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PRELIMINARY RESULTS OF FISH FERTILIZER EFFECTS ON LETTUCE

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Abstract

To address the latest environmental and pollution challenges, as well as the growing need for food due to a progressively expanding population, this study proposes an alternative approach to increase food production by testing residual materials from the blue value chain for their potential use in farming and agriculture. For this purpose, the experimental framework involved establishing a greenhouse potted lettuce crop with the curly variety 'Simona'. We considered four experimental variants, two of which involved organic fertilization treatments using fish residues, i.e., cod bone powder (F1) and common ling bone powder (F2), one with mineral fertilizer (F3), and a control (C). The experimental monitoring period was set from November 14, 2022, to January 4, 2023. The lettuce crop was evaluated for physiological and chemical characteristics. Fish-based fertilizers showed results similar to those of the variant with mineral fertilizer, but superior to the control variant. Consequently, fish-derived organic fertilizers could be an effective alternative to mineral fertilizers for greenhouse-grown lettuce.

***Key words**: greenhouse, lettuce, fertilizer, blue bioeconomy.*

INTRODUCTION

According to the United Nations, the global population exceeded 8000 million by the end of 2022. World population growth is exponential, and according to the FAO, 870 million people in this population do not have enough food to eat (Oluwole et al., 2023).

This problem is compounded by environmental issues, with agriculture leading to environmental pollution at all levels: air, soil, and water. In agriculture and horticultural production, water is consumed in significant quantities (Liu et al., 2022) and is a focal point because some countries are experiencing a severe water deficit or have even reached 'Day Zero' (Chen et al., 2019; Millington & Scheba, 2020). The primary agricultural water pollutants are mineral nitrogen and pesticide used extensively in plant protection and fertilization, often applied excessively (Savcı, 2012).

New European policies aim at addressing these concerns, *e.g.*, through promoting soil health and organic growing (EU2020). This includes increased interest in the use of various types of organic waste, such as fish residues, manure, sewage sludge, etc. These efforts are strengthened by bioeconomy and circular economy concepts, which may increase sustainability and preserve biodiversity by replacing mineral inputs with adverse effects (Løes & Adler, 2019; Ahuja et al., 2020; Coppola et al., 2021; Cardarelli et al., 2023).

Fish-based fertilizers can be one example of a bioeconomic solution, where residual materials which are currently wasted, may replace mineral fertilizers which are getting scarce (phosphorus) and/or require high energy consumption (nitrogen). By-products from the fish processing industry contain valuable nutrients, making a compelling case for their use as fertilizers (Illera-Vives et al., 2015). Furthermore, the use of fish remains is not a new concept. Ahuja et al. (2020) noted their historical utilization by Egyptians, Incas, and Mayans, as well as their traditional application in coastal crop cultivation. Fish meal produced in Norway has a modern counterpart in the form of commercial fertilizer products (Ahuja et al., 2020). These products are formulated using various fish-derived components and are approved for use in certified organic farming (Illera-Vives et al., 2015). It is necessary to develop a stable formula for the processing of such residues to ensure that the derived fertilizers do not have harmful effects on crops, including issues like phytotoxicity and organic microcontaminants, which can have metabolic and phenotypic implications for the plants (Matamoros et al., 2021). These processed fish residues can be found in various forms, *e.g.*, emulsion, hydrolysate, compost, digestate (Ahuja et al., 2020).

Fish residual materials have been successfully used as fertilizers in various forms and within different culture systems. Some of the most popular methods include aquaponics (Cohen et al., 2018) and hydroponics (Ahmed et al., 2021). Fish emulsions and hydrolysates are typically used in liquid form, but dried, powdered fish residues also showed positive results when used for biosolarization (Zou et al., 2023), and had significant growth effects in recent field experiments (Løes et al., 2022). Illera-Vives et al. (2015) reported positive results using compost from fish residue and seaweed on tomato and lettuce as a second crop. They recommended composting as one of the most cost-effective methods for stabilizing waste materials. Xu & Mou (2017) concluded that fish-derived protein hydrolysates can enhance lettuce growth, increase leaf water content, boost leaf chlorophyll content, and improve gas exchange. Muscolo et al. (2022) achieved favourable results in the cultivation of Tropea red onion (*Allium cepa*) using an extract from solid residues of anchovy fillet waste compared to commonly used mineral and organic fertilizers.

Fish-based fertilizers offer a sustainable solution for the future, and their applicability can be scalable with the right formulation. However, despite their many benefits, there are some impediments, including high salt content, which is a challenge for the growth of various plant species, particularly lettuce, known for its sensitivity to elevated salt levels. In this context, the aim of the study was to assess the impact of fish powder fertilizers on potted lettuce growth in a protected indoor environment (greenhouse).

MATERIALS AND METHODS

Experiment design

The experiment was conducted in the research greenhouse at the Research Center for Food Quality and Agricultural Products, USAMV Bucharest. We chose a Romanian variety of curly lettuce, 'Simona', which was started from seeds purchased from a local market.

The experimental treatments, related to the types of fertilizers used, included: (C) Control, using peat substrate (OPM 540 W, Kekkilä-BVB, Finland) and perlite; (F1) Peat amended with cod (*Gadus morhua*) bone powder; (F2) Peat amended with common ling (*Molva molva*) bone powder; (F3) Peat amended with a commercial mineral fertilizer.

The characterization of the fertilizers used in the current experiment can be found more detailed in our previous paper (Moloșag et al., 2023). The amounts of fish-based fertilizers were 30 g/pot of cod bone powder and 40 g/pot of common ling bone powder, and these fertilizers were used only at the beginning of the experiment, when the substrate was prepared.

The experiment began by marking the pots and preparing the substrate, using a peat/perlite ratio of 4/1 (v/v). After thorough mixing, the substrate was evenly distributed among 40 pots of 3 L each, with 10 pots designated for each fertilizer variant (C, F1, F2, and F3). The required amount of fertilizer for each variant was then added and thoroughly mixed. The materials and relevant stages of the experimental procedure are shown in Figure 1. Initially, each pot was watered with 1000 mL of tap water to moisten the peat. Before the seedlings were transplanted, a total of 2.8 L of water was applied to the pots based on their appearance and moisture requirements. The substrate was prepared 51 days before the seedlings were transplanted to allow the fish powder to partially degrade. During this period the substrate was kept relatively moist, and its pH values after 51 days were as follows: 5.90±0.02 for C, 6.94±0.03 for F1, 7.02±0.02 for F2, and 6.23±0.02 for F3.

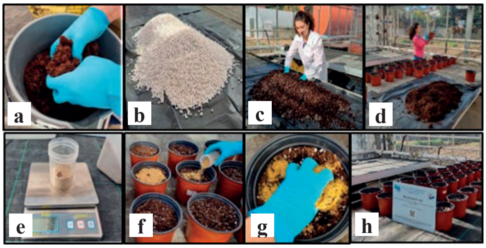

Figure 1. Peat (a); perlite (b); preparation of peat/perlite substate (4/1 v/v) (c); adding the substrate to the pots (d); weighing the fish-based fertilizer (e); adding the fertilizer over the substrate (f); mixing the fertilizer with the substrate (g); pots containing mixtures of fertilizer and substrate (h)

Lettuce seedling experiments

On October 18, 2022, the seeds of the commercial lettuce variety 'Simona' were sown in the control substrate. The seeds emerged after three days, corresponding to code BBCH 09, which indicates cotyledons breaking through the soil surface. On October 28, 2022, when the first true leaf was unfolded (Figure 2d), corresponding to BBCH code 11, the seedlings were transplanted into the 72-cell tray (Figure 2a-c). On November 14, 2022 (initial time, T_0), when the third true leaf was unfolded (Figure 2e), corresponding to BBCH code 13, the seedlings were transplanted (1 plant/pot) into the experimental pots (Figure 2f). The newly transplanted plants were watered with 200 mL water/pot and the selected growth parameters started to be monitored. The experiment lasted until January 4, 2022 (final time, T_f).

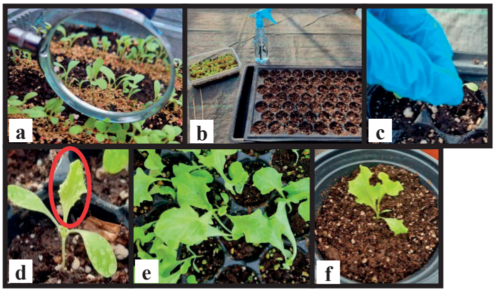

Figure 2. Lettuce seedlings grown in the peat/perlite substrate (28/10/2022) (a); 72 cell tray containing peat/perlite substrate (4/1 v/v) (b); transplanting lettuce seedlings into cells (28/10/2022) (c); first true leaf (31/10/2022) (d); third true leaf (14/11/2022) (e); lettuce seedlings transplanted into pots (14/11/2022) (f)

Substrate monitoring and characterization

The following parameters of the substrate were measured in the greenhouse: substrate moisture (*M*, %) using a MO750 Soil Moisture Meter (EXTECH Instruments, Nashua, NH, USA), electrical conductivity (*EC*, mS/cm) and temperature (*t*, °C) using a HI-98331 Groline Direct Soil Conductivity (EC) & Temperature Tester (Hanna Instruments Smithfield, RI, USA).

Substrate samples were monitored for total carbon content (*TC*, %) and total nitrogen content (*TN*, %) using the Dumas method (Moț et al., 2022) and measured with an EA 3100 elemental analyzer (Eurovector, Pavia, PV, Italy). Dry matter (*DM*, %) was determined using a Memmert Universal Oven (Memmert, Schwabach, Germany).

Plant monitoring and characterization

The number of leaves (*NL*) and plant height (*H*, cm) were monitored in the greenhouse (Drăghici et al., 2016). To calculate the specific leaf area (*SLA*, cm^2/g), which is the ratio of leaf area (*LA*, cm^2) to dry matter content (*DM*, g), lettuce leaf analyses were performed using WinFolia software (LA2400, Regent Instruments, Sainte-Foy, Quebec, Canada) and an Epson Expression 11000 XL scanner to obtain *LA* values, along with a Memmert Universal Oven (Memmert, Schwabach, Germany) for *DM* measurements.

Total carbon content (*TC*, %) and total nitrogen content (*TN*, %) in the leaves were determined using the Dumas method (Moț et al., 2022) and measured with an EA 3100 elemental analyzer (Eurovector, Pavia, PV, Italy).

The analyses were carried out in the laboratories of the Research Center for Studies of Food Quality and Agricultural Products at the start and end of the experiment.

Statistical analysis

The data were analyzed with IBM SPSS statistical software, and Duncan's Multiple Range Test was applied to determine if the means of selected parameters for the four experimental variants were significantly different ($p < 0.05$) or not (Moț et al., 2021).

RESULTS AND DISCUSSIONS

Substrate characterization

The lettuce crop faced unfavourable temperature conditions with temperatures soaring up to 31°C in the greenhouse, hindering its growth. In this study, crops were cultivated in a greenhouse using natural light. Nevertheless, the distribution of light within the greenhouse was influenced by various factors including nearby structures and seasonal changes. The light distribution also influenced the substrate moisture content (*M*). Despite being irrigated based on its needs, the control substrate exhibited lower *M* levels (9.86±0.91%) at the end of the experiment (M_f) compared to the variants containing fish fertilizers, which had *M* values of 21.63±1.58% for F1 and 20.88±5.55% for F2 (Table 1). These values are notably lower compared to those reported by Martins et al. (2023), who recorded substrate moisture values exceeding 70%. However, they are similar to the findings of Matamoros et al. (2021) for the organic fraction of municipal solid waste (OFMSW) used as a substrate in their experiment, which had a moisture value of 23%.

Electrical conductivity (*EC*) represents the total ion concentration of a solution (Samarakoon et al., 2020). It can hinder plant growth and development when its values do not meet the plant requirements. Numerous studies have been conducted to determine the most suitable *EC* for growing lettuce, particularly in a hydroponic system. The studies concluded that lettuce was moderately sensitive to salinity (Andriolo et al., 2005; Ünlükara et al., 2008; Martins et al., 2023), with greater sensitivity observed in the early stages of development (Martins et al., 2023). In our investigation, *EC* values (Table 1) varied for the fish-fertilized variants for T_0, showing significantly different values for F1 (0.79±0.12 mS/cm) and F2 (1.08±0.27 mS/cm) compared to the control (0.12±0.02 mS/cm). Data summarized in Table 2 indicate a significant decrease in *EC* for F1 and F2 at the end of the experiment. Martins et al. (2023) reported *EC* values of 2.44-2.79 mS/cm and even above 3.2 mS/cm in the early stage of one of the experiments, without the lettuce seedlings showing visual symptoms of excess salts.

Abou-Hadid et al. (1996) concluded that fresh weight yield decreased with an increase in *EC* of the nutrient solution in a hydroponic system. They also determined that the microelements in lettuce plants were affected not only by *EC* levels but also by variety. Samarakoon et al. in 2019 found that the optimum lettuce yield was obtained at 1.8 mS/cm for several cultivars in two growing seasons.

The mean values of substrate temperature specified in Table 1, *i.e.*, 20.9-22.6°C, are normal in the greenhouse.

Carbon and nitrogen are extremely important for the proper development of plants (Cardarelli et al., 2023). Carbon is found in different forms, and the available carbon, in the appropriate doses, can regulate excess nitrate in the soil, but an oversupply of it can create soil hypoxia, which is detrimental to plant growth or even lethal (Qin et al., 2019). Data summarized in Table 2 highlight a decrease in total carbon content (*TC*) and an increase in total nitrogen content (*TN*) of the substrate at the end of the experiment. The most significant difference between the mean values of *TC* was for T_0-F2 (40.00±1.07%) and T_f-F2 (32.85±0.67%).

Table 1. Moisture content, electrical conductivity, and temperature of the substrate for different fertilization variants, at initial (T_0) and final time (T_f)

Variant	*M* (%)	*EC* (mS/cm)	*t* (°C)
T_0-C	11.26±1.88^{a}	0.12±0.02^{ab}	21.9±0.2^{bc}
T_0-F1	21.9±2.00^{cd}	0.79±0.12^{d}	21.8±0.4^{b}
T_0-F2	24.13±2.34^{d}	1.08±0.27^{e}	22.1±0.2^{c}
T_0-F3	9.21±1.54^{a}	0.07±0.02^{a}	22.6±0.5^{b}
T_f-C	9.86±0.91^{a}	0.08±0.02^{a}	20.9±0.1^{a}
T_f-F1	21.63±1.58^{c}	0.33±0.12^{c}	21.2±0.3^{a}
T_f-F2	20.88±5.55^{c}	0.32±0.18^{c}	21.1±0.2^{a}
T_f-F3	17.89±2.94^{b}	0.21±0.07^{bc}	21.1±0.3^{a}

Different letters in the same column indicate a significant difference ($p < 0.05$).

Table 2. Total carbon content and total nitrogen content of the substrate for different fertilization variants, at initial (T_0) and final time (T_f)

Variant	*TC* (%)	*TN* (%)
T_0-C	40.20±0.29^{d}	0.71±0.05^{a}
T_0-F1	37.58±0.98^{c}	1.33±0.05^{bc}
T_0-F2	40.00 ±1.07^{d}	1.40 ±0.15^{c}
T_0-F3	37.62 ± 0.43^{c}	0.68±0.05^{a}
T_f-C	37.73±0.25^{c}	1.25±0.00^{b}
T_f-F1	37.04±0.22^{c}	1.69±0.03^{d}
T_f-F2	32.85±0.67^{a}	1.42±0.01^{c}
T_f-F3	35.27±0.20^{b}	1.38±0.16^{bc}

Different letters in the same column indicate a significant difference ($p < 0.05$).

Plant characterization

The number of lettuce leaves (*NL*) showed significant differences for all fertilized variants compared to the control at the end of the experiment (Table 3). At the end of the experiment, F1 showed the highest value (19.9±2.2) from the fertilized variants compared to those of F2 (17.6±2.5) and F3 (18.2±3.7). Our results are similar to those of Islam et al. (2021) and very slightly different from those reported by Xu & Mou (2017).

In terms of the plant height (*H*), significant differences were for all fertilized variants between the initial and final time, but not between variants (Figure 3). However, the highest value was recorded for F3 (18.9±3.9 cm) and the lowest for C (9.0±1.6 cm). The values of this growth indicator obtained by Vetrano et al. (2020) in a floating system were higher, varying between 23.6 cm and 27.4 cm, depending on the treatment applied. Also, *H* values obtained in this study are similar to those reported by Islam et al. (2021) for *Lactuca sativa* cv. Green Wave (18.63 ± 1.8 cm) and *Lactuca sativa* cv. New Red Fire (16.95 ± 0.76 cm) grown in a mixture of soil (20%), vermicompost (40%), and spent mushroom compost (40%).

Specific leaf area (*SLA*) or specific foliar area (*SFA*) is a representative element when referring to the plant growth (Schneider et al., 2018). It is an indicator of the plant photosynthetic capacity, explaining growth variations influenced by the action of different environments, the latter also impacting leaf density and/or thickness (morphological traits) (Liu et al., 2016; de Ávila Silva et al., 2021). A study focused on salt stress tolerance showed that lettuce leaf morphology, represented by petiole thickening, changed under stress conditions, *i.e.*, *SLA* value decreased (Vetrano et al., 2020). But when taking into account the transplant quality, a low *SLA* value indicates a superior quality of the plant material (Spalholz & Hernández, 2018). Regarding *SLA* (Table 3), significant differences were between T_0 and T_f and between fertilizer variants and control variant for T_f. Vetrano et al. (2020) reported a mean *SLA* value of 703.2 cm^2/g in the unstressed lettuce plants and Schneider et al. (2018) obtained a mean value of 400 cm^2/g. Xu & Mou (2017) concluded that fish-derived protein hydrolysates influenced positively leaf juiciness and leaf water content but had no effect on *SLA*, the latter having values between 302±18 cm^2/g for the control and 330±13 cm^2/g for the variants where the treatment was applied. Salinas et al. (2019) in their attempt to quantify changes in biomass accumulation of lettuce following amino acid treatments obtained results similar to ours.

In terms of total carbon and nitrogen contents of lettuce leaves (*TC* and *TN*), significant differences were recorded between T_0 and T_f (Table 4). Tabulated data highlight a decrease in *TC* and an increase in *TN* at the end of the experiment, when the lowest levels of *TC* and *TN* were for F1 (32.58±0.19%) and F2 (5.78±0.04%), respectively.

Table 3. Number of leaves, plant height, and specific leaf area of lettuce for different fertilization variants, at initial (T_0) and final time (T_f)

Variant	*NL*	*H* (cm)	*SLA* (cm^2/g)
T_0-C	3.4 ± 0.5^{a}	8.3 ± 0.8^{a}	12.17 ± 3.63^{a}
T_0-F1	3.6 ± 0.5^{a}	8.7 ± 0.5^{a}	12.17 ± 3.63^{a}
T_0-F2	3.8 ± 0.4^{a}	8.0 ± 0.6^{a}	12.17 ± 3.63^{a}
T_0-F3	4.0 ± 0.0^{a}	8.8 ± 1.1^{a}	12.17 ± 3.63^{a}
T_f-C	4.6 ± 0.5^{a}	9.0 ± 1.6^{a}	44.37 ± 14.93^{b}
T_f-F1	19.9 ± 2.2^{c}	18.8 ± 2.7^{b}	99.48 ± 40.04^{c}
T_f-F2	17.6 ± 2.5^{b}	18.3 ± 2.0^{b}	107.12 ± 33.71^{c}
T_f-F3	18.2 ± 3.7^{b}	18.9 ± 3.9^{b}	108.48 ± 11.28^{c}

Different letters in the same column indicate a significant difference ($p < 0.05$).

Table 4. Total carbon content and total nitrogen content in the plant leaves for different fertilization variants, at initial (T_0) and final time (T_f)

Variant	*TC* (%)	*TN* (%)
T_0-C,F1,F2,F3	36.53 ± 0.54^{d}	5.40 ± 0.20^{a}
T_f-C	34.13 ± 0.22^{c}	6.18 ± 0.10^{cd}
T_f-F1	32.58 ± 0.19^{a}	6.02 ± 0.06^{c}
T_f-F2	33.40 ± 0.08^{b}	5.78 ± 0.04^{b}
T_f-F3	34.57 ± 0.46^{c}	6.37 ± 0.10^{d}

Different letters in the same column indicate a significant difference ($p < 0.05$).

CONCLUSIONS

The mean values of selected lettuce growth parameters *i.e.*, number of leaves (*NL*), plant height (*H*), and specific leaf area (*SLA*), for fertilizer variants (F1, F2, and F3) were significantly higher than those for control variant (C). The mean value of *NL* for F1 (19.9) was significantly higher than those for F2 (17.6) and F3 (18.2), which were similar, whereas the mean values of *H* (18.3-18.9 cm) and *SLA* (99.48-108.48 cm^2/g) for all fertilizer variants were similar.

Considering the results obtained for the variants fertilized with fish residues compared to conventional fertilization we can conclude that fish-based fertilizers could partially replace the mineral fertilizers and can be a more efficient and environmentally friendly alternative. However, further studies with better controlled environmental conditions and monitoring of more parameters are needed.

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