in six times during the growing season, the first dose after 21 days from transplanting and the second dose after 10 days from the first dose, then every 10 days by intervals combined with spraying the plants with ***Spirulina Platensis*** foliar extract at concentration 2 cm3/ 1 liter of water four times, starting after 21 days from transplanting and every 15 days by intervals was recommended to obtain good vegetative growth, higher yield and its components traits as well as good chemical composition traits with best quality.

Keywords: Human urine, Lettuce, *Spirulina Platensis*, Foliar spray.

Introduction

Lettuce **(*Lactuca sativa, L.*)** is the most important vegetable crops in Egypt for both farmers and consumers. For farmers, it is considered as a fast cash crop in the winter season. For consumers, lettuce and rocket are eaten fresh and salad, where it is rich in vitamins and minerals; therefore it is the most used species in aquaponic systems **(Kim *et al.*, 2018).** Lettuce has a rich nutritional profile including vitamins A, C, B complex, and K as well as a substantial number of secondary metabolites such as phenolic acids, flavonoids, carotenoids, and folate which have been found to promote good health **(Bunning and Kendall, 2012).** The phenolic compounds in lettuce produce antioxidant activity that produces free radicals with scavenging ability. It has been reported that extracts from lettuce leaves reduce inflammatory and oxidative stress in murine monocyte and macrophage cells by decreasing reactive oxygen species and nitric oxide release **(Zapata-Vahos *et al.*, 2020).**

The fertilizers used in vegetable growing increase yield and production in important levels. However, the use of excess fertilizer in agriculture, especially chemical fertilizers, causes the increase in the level of pollutants, the decrease in soil fertility, the increase in soil and groundwater pollution and increase in nitrate concentration of vegetable leaves such as lettuce and rocket **(Hernandez *et al.*, 2010: Saleh *et al.*, 2016).**

Vegetables occupy an important place in human nutrition especially in terms of mineral substances and vitamins they contain. It is stated that vegetables can provide more than 70.8% of daily

nitrate intake **(Gangolli *et al.*, 1994; Premuzic *et al.*, 2004).** Especially leafy vegetables as lettuce are known to accumulate more nitrates. Although it has a short growing period, high nitrate intake occurs due to the need for high doses of N in lettuce **(Chiesa *et al.*, 2009; Saleh *et al.*, 2016).** In the other hand, excessive nitrate has harmful effects for human health and environment. Increasing demand for agricultural products that do not contain chemical residues enabled production models to reduce this problem in production. N doses suitable for lettuce cultivation or organic production systems should be preferred to prevent nitrate accumulation **(Pôrto *et al.*, 2008).** Optimal fertilization application and use of N, P and K have important to increase yield and quality of agriculture crops, and decreased production costs **(Zandvakili *et al.*, 2019).** It has been reported that inorganic fertilization causes three times more nitrate accumulation in lettuce and salads than organic fertilization **(Özgen and Sekerci 2011).** In addition, the physical and chemical structure of the soil can be improved by increasing microorganism activities with the use of organic fertilizers **(Özer, 2016).** As a result we should use of un-traditional nitrogen sources such as human urine to improve the vegetative growth and yield and its components as well as quality traits of some leafy vegetable crops such as lettuce and rocket.

Human urine is a source of macro nutrients such as N, P, K and other trace elements required by plants. A healthy human being will only excrete what they have consumed. Most of the food taken comes from agricultural and animal products. These are broken down, retained in the body to build news cells or excreted as waste. If the waste is inadequately or improperly disposed, it leads to the contamination of food and water. Urine is organic and therefore does not pose any danger to the environment. Unlike feces, urine does not contain pathogens except intestinal micro-organisms which are almost sterile. When source separated from excrement, it is safe to use following right instructions. **(Mauerer *et al.*, 2018).**

Human Urine is a well-balanced nitrogen-rich quick acting liquid fertilizer (**Richert *et al.*, 2010**). Different crops respond differently to urine application. Pot trials have been conducted with human urine as fertilizer source on cabbage, spinach, maize and tomato production **(Gensch *et al.*, 2011).**

On the other hand, we can use some growth stimulants such as *Spirulina Platensis* foliar extract to improve the vegetative growth and yield and its components as well as chemical composition of some leafy vegetable crops such as lettuce (Eden variety). **(Siringi *et al.*, 2022)** found that the application of (*Spirulina platensis*) foliar spray has a positive effect on the growth performance, yield and biochemical properties of lettuce (*Lactuca sativa, L.*) grown under soilless culture conditions. Therefore, the main objectives of this study are: -

1- Study the effect of use of un-traditional nitrogen sources such as human urine and spraying with some growth stimulants such as *Spirulina Platensis* foliar extract on vegetative growth, yield and its components and quality traits of some leafy vegetable crops such as lettuce (Eden variety).

Materials and Methods

3.1 Pot Experiment:

This experiment was carried out during the two successive growing winter seasons of 2020/2021 and 2021/2022 at the Agricultural Research Center, Giza Governorate, Egypt, to study the use of un-traditional nitrogen sources such as human urine and spraying the plant foliage with some growth stimulants such as *Spirulina Platensis* foliar extract to improve the vegetative growth, yield and its components as well as chemical composition of some leafy vegetable crops such as lettuce Eden variety (***Lactuca sativa, L.).***

In 2020/2021 and 2021/2022 growing winter seasons, lettuce seeds were sown directly in the nursery on first of October in the first and second season. Nursing period lasted after 30 days each seedlings were individually transplanted in the black polyethylene bag 25 x 25cm, where 10 kg of soil mixture (sand and compost, 1:3-V:V) was added to the black polyethylene bag. Other normal agricultural practices for lettuce production were carried out as a recommended in the district. The crop was harvested on January. The physical and chemical characteristics of the growing media were analyzed as described in **Table (1).**

Table 1. Chemical and physical analysis of the growing media as average of both seasons:

Chemical analysis					
Cations meg/Liter			Anions meg/Liter		
Cations	**Before sowing**	**After sowing**	**Anions**	**Before sowing**	**After sowing**
Ca++	3	3.6	$co3^{--}$	0	**0**
Mg++	0.8	0.8	$Hco3^{-}$	0.2	**0.2**
Na+ K+	0.2	0.4	Cl^{-}	0.5	**0.1**
	0.3	0.3	$SO4^{--}$	3.6	**3.9**
Minor Elements (ppm)			Major Elements (ppm)		
Element	**Before sowing**	**After sowing**	**Element**	**Before sowing**	**After sowing**
Available Fe	6.3	6.4	Available N	20	**20**
Available Cu	0.14	0.19	Available P	36	**38**

Available Zn	1.2	1.3	Available K	288	176
Available Mn	3.2	3.6			
Physical analysis		**Organic Matter**	**Soil PH**	**E.C, ds/m**	**CaCo3 (%)**
Coarse sand	7.14%		**Before sowing: 7.4**	**Before sowing: 0.43**	**Before sowing: 1.2**
Fine sand	17.26%	**Before sowing: 2.16%**			
Silt	23.20%				
Clay	52.40%	**After sowing: 2.16%**	**After sowing: 7.5**	**After sowing: 0.51**	**After sowing: 1.2**

This experiment consisting of two main factors:

1- Human urine fertilization:

The experimental treatments of human urine and chemical fertilization in single or combination application were conducted as follows:

(A) Five treatments of Lettuce:

1- 6.25 g ammonium nitrate plus 600 ml of water per pot (The control treatment); added in six times during the growing season, the first dose after 21 days from transplanting and the second dose after 10 days from the first dose, then every 10 days by intervals (100% meneral nitrogen from the recommended dose 200kg/fed ammonium nitrate 33% N).

2- 4.69 g ammonium nitrate plus (0.15 ml of human urine was diluted with 600 ml of water) per pot; added in six times during the growing season, the first dose after 21 days from transplanting and the second dose after 10 days from the first dose, then every 10 days by intervals (75% meneral nitrogen + 25% human urine-N).

3- 3.3 g ammonium nitrate plus (0.3 ml of human urine was diluted with 600 ml of water) per pot; added in six times during the growing season, the first dose after 21 days from transplanting and the second dose after 10 days from the first dose, then every 10 days by intervals (50% meneral nitrogen + 50% human urine-N).

4- 1.56 g ammonium nitrate plus (0.45 of human urine was diluted with 600 ml of water) per pot; added in six times during the growing season, the first dose after 21 days from transplanting and the second dose after 10 days from the first dose, then every 10 days by intervals (25% meneral nitrogen + 75% human urine-N).

5- 0.6 ml of human urine was diluted with 600 ml of water per pot; added in six times during the growing season, the first dose after 21 days from transplanting and the second dose after 10 days from the first dose, then every 10 days by intervals (100% human urine-N).

2- ***Spirulina Platensis* foliar extract:**

Extract of *Spirulina Platensis* was used as foliar spray treatments as follows:

1- Spraying with distilled water, without any spraying of *Spirulina Platensis* foliar extract (The control treatment) per pot.

2- Spraying with *Spirulina Platensis* foliar extract at concentration of 1 cm^3/ 1 liter of water per pot.

3- Spraying with *Spirulina Platensis* foliar extract at concentration of 2 cm^3/ 1 liter of water per pot.

Determination of Physical and Chemical Composition of Human Urine:

Table 2. Some chemical characteristics of urine used in the study.

Parameters	Maximum	Minimum	Mean
pH	**9.30**	**9.13**	**9.34**
EC (dS/cm)	**15.00**	**12.00**	**13.5**
Total Nitrogen (g/l)	**3.48**	**3.66**	**3.57**
Total Phosphorus (%)	**0.50**	**0.47**	**0.49**
Total Potassium (%)	**1.59**	**1. 47**	**1.53**
Ammonium Nitrogen (g/l)	**2.62**	**2.58**	**2.59**
Nitrate Nitrogen (g/l)	**0.04**	**0.04**	**0.04**
Available Potassium (g/l)	**0.18**	**0.18**	**0.18**
Available Phosphorus (%)	**0.011**	**0.012**	**0.012**
Heavy metals in human urine			
Total Pb Ug/ml (PPm)	**No**	**No**	**No**
Total Cd Ug/ml (PPm)	**No**	**No**	**No**
Total Ni Ug/ml (PPm)	**No**	**No**	**No**
Total Co Ug/ml (PPm)	**No**	**No**	**No**

Determination of Chemical Composition of *Spirulina Platensis* algae extract: The blue green algae *Spirulina platensis* was massively produced at Algae Biotechnology Unit, National Research Center (NRC) in continuous cultures. Algae extract was subjected to the chemical analysis according to

(**Chapman, 1978**). Chemical analysis concerning available macro- and micro-nutrients is listed in **Table (3).** Furthermore, amino acid content **Table (4)** was determined according to **(AOAC, 2012).**

Table 3. Available macro and micro-nutrients of the used algae extract. **ppm (%)**

Total protein	Cu	Zn	Mn	Fe	Ca	Na	Mg	K	P	N
19.06	18	21	68	1936	0.33	0.01	0.22	2.13	2.22	13.30

Table 4. Amino acid content of the used *Spirulina Platensis* algae extract.

Amino acid	%	Amino acid	%
Aspartic (ASP)	1.85	Isoleucine	0.71
Threonine (THR)	0.83	Leucine (LEU)	1.29
Serine (SER)	0.7	Tyrosine (TYR)	0.53
Glutamic (GLU)	2.24	Phenylalanine (PHE)	0.87
Proline (PRO)	0.67	Histidine (HIS)	0.24
Glycine (GLY)	1.07	Lysine (LYS)	0.7
Alanine (ALA)	1.55	Arginine (ARG)	0.98
Valine (VAL)	1.11	Cysteine (CYC)	0.22
Methionine (MET)	0.33	Total amino acids	15.89

Table 5. Quantification hormones of *Spirulina Platensis* algae extract.

Indole acetic acid (mg/g)	Indole butyric acid (mg/g)	Gibberellic acid (mg/g)
13.662	3.248	1.1917

Quantification of hormones **Table (5),** indole acetic acid, indole butyric acid and gibberellic acid was performed by High Performance Liquid Chromatography (HPLC), LC-10AD, Shimadzu, Japan 26. Algae extract was sprayed on the plants at the rate of 1 and 2 cm^3/liter of water, 30 and 45 days after sowing.

3.2 Experimental design:

A split plot design with four replicates was adopted. The mineral fertilizer and human urine treatments were arranged in the main plot, while foliar spraying by (*Spirulina Platensis)* extracts were randomly distributed in the sub-plots. Each experimental plot consisted of five pots. This experiment included 15 treatments which were the combination of five fertilizer treatments and three foliar spray treatments by (*Spirulina Platensis)* extract application were as follows:-

1- 100% mineral nitrogen fertilizer from recommended dose (200kg ammonium nitrate per fed) as control.
2- 75% mineral nitrogen fertilizer plus 25% human urine.
3- 50% mineral nitrogen fertilizer plus 50% human urine.
4- 25% mineral nitrogen fertilizer plus 75% human urine.
5- 100% of human urine.
6- 100% mineral nitrogen fertilizer from recommended dose (200kg ammonium nitrate per fed) plus spraying with *S. Platensis* as foliar extract at concentration of 1 cm3/ 1 liter of water.
7- 75% mineral nitrogen fertilizer plus 25% human urine plus spraying with *S. Platensis* as foliar extract at concentration of 1 cm3/ 1 liter of water.
8- 50% mineral nitrogen fertilizer plus 50% human urine plus spraying with *S. Platensis* as foliar extract at concentration of 1 cm3/ 1 liter of water.
9- 25% mineral nitrogen fertilizer plus 75% human urine plus spraying with *S. Platensis* as foliar extract at concentration of 1 cm3/ 1 liter of water.
10- 100% human urine plus spraying with *S. Platensis* as foliar extract at concentration of 1 cm3/ 1 liter of water.
11-100% mineral nitrogen fertilizer from recommended dose (200kg ammonium nitrate per fed) plus spraying with *S. Platensis* as foliar extract at concentration of 2cm3/ 1 liter of water.
12- 75% mineral nitrogen fertilizer plus 25% human urine plus spraying with S. Platensis as foliar extract at concentration of 2 cm3/ 1 liter of water.
13- 50% mineral nitrogen fertilizer plus 50% human urine plus spraying with S. Platensis as foliar extract at concentration of 2 cm3/ 1 liter of water.

14- 25% mineral nitrogen fertilizer plus 75% human urine plus spraying with S. Platensis as foliar extract at concentration of 2 cm3/ 1 liter of water.
15- 100% human urine plus spraying with S. Platensis as foliar extract at concentration of 2 cm3/ 1 liter of water.

3.3 Data recorded:-
Data on vegetative growth, yield and its components as well as chemical composition characteristics were recorded as follows:

3.3.1 Vegetative growth traits:
3.3.1.1 Lettuce plant:
Three plants from each experimental plot were taken randomly at the maturity stage, i.e., 80 days after transplanting and the following data were recorded:
1. Plant height (cm): It was measured from the joint point of stem with roots to the end of the higher point of plant.
2. No. of the outer leaves /plant:
3. Leaves dry matter ratio (%): The dry matter percentage of the leaves was determined in leaves representative samples after drying the fresh samples in an oven at 70°C to constant weight to determine dry weight percentage.
4. Dry matter content in the leaves (g /plant^{-1}): It was determined according to total fresh weight of plant and dry matter percentage.
5. Total chlorophyll reading (SPAD): Chlorophyll reading of the three outer mature leaves was measured at 60 days from transplanting
Using Minolta chlorophyll meter SPAD- 502 according to **Yadava (1986).**

3.3.2 Yield and its component traits:
3.3.2.1 Lettuce plant: Three plants from each plot were taken at maturity stage to evaluate the following characters:

1. Plant weight (g):
2. Weight of outer leaves (g/ plant):
3. Head weight (g):
4. Head Length (cm):
5. Head diameter (cm):
6. Total yield (ton/ fed^{-1}): Total yield of each pot was taken and total yield per feddan was determined.

3.3.3 Chemical composition of plant foliage:
Dry samples of plant foliage were group and then 0.2 g of each one was digested in sulphuric and perocloric acids at ratio 2:1 by volume and then used for determining the following chemical constituents:
1. Total Nitrogen (N %): It was determined in the digested dry matter of plant leaves using microkjeldahl method according to **Pregl (1945).**
2. Phosphorus content (P %): It was estimated the colorimetric determination of (P) by using spectrophotometer method as described by **Murphy and Riley (1962)** as modified by **John (1970).**
3. Potassium content (K %): It was determined by using flame- photometer method as described by **Brown and Lilleland (1964).**
4. Total carbohydrates (%): It was determined colorimetrically in the dry matter of leaves samples as (g glucose/100g) according to **Herbert *et al.*, (1971)** as modified by **Michel *et al.*, (1956).**
5. Nitrate content (No_3-N mg/kg): It was determined according to the methods as described by **Singh, (1988).**

3.4 Statistical analysis:
All obtained data were subjected to statistical analysis according to **Snedecor and Cochran (1991)** and L.S.D multiple range tests at 5% level was used to compare between treatment means.

Results and Discussion

4.1 Vegetative growth traits:

4.1.1 Lettuce plant:
4.1.1.1 Effect of human urine fertilization treatments:
Concerning the effect of human urine fertilization treatments:, i.e., 4.69 g ammonium nitrate plus (0.15 ml of human urine was diluted with 600 ml of water) per pot (75% menral nitrogen + 25% human urine-N), 3.3 g ammonium nitrate plus (0.3 ml of human was diluted with 600 ml of water) per pot (50% menral nitrogen + 50% human urine-N), 1.56 g ammonium nitrate plus (0.45 of human urine was diluted with 600 ml of water) per pot(25% menral nitrogen + 75% human urine-N) and 0.6 ml of human urine was diluted with 600 ml of water per pot as well as the control of treatment(6.25 g ammonium nitrate plus 600 ml of water per pot) (100% menral nitrogen from recommended dose), it is obvious from data in **Table (6)** that vegetative growth characteristics were significantly increased when the plant were supplement with all human urine treatments compared with the control treatment (100% menral nitrogen from recommended dose)during the both seasons of this study. In this respect, lettuce plant treated with 3.3 g ammonium nitrate plus (0.3 ml of human was diluted with 600 ml of water) per pot (50% menral nitrogen + 50% human urine from the recommended dose)beside the plants six times during the growing season, the first dose after 21 days from transplanting and the second dose after 10 days from the first dose, then every 10 days by interval during the growing seasons followed by using 0.6 ml of human urine was diluted with 600 ml of water per pot (100% human urine) during both seasons and 1.56 g ammonium nitrate plus (0.45 ml of human urine was diluted with 600 ml of water) per pot(25% menral nitrogen + 75% human urine) during

both seasons gave the highest values of all studied vegetative growth traits. In this concern, the increments in different studied vegetative growth aspects as a result of using human urine fertilization treatments application may be due to the main role of human urine as natural fertilizer contains high levels of macro and micro nutrients **(Table 6)** that can substitute synthetic fertilizer for leafy vegetable crops. Similar results have been reported by **Adjoa, (2013); Ranasinghe *et al.*, (2016); Chrispim *et al.*, (2017); Sheneni *et al.*, (2018) and Mauerer *et al.*, (2018).**

4.1.1.2 Effect of foliar spraying with *Spirulina Platensis* extract:

With regard to the effect of foliar spraying with *Spirulina Platensis* extract, the same data in **Table (6)** indicate also that vegetative growth characteristics of lettuce plants expressed as plant height, number of outer leaves/plant, total chlorophyll reading (SPAD), leaves dry matter content (g /plant^{-1}) and leaves dry matter ratio (%) were significantly increased when the plants were foliar spraying with *Spirulina Platensis* extract at concentration of 1 cm^3/ 1 liter of water and at 2 cm^3/ 1 liter of water; added in four times, starting after 21 days from transplanting and every 15 days by intervals compared with the control treatment (Spraying with distilled water) during the two seasons of growth. In this concept, spraying the plants with *Spirulina Platensis* extract at 2 cm^3/ 1 liter of water exhibited the highest values in all measured vegetative growth traits followed by spraying at concentration of 1 cm^3/ 1 liter of water in the both seasons. This was true during both seasons of this study. In this connection, the positive effect of foliar spray with *Spirulina Platensis* extract compared with the other foliar spray treatments and control treatment (Spraying with distilled water) may be due to significantly increases photosynthetic pigments content regard with vegetative growth and improves the yield and quality of lettuce plants in terms of antioxidant contents in the plant. These results are in harmony with the findings of other researchers **Hassan *et al.*, (2017); Abdallah *et al.*, (2019); Godlewska *et al.*, (2019) and Siringi *et al.*, (2022).**

4.1.1.3 Effect of the interaction:

As for the effect of the interaction treatments between human urine fertilization and foliar spray with *Spirulina Platensis* extract on vegetative growth characteristics of lettuce plants expressed as plant height, number of outer leaves/plant, total chlorophyll reading (SPAD), leaves dry matter content (g /plant^{-1}) and leaves dry matter ratio (%), the same data in **Table (6)** revealed that the highest values in all measured growth traits were recorded as a result of application 3.3 g ammonium nitrate plus (0.3 ml of human urine was diluted with 600 ml of water) / pot to the soil (50% menral nitrogen + 50% human urine from the recommended dose) combined with foliar spray with *Spirulina Platensis* extract at concentration of 2 cm^3/ 1 liter of water. These obtained results are true during both 2020/2021 and 2021/2022 growing winter seasons of this study.

4.2 Yield and its components traits:

4.2.1 Lettuce plant:

4.2.1.1 Effect of human urine fertilization treatments:

Data are presented in **Table (7)** showed the effect of human urine fertilization on lettuce yield and its components traits expressed as plant weight (g), outer leaves weight (g /plant), head weight (g), head length (cm), head diameter (cm) and total yield (ton/ fed^{-1}) were significantly affected as a result of fertilization by human urine , i.e., 4.69g ammonium nitrate plus (0.15 ml of human urine was diluted with 600 ml of water) per pot (75% menral nitrogen + 25% human urine), 3.3 g ammonium nitrate plus (0.3 ml of human was diluted with 600 ml of water) per pot (50% menral nitrogen + 50% human urine), 1.56 g ammonium nitrate plus (0.45 of human urine was diluted with 600 ml of water) per pot (25% menral nitrogen + 75% human urine) and 0.6 ml of human urine was diluted with 600 ml of water per pot as well as the control of treatment(6.25 g ammonium nitrate plus 600 ml of water per pot) (100% menral nitrogen from the recommended dose) beside the lettuce plants; added in add on six times during the growing season, the first dose after 21 days from transplanting and the second dose after 10 days from the first dose, then every 10 days by interval compared with the control treatment (6.25 g ammonium nitrate plus 600 ml of water per pot) (100% menral nitrogen from the recommended dose). Such trend was true during both seasons of study. In this regard, using 3.3 g ammonium nitrate plus (0.3 ml of human was diluted with 600 ml of water) per pot (50% menral nitrogen + 50% human urine), followed by 0.6 ml of human urine was diluted with 600 ml of water per pot (100% human urine-N) and 1.56 g ammonium nitrate plus (0.45 of human urine was diluted with 600 ml of water) per pot (25% menral nitrogen + 75% human urine-N) exhibited the highest values of yield and its components traits compared with the control treatment (100% menral nitrogen from the recommended dose) and other treatments. Meanwhile, the lowest values of total yield and its components characters were obtained from the treatment which fertilized with the recommended dose of menral nitrogen (200 kg ammonium nitrate/fed). Moreover, such increases in yield and its components as a result of using human urine as natural fertilizer contains high levels of macro and micro- nutrients that can substitute synthetic fertilizer for leafy vegetable crops which connected with increasing the vegetative growth traits **(Table 6)** and increasing the chemical composition of plant foliage **(Table 8)** which in turn positively effect on produced

yield and its components. These results agreed with the data reported by **Shingiro *et al.*, (2020); El-Nakhel *et al.*, (2021); Jurga *et al.*, (2021)** and **Wilde *et al.*, (2022).**

4.2.1.2 Effect of foliar spraying with *Spirulina Platensis* extract:

As for, the effect of foliar spray with *Spirulina Platensis* extract on yield and its components traits, the same data in **Table (7)** indicated clearly that yield and its components expressed as plant weight (g), outer leaves weight (g/plant), head weight (g), head length (cm), head diameter (cm) and total yield (ton/ fed^{-1}) were significantly increased as a result of spraying with *Spirulina Platensis* foliar extract at concentration of 1 cm^3/ 1 liter of water and at 2 cm^3/ 1 liter of water; added in four times during the growing season, the first dose after 21 days from transplanting and every 15 days by interval compared with the control treatment (Spraying with distilled water) during the two seasons of growth, except outer leaves weight and head length during the two seasons and head diameter in the second season only, which did not reach the level of significant at 5% level. In this regard, the highest values of all yield and its components traits were obtained as a result of using *Spirulina Platensis* extract at concentration of 2 cm^3/ 1 liter of water followed by using *Spirulina Platensis* extract at concentration of 1 cm^3/ 1 liter of water per pot. Such increments in yield and its components traits as a result of spraying the plants with *Spirulina Platensis* extract are connected with the improvement of plant vegetative growth parameters **(Table 6).** In addition its effect on increasing in chemical constituents, i.e., total chlorophyll reading and macronutrients **(Table 8)** which affected plant growth and in turn increased its productivity. Similar results have been reported by **El-Mahdy *et al.*, (2017); Godlewska *et al.*, (2019); Shams and Morsy, (2019); Uddin *et al.*, (2019) and Hoa *et al.*, (2022).**

4.2.1.3 Effect of the interaction:

Regarding with, the effect of the interaction treatments between human urine fertilization and foliar spray with *Spirulina Platensis* extract on yield and its components traits, i.e., plant weight (g), outer leaves weight (g/plant), head weight (g), head length (cm), head diameter (cm), total yield (ton/ fed^{-1}). Such data in **Table (7)** revealed that the highest values in all measured yield and its attributed traits were recorded as a result of application 3.3 g ammonium nitrate plus (0.3 ml of human was diluted with 600 ml of water) per pot to the soil (50% menral nitrogen + 50% human urine-N) combined with foliar spray with *Spirulina Platensis* extract at concentration of 2 cm^3/ 1 liter of water per pot, followed by using 0.6 ml of human urine was diluted with 600 ml of water per pot (100% of human urine-N) combined with foliar spray with *Spirulina Platensis* extract at concentration of 2 cm^3/ 1 liter of water per pot, as well as (50% menral nitrogen + 50% human urine-N) plus foliar spray with *Spirulina Platensis* extract at concentration of 1 cm^3/ 1 liter of water, as compared with all other treatments in both seasons of growth.

4.3 Chemical composition of plant foliage:

4.2.1 Lettuce plant:

4.2.1.1 Effect of human urine fertilization treatments:

Data are presented in **Table (8)** show the effect of human urine fertilization treatments on chemical composition of lettuce plant foliage. Such data show clearly that chemical constituents of plant foliage i.e., Macro elements (N, P and K percentage) and total carbohydrates (%) were significantly increased, when the plant were supplement with all human urine treatments compared with the control treatment (100% menral nitrogen from recommended dose, 200kg ammonium nitrate /fed) during the both seasons of this study. On the other hand, nitrate contents (No_3 mg/kg dry weight) in lettuce plants were significantly increased by addition of the mineral N fertilizer sources (Ammonium Nitrate or Ammonium Sulphate) compared to all human urine treatments, which decrees the nitrate contents in lettuce plants during the two growing winter seasons. In this respect, lettuce plants treated with 3.3 g ammonium nitrate plus (0.3 ml of human was diluted with 600 ml of water) per pot (50% menral nitrogen + 50% human urine-N) gave the highest values of all determined chemical constituents, except nitrate content which decreased, followed by those treated with 0.6 ml of human urine was diluted with 600 ml of water (100% human urine) during both seasons of this investigation. Such in all assayed chemical constituents, i.e., Macro elements (N, P and K percentage), nitrates contents and total carbohydrates (%) as a result of application of human urine fertilization treatments may be attributed to the application of effective microorganisms makes such macro-elements in the soil more available for plant absorption and consequently increased its accumulation and its content in plant leaves. In addition to, the use of pure human urine as fertilizer without use any chemical fertilizers reduced nitrates contents in lettuce plants. Obtained results are in agreement with those reported by **Amoah *et al.*, (2017); Mauerer *et al.*, (2018); Viskari *et al.*, (2018) and El-Nakhel *et al.*, (2021).**

4.2.1.2 Effect of foliar spraying with *Spirulina Platensis* extract:

With regard to the effect of foliar spray with *Spirulina Platensis* extract on chemical composition traits, the same data in **Table (8)** reveal that all measured chemical constituent, i.e., N(%), P(%), K(%) and total carbohydrate (%) were significantly increased as a result of spraying the plants three times during the growing seasons starting after three

weeks from transplanting and every 10days by using *Spirulina Platensis* foliar extract at concentration of 1 cm^3/ 1 liter of water and 2 cm^3/ 1 liter of water per pot, compared with the control treatment (Spraying with distilled water) during the two seasons of growth. Whereas, nitrate content was significantly decreased as a result of spraying the plants with *Spirulina Platensis* foliar extract at any used concentrations compared with the control treatment (Spraying with distilled water). In this connection, the highest values for all determined chemical constituents were obtained as a result of using *Spirulina Platensis* extract at concentration of 2 cm^3/ 1 liter of water followed by using *Spirulina Platensis* extract at concentration of 1 cm^3/ 1 liter of water per pot. Obtained results are true during both seasons of study. The increases in assayed chemical constituents as a result of foliar spray with such tested growth stimulating substances may be attributed to its effect on increasing the vegetative growth in turn increased the capability of plant absorption and assimilation of different chemical constituents. In this connection the superiority of foliar spray with *Spirulina Platensis* extract may be attributed to its effect on photosynthetic as simulates through photosynthetic process which in turn effect on the chemical composition of plant foliage and in turn increased plant growth. Obtained results are coincided with those reported by **Yassen *et al.*, (2019); Aboud and Abd-Alrahman (2021); Bella *et al.*, (2021) and Shedeed *et al.*, (2022).**

4.2.1.3 Effect of the interaction:

As for the effect of the interaction treatments between human urine fertilization and foliar spray with *Spirulina Platensis* extract on chemical composition traits, the same data in **Table (8)** show clearly that all measured chemical constituent, i.e., N%, P%, K%, nitrate contents and total carbohydrate (%) were significantly affected as a result of the interaction treatments between human urine fertilization and foliar spray with *Spirulina Platensis* extract during both 2020/2021 and 2021/2022 seasons of growth. In this regard, chemical constituent, i.e., N%, P%, K%, and total carbohydrate (%) of lettuce plant treated with 3.3 g ammonium nitrate plus (0.3 ml of human was diluted with 600 ml of water) (50% menral nitrogen + 50% human urine-N) and foliar spray with *Spirulina Platensis* extract at concentration of 2 cm^3/ 1 liter of water per pot gave the highest values of all determined chemical constituents followed by those treated with 0.6 ml of human urine was diluted with 600 ml of water (100% of human urine –N) plus foliar spray with *Spirulina Platensis* extract at concentration of 2 cm^3/ 1 liter of water per pot during both seasons of this investigation. While, lettuce crop treated with (100% of human urine –N) 0.6 ml of human urine was diluted with 600 ml of water plus foliar spray with *Spirulina Platensis* extract at concentration of 2 cm^3/ 1 liter of water per pot gave the lowest values for nitrates contents compared with other interaction treatments during both seasons of growth.

Table 6. Effect of human urine fertilization and foliar spray with *Spirulina Platensis* extract as well as their interaction on vegetative growth characteristics of lettuce plants during 2020/2021 and 2021/2022 winter seasons.

Treatments		First Season2020/2021					Second Season2021/2022				
Fertilization	Foliar	Plant	No. of	Total	Leaves	Leaves /plant^{-1}	Plant	No. of	Total	Leaves	Leaves dry /plant^{-1}
100% menral		19.44	5.44	24.84	8.09	24.52	20.11	5.11	27.53	9.57	25.7
75% menral-N +		20.22	5.22	25.47	8.50	27.33	22.00	5.22	29.83	9.69	28.2
50% menral-N +		22.66	6.55	28.05	9.26	28.5	24.33	6.77	34.03	11.26	27.4
25% menral-N +		20.88	5.33	26.62	8.49	26.2	22.33	5.33	31.03	9.69	26.1
100 % human urine		21.22	5.55	27.02	8.62	25.5	23.11	6.44	33.00	10.47	26.0
LSD at 5%			0.28	0.7	0.45	1.29	1.48	0.31	1.1	0.47	1.33
	Control	20.00	5.13	26.04	8.29	27.2	21.40	4.93	30.78	9.82	27.8
	S.P(1cm^3/L)	20.93	5.46	26.41	8.58	28.6	22.33	5.80	31.20	10.11	28.9
	S.P(2cm^3/L)	21.73	6.26	26.76	8.91	29.4	23.40	6.60	31.88	10.77	30.2
LSD at 5%			0.28	0.5	0.45	1.27	1.45	0.28	0.8	0.46	1.31
100% menral nitrogen fertilizer	Control	18.34	5.00	24.34	7.71	22.20	18.67	3.67	27	9.34	27.49
	S.P(1cm^3/L)	19.67	5.34	25.07	8.22	25.67	20.67	5.00	27.77	9.66	31.52
	S.P(2cm^3/L)	20.34	6.00	25.14	8.38	31.34	21.00	6.67	27.84	9.74	38.05
75% menral + 25% human urine	Control	19.34	4.67	25.04	8.22	25.89	21.34	5.00	29.7	9.9	32.41
	S.P(1cm^3/L)	20.34	5.00	25.1	8.54	27.16	22.00	5.00	29.67	9.46	31.72
	S.P(2cm^3/L)	21.00	6.00	26.3	8.76	33.23	22.67	5.67	30.14	9.72	38.07
50% menral + 50% human urine	Control	21.67	6.00	27.84	9.03	36.81	23.34	5.67	33.77	10.43	44.50
	S.P(1cm^3/L)	22.00	6.34	28.07	9.21	38.31	24.00	7.00	34.17	11.24	47.88
	S.P(2cm^3/L)	24.34	7.34	28.27	9.57	39.81	25.67	7.67	34.77	12.1	52.84
25% menral-N + 75% human urine	Control	19.67	5.00	26.3	8.27	30.16	21.67	5.00	30.7	9.54	36
	S.P(1cm^3/L)	21.34	5.34	26.7	8.38	31.50	22.00	5.34	31.2	9.66	37.06
	S.P(2cm^3/L)	21.67	5.67	26.87	8.85	33.98	23.34	5.67	31.2	9.82	39.74
100 % of human urine	Control	21.00	5.00	26.7	8.26	33.45	22.00	5.34	32.74	9.92	40.74
	S.P(1cm^3/L)	21.34	5.34	27.14	8.58	34.74	23.00	6.67	33.2	10.03	41.29
	S.P(2cm^3/L)	21.34	6.34	27.24	9.05	36.65	24.34	7.34	33.07	11.49	48.41
LSD at 5%			0.77	1.8	0.52	2.72	2.66	1.2	2.89	1.65	3.89

Table 7. Effect of human urine fertilization and foliar spray with *Spirulina Platensis* extract as well as their interaction on yield and its components traits of lettuce plants during 2020/2021 and 2021/2022 winter seasons.

Treatments		First Season2020/2021						Second Season2021/2022					
Fertilization	Foliar spray	Plant weight	Outer leaves	Head weight	Head Length	Head diameter	Total yield	Plant weight	Outer leaves	Head weight	Head Length	Head diameter	Total yield
100% menral nitrogen fertilizer		327.44	81.77	245.67	9.48	9.033	21.2	337.11	68.44	268.67	10.14	9.68	22.3
75% menral-N + 25% human urine		337.44	80.55	256.67	9.97	10.04	18.6	351.44	75.78	272.56	11.11	10.47	20.3
50% menral-N + 50% human urine		413.22	116.22	304.89	11.47	10.38	26.8	429.78	113.22	343.78	12.14	11.45	24.2
25% menral-N + 75% human urine		374.89	90.22	276.67	10.63	10.06	23.2	388.56	79.11	280.22	11.27	10.31	22.3
100 % human urine		405.00	108.11	297.00	10.75	10.17	24.5	414.56	102.33	317.44	11.66	10.85	23.8
LSD at 5%			16.1	43.2	1.8	0.78	1.9	34.2	17.3	45.2	1.6	0.67	1.8
	Control	357.67	89.00	261.73	9.99	9.447	22.1	367.33	79.13	274.33	10.67	10.29	24.5
	S.P(1cm^3/L)	365.47	94.73	268.80	10.36	9.933	23.2	376.73	87.80	289.80	11.40	10.62	25.5
	S.P(2cm^3/L)	391.67	102.40	298.00	11.15	10.32	24.4	409.00	96.40	325.67	11.77	10.76	26.8
LSD at 5%			16.1	43.2	1.8	0.78	1.9	34.2	17.3	45.2	1.6	0.67	1.8
100% menral nitrogen fertilizer	Control	296	76.00	220	9.44	8.4	19.89	294.34	62.00	225.67	9.87	9.27	19.78
	S.P(1cm^3/L	312.34	78.00	234.34	9.47	9.27	20.97	326.34	68.67	264.34	10.4	9.67	21.93
	S.P(2cm^3/L	374	91.34	282.67	9.57	9.44	25.13	390.67	74.67	316	10.17	10.14	26.25
75% menral + 25% human urine	Control	315	77.00	238	9.3	9.44	21.17	327.34	72.67	253	10.74	10.14	22
	S.P(1cm^3/L	318	79.67	238.34	9.7	10.34	21.37	335.34	74.34	262.67	11.3	10.54	22.51
	S.P(2cm^3/L	379.3	85.0	293.6	10.9	10.3	25.4	391.6	80.3	302	11.3	10.7	26.3
50% menral + 50% human urine	Control	67.407	112.34	299	10.77	10.1	27.35	426	100.00	331	11.57	11.4	28.63
	S.P(1cm^3/L	416	115.34	306	11.14	10.14	27.95	426.67	110.67	334	12.4	11.2	28.63
	S.P(2cm^3/L	418	121.00	309.67	12.5	10.94	27.95	436.67	129.00	366.34	12.77	11.87	30.00
25% menral-N + 75% human urine	Control	364.67	80.67	256.67	10.44	9.9	24.46	377.34	71.34	263.34	10.04	10.04	25.33
	S.P(1cm^3/L	376	94.67	270	10.87	9.54	25.27	383.67	80.67	272.67	11.67	10.24	25.74
	S.P(2cm^3/L	384	95.34	303.34	11.2	10.77	25.80	404.67	85.34	304.67	12.1	10.67	27.15
100 % of human urine	Control	405	99.00	295	10.04	9.4	27.22	410.67	89.67	297.67	11.14	10.64	27.55
	S.P(1cm^3/L	405	106.00	295.34	10.67	10.4	27.22	411.67	104.67	315.34	11.24	10.97	27.62
	S.P(2cm^3/L)	405	119.34	300.67	11.57	10.14	27.22	421.34	112.67	339.34	12.6	10.97	28.29
LSD at 5%			18.1	45.2	1.9	0.75	1.7	34.2	17.3	45.2	1.6	0.65	1.9

Table 8. Effect of human urine fertilization and foliar spray with *Spirulina Platensis* extract as well as their interaction on chemical composition of lettuce plants during 2020/2021 and 2021/2022 winter seasons.

Treatments		First Season2020/2021					Second Season2021/2022				
Fertilization	Foliar spray	N%	P%	K%	Total carbohydrates	No3 (mg/kg)	N%	P%	K%	Total carbohydrates	No3 (mg/kg)
100% menral nitrogen fertilizer		1.43	0.28	1.52	9.29	386.67	1.80	0.35	1.54	10.16	444.00
75% menral-N + 25% human urine		1.67	0.32	1.59	10.20	372.66	2.07	0.35	1.65	11.08	420.33
50% menral -N+ 50% human urine		2.03	0.42	1.72	13.15	352.66	2.32	0.46	1.83	13.98	376.33
25% menral -N+ 75% human urine		1.58	0.32	1.63	10.98	329.66	1.98	0.36	1.70	11.87	348.00
100 % human urine		1.87	0.40	1.67	11.93	285.33	2.21	0.42	1.76	12.85	298.00
LSD at 5%			0.06	0.16	1.24	26.7	0.24	0.07	0.09	1.30	29.6
	Control	1.59	0.32	1.55	10.46	382.20	1.93	0.36	1.63	11.28	408.40
	S.P(1cm^3/L)	1.71	0.34	1.63	11.04	351.40	2.08	0.38	1.71	11.88	372.60
	S.P(2cm^3/L)	1.84	0.38	1.70	11.82	302.60	2.21	0.43	1.75	12.80	351.00
LSD at 5%			0.06	0.19	1.26	26.7	0.26	0.07	0.09	1.35	28.7
100% menral nitrogen fertilizer	Control	1.24	0.25	1.43	8.86	421	1.62	0.30	1.47	9.51	486
	S.P(1cm^3/L)	1.47	0.29	1.52	9.21	392	1.85	0.32	1.58	10.12	431
	S.P(2cm^3/L)	1.59	0.32	1.61	9.81	347	1.93	0.34	1.65	10.91	415
75% menral + 25% human urine	Control	1.56	0.28	1.51	9.65	408	1.94	0.32	1.59	10.71	452
	S.P(1cm^3/L)	1.63	0.32	1.59	10.05	386	2.08	0.35	1.66	10.91	413
	S.P(2cm^3/L)	1.84	0.37	1.67	10.91	324	2.19	0.39	1.72	11.84	396
50% menral + 50% human urine	Control	1.95	0.39	1.67	12.51	390	2.18	0.43	1.79	13.12	398
	S.P(1cm^3/L)	2.03	0.42	1.72	13.12	356	2.32	0.47	1.85	13.89	372
	S.P(2cm^3/L)	2.11	0.46	1.79	13.83	312	2.46	0.49	1.87	14.91	359
25% menral-N + 75% human urine	Control	1.48	0.31	1.56	10.18	373	1.86	0.34	1.63	11.21	381
	S.P(1cm^3/L)	1.55	0.34	1.63	10.92	332	1.97	0.37	1.72	11.75	346
	S.P(2cm^3/L)	1.71	0.36	1.70	11.84	284	2.11	0.38	1.77	12.67	317
100 % of human urine	Control	1.76	0.40	1.60	11.11	319	2.05	0.41	1.69	12.09	325
	S.P(1cm^3/L)	1.87	0.39	1.69	11.94	291	2.21	0.42	1.78	12.81	301
	S.P(2cm^3/L)	1.98	0.43	1.74	12.75	246	2.37	0.45	1.82	13.56	268
LSD at 5%			0.07	0.25	2.42	33.8	0.28	0.05	0.09	1.64	28.5

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تأثير التسميد بالبول البشري والرش الورقي بمستخلص سبيرولينا بلاتنسيس على نمو ومحصول وجودة نباتات الخس في الأصص

محمود عبد العزيز محمد عبد المطلب *، لطفى عبد الفتاح بدر*، أحمد سعيد أبو زيد *

***قسم البساتين– كلية الزراعة – جامعة بنها.**

*** قسم الأراضي والمياه – كلية الزراعة – جامعة بنها.**

**** الإدارة المركزية للأراضى والمياه والبيئة، وزارة الزراعة وإستصلاح الأراضى الدقى, جيزة.**

تم إجراء تجربتين من تجارب الأصص خلال موسمي الشتاء 2020–2021 و 2021–2022 في مركز البحوث الزراعية بمحافظة الجيزة ، مصر ، بهدف دراسة استخدام بعض مصادر النيتروجين غير التقليدية مثل البول البشري والرش بعض منشطات النمو مثل المستخلص الورقي سبيرولينا بلاتنسيس لتحسين النمو الخضري والمحصول ومكوناته وكذلك صفات التركيب الكيميائي لبعض محاصيل الخضر الورقية مثل الخس (*Lactuca sativa, L.*) والجرجير (*Eruca sativa, L.*). اشتملت هذه التجربة على 15 معاملة ، عبارة عن مزيج من خمسة معاملات بالبول البشري والأسمدة وثلاث معاملات بالرش الورقي باستخدام مستخلص سبيرولينا بلاتنسيس. تمت إضافة معاملات التسميد البشري للبول بجوار النباتات مقسمة علي ست دفعات خلال موسم النمو: الدفعة الأولى بعد 21 يومًا من الزراعة والدفعة الثانية بعد 10 أيام من الدفعة الأولى ، ثم كل 10 أيام على فترات. وتمت إضافة الرش الورقي بواسطة الرش بمستخلص سبيرولينا بلاتنسيس أربع مرات خلال موسم النمو ، الدفعة الأولى بعد 21 يومًا من الزراعة وكل 15 يومًا على فترات. تم استخدام تصميم القطعة المنشقة باستخدام ثلاث مكررات ، حيث تم وضع السماد المعدني ومعاملات البول البشري في القطع الرئيسية وتم توزيع معاملات الرش الورقي باستخدام مستخلص سبيرولينا بلاتنسيس بشكل عشوائي في القطع الفرعية. أظهرت النتائج المتحصل عليها أن نباتات الخس التي تمت معاملتها بـ 3.3 جم نترات الأمونيوم مضافاً إليها (0.3 مل من بول الإنسان المخفف بـ 600 مل من الماء) لكل أصيص بحيث يتم إضافتها علي ست مرات خلال موسم النمو: الدفعة الأولى بعد 21 يومًا من الزراعة والدفعة الثانية بعد 10 أيام من الدفعة الأولى ، ثم كل 10 أيام على فترات متقطعة مع رش أوراق النباتات بمستخلص سبيرولينا بلاتنسيس بتركيز 0.5 مجم / 12 مل من الماء: تضاف علي أربع مرات ، تبدأ بعد 21 يومًا من الزرع وكل 15 يومًا على فترات متقطعة للحصول على نمو خضري جيد ، محصول أعلى بالإضافة إلى صفات جيدة للتركيب الكيميائي وبأفضل جودة.