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Article

# Impact of Solid Grain Waste Digestate on Biometrics and Photosynthetic Parameters of Tomato (L. *Lycopersicon esculentum*) Seedlings †

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**Abstract:** Anaerobic digestate has been commonly used for the cultivation of vegetable seedlings and as one of the measures for improving the peat substrate. Studies have shown that there is no further research made about the effect of anaerobic digestate on greenhouse vegetable seedlings. The main objective was to investigate the effect of the additional insertion of different rates of solid grain waste digestate in the peat substrate on tomato seedling quality. The results showed that 10 % of solid grain waste digestate (peat-digestate) application with transplanted seedlings had better biometrical measures and photosynthetic parameters of tomato seedlings compared with the control variant.

**Keywords:** biometrics; photosynthetic parameters; seedlings; solid grain waste digestate; tomato

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## 1. Introduction

The cultivation of qualitative vegetable seedlings is important in agriculture because planting healthy seedlings not only reduces the use of pesticides and chemical fertilizers but also ensures a higher vegetable yield [1]. The quality of vegetable seedlings is characterized by morphological features, physiological and biochemical indicators [2]. The morphological characteristics of quality greenhouse plant seedlings are not elongated, are compact, with short internodes, dark green leaves, and roots are white, and intact, covering the entire peat substrate in the pot. Many factors can affect the quality of seedlings, such as light, temperature, humidity, fertilizers, substrate, etc. [3]. Studies have shown that the best and most sustainable way to use various biological wastes is to turn them into organic fertilizers. In recent years, the use of biosubstrate (digestate) obtained during the biogas and bioethanol production process in agriculture, has been increasing. Biogas digestate is rich in essential elements and nutrients: nitrogen (N), phosphorus (P), potassium (K), amino acids, vitamins, and some beneficial microorganisms [4], as well has a positive effect on soil properties and plant nutrition [5]. According to Jain and other scientists [6] digestate could replace about 7% of inorganic fertilizers that are currently used worldwide. It has also been proven that digestate has better properties as a fertilizer than manure [7]. The latest agro-environmental requirements, the challenges of soil degradation, and decreasing plant productivity, encourage farms to produce agricultural products with limiting the use of synthetic and chemical fertilizers. The development of resource-efficient fertilizers for horticultural crops is an important step toward more sustainable food production needed to meet the challenges of the future.

## 2. Materials and Methods

*2.1. Research Conditions*

The investigation was carried out at the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry in unheated greenhouses covered with a double polymer film. Two factors were investigated: seedlings establishment method (transplanted and direct sowed in the pod seedlings) and different substrates: control (peat, Profi 1), peat +10% peat-digestate, peat+20% peat-digestate. The transplanted tomatoes were sown in the middle of February and direct sowed in the pod seedlings–at the end of February, both were kept under the same conditions in a heated nursery. The plants were watered when necessary. The seedlings were cultivated for 60 days. The investigation object was the variant "Brooklyn H". At the beginning of May, the seedlings were transported to unheated greenhouses. Three replications were done in a randomized block design.


**Figure 1.** Tomato seedlings grown in solid grain waste digestate (peat-digestate) and peat substrate's mixtures: control (peat), peat +10% peat-digestate, peat+20% peat-digestate**.**

*2.2. Biometric Measurements*

The biometrical measurements were carried out at the seedling full development. The seedling height was measured to the tip of the youngest leaf. The leaf area of seedlings was measured by "WinDias" leaf area meter (Delta-T Devices Ltd., UK).

*2.3. Non-Destructive Measurements*

Non-destructive measurements of leaf chlorophyll (CHL), nitrogen balance (NBI) and flavanoid (FL) indexes were performed using the Dualex 4 Scientific® (FORCE-A, Orsay, France) meter. Tomoto seedlings leaf, stem, roots and all plant fresh mass was also evaluted by calculating their weight (g) [8–10].

*2.4. Determination of Photosynthetic Parameters*

Photosynthetic rate (Pr, μmol $CO_2$ $m^{-2}$ $s^{-1}$), transpiration rate (Tr, mmol $H_2O$ $m^{-2}$ $s^{-1}$), stomatal conductance ($g_s$, mol $H_2O$ $m^{-2}$ $s^{-1}$), intercellular to ambient $CO_2$ concentration ($C_i/C_a$**)** was determined 9:00-11:00 am by using an LI-6400XT portable open flow gas exchange system (Li-COR 6400XT Biosciences, Lincoln, USA). For measurements were chosen two most developed leafs from the plant, tree plants were measured for one minute. Reference air [$CO_2$] (400 μmol $mol^{-1}$), light intensity (1000 μmol $m^{-2}$ $s^{-1}$), and the flow rate of gas pump (500 mmol $s^{-1}$) were set [11].

*2.5. Statistical Analysis*

Research data was performed using a two-factor analysis of variance (ANOVA) using the computer program STATISTICA (STATISTICA 10) for statistical and data analysis [12]. The data presented as the mean of three replicates (n = 3) linked to the sampling points. The statistical reliability of the differences between means assessed by Tukey's significant difference test ($p < 0.05$).

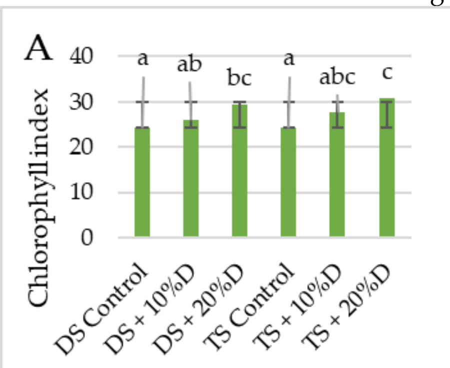
## 3. Results and Discussion

**Table 1.** The effect of solid grain waste digestate (peat-digestate) and peat substrate's mixture on biometric parameters of tomato seedlings.

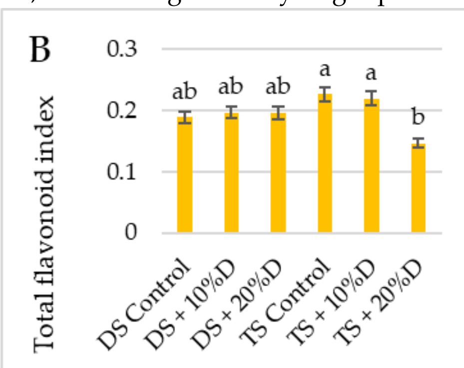
| Treatment | Plant height, cm | Hypocotyl lenght, cm | Stem diameter, mm | Number of leaves, unit | Leaf area, $cm^2$ |
|---|---|---|---|---|---|
| Direct seeding | | | | | |
| (Control/Peat substrate) | 24.50d | 3.40b | 4.37c | 5.27a | 189.01d |
| Peat + 10% peat-digestate | 19.20a | 2.90ab | 3.77a | 4.40d | 97.04b |
| Peat + 20% peat-digestate | 12.67c | 2.73ab | 2.70b | 3.80c | 29.51a |
| Transplanting | | | | | |
| (Control /Peat substrate) | 40.30b | 1.63c | 5.90e | 7.33b | 667.56e |
| Peat + 10% peat-digestate | 38.67b | 2.40a | 5.37d | 7.50b | 699.04f |
| Peat + 20% peat-digestate | 18.47a | 1.47c | 3.73a | 5.67a | 177.63c |

Means with different letters are significantly different at the $p < 0.05$ level according to Tukey's significant difference test.

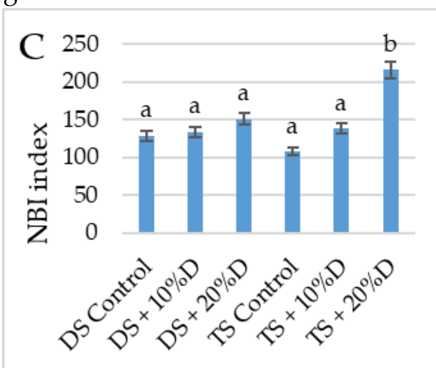
Solid grain waste digestate (peat-digestate) with peat substrate's mixture and seedlings establishment method influenced the biometric parameters of the seedlings. The results showed that 10 % of peat-digestate application with transplanted seedlings had a significant effect on plant height, leaf number, leaf area, and all plant fresh mass of tomato seedlings compared with the control variant (Table 1). Comparing direct sowed in the pod seedlings with transplanted seedlings significantly the largest area of leaves was formed by transplanted seedlings. These transplanted seedlings were more developed, compact, not elongated, and had significantly larger plant height.




(**a**)




(**b**)




(**c**)

**Figure 2.** The effect of solid grain waste digestate (peat-digestate) and peat substrate's mixture of tomato seedlings Chlorophyll (A), Total flavonoid (B) and NBI indexes (B): DS Control – direct seeding (Control/Peat); DS + 10%D – direct seeding + 10% peat-digestate; DS + 20%–direct seeding + 20% peat-digestate; TS–transplanting (Control /Peat); TS +10%D–transplanting + 10% peat-digestate; TS +20%D–transplanting + 20% peat-digestate. Means with different letters are significantly different at the $p < 0.05$ leve–l according to Tukey's significant difference test. Error bars showed in percentage.

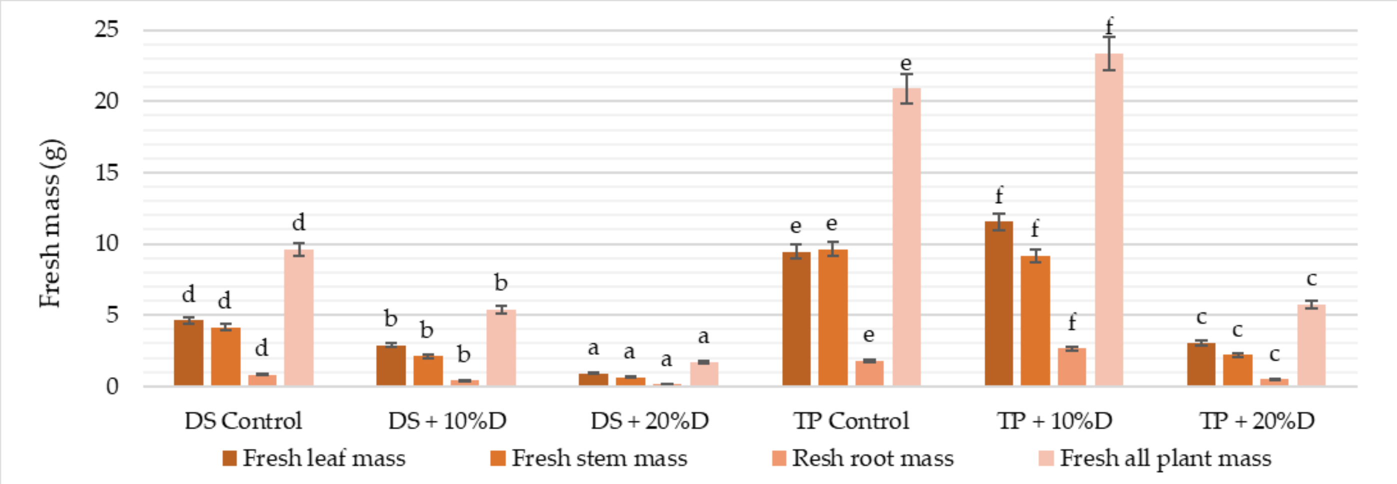



**Figure 3.** The effect of solid grain waste digestate and peat substrate's mixture of tomato seedlings fresh leaf, stem, root and all plant masses. DS Control–direct seeding (Control/Peat); DS + 10%D–direct seeding + 10% peat-digestate; DS + 20%–direct seeding + 20% peat-digestate; TS–transplanting (Control /Peat); TS +10%D–transplanting + 10% peat-digestate; TS +20%D–transplanting + 20% peat-digestate. Means with different letters are significantly different at the $p < 0.05$ level according to Tukey's significant difference test. Error bars showed in percentage.

The transplanted control variant and 10 % solid grain waste digestate (peat-digestate) with peat substrate mixture had 2.2-2.4 times higher fresh leaf mass compared with direct sowed in the pod seedlings (Figure **3**).

The chlorophyll and NBI index of transplanted tomato seedlings were much affected by these substrates. The addition of more peat-digestate to the peat substrate increased the chlorophyll index up to 10.5-21.1% in the seedlings compared to peat without it (Figure 2). NBI index in transplanted seedlings with 20% peat-digestate and peat mixture was 40.4 % higher comparing with other variants (Figure 2). The seedlings establishment method had a significant an effect on seedlings, however, the total flavonoid content in tomato seedlings had no significant difference (Figure 2).

**Table 2.** The effect of solid grain waste digestate and peat substrate's mixture on photosynthetic parameters of tomato seedlings.

| **Treatments** | **Photosynthetic rate, (μmol $CO_2$ $m^{-2}$ $s^{-1}$)** | **Stomatal conductance, ($H_20$ mol $m^{-2}$ $s^{-1}$)** | **Intercellular $CO_2$, (μmol $CO_2$ $mol^{-1}$)** | **Transpiration rate, (Mmol $H_20$ $m^{-2}$ $s^{-1}$)** |
|---|---|---|---|---|
| Direct seeding | | | | |
| (Control/Peat substrate) | 7.18b | 0.021a | 166.76c | 0.81a |
| Peat + 10% peat-digestate | 5.62ab | 0.014a | 248.71a | 0.58a |
| Peat + 20% peat-digestate | 4.50a | 0.011a | 278.83ab | 0.48a |
| Transplanting | | | | |
| (Control/Peat substrate) | 9.73d | 0.41d | 339.74b | 2.83c |
| Peat + 10% peat-digestate | 14.77c | 0.29c | 288.54ab | 2.36b |
| Peat + 20% peat-digestate | 14.31c | 0.22b | 265.32a | 1.93b |

Means with different letters are significantly different at the $p < 0.05$ level according to Tukey's significant difference test.

The highest photosynthetic parameters were found in the leaves of transplanted seedlings + 10% peat-digestate. The photosynthetic rate was up to 31.9-50.3 % increase and intercellular $CO_2$ was 1.7 times higher compared with other experiment variants. Transplanted seedlings excelled in higher stomatal conductance (19.5-37.3 %) and 3.4- 6.9 times better transpiration rate (Table 2).

## 4. Conclusions

The insertion of solid grain waste digestate (peat-digestate) into the peat substrate influenced the biometric parameters of tomato seedlings, their physiological parameters, and non-destructive measures. Transplanted seedlings grown in peat-digestate were higher, had more leaves and a larger leaf area, so as fresh all plant mass. The addition of peat-digestate to the peat substrate resulted in better chlorophyll and nitrogen balance index (NBI) parameters in the tomato seedlings. The positive effect of the mixture of peat-digestate into peat substrate on photosynthetic parameters in the tomato seedling was observed. The higher amount of peat-digestate in the peat increased the photosynthetic rate and intercellular $CO_2$. Transplanted seedlings showed a significantly higher transpiration rate and stomatal conductance increase in the leaves of seedlings. They, as well, formed the best and showed the best results from all the experiment variants.

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## References

1. Feng, J.; Xiangpei, H. 2021. An IoT-based Hierarchical Control Method for Greenhouse Seedling Production. Procedia Computer Science 192: P. 1954-1963.
2. Aragão, V.P.M., Navarro, B.V., Passamani, L.Z., Macedo, A.F., Floh, E.I.S., Silveira, V. and Santa-Catarina, C., 2015. Free amino acids, polyamines, soluble sugars and proteins during seed germination and early seedling growth of Cedrela fissilis Vellozo (Meliaceae), an endangered hardwood species from the Atlantic Forest in Brazil. Theoretical and Experimental Plant Physiology, 27, p.157-169.
3. Paul, L.C., Metzger, J.D. 2005. Impact of vermicompost on vegetable transplant quality. Hort-Science, 40 (7), p. 2020–2023.
4. Chong, C.C., Cheng, Y.W., Ishak, S., Lam, M.K., Lim, J.W., Tan, I.S., Show, P.L. and Lee, K.T., 2022. Anaerobic digestate as a low-cost nutrient source for sustainable microalgae cultivation: A way forward through waste valorization approach. Science of the Total Environment, 803, p.150070.
5. Panuccio, M.R., Attinà, E., Basile, C., Mallamaci, C., Muscolo, A. 2016. Use of recalcitrant agriculture wastes to produce biogas and feasible biofertilizer. Waste Biomass Valor. 7: P. 267–280.
6. Jain, S., Newman, D., Nzihou, A., Dekker, H., Le Feuvre, P., Richte, H.; et al. 2019. Global Potential of Biogas. (Doctoral dissertation). (London: World Biogas Association).
7. Lind, P.O, Hultberg, M., Bergstrand, K-J., Larsson-Jönsson, H., Caspersen, S., Asp, H. 2020. Biogas digestate in vegetable hydroponic production: pH dynamics and pH management by controlled nitrification. Waste Biomass Valor.
8. Hüner, P.A.; Dahal, K.; Bode, R.; Kurepin, L.V.; Ivanov, A.G. Photosynthetic Acclimation, Vernalization, Crop Productivity and 'the Grand Design of Photosynthesis. Journal of Plant Physiology 2016, 203(20), 29-43. https://doi.org/10.1016/j.jplph.2016.04.006.
9. Sala, F.; Arsene, G.G.; Iordănescu, O.; Boldea, M. Leaf Area Constant Model in Optimizing Foliar Area Measurement in Plants: A Case Study in Apple Tree. Scientia Horticulturae **2015**, *193*, 218–224. https://doi.org/10.1016/j.scienta.2015.07.008.
10. Merzlyak, M.N.; Gitelson, A.A.; Chivkunova, O.B.; Rakitin, V.Y. Non-destructive optical detection of pigment changes during leaf senescence and fruit ripening. Physiologia Plantarum, **1999**, *106*, 135–141.
11. Laužikė, K.; Uselis, N.; Kviklys, D.; Samuolienė, G. Orchard planting density and tree development stage affects physiological processes of apple (Malus domestica Borkh.) tree. Agronomy 2020, 10(12), 1912.
12. Sakalauskas, V. 2003. Duomenų analizė su STATISTIKA. Vilnius: Margi raštai. p. 235.