Application of human urine from dry toilets as natural fertilizer in onion (*Allium cepa* L.) cultivation – an chlorophyll indicator study

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Abstract
Fermented human urine from the model dry toilet facility was used as natural fertilizer in small-scale cultivation of onion (*Allium cepa* L.). Model agricultural field was constructed assuming *Latin square* approach. The 6 fertilization modes were tested: mineral fertilizer (ammonium nitrate 170 kg N/ha), fermented human urine (60, 120, 170, 240 kg N/ha), as well as cultivation without any fertilization (reference cultivation sectors). Fertilization effects were quantified in respect to chlorophyll (a, b, total) content in the green part of the plant. The lowest chlorophyll contents corresponded to non-fertilized plants and to plants fertilized only with NH_4NO_3. All four plant samples representing the fermented human urine application demonstrated distinctly higher all chlorophyll contents. Fermented human urine dose of 120 kg N/ha turned out to be the best for all chlorophyll contents in the *Allium cepa* L..

Fermented human urine, natural fertilizer, onion Allium cepa L., chlorophyll, nutrients bioassimilation

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Introduction

Scientific research confirmed that improper and excessive use of mineral fertilizers is responsible for soil degradation, especially in its organic fraction, resulting in systematically decreasing crop yields [1]. To counteract this tendency ecological cultivation methods are applied [2], including human urine application [3-8], especially collected from the dry toilet systems [9-10]. The method is economical, ecological and relatively simple in use. Based on the global research results, one can suppose that this form of fertilization can be a promising alternative of fertilizing in the near future. However, specific requirements of small-scale cultivation fields require new research providing reliable data concerning optimal dose and application form of this natural fertilizer. The work focused on selected aspects of application of the post fermented human urine as natural fertilizer in small-scale gardening (prospects and limitations), with special emphasis on selected natural indicator of crops properties (chlorophyll content).

Materials, cultivation methods

Onion (*Allium cepa* L.) is one of the most commonly cultivated vegetables worldwide, known from its scientifically verified antioxidant properties (effect of presence of flavonoids – flavonols, anthocyanins – and other phenolics). Some other valuable compounds like polysaccharides, minerals, selected vitamins and other nutrients were also detected in onion [11-24].

Onion was used as a research object focusing on the effect of natural fertilizer (fermented human urine) dosage and its distribution method on chlorophyll (a, b, total) content in its green part.

According to previous research results in the field existence of some long-term correlations between water and various mineral contents, NO_3-N, bioavailable phosphorus and K in the cultivation area and onion crops quality have been demonstrated. Nutrients accumulated in the soil could also indicate some negative impact on the plant's mineral composition, thus scientifically-based program for sustainable onion fertilization can be elaborated. Application of classical organic fertilizer (organic materials with addition of minerals: illite and mountainous soil) provided stable NO_3-N concentration, affecting also number of natural soil microorganisms. Contrary, application of chemical fertilizer (reference system) resulted in appearance of rapid oscillation of this concentration leading to unstable growth conditions. Effect of biostimulant on onion growth conditions is also reported [25-27].

Chlorophyll, more precisely mixture of chlorophyll a and chlorophyll b, is a complex bioorganic, strong antioxidant substance, from chemical point of view similar to hemoglobin. It demonstrates some advantageous effects including annihilation of some xenobiotics, blood detoxification possibility, skin cleaning and metabolism adjustment [28].

Chlorophyll was purposefully selected as an convenient indicator of plant quality and primary production [29, 30, 31], providing direct information about nutrients bioassimilation [32, 33, 34], thus biological efficiency of the fertilizer dose/form variant. Since chlorophyll concentration attains a plateau in properly and satisfactory fertilized crops it can be used as an convenient indicator of fertilization progress directly [35]. Moreover, because of its properties it can be also used as useful indicator of: food processing (freezing) [36, 37], plant sensitivity towards UV-B radiation [38], chilling stress in plants [39], plant growth [40], drought tolerance [41], environmental stress [42], flood adaptation [43] or potato tuber yield in the water-shortage conditions [44].

Human urine [45-48] was collected using modified male lavatory, reflecting the idea of separating dry toilet, and stored hermetically sealed for 3 months in temperature 20ºC for natural fermentation run (finally resulted in 5.6 g total Kjeldhal N/dm^3). Experimental cultivation field covered 36 sectors – each of 1 m^2, 10 rows each with 10 plants, with ca. 10 cm distances between the plant rows. Test time (cultivation) was 14 weeks. Model agricultural field was constructed assuming *Latin square* approach (Fig. 1a). The 6 fertilization modes were tested applying: mineral fertilizer (ammonium nitrate 170 kg N/ha), fermented human urine (60, 120, 170, 240 kg N/ha), as well as no fertilization (reference cultivation sectors). Mineral fertilizer (NH_4NO_3) was dosed as granules while natural fertilizer represented by fermented human urine – in a liquid form after water dilution of original urine content in proportion of 1 : 5 (volume based). Each fertilization mode was repeated 6 times to fulfill the mentioned 36 sectors (Fig. 1b).

Analysis and results

Chlorophyll concentration in green parts (Fig. 1c) of the onion was determined with analytical method (Fig. 1d). The following procedure was used. Precisely determined mass of green part (0.5 g) from each sample was isolated and homogenized in 25 cm^3 of acetone (cooled to 0°C) with the use of manual triturate in a laboratory mortar. Resulting suspension was filtered through double layer gauze into laboratory measuring flask (100 cm^3) insulated with aluminum leaf. The resulting deposit was washed with small portions of acetone up till whole chlorophyll content had been leached. Volume of the post-leaching extract was measured and supplemented with distilled water to obtain 80% aqueous solution (volume based). Prepared this way plant samples (18 – 3 samples representing each from 6 fertilization modes) were analyzed using spectrophotometer UV/VIS Cary 50 Scan Varian with the spectrophotometric method. Analysis within the 400–700 nm range was done in each case (Fig. 2). For determination of chlorophyll a, b and total chlorophyll contents the absorbance values corresponding to 645 and 663 nm wavelengths (including contribution of aqueous/acetone solution background) were used for the calculations with the following equations:

$$c_a = 12.7A_{663\,nm} - 2.7A_{645\,nm}$$

$$c_b = 22.9A_{645\,nm} - 4.7A_{663\,nm}$$

$$c_a + c_b = 20.2A_{645\,nm} + 8.0A_{663\,nm}$$

Calculated values of chlorophyll a, b, as well as (a+b) (total) contents, corresponding to all six fertilization modes, as well as full graphical results of spectrophotometric analysis within the 400–700 nm range with characteristic chlorophyll peaks are presented in Table 1 and in Fig. 2, correspondingly.

a b

c d

Fig. 1 Small farm/garden scale experimental cultivation of onion (*Allium cepa* L.) – practical tests of fermented human urine application as an natural fertilizer: a) model agricultural field constructed assuming *Latin square* approach before experiment, b) model agricultural field with the onion (*Allium cepa* L.) mature crops, c) green part of onion (*Allium cepa* L.), d) samples of onion crops before chlorophyll leaching followed by spectrophotometric analyses.

Table 1
Effect of fertilization mode on chlorophyll content in *Allium cepa* L. (chlorophyll a, chlorophyll b, total content of chlorophyll a and b).

Fertilization mode		Absorbance at:		Chlorophyll content [mg/dm^3]		
		645 nm	663 nm	a	b	(a + b)
1	Mineral fertilizer NH_4NO_3	0.163	0.245	2.67	2.58	5.25
2	None fertilizer	0.162	0.244	2.66	2.56	5.22
3	Fermented urine – 60 kgN/ha	0.175	0.285	3.15	2.67	5.82
4	Fermented urine – 120 kgN/ha	0.215	0.383	4.28	3.12	7.40
5	Fermented urine – 170 kgN/ha	0.177	0.294	3.26	2.67	5.93
6	Fermented urine – 240 kgN/ha	0.190	0.320	3.55	2.85	6.40

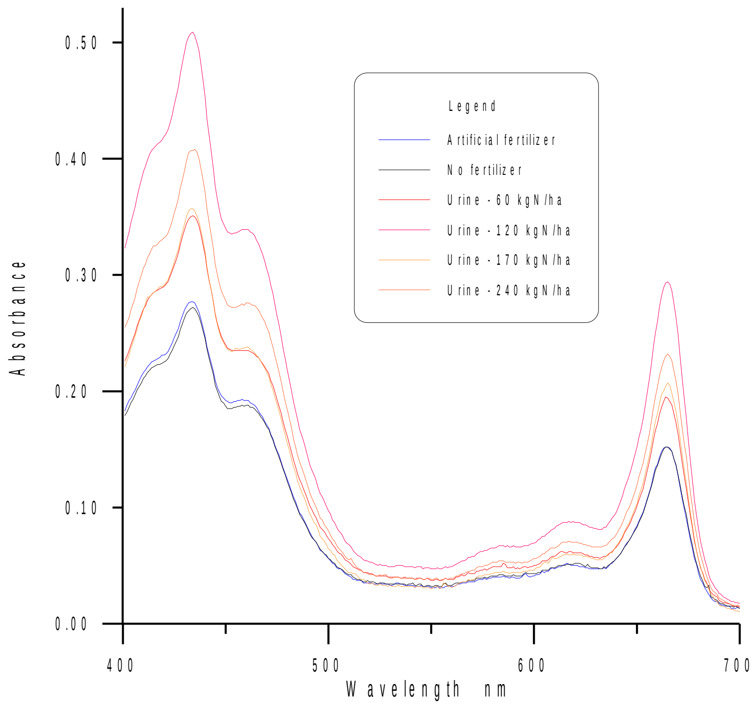

Fig. 2 Effect of fertilization mode on chlorophyll content in *Allium cepa* L. (absorbance within 400 – 700 nm wavelength).

Discussion

Based on spectrophotometer analysis one was able to determine some correlation between fertilizer type (natural, mineral – indirectly distribution form: granules, liquid solution) and its dose on chlorophyll a, b and (a+b) contents in the *Allium cepa* L. In all 6 sample types characteristic peaks occurred at: 434, 460, 584, 617 and 665 nm wavelength, representing characteristic absorption spectra for chlorophyll. However, diversification in absorbance was clearly observed – the highest absorbance values were attributed to sample representing fermented human urine as an natural fertilizer, with doze of 120 kgN/ha. The lowest absorbance values, practically overlapping, suggesting the lowest chlorophyll contents, corresponded to non-fertilized plants (chlorophyll a: 2.66 mg/dm^3, chlorophyll b: 2.56 mg/dm^3, total chlorophyll: 5.22 mg/dm^3), as well as to plants fertilized with ammonium nitrate (chlorophyll a: 2.67 mg/dm^3, chlorophyll b: 2.58 mg/dm^3, total chlorophyll: 5.25 mg/dm^3). All four samples representing results of fermented human urine application demonstrated distinctly higher chlorophyll contents (chlorophyll a: 3.15–4.28 mg/dm^3, chlorophyll b: 2.67–3.12 mg/dm^3, total chlorophyll: 5.82–7.40 mg/dm^3), what speaks for better condition of plants and can be regarded as an evident proof of (fermented) human urine superiority over classical mineral fertilizers like ammonium nitrate. Higher chlorophyll content can be used as an indicator of nutritional advantages, better and higher N bioavailability. Moreover, its higher content is also favorable in respect to *Allium cepa* L. taste and nutrient properties. Fermented human urine dose of 120 kgN/ha also turned out to be better for chlorophyll a, b and total chlorophyll contents in the *Allium cepa* L. than recommended by scientific reports dose of 170 kgN/ha in a form of artificial mineral fertilizer NH_4NO_3. Similar effect of increase in chlorophyll content in onion by addition of zinc and selenium was also reported in literature [28].

It should be noted, that chlorophyll content can be practically interpreted as indirect indicator of photosynthesis efficiency in a given plant [49]. This way any disturbances in growth processes (correlated with N content in plant green leaves), represented by lower or oscillating chlorophyll contents, can be quickly identified. Chlorophyll concentration in green parts (like leaves, stems, etc.) is also some form of quantitatively measure of soil and air nitrogen supply to the plant metabolic system and its bioavailability for the given plant [50] cultivated under precisely specified conditions (including: climate, humidity and fertilization mode). It can be thus regarded as a reliable base for determination of optimal fertilization dose and dosing frequency (higher current chlorophyll level – lower fertilizer doses are necessary).

Chlorophyll content is also one of the indicators of “plant green covering” – complex group of properties including, besides chlorophyll concentration, also leaf surface, size, morphology and its physiological state. These together provide one with more thorough information about possibility of photosynthesis process in a given plant, photosynthesis-active energy level, as well as of many dependent subprocesses [51, 52], determining together primary production of a given ecosystem. Higher chlorophyll concentration demonstrates that higher biomass content and higher N content in leaves is attainable – higher fractions from N doses in fertilizer are better assimilated [53, 54].

One should also take under consideration that chlorophyll content is also strongly dependent on total precipitation, temperature and weather conditions (including light intensity and plant’s exposition time), content of nutrients (N, S, as well as Mg, K) in the soil, as well as plant type [55-58]. Genetically determined intrinsic chlorophyll content attributed to a given plant type can be, however, effectively modified by environmental factors. Thus proper manipulation in fertilization schedule can effectively improve chlorophyll content in the plant with all positive consequences. The research proved that one of such effective methods for chlorophyll content determination/upgrading is application of fermented human urine as a natural, fully quality fertilizer. Changes in chlorophyll contents can be correlated with plant’s health. Optimal nitrogen content for the plant is determined, among others, by the plant’s mass [59, 60].

Conclusions

Practical applicability of fermented human urine as natural fertilizer in small-scale onion (*Allium cepa* L.) cultivation was tested. Within the range tested (60–240 kgN/ha) the best turned out to be dose 120 kgN/ha, corresponding to the highest chlorophyll a, b and total chlorophyll concentrations in the green parts of the plant. Both non-fertilized plants and fertilized with non-natural fertilizer (inorganic NH_4NO_3) represented considerable lower and practically overlapping chlorophyll concentration values (a, b, total). Fermented human urine can be thus recommended as better and cheaper source of more effectively available nutrients in agriculture. Changes in chlorophyll content can be also a result of nutrients deficiency, solar radiation accessibility, as well as other natural and anthropogenic (e.g. herbicides presence and concentration) factors. Based on this some optimal fertilization strategy can be developed, focusing mainly on rational nutrients dosing thus their overdosing prevention (eutrophication hindering).

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