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Vegetative growth response of beets and lettuce to stored human urine

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Abstract. In this work, we present the experimental results of the effect of stored human urine (SHU) on the growth of beets (*Beta vulgaris* L) and lettuce (*Lactuca sativa* L). We apply different amounts of SHU according to the recommended dose of nitrogen, considering soil from farmland and vermiculite as substrates. The last allows us to determine with high precision the isolated effect of SHU over the vegetative development of beet plants, without considering other nutrients present in common soils. Experimental results demonstrate that the application of SHU has no significant effects on lettuce vegetative growth under our soil conditions. In contrast, SHU can be used successfully as a fertilizer of beets. The optimum dose was found at 120 kg N ha^{-1} and resulted in average dry weight of 125 g. However, if the dose exceeds the optimum levels, the growth of the plant is inhibited. Beets fertilized with SHU does not pose any hygienic risk for human consumption. Our findings represent a promising alternative to propose expanding the use of SHU as fertilizer in medium-sized greenhouses and to provide benefits to families in rural areas, with little or no available water supplies.

Key words: lettuce, beets, human urine, fertilizer, nitrogen.

INTRODUCTION

The production of food in the field requires water and fertilizers. However, there are large areas of water scarcity in the world that affect millions of people, many of whom are poor and disadvantaged. Therefore, there is a growing need to use fertilizer and water in smarter ways to improve the yield production of vegetables. Among other requirements, the macronutrients as nitrogen (N), phosphorus (P), and potassium (K) are essential for the plant development, including its growth and nutritional content (White et al., 2010; Vatansever et al., 2017). Therefore, fertilizers become indispensable for agriculture practices. Except for legumes (Bibi et al., 2016), many crops have benefited from fertilization with nitrogen (Pavlou et al., 2007; Liu et al., 2014), but in parallel several environmental and health problems are attributed to the misuse and excessive use of commercial fertilizers (van der Ploeg et al., 2001). The search for alternative fertilizers, economically accessible for people living in rural regions, suggests the application of human urine as a nitrogen source. In the literature there is a vast amount of work dealing with the treatment and utilization of human urine for agricultural purposes (Maurer et al., 2006; Pradhan et al., 2007; Pradhan et al., 2010; Richert et al., 2010; Wohlsager et al., 2010; Makaya et al., 2014; Andersson et al., 2015). The urine is a natural by-product of human metabolism, constituted by 91–96% of water, salts, organic compounds. Urine is widely available; an adult person produces around 1.3 L of urine per day. Likewise, the chemical composition of human urine depends on diet and health factors. Urine contains large quantities of N (90%), K (65%), and P (55%) (Heinonen-Tanski et al., 2005), which are favorable for vegetative plant development (Nagy & Zseni, 2017). Perhaps, one of the main concerns of consuming vegetables fertilized with human urine are the hygienic and taste properties. For the first issue, the urine of healthy persons is considered sterile up to it flows through the urethra. It is well known that urethra is covered by epithelial cells that contain bacteria (Colleen et al., 1980). Usually, freshly dejected urine contains < 10,000 bacteria in one mL (Tortora et al., 1989). The persistence of bacteria in urine depends on pH (Thornton et al., 2018), while the viruses are more frequently related to temperature (Höglund et al., 2001; Vinnerås et al., 2008). The pH of freshly dejected urine is ~6.5, and after urea hydrolysis, urine could reach up a pH ~9 enabling urine sanitization (Höglund, 2001; Senecal et al., 2018; Thornton et al., 2018). Therefore, it is recommended to store urine for up to 6 months to guarantees an increase in pH, and consequently, neutralize all pathogens (Bischel et al., 2015). In this regard, human treated urine can be seen as a low-cost solution, available on each house, and requiring a minimum amount of water.

The goals of this research are to evaluate the use of stored human urine as fertilizer on (i) vegetative development of beet (*Beta vulgaris* L.) and lettuce (*Lactuca sativa* L.) plants. (ii) Test the hygienic quality of the plants to ensure no risk for human consumption and (iii) determine to some extent if the farm soil is suitable for the agriculture production of these vegetables. This paper focuses on testing the effect of different doses of nitrogen contained in urine on the growth of plants. We compare the yields of beets when vermiculite and farm soil substrates are used.

The state of Puebla occupies the second and third place in beet and lettuce production at national level. These plants grown in domestic or regular size farms, with the potential of benefiting families in rural areas of Puebla. The quality of the irrigation water compromises the production of these vegetables. In Puebla, only one-third of the 2,248 municipalities carry out water treatment. Then it is crucial to ensure and measure the hygienic quality of urine, substrate, harvested plants, and water used for irrigation.

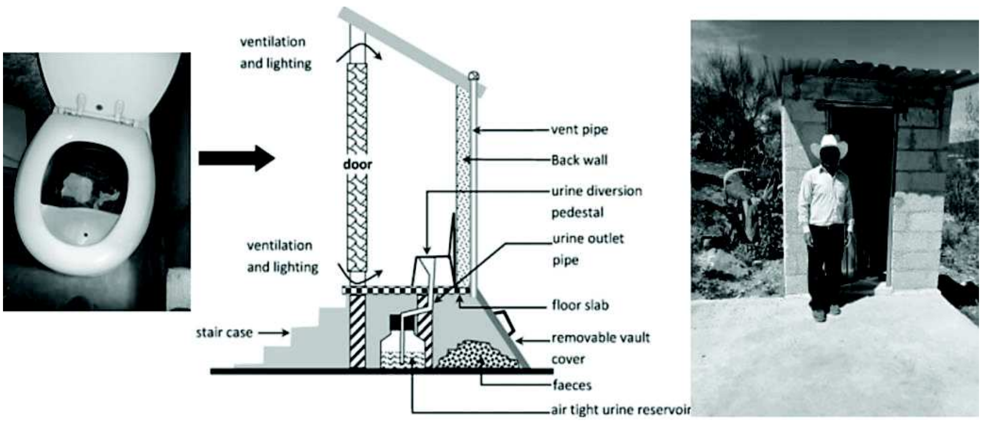
MATERIALS AND METHODS

Temporal and spatial domains for the study

We conducted experiments on vegetative development in three stages. From July to August 2017 (60 days) the lettuce growth (weight, size) was studied using farm soil as a substrate. Two additional experiments were performed to evaluate the growth (weight) of beets. From September to December 2017 (73 days) using farm soil as substrate, and from June to August 2018 (62 days) using vermiculite as a substrate. The experiments were carried out at the Greenhouse of the University of Puebla, located at 19.00 °N latitude and -98.20ºW longitude with an area of 24 m^2 at an altitude of 2,135 MASL. These facilities are suitable for the development of plants, equipped with the essential requirements to grow plants, including an entrance locker to minimize contamination by external agents.

The urine and soil samples were collected from San Bernardino Tepene, located in the region between the Sierra del Tentzo and the Valsequillo depression, as shown in Fig. 1. The area, in general, presents rugged hills, calcareous and barren, limestone, and arid hills that rise on the Poblana plateau. According to the World Reference Base (Martínez-Villegas, 2007; FAO, 2015), these semi-arid areas are classified as regosols, which usually allow for incipient production and are complicated to handle. On average, these soils have between 167 to 200 mg N m^{-3} (Batjes, 1996; Premanandarajah, 2017). Besides, these soils have a low moisture retention capacity. Consequently, techniques such as drip or spray are required, which is not an economically viable alternative for the inhabitants. The weather in this region is identified as CWA (Köpen et al., 1918); this corresponds to temperate sub-humid climate, with rains (800 mm yr^{-1}) between May and September. The mean temperature of the warmest month (May) exceeds 23 °C.

a)



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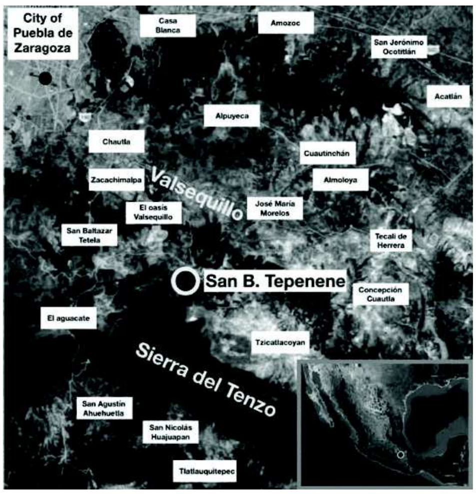


Figure 1. a) From left to right: UDDT, schematic design and, the facility installed in b) San Bernardino Tepenene, Puebla (18.87°N, -98.09ºW), ubicated among the Sierra del Tenzo and Valsequillo depression.

Sampling

The farm soil and commercial vermiculite were the two types of substrates used in this project. As it is intended that the results of this work can be applied in situ, soil samples were collected directly from the family farm of San Bernardino Tepenene. Soil samples were taken later to our greenhouse at the University of Puebla. The other substrate used in our experiments was the Vermiculite, collected from the Agronomy Department of the University of Puebla, in the commercial form 'Agrolite' in 100 L bags, and later analyzed and put in the corresponding 6 L pots.

Lettuce and beets seedlings were carefully obtained two weeks after germination. Intending to simulate the farmer's behavior of acquiring seedlings for cultivation, and at the same time, it is more favorable to start the experiment with ready-made seedlings than trying first to achieve germination of the corresponding seed in the pots. These facts are an advantage for us in comparison with the procedures of other authors (Taylor, 1997). Another essential element in our research was the water, which comes from a University cistern. Microbiological analysis and physical-chemical parameters were measured to inspect the microorganisms or variables that have relevance in the results of the experiment.

The urine was collected in a familiar ecological toilet with urine separation, known as Urine-diverting dry toilet (UDDT), and ubicated at the community of San Bernardino Tepenene in Puebla (18.87ºN, -98.09ºW). Fig. 1, a shows the UDDT, the design and the facility. These follow the literature recommendations (Larsen et al., 1997). We collected urine from all family members. Recent studies indicate that there is no difference between the effect that urine of men and women can cause in vegetable development (Duniya, 2018). Urine management and storage is based on other experiments (von Münch, 2011).

Experimental design and treatments

Sixty kilograms of the farm soil sample was deposited on a clean surface to homogenize while water was added up to reach 60% moisture in the soil sample. Each tool used for the substrate manipulation was previously cleaned to reduce the contamination vector for the plants, and avoid interference with the further microbiological analyses. The soil then was ready to be transferred into equal plastic containers, 6 L pots. These will serve, as explained later, to perform experiments with lettuce and beets. Also, we collect vermiculite in commercial form 'Agrolite' in 100 L bags. Both farm soil samples and vermiculite were subjected to various tests to measure different parameters.

It is recommended to store urine up to six months to reduce the levels of risk agents (e.g., pathogens) that could make the consumption of vegetables fertilized with urine dangerous (Wielemaker et al., 2018). Also, the World Health Organization (WHO) recommends reaching elevated pH (~9) and high ammonium ($NH4^+$) concentration, in combination with warm temperatures. For our experiment, the collected urine was stored in a sealed (20 L) container for six months at room temperature (20.6 ± 2.6 °C). The container was collocated in the shade to ensure a fresh environment to reduce the risk of nitrogen evaporation and bad odors. Hereafter, we use the term urine dose, as the amount of nitrogen per hectare (kg N ha^{-1}) supplied to the plant. The urine dose is considered our study factor; this means we analyzed the vegetative response to different doses of SHU. Based on the literature (Mnkeni et al., 2008; Andersson et al., 2015; Mamani-Mamani et al., 2015), we selected doses for our experimental vegetables. Table 1 shows the different doses applied since the first day up to the harvesting day, known as treatments, and their corresponding equivalences in liters and nitrogen grams. Table 1, a reveals four treatments (LD_1, …, LD_4) for lettuce cultivated in pots filled with farm soil, and Table 1, b presents the treatments (BD_1, ..., BD_5) for beets grown in both farm soil and vermiculite. The values summarized in Table 1, correspond to the suggested amounts of nitrogen by a hectare (kg N ha^{-1}). The equivalent doses for our plants were calculated based on our pot dimensions. Each pot has a soil surface ~0.062 m^2, in agreement withthis, we calculate the total equivalent liters (L) and grams of nitrogen (g N) that must receive each plant. The second and third columns of Tables 1, a and 1, b show the equivalences. Equivalences are intending to make more reliable the possibility of practical consulting from the population. Then each total dose is divided into three identical proportions that are supplied to plants every ~15 days (on days 1, 15 and, 30). These, in turn, were previously diluted with water (1:4), with the aim not damage the plant with the direct application of urine. Finally, pour the mixture around the base of the seedling stem.

Table 1. Treatments for cultivated (a) lettuce in farm soil and (b) beets in farm soil or vermiculite as substrate. The dose and their corresponding equivalence in each pot (~0.062 m^2) are given

a)

	Lettuce		
	(kg N ha^{-1})	(L)	(g N)
LD_1	0	0	0
LD_2	66	0.09	0.40
LD_3	132	0.18	0.81
LD_4	198	0.28	1.21

b)

	Beets		
	(kg N ha^{-1})	(L)	(g N)
BD_1	0	0	0
BD_2	30	0.04	0.18
BD_3	60	0.08	0.36
BD_4	120	0.17	0.73
BD_5	240	0.33	1.46

Experimental Units

For the first experiment, we select from a sample of 100 units the 32 of best lettuce specimens, those that present at our discretion the better vegetative development. Using the R package ´agricolae´, randomly formed four groups (LD1, LD2, LD3, LD4) corresponding to the respective doses shown in Table 1, a. These, in turn, each one consisting of (8) lettuce plants, planted in their corresponding pots filled with farm soil and positioned randomly in four rows in the table of greenhouse facility. Lettuce plants received their respective doses of SHU on days 1, 15, and 30. Likewise, each pot was irrigated with 500 mL of water, in which case the precaution was taken to return the leachate to the pot. The lettuce plants were harvested at day 60, and the dry weight of the plant, the width, and length of the leaves were measured.

Similarly, for the second experiment, we selected thirty (30) of the best beet specimens from 100 units and, form five groups, each one of 6 elements. The corresponding dosages of SHU, shown in Table 1, b, were supplied on days 1, 15, and 30. Again, each pot filled with farm soil was irrigated with 500 mL of water provided every two days. Finally, on day 73, beets were harvested, and the dry weight parameter was measured.

For the third experiment, we selected the best forty (40) beets, from a sample of 100 units, and five groups were formed, with eight members each. However, this time, beets were planted on vermiculite, and 1 mL of nutritional solution (Qfuska Foliar; 5N-15P-5K) was added on the first day. Then we add the respective 500 mL of water every two days, as well as the application of SHU on days 1, 15, and 32. Finally, harvests on day 62 and measure the dry weight of the plant.

Response variables

The response variables chosen in this work, correspond to those that best reflect and characterize the object of study. These are the dry weight of the plant and, length and width of the plant's leaf. All of them measured at the harvest day. For each leaf, dimensions were calculated using a meter rule (± 0.5 mm), and for each plant and dose, the average length and width were calculated. After measuring the fresh plant dimensions, we proceed to wash the plant and start the drying process. The process consists of collocating the plant in a paper bag inside an oven set to low heat (140 °F or 60 °C). The plants dry up them get cool and measured on a scale (Ohaus EX223 milligram laboratory balance). The same process is repeated for each plant per treatment to obtain dry weight averages. However, we must warn that there is a possibility that a few plants die during the experiment. We will make sure that this has nothing to do with the increase in the dose of urine, but other random factors. Research on the correlation of urine dose and plant development was done using statistical analysis. We explore the data distribution and performed variance analysis (ANOVA). Also, the Tukey test incorporated in analysis tools in R allows for multiple comparisons between the averages of each treatment. It was interesting to propose vermiculite as a substrate to study, without the effect of other organic substances, the correlation between the response variables, and the different doses of stored human urine. The significance level requires to have p-values > 0.05 to declare significance.

Response variables

Table 2 summarizes the measured parameters in sample elements: urine, farm soil, plants, water, and soil. The study of hygienic quality of the plants results in negative for coliforms, Salmonella, molds, and yeasts. The urine quality results, show as expected, absence of E. coli. This bacterium typically has short survival in the urine. Then, this is not a suitable indicator of fecal contamination. Other indicators in urine, like gram-negative bacteria; Salmonella, and Aerobic mesophilic bacteria (BMA) resulted in negative, under the standard norms, indicating a low risk of gastrointestinal infections. These results are coincident with the results obtained for urine stored at 20 °C (Senecal et al., 2018), were no risk to health was found. Transmitted diseases via urine are considered a limited risk in tempered countries. The measured amount of nitrogen in SHU was 4.37 $g\ L^{-1}$, similar to 4.03 $g\ L^{-1}$ in Bolivian study (Mammani et al., 2015). Water measurements show this element, in general, is adequate for plant irrigation. Electric conductivity (EC), which is a good proxy of water salinity, is equal to 1.25 dSm^{-1}. Indicating there is slight to moderate restriction of use, according to the recommended levels ($0.7 < EC < 3.0$) from FAO Soils Bulletin 10 (FAO, 1970). Most plants are sensitive to sodium and chloride irrigation. Ions results were $Na^{+1} = 94.3\ mg\ L^{-1} \sim 4.1\ mmol\ L^{-1}$. Meaning a slight to moderate degree of restriction of use as irrigation water, while in case of $Cl^{-1} = 84.0\ mg\ L^{-1} \sim 2.4\ mmol\ L^{-1}$ indicate none degree of restriction on use. Worth noting that no health risk elements were found in water samples.

Table 2. Summary of resulting parameters measured in sampling elements (urine, farm soil, water, and plant) and their corresponding technique

Parameter			Element			
	Units	Method	Urine	Farm Soil	Water	Plant(2)
pH		NMX-AA-008	8.94	8.28	7.10	
Electric Conductivity	**	Conductometer	31.2		1.25	
Calcium (Ca^{2+})	*	EDTA method	289.9	280.54	130.4	
Magnesium (Mg^{2+})	*	EDTA method	50.7		26.0	
Sodium (Na^{1+})	*	Flamometry	1400.0		94.3	
Potassium (K^{1+})	*	Flamometry	1860.0		13.4	
Sulfate (SO_4^{-2})	*	NMX-AA-074	710.0		232.0	
Phosphates (PO_4^{-3})	*	NMX-AA-029	180.9		2.6	
Carbonates (CO_3^{-2})	*	Volumetry	576.0		25.6	
Bicarbonates (HCO_3^{-1})	*	Volumetry	90.0		366.0	
Chlorides (Cl^{-1})	*	NMX-AA-073	367.5	53.17	84.0	
Fe	*	Atomic Absorption	8.9	54411.5		
Cu	*	Atomic Absorption	0.0	31.6		
Mn	*	Atomic Absorption	2.4	326.5		
Zn	*	Atomic Absorption	0.1	81.6		
Coliforms		NOM112-SSA1-94	(-)	(-)	(-)	(-)
AMB		NOM092-SSA1-94	(-)	(-)	(-)	(-)
Salmonella		NOM114-SSA1-94	(-)	(-)	(-)	(-)
Molds and yeast		NOM111-SSA1-94	(-)	(-)	(-)	(-)

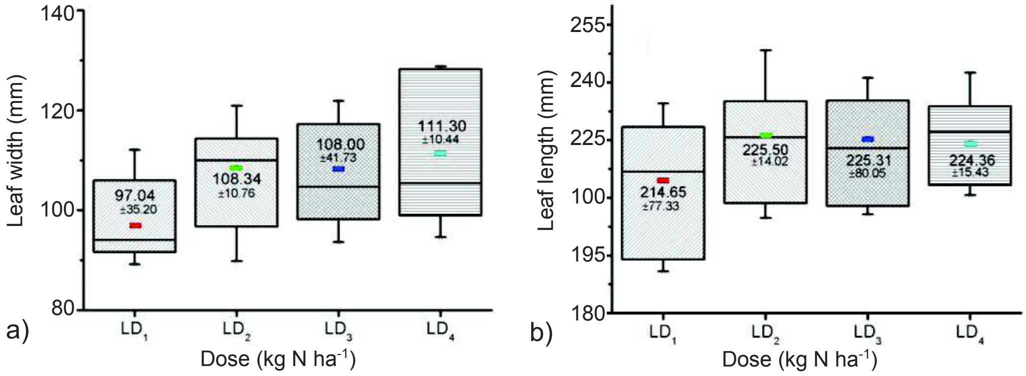
(1) farm soil sample measurements; (2) both lettuce and beets show the same results; AMB = Aerobic Mesophilic Bacteria, and (-) stands for negative; Units equivalences: * (mg L^{-1}), ** (dS m^{-1}).

Lettuce

Lettuce dose-response results of leaf dimensions (width and length) and the plant dry weight are shown in Fig. 2. It is observed that none of these variables has a significant increase with doses. We removed two data points from Fig. 2 these were found in LD_1 and LD_2 treatments, and both correspond to plants that died. The analysis of the dry weight of the plant, Fig. 2, c, shows that the variation of the measurements increases as the dose increase, and we cannot detect a significative change in this variable with the increase in dose. From the analysis of variance, *p*-values of 0.308, 0.412 and, 0.258 were obtained, these correspond to the width, length and dry weight of the plant respectively. This means that there is no significant influence of dose on each one of the response variables for the lettuce.

Beets

In contrast to the development of lettuce under SHU, in case of beet fertilization with SHU, we can observe from Fig. 3, that each dose benefits the plant development. Mainly, the fourth dose (BD_4) generates a substantial increase in the dry weight of the plant. However, at the same time, it is to remark that the growth of the plant is inhibited for the higher concentration dose (e.g., BD_5). To interpret the results more transparent, we have removed one data point, this accounts for BD_3 treatment, and corresponds to died plant. The results of the statistical analysis show that the BD_4 dose significantly affects the dry weight of the plant (p-value = 1.99×10^{-4}). Moreover, also Tukey test results show, from the mean values of treatments, two groups, G_1 and G_2, which means that there is a significative difference between BD_4 treatment and the rest of the treatments, as seen in Fig. 3.



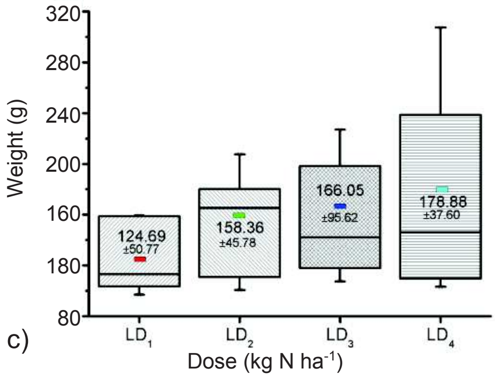


Figure 2. Variation of mean (a) width, (b) length, and (c) dry weight as a function of the supplied dose of SHU in 32 units of Lettuce plants cultivated in pots filled with farm soil substrate. Each box displays the mean value and its corresponding standard deviation. A horizontal line represents the median, and the whiskers correspond to the minimum and maximum values, respectively.

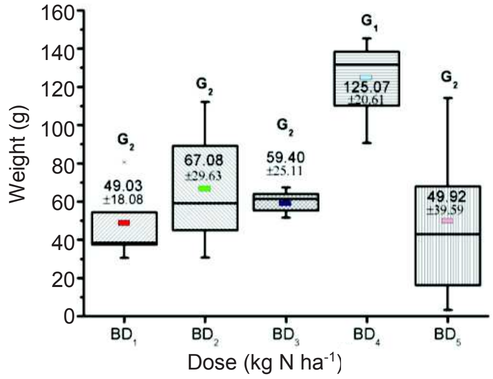
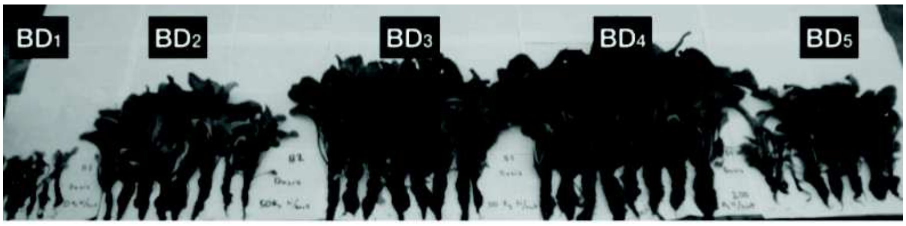


Figure 3. Variation of the mean dry weight of beets (30 units) cultivated in pots filled with farm soil as a function of the supplied dose of SHU. The mean dry weight of beets (filled squares), standard deviation, median (horizontal line), and their Tukey group (G_i) are shown for each dose.

Fig. 4, a shows the development of the beets under different treatments using vermiculite as a substrate. We could appreciate that BD_4 treatment provides the best yield. Fig. 4. shows the corresponding mean dry weight of beets at different doses of SHU. From BD_1 up to BD_4 dose, an increase in the dry weight of beets was shown, similar to Mnkeni et al., (2008), and also agrees that after BD_4 dose, the growth of the plant is inhibited. The maximum mean dry weight (123.52 ± 21.14 g) for beets cultivated with vermiculite differs a 2% from the maximum mean dry weight (125.07 ± 20.06 g) for beets grown with farm soil. The difference could be explained by the farm soil nutrients, absent in vermiculite. As we already mentioned, the farm soil corresponds to a region where the regosols are the characteristic soil type, deficient in nutrients, and high in calcium.

a)



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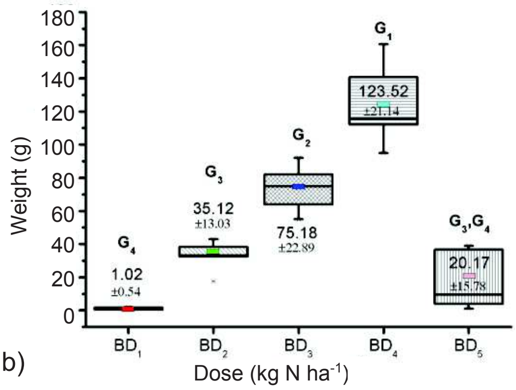

Figure 4. Shows (40 units) beets (a) development and their corresponding (b) mean dry weight at different doses of SHU using vermiculite as substrate. The mean dry-weight of beets (filled squares), standard deviation, median (horizontal line) and their Tukey group (G_i) are shown for each dose.

Statistical analysis of the mean dry weight of beets confirms that each dose (except for BD_5) significantly affects the dry weight of the plant. The calculated p-value = 1.45×10^{-15} is eleven orders of magnitude smaller than the corresponding p-value for farm soil. This fact, confirms our experimental hypothesis about the vermiculite allows us to study the potential impact SHU supply without considering the soil nutrients. Fig. 4, b shows the mean dry weight of beets at different doses of SHU, and using vermiculite as a substrate. A filled square represents the mean dry weight of beets. And with the aim of more relaxed reading, the numerical value and the standard deviation were added to the side. The corresponding Tukey group (G_i) for each dose is also shown. Tukey test enables us to detect four groups (G_1, G_2, G_3, G_4). Except for the higher dose (BD_5), which shows the coupling of two groups (G_3, G_4) and reduces the development of the plant, all others contain only one group.

In summary, we found that lettuce yield was not benefited under any treatment. While for beet, it was observed that increasing the dose (up to a specific critical value) increases the dry weight of the plant. This difference could be explained since the plants, with different physiology, respond differently to certain factors, specifically to the salinity of the soil, and this, in turn, could help to explain the observed differences. Lettuce, is sensitive to salinity (Ayers et al., 1951; Osawa, 1965; Bernstein et al., 1974), while the beet is tolerant (Bower et al., 1954). Finally, the extrapolation of these results to the region, or over the world, should be taken with cautions. Since soils can be different and characteristics of human urine in terms of the critical nutrients can vary, place to place. The variation of nutrient contents in human urine is attributable to the environmental conditions, physical activity, as well as the diet of the urine donor (Rose et al., 2015). Studies in Finland (Pradhan et al., 2007) and Africa (Mnkeni et al., 2008) report 8.3 g N L^{-1} and 7.4 g N L^{-1} respectively, while in Bolivian study (Mamani et al., 2015) 4.2 g N L^{-1}, similar than our urine measurements (4.37 g N L^{-1}), in contrast, Sweden studies (Kirchmann & Pettersson, 1995) reported 1.79 g L^{-1} to 2.9 g N L^{-1}.

CONCLUSIONS

Based on a review of literature, the lettuce and beets in pots were fertilized with SHU doses. Results show that SHU as fertilizer at the 120 kg N ha^{-1} dose increases the weight of the beet plant and that higher doses inhibit the growth of the plant. The results on the dry weight of beet with both farm soil and vermiculite, allow us to establish with accuracy that the SHU correlates with the growth of the beet plant. In contrast, lettuce did not experience significant changes when fertilized with SHU. We also verify that our experimental samples of farm soil contribute slightly to the vegetative development of the plant when used in combination with the SHU. In other words, the only use of SHU does not maximize beet growth. The farm soil composition improved the dry weight of the plant by 2%; the small difference is due to the lack of farm soil nutrients in regosols founded in the region. However, we could speculate that the farm soil composition considered represents a promising substrate. When, compared with other results (Mnkeni et al., 2008), that uses similar doses of nitrogen on beets, they reported four times lower dry weight of beets.

Therefore, we can carry out the plantation on a larger scale for the production of beets at the local level, on the farm soil, under the explained warnings. In this regard, once the appropriate dose for the beet was identified. We proceed to estimate the possible economic benefits of using human urine as a fertilizer instead of commercial fertilizer, resulting in saving about 780 USD $yr^{-1}ha^{-1}$. This number is a hopeful result for families in peripheral zones of Puebla. However, the urine required to fertilize this same area requires the daily contribution of 50 people, ~12–13 families, and leads to other problems of logistics, distribution, storage, and sanitization, that need further research. Besides, beets are close to the ground and, therefore, insects, and microbial contamination can play an important role. In this regard, our research group is conducting more studies to include these aspects.

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