EDITORIAL CSIC

Spanish Journal of Agricultural Research
24 (1) January-March 2026, 21789
ISSN-L: 1695-971X, eISSN: 2171-9292
https://doi.org/10.5424/sjar/2026241-21789

ARTICLE

RESEARCH ARTICLE

Growth response of barley to short-term combined application of sewage sludge and conventional fertilizer

Respuesta del crecimiento de la cebada a la aplicación combinada a corto plazo de fangos de depuradora y fertilización convencional

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Abstract: *Aim of study:* Agricultural nitrogen (N) losses from synthetic fertilizer significantly impact soil-plant-water system. The use of sewage sludge (SS) as a fertilizer presents a cost-effective and sustainable alternative, aiming to reduce dependence on chemical fertilizers, while boosting crop productivity. *Area of study:* The research was conducted at the National Research Institute of Rural Engineering, Water and Forests, Tunisia. *Material and methods:* A greenhouse experiment was conducted in rhizotrons to assess the impact of SS applied alone (T3: 100% N SS) or combined with three levels of mineral fertilizer (MF) (T4: 30% N MF + 70 % N SS, T5: 50 % N MF + 50 % N SS, and T6: 70% N MF + 30% N SS) on barley (*Hordeum vulgare* L.) growth and production, compared with the sole application of MF (T2), and an unfertilized control (T1). *Main results:* SS, whether applied alone or in combination with MF, significantly promoted tillering and shoot growth compared to control and chemically amended plants. The increases in the tiller number reached 90% and 56% in T4, compared to T1 and T2, respectively. Biomass production was also highest in SS treatments. The greatest above-ground biomass accumulation occurred in T4 and T3, with increases of 119% and 78% over T1 and T2, respectively. *Research highlights:* When applied in strict compliance with regulatory standards, SS demonstrates significant agronomic value by improving forage biomass yield and productivity at a lower cost, while reducing reliance on synthetic fertilizers.

Keywords: forage biomass; germination; production; root development; sewage sludge; synthetic fertilization.

Resumen: *Objetivo del estudio:* Las pérdidas agrícolas de nitrógeno derivadas de fertilizantes sintéticos afectan significativamente el sistema suelo–planta–agua. El uso de fangos de depuradora (SS) como fertilizante constituye una alternativa sostenible y rentable, orientada a reducir la dependencia de fertilizantes químicos y, al mismo tiempo, mejorar la productividad de los cultivos. *Área del estudio:* La investigación se llevó a cabo en el Instituto Nacional de Investigación en Ingeniería Rural, Agua y Bosques (Institut National de Recherche en Génie Rural, Eau et Forêt - INRGREF), Túnez. *Material y métodos:* Se realizó un experimento en invernadero utilizando rizotrones para evaluar el impacto de la aplicación de SS solos (T3: 100 % del N SS) o combinados con tres niveles de fertilización mineral (MF) (T4: 30 % N MF + 70 % N SS; T5: 50 % N MF + 50 % N SS; y T6: 70 % N MF + 30 % N SS) sobre el crecimiento y la producción de la cebada (*Hordeum vulgare* L.), en comparación con la aplicación exclusiva de MF (T2) y un control sin fertilización (T1). *Resultados principales:* Los fangos de depuradora, aplicados solos o en combinación con MF, promovieron significativamente el macollamiento y el crecimiento aéreo en comparación con el control y las plantas fertilizadas únicamente con fertilizantes químicos. El número de macollos aumentó hasta un 90 % y el 56 % en el tratamiento T4, en comparación con T1 y T2, respectivamente. La producción de biomasa también fue mayor en los tratamientos con lodos. La mayor acumulación de biomasa aérea se observó en T4 y T3, con incrementos del 119 % y 78 % respecto a T1 y T2, respectivamente. *Aspectos destacados de la investigación:* Cuando se aplican en estricto cumplimiento de las normativas vigentes, los fangos de depuradora presentan un valor agronómico significativo, al mejorar el rendimiento y la productividad de la biomasa forrajera a menor costo y reducir, al mismo tiempo, la dependencia de fertilizantes sintéticos.

Palabras clave: biomasa forrajera; desarrollo radicular; fangos de depuradora; fertilización sintética; germinación; producción.

Received: 12-05-2025 / **Accepted**: 15-10-2025 / **Published**: 26-06-2026

Citation: Kchaou, R; Rahmani, A; Marzougui, N; BenYoussef, S; Hachicha, M (2026). Growth response of barley to short-term combined application of sewage sludge and conventional fertilizer. Spanish Journal of Agricultural Research, Volume 24, Issue 1, 21789. https://doi.org/10.5424/sjar/2026241-21789

Supplementary information ↓

1. INTRODUCTION

Intensive agricultural systems rely heavily on agrochemical inputs, including pesticides and synthetic fertilizers, employed to boost crop productivity. However, such agricultural practices exert significant pressure on natural resources, leading particularly to reduced soil health. A variety of organic amendments, including green and farmyard manure, straw incorporation, and sewage sludge (SS), have been widely advocated as sustainable soil management strategies for enhancing soil fertility and boosting crop yield, while reducing reliance on synthetic fertilizers (Alvarengaa et al., 2015).

Sewage sludge (biosolids) is a by-product of sewage treatment in wastewater treatment plants resulting from aerobic or anaerobic digestion processes. The substantial global production of SS raised the need for efficient and sustainable management strategies of this organic waste (Grobelak et al., 2019). One of the key strategies for SS management disposal is its agricultural application as an organic soil amendment. Due to its high content in macro and micronutrients along with organic matter, SS is increasingly recognized as both a valuable alternative to synthetic fertilizers and an effective conditioner that improve soil physico-chemical properties (Fijałkowski & Kwarciak-Kozłowska, 2021).

In Tunisia, the agricultural use of SS remains limited. Despite raising production volumes, the areas amended with SS remain limited, with only 18.6% of dried SS being used in 2016 (The National Sanitation

Utility, 2016), a figure that declined sharply to just 3% in 2020 (The National Sanitation Utility, 2020). The current recovery rate is particularly low, with only 7,275 t of SS being recycled annually compared to a total production of 187000 m^3 of solar-dried SS coming from 125 wastewater treatment plants (The National Sanitation Utility, 2022).

Numerous studies have highlighted the agronomic benefits of SS on the soil-plant system. As a source of valuable elements, SS can serve as an amendment to improve soil structure, nutrient availability, and water retention. (García et al., 2004; Singh & Agrawal, 2008; Kayikcioglu et al., 2019). It also contributes to maintaining soil organic matter (Albiach et al., 2001), thereby reducing dependence on chemical fertilizer. Furthermore, considerable research has been conducted worldwide on the effect of SS as a nitrogen (N) fertilizer source on many crops. In most cases, under various climatic and soil conditions, applying SS, in strict compliance with standards, led to improved crop productivity, especially for forage crops (Eid et al., 2020; Ondreičková et al., 2021). For instance, Kchaou et al. (2018) demonstrated that incremental application of urban SS (6, 12 and 18 t ha-1) significantly improved triticale plant growth through increasing leaf area index, tillering capacity, accumulated aboveground dry matter, and plant height, with no crop damage or toxic effects. Furthermore, several works have shown that triticale's growth and yield under various SS application rates were similar to, or in some cases exceeded, those achieved with mineral fertilizers (MF) alone. Jatav et al. (2022) stated that applying 20 or 30 Mg ha^{-1} SS in combination with 100% MF significantly increased the productivity of the rice-wheat system compared to sole application of MF. However, they showed that combining these SS rates with recommended or reduced fertilizer doses led to increased heavy metal concentrations in both harvested grains and soil, without exceeding acceptable safety thresholds. Ondreičková et al. (2021) found that barley plant height increased with rising SS rates, while thousand seed weight increased significantly at 5 t ha^{-1}, but decreased at 15 t ha^{-1}. Eid et al. (2020) attributed this decline to the phytotoxic effects of certain heavy metals accumulating at elevated SS application rates, which may disrupt critical plant metabolic processes and ultimately impair growth performance. In a repeated annual study, Antolin et al. (2005) demonstrated that successive SS applications over three years enhanced barley grain yield, dry matter production, and leaf protein content, along with improvements in soil chemical, microbiological, and biochemical characteristics. Nonetheless, they noted a significant accumulation of heavy metal concentrations in barley grains. This finding aligns with those of Fernandez et al. (2009) who reported that high cumulative application rates of composted and thermally dried SS (80 t ha^{-1}) significantly reduced barley yield, whereas moderate annual cumulative rates (20 t ha^{-1}) improved yield performance. Al Zoubi et al. (2008) confirmed these observations, showing that SS application within the 10-34 t ha^{-1} range enhanced wheat, maize, and vetch productivity, while maintaining soil and crop heavy metal concentrations below phytotoxic thresholds, suggesting that, under controlled conditions and with low-metal-content SS, long-term SS application poses minimal environmental risks.

While numerous studies have explored the potential of SS as a crop fertilizer, limited attention has been given to its impact on root systems. Indeed, most research on root development relies on destructive sampling methods, such as measuring fresh and dry root weight at harvest, which do not allow monitoring of root growth dynamics throughout the plant's life cycle. Rhizotrons, featuring a transparent viewing surface, serve as a contemporary, non-destructive alternative, that enables direct root growth observation. Introduced in the early 1960s, rhizotrons have been used to investigate seasonal and diurnal root growth patterns, cultivar differences, and the influence of soil treatments on root behaviour and water uptake (Klepper & Kaspar, 1994). Beyond root phenotyping, rhizotrons provide a powerful platform for investigating the spatio-temporal dynamics within the rhizosphere. They also enable for detailed analysis of physical transport processes, such as the diffusion and mass flow of liquids and gases in soil. Furthermore, rhizotrons facilitate the identification and characterization of root system architecture, thereby enhancing understanding of plant fitness, crop performance and grain yield (Sharma et al., 2024).

Building upon the above foundations, the present study aims to evaluate the impact of combined SS and MF application on both root and shoot growth, as well as the overall production of barley (*Hordeum vulgare* L.) plants in rhizotrons, compared to sole application of MF or SS and an unfertilized control.

2. MATERIAL AND METHODS

2.1. Phytotoxicity test

A seed germination assay was carried out in the laboratory to evaluate the impact of urban SS on barley seed germination compared to the control. The SS used for both the germination and growth tests was collected from the drying beds of the Mrezga wastewater treatment plant (WWTP), located in the governorate of Nabeul, Tunisia. Prior to its application, SS was aerobically digested and air-dried. Its main physicochemical and bacteriological characteristics were determined and compared with the Tunisian standard (NT 106.20) (2002) (Table 1).

Table 1. Main physico-chemical and biological characteristics of used sewage sludge (SS) from the Mrezga wastewater treatment plant (WWTP), compared to those set by the Tunisian Standard (NT) 106.20 (2002).

Parameters	Used SS	NT106.20 (2002)
pH	6.8	-
Dry matter (%)	90	-
Organic matter (g/kg DM)	572	-
Total Carbon (g /kg DM)	336	-
Total Nitrogen (g N/kg DM)	17.8	-
Total Phosphorus (g P/kg DM)	9.47	-
Cadmium (mg/kg DM)	< 0,553	20
Copper (mg/kg DM)	201.1	1000
Lead (mg/kg DM)	29.67	800
Nickel (mg/kg DM)	< 2.779	200
Zinc (mg/mg DM)	522.2	2000
Chromium (mg/kg DM)	33,59	500
Mercury (µg/kg DM)	< 1.0	-
Fecal Coliforms (Germs/g)	$2{,}1.\ 10^9$	$<2.10^6$
Nematode Eggs	Presence	-

Source: WWTP-Mrezga, Nabeul.

Characterization results showed that the SS contained a significant amount of organic matter (OM) and N, while phosphorus (P) was comparatively lower. The SS exhibited a near-neutral pH. Although heavy metals were detected, their concentrations remained well below the permissible limits for agricultural SS reuse, and were substantially lower than those specified by the Tunisian Standard NT 106.20 (2002).

The soil used in this study (for both the germination and growth tests) was taken from a depth of 10-15 cm at the Oued Souhil experimental station of the National Research Institute of Rural Engineering, Water and Forests (INRGREF), located in Nabeul. It is classified as an alkaline sandy soil (pH 8.3), poor in organic matter content and active limestone (6.5%), with an electrical conductivity of 1.3 mmhos/cm.

The phytotoxicity of SS was assessed according to the method of Zucconi et al. (1981), which evaluated the germination capacity of barley seeds in the presence of different concentrations of SS [0% (T1); 0.5% (T2); 0.8% (T3); 1.2% (T4); 1.7% (T5) in SS-soil mixtures].

The preparation of the extracts involved the following steps: (i) adding 100 ml of distilled water to the different soil-SS mixtures (i.e., in g: 10-0; 9.95-0.05; 9.92-0.08 and 9.88-0.12; 9.83-0.17) to achieve a ratio of 1/10 (m/v), (ii) shaking the mixtures for one hour, and (iii) filtering them. Finally, ten barley seeds were placed in Petri dishes, lined with filter paper, moistened with5 ml of each extract, and incubated in the dark at 25 °C for one week. Each treatment was conducted with four replicates.

Germination percentage (G) and root elongation (E) compared to the control (unamended soil extract), and germination index (GI) were calculated using the following formulas (Zucconi et al., 1981):

After assessing phytotoxicity through the GI, the half-maximal effective concentration (EC50) was calculated to determine the SS concentration at which 50% of the maximum effect was observed on the measured parameters (E, G, GI). This analysis provides critical insight into the SS rate required to induce 50% of the observed maximum effect (Parveen et al., 2024).

2.2. Experimental design

A greenhouse trial was conducted at the National Research Institute of Rural Engineering, Water and Forests Tunis (INRGREF) to evaluate the effect of SS on barley growth and production, using rhizotrons. The rhizotrons consisted of parallelepipedic wooden boxes (26 cm x 20.5 cm x 6.5 cm), with a removable transparent plexiglass front, allowing continuous visual observations of root system changes in response to SS treatments.

Twenty-four rhizotrons were randomly assigned to six treatments, each with four replicates (one rhizotron per replicate). The treatments included: a control with no application of SS or MF (T1); 100% N supplied from MF (T2); 100% N from SS alone (T3); and three combined treatments: 30% N from MF with 70 % N from SS (T4), 50% N from each source (T5), and 70% N from MF with 30% N from SS (T6). The corresponding SS amendment rates were 12, 8, 6, and 4 t ha^{-1} for T3, T4, T5 and T6.

The sole application rate of MF (T2) was determined based on the agronomic N requirement of barley, i.e., 67 kg ha^{-1}. For SS, application rates were calculated according to the crop's N demand and the estimated amount of plant-available N released through the mineralization of organic matter. Although mineralized N was not directly measured, the carbon-to-N (C/N) ratio of the SS was used as a key indicator to estimate its N release potential. The applied SS had a relatively narrow C/N ratio (average of 16), promoting rapid decomposition and mineralization of N (≥ 30%), thereby contributing to enhanced soil N availability in both the short and long term (Azam & Lodhi, 2001, Bourioug et al., 2014; Rigby et al., 2016).

The SS used was applied three weeks before barley seeding. The MF was administered in two equal split doses: the first when the third leaf had emerged, and the second at the 1 cm ear stage, to minimize the risk of excessive nitrate accumulation in soil. Sowing was performed on December 22, 2021.

Before sowing, a layer of gravel was first added in the bottom of each rhizotron, followed by 4 kg of soil and watered to field capacity. After planting (four seeds per rhizotron), the rhizotrons were placed on supports at 45° angle to the vertical, with the transparent face oriented downwards, to promote root development along the front plate through gravitropism.

2.3. Plant measurements

Throughout the experiment, different plant growth parameters were monitored every two weeks, including shoot height, root growth, leaf, tiller (fertile and sterile) and ear count, and the evolution of the chlorophyll index (ChI) (measured non-destructively using the Minolta SPAD-502 meters). Plant height was initially determined from the collar to the tip of the taller leaf; at the heading stage, it was measured from the collar to the top of the terminal spike (excluding the beards). Root development was monitored every two weeks by manually tracing visible root outlines on transparent plastic sheets placed against the transparent side of the rhizotrons. These tracings were subsequently scanned and used as a qualitative visual indicator of treatment-induced variations in root system density and architecture, as well as their spatial and temporal distribution. At the final harvest, the fresh and dry weights of shoots (straw and grain) and roots (after 72 h of drying in an oven at 60 °C) were measured.

2.4. Statistical analysis

The collected data were analysed statistically using analysis of variance (ANOVA) performed with the Statistical Package for Social Sciences software (SPSS 20.0 for Windows). The means of four replicates were compared using Duncan's multiple range test at a 5% significance level. Average values per treatment were labelled with lowercase letters (a, b, c) to indicate statistically significant differences. The letter "a" indicates the highest average. Treatments sharing the same letter are not significantly different, while those labelled with different letters show statistically significant differences. Relationships between variables were assessed through Pearson correlation analysis, also conducted using SPSS. Additionally, the data were subjected to principal component analysis (PCA) to visualize the grouping and differences between the various treatments. The PCA was performed using SRplot, a free online platform for data visualization and graphing (Tang et al., 2023).

3. RESULTS

3.1. Effect of sewage sludge on seed germination

The GI is widely used as an indicator to evaluate the phytotoxicity of SS. Compared to the control, it is calculated based on the relative E and seed G in response to SS extract. Figure 1 illustrated the variation in the GI of barley seeds across different SS treatments. The results showed an increase in GI with rising SS concentrations (T2, T3, T4) compared to the control (T1). These increases ranged from 9% (in T3) to 50% (in T2).

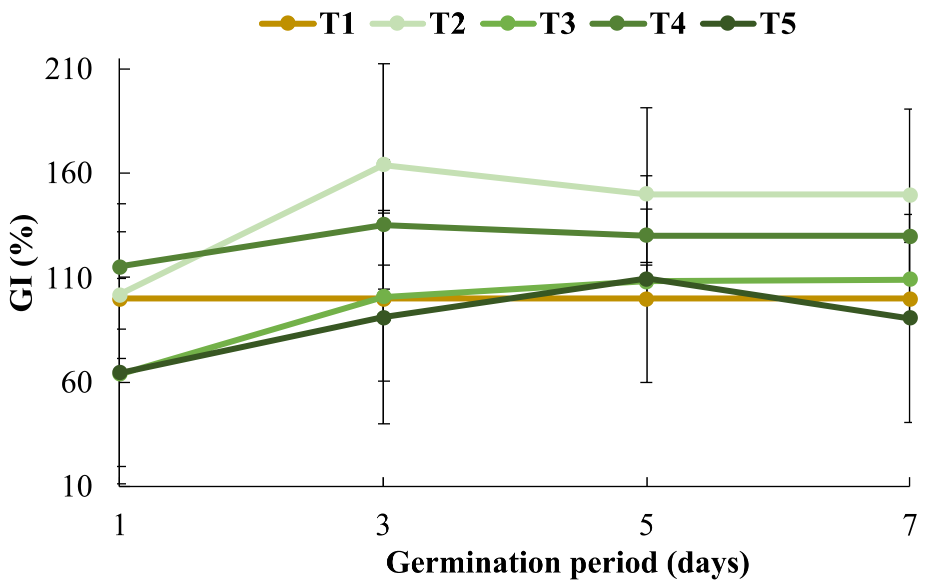

Figure 1. Germination indexes (GI) of barley seeds exposed to different sewage sludge concentrations (T2, T3, T4, and T5) extracts, compared to the control (T1).

The values are mean of four replicates. Treatments: T1: 0%; T2: 0.5%; T3: 0.8%; T4: 1.2%; T5: 1.7% of SS-soil mixtures.

The EC50 analysis revealed that the maximum effects on G (%), E (%), and GI (%) were 161.54%, 179.48%, and 196.36%, respectively, with corresponding EC50 values of 80.77%, 89.74%, and 98.18%. These results identify the optimal SS concentrations for achieving agronomic benefits without phytotoxicity. Notably, T3 (0.8% SS-soil mixture) reached a G of 92.31%, nearing its EC50 value of 80.77%. Similarly, T5 (1.7% SS-soil mixture) resulted in E and GI values of 84.36% and 98.07%, respectively, nearing their EC50 values of 89.74% and 98.18%.

3.2. Effect of sewage sludge on plant growth

The effect of SS on barley shoot height was monitored at various growth stages, with final measurements taken at harvest (Figure 2a). Although the differences were not statistically significant, a moderate increase in plant height was consistently observed in all SS amended treatments (T3-T6), compared to both the unfertilized treatment (T1) and the sole mineral treatment (T2). Notably, the T5 treatment (50% N from each source) showed similar results to T6 (70% N from MF + 30% N from SS), indicating limited additional benefit from increasing the proportion of MF. The highest increase, 15%, was recorded in T3 (100% N from SS) and T4 (70% N from SS + 30% N from MF) compared to the control (T1).

Regarding tiller development, barley plants showed only a limited response to MF alone (T2), with no significant differences between control (T1) and T2 (Figure 2b). In both treatments, only 30% of tillers were fertile. In contrast, treatments involving SS and SS+MF treatments, promoted tiller development, by increasing tiller number per plant, compared to both the unfertilized treatment (T1) and the sole conventional fertilization (T2). In Addition, the number of fertile tillers (FT) increased significantly with higher SS application rates. The highest increases, 94% and 83%, were recorded with T3 (100% SS) and T4 (70% SS+30% MF), respectively, compared to the control (T1).

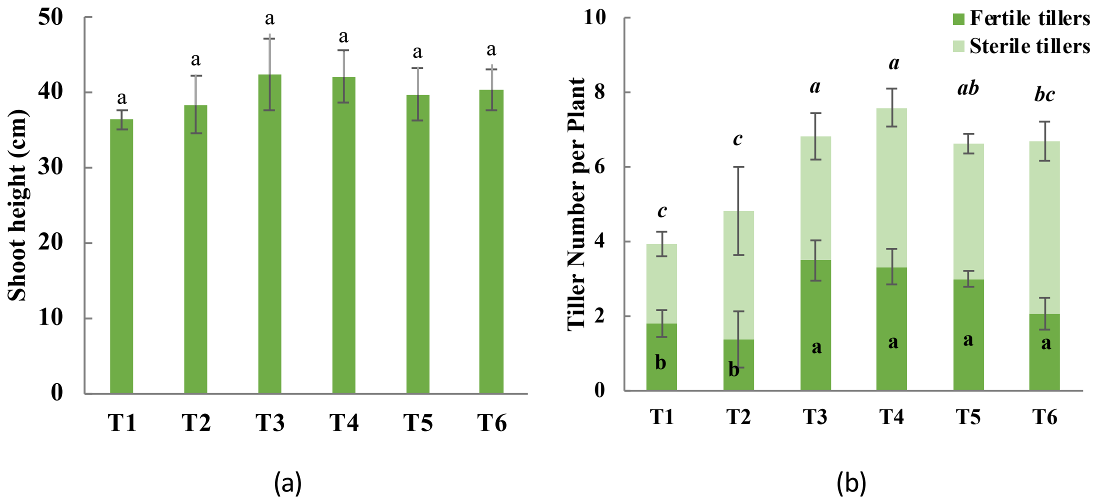

Figure 2. Average shoot height (cm) (a) and tiller number (b) in each treatment (Mean values of four replicates).

Treatments: T1: 0 N; T2: 100% N MF; T3:100% N SS; T4: 30% N MF + 70% N SS; T5: 50% N MF + 50% N SS; T6: 70% N MF + 30% N SS. (a, b, c), (**a, b, c**) and (***a, b, c***) indicate significant differences in shoot height, fertile tiller number, and total tiller number respectively. Average values followed by the same letter are not significantly different at $p < 0.05$.

Monitoring of the ChI throughout the experimental period and across different treatments revealed that the addition of SS at increasing rates (T6, T5, T4 and T3) led to a significant increase in leaf chlorophyll content, compared to both control (T1) and MF (T2) (Figure 3). The ChI increased by 50% and by 17% in T3 (sole application of SS), compared to the control (T1) and MF alone (T2), respectively.

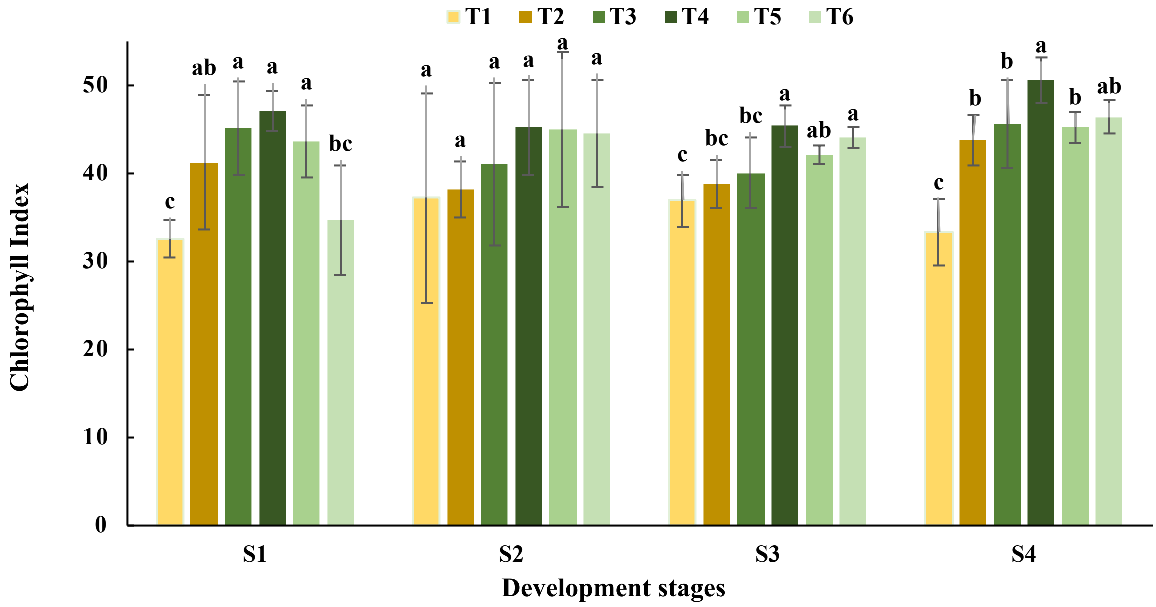

Figure 3. Chlorophyll index throughout the development cycle of barley in each treatment.

Treatments: T1: 0 N; T2: 100% N from MF; T3: 100% N from SS; T4: 30% N from MF + 70% N from SS; T5: 50% N from MF + 50% N from SS; T6: 70% N from MF + 30% N from SS. For each stage, (a,b,c) indicate significant differences in Chlorophyll index between treatments. Average values followed by the same letter are not significantly different at $p < 0.05$.

Root growth exhibited a similar trend: the lowest growth was recorded in the unfertilized treatment (T1), followed by the sole MF treatment (T2). Conversely, all SS-based treatments (T3-T6), significantly promoted root development. The most substantial growth was observed at the highest SS application rates (T3 and T4) (Figure 4).

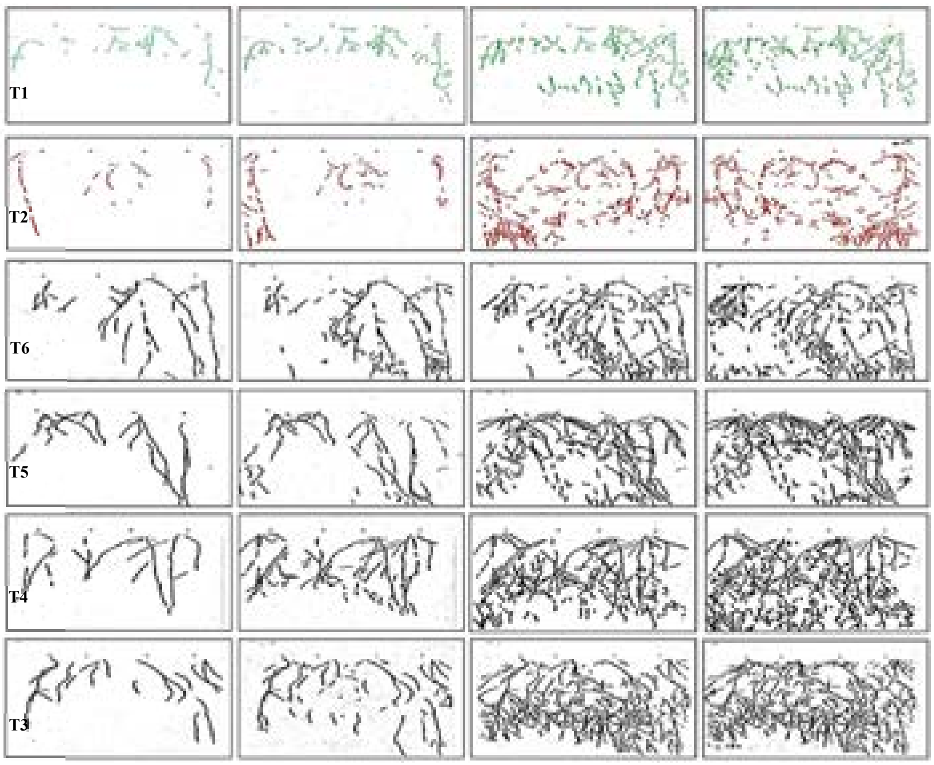

Figure 4. Root growth throughout the development cycle of barley in each treatment.

Treatments: T1: 0 N; T2: 100% N from MF; T3: 100% N from SS; T4: 30% N from MF + 70% N from SS; T5: 50% N from MF + 50% N from SS; T6: 70% N from MF + 30% N from SS.

3.3. Effect of sewage sludge on plant production

Shoot dry matter production was significantly higher in SS-amended treatments (T3-T6) compared to both the control (T1) and MF (T2) (Figure 5). Among the SS treatments (T3-T6), the highest increases, 114% and 120%, occurred in T3 (100% SS) and T4 (70% SS + 30 MF), respectively, compared to the control. MF alone (T2) produced only a 22% increase in shoot biomass (SHB) (Fig. 5). Similarly, the highest root dry matter was recorded in T3 (100% SS) and T4 (70% SS + 30% MF). However, T5, T6, and T2, all with over 50% MF, yielded root biomass (RB) similar to the control.

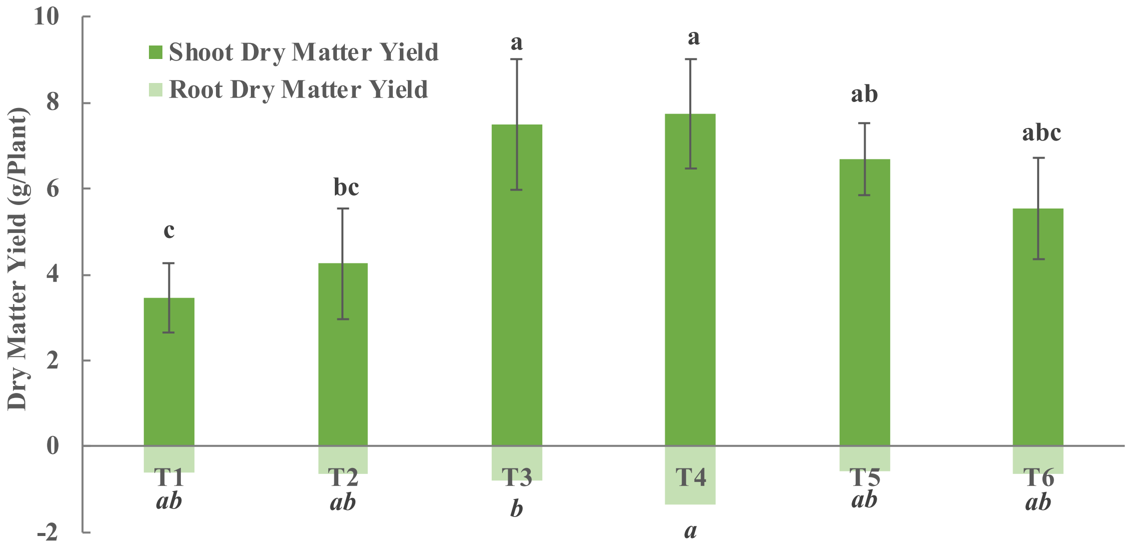

Figure 5. Dry matter production in each treatment (Mean values of four replicates).

Treatments: T1: 0 N; T2: 100% N from MF; T3: 100% N from SS; T4: 30% N from MF + 70% N from SS; T5: 50% N from MF + 50% N from SS; T6: 70% N from MF + 30% N from SS. (a, b, c) indicate significant differences in shoot dry matter production between treatments; (a, b, c) indicate significant differences in root dry matter production between treatments. Average values followed by the same letter are not significantly different at $p < 0.05$.

The PCA biplot illustrated (Figure 6) the impact of SS and MF on key growth and production parameters, including FT, sterile (ST), and total tillers (TT), Chl, RB and SHB. The first two principal components (PC1 and PC2) account for 82.3% of the total variation, with PC1 ex-plaining 63.7% and PC2 explaining 18.6%, confirming the effectiveness of the PCA in summarizing the relationships among the different treatments (T1 to T6). PCA results con-firmed that treatments involving SS (T3-T6), were closely associated with enhanced biomass and plant development (SHB, RB, FT). Interestingly, T6 (70% MF + 30% SS) and T2 (100% MF) showed similar patterns and clustered closely in the PCA plot, whereas T1 and T2 appeared in the bot-tom-left quadrant, indicating a relatively weaker effect of MF, even at full (T2) or high application (T6) rates, particularly on shoot growth and chlorophyll content.

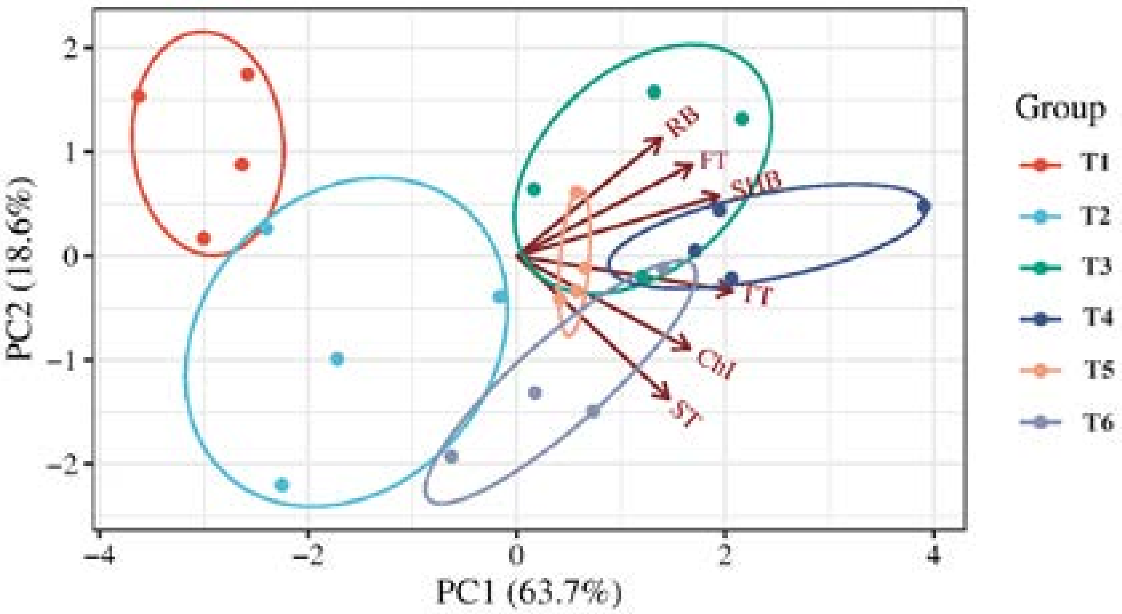

Figure 6. PCA biplot of barley growth and production traits under different treatments.

4. DISCUSSION

Sewage sludge treatments increased the GI, with the highest value observed at the lowest application rate (T2). This improvement suggests the absence of phytotoxic effects, as supported by Saidi et al. (2009), and may be attributed to the stimulatory properties of SS on seed germination. According to Barral & Paradelo (2011), an organic amendment with a GI above 80% is considered non-phytotoxic, whereas lower values suggest potential toxicity. Furthermore, a GI exceeding 100% indicates the presence of stimulatory compounds in the compost aqueous extracts (Moldes et al., 2007). These findings confirm that the SS used in this experiment was not phytotoxic to barley seed germination. By contrast, specific concentrations (T2, T3, and T4) exerted a promotive effect. Similar observations were reported by Kominko et al. (2022), who found that GI values for watercress, sorghum, and mustard ranged from 50% to 92% with different SS rates, suggesting moderate to no toxicity. Our results also align with those of Karoune (2008), who showed that organic amendments, particularly SS, enhanced germination and vigour of cork oak acorns across various application rates (20% to 80%). However, excessive SS application may reduce seed germination due to the accumulation of high levels of heavy metals (Kesherwani et al., 2022). EC50 analysis revealed that a 0.8% SS concentration elicited half of the maximum germination response, in line with Nunes et al. (2021), who reported that moderate SS levels enhanced seed germination without adverse effects. Moreover, E and GI continued to improve even at the highest concentration tested (1.7%), suggesting that this rate approaches optimal level for these growth parameters. Supporting these finding, Eid et al. (2017) observed that comparable SS rates promoted root development and crop quality without inducing harmful accumulation of heavy metals. These insights are critical for optimizing SS application in agriculture. Determining EC50 values allows for the establishment of effective and safe application rates that maximize agronomic benefits while minimizing environmental risks, such as contaminant accumulation or nutrient imbalance. This is consistent with the findings of Hamdi et al. (2019), who highlighted the importance of adapting SS application to specific soil and environmental conditions to improve soil fertility and crop productivity.

Despite the response being non-significant, a consistent increase in shoot height was observed in all SS amended-treatments, particularly in T3 (100% N from SS) and T4 (70% SS + 30% MF). These results are in accordance with those of Jatav et al. (2022), who found that both sole and joint applications of SS with MF positively impacted growth and production in a rice–wheat cropping system. Their study reported that applying 30 Mg SS ha^{-1}, combined with a recommended or low conventional fertilizer rates, led to an increase in plant height by approximately 14.9% and 13.9%, and grain yield by around 8.1% and

18.9% for rice and wheat, respectively, compared to sole application of MF. Similarly, Shan et al. (2021) observed improvements in rice yield, root growth, and activity following SS application, particularly during the tillering, jointing, and maturity stages. According to Latare et al. (2014), the combined use of SS and MF enhances soil fertility by improving N and micronutrient availability, thereby promoting plant development.

Sewage sludge also enhanced the chlorophyll index and tiller development, more effectively than conventional MF. This beneficial effect can be attributed to the sludge's rich content of various key nutrients, such as N, P, magnesium (Mg), zinc (Zn) that are crucial for plant development, but often lacking in MF. Our results are corroborated by Boudjabi et al. (2019), who reported that the increase in tillering number in wheat was linked to the nutrient input from SS, especially N, which enhanced N content in plant tissues, supported cellular integrity, and promoted aerial growth, factors contributing to improve the plant's tillering capacity. Likewise, Kchaou et al. (2018) reported that SS amendments improved triticale development by increasing plant height, leaf number, and chlorophyll index throughout the growth cycle. In the same way, Korboulewsky et al. (2002) and Orman et al. (2014) have highlighted the contribution of SS-derived elements like Zn, and Mg to chlorophyll synthesis.

Sewage sludge application also promoted root growth, indicating the absence of heavy metal stress in the soil environment, which could otherwise negatively impact plant growth and biomass accumulation (Seleiman et al., 2013). Our findings agree with Boudjabi et al. (2019), who found that SS improved root length in durum wheat, and with Shan et al. (2021), who observed increases in total root length, surface area, diameter, and volume in rice. Root activity also increased with higher SS doses. According to Latare et al. (2014), these improvements can be attributed to the structural effect of SS on soil physical properties, as well as improvement in soil chemical characteristics.

Similar patterns were observed for barley biomass production. Although MF (T2) led to a slight increase in shoot biomass, the effect was notably less pronounced than in SS treatments, particularly T3 and T4. Similarly, Seleiman et al. (2013) showed an increase on biomass accumulation of different crops including maize, rye grass, and hemp fertilized with SS greater than that with synthetic fertilizers. The enhancements in shoot biomass under T3 and T4 can be attributed to the increases in root development observed in these treatments. The positive correlation (r = 0.694) between root and shoot production supports the idea that SS fosters vigorous root growth, enabling plants to explore deeper soil layers and absorb more water and essential nutrients (Boudjabi et al., 2019). Similar findings have been reported by Kukkola et al. (2000), Biernacki & Lovett-Doust (2002) and Orman et al. (2014), who highlighted the crucial role of root system in plant growth and development, noting that any alteration to root structure can affect other plant organs. These studies also linked root damage to heavy metals accumulation, particularly Ni, Cu, and Cr, when SS was applied to soil at rates exceeding 40 t/ha. Similarly, Yagmur et al. (2017) found that increasing SS rates (0, 5, 10, 15, 25, 30 t ha^{-1}) did not lead to elevated heavy metals (Al, Cd, Cr, Ni, Pb, and Cu) concentrations in triticale straw. In addition, they revealed that straw biomass obtained after application of 30 t ha^{-1} of SS could be safely recommended for animal feed, without posing heavy metal risks. Consistently, Eid et al. 2020 reported that all SS amendment improved the postharvest soil properties and enhanced barley growth, with the most pronounced effect observed at a dose of 40 g/kg. At this rate, the soil organic matter content increased to 6.7% compared with 1.4% in the control. Heavy metal concentrations in postharvest soils remained within permissible limits for agricultural use across all treatments, except for Co. Moreover, all SS rates enhanced barley growth and yield traits, including shoot and root lengths, dry biomass, spike weight and spike number per plant. Up to the 40 g/kg dose, heavy metal in harvested plant tissues remained within safe thresholds, with most metals retained in the roots. Collectively, these findings suggest that only the land application of inappropriate or excessive rates of SS may lead to the accumulation of heavy metals in agricultural soils, thereby causing soil contamination and reducing crop productivity (Kesherwani et al., 2022). Importantly, this does not exclude the long-term risks associated with the cumulative build-up of toxic elements which may negatively impact groundwater, soil health, plant development, and untimely the food chain and environment.

The PCA analyses confirmed that both SS and SS+MF treatments significantly improved barley performance, particularly in terms of biomass and tillering capacity. These improvements can be attributed to the higher nutrient SS efficiency and retention compared to MF alone. Our findings align with previous findings (Singh & Agrawal, 2008; Boudjabi et al., 2019) demonstrating the positive impact of nutrient-rich SS on soil fertility and crop performance. These results are consistent with studies by Latare et al. (2014) and Wang et al. (2021), which showed that combining SS with MF reduced nutrient losses and enhanced plant growth and reduces nutrient losses. Our findings support the literature (e.g., Guo 2020), highlighting the agronomic and environmental benefits of organic amendments like SS in sustainable agriculture.

Overall, visual assessments and PCA analyses confirmed that SS treatments had a consistently positive effect on barley growth parameters. MF had a comparatively lower impact, highlighting its limited effectiveness compared to SS-based treatments. These results corroborated those of Antolin et al. (2005), who found that SS is more effective than nitrogenous MF for improving barley yield. Similar conclusions were drawn by Kchaou et al. (2018, 2020), who reported yield increases of up to 62% in triticale following SS application of 6 t ha^{-1}. The reduced efficiency of MF may be due to nutritional imbalance, since only N was supplied, or to nutrient losses via leaching or volatilization. Andrés et al. (2010) and Yagmur et al. (2017) reported similar outcomes in kenaf crops, while Wang et al. (2021) noted that N leaching losses can reach 32.14%. In contrast, the superior performance of SS highlights its efficiency as a nutrient source, particularly for N, P, Mg, Zn, and Cu, which are often lacking in MF but essential for chlorophyll synthesis and overall plant health (Korboulewsky et al., 2002; Balkrishna et al., 2025).

The benefits of SS amendment can be mainly attributed to the rapid mineralization of organic N released during the decomposition of organic matter in SS, characterized by high N content and a narrow C/N ratio (Azam & Lodhi, 2001). As highlighted by Bourioug et al. (2014) and Rigby et al. (2016), SS with a high C/N ratio (typically >20) tends to promote N immobilization, thereby delaying its availability to plants. In contrast, SS with a narrow C/N ratio (< 25), such as that used in our study (average C/N = 16), promotes rapid decomposition and mineralization of N within just few weeks after application, enhancing soil N availability in both the short and longer term. Consistently, Kchaou et al. (2010), using direct method based on ^{15}N isotope tracing, demonstrated that ^{15}N-labeled urban digested SS, characterized by a narrow C/N ratio and low heavy metal content, exhibited N dynamics comparable to those of urea fertilization, achieving a total N recovery of approximately 27%. The availability of N from SS to crops is influenced by several factors beyond its intrinsic properties, particularly soil characteristics and the type of SS treatment. Correa et al. (2006) reported that digested SS exhibited nearly twice the N mineralization rate of composted SS when applied at comparable N levels. Similarly, Yoshida et al. (2015) revealed that limed SS could release 1.5 to 6 times more mineral N than digested or dried and dewatered SS, respectively. In addition to SS treatment, soil properties, especially pH, and the availability of key compounds such as ammonium, nitrate significantly influence N dynamics in soils amended with various types of stabilized SS. For instance, Correa et al. (2006) found that digested SS released higher amounts of NH_4^+ and NO_3^- in a clayey Ustox Oxisol compared to a sandy Orthod Spodosol. Huang & Chen (2009) reported that applying composted SS to acidic soils led to lower nitrification rates than in neutral to alkaline soils. Consistently, Giannopoulos et al. (2025) demonstrated that low soil pH inhibited NH_4^+ oxidation, while high pH limited NO_3^- reduction. Their findings also revealed that, regardless of soil type or SS treatment, SS-amended soils exhibited lower N_2O emission factors and maintained a higher pool of mineralizable N than those receiving MF. These combined effects on N availability, particularly the use of digested SS with a narrow C/N, applied to an alkaline soil (with basic pH), may partly account for the high performance and efficiency of SS observed across all evaluated growth and yield parameters throughout our study.

Although our results provide valuable insights under controlled conditions, several important factors must be considered before applying these findings to agricultural systems. Variability in SS composition (water, mineral, and chemical content), difference in soil characteristics (texture, cation exchange capacity, and mineral composition), across different sites, along with varying field management practices including SS application methods (surface spreading vs. incorporation into the soil) and water regimes (rain-fed vs. irrigated, freshwater vs. saline water) can probably influence SS behavior and subsequent nutrient bioavailability and mobility, as well as the fate of potential contaminants. These complex interactions among SS, soil, water, and plant systems underscore the necessity for long-term, *in situ* studies to validate and extend the findings of this greenhouse experiment.

5. CONCLUSIONS

Sewage sludge amendments both sole and combined, with MF, significantly enhanced barley growth and biomass production. In all cases, SS consistently produced better results than pure mineral fertilization. Due to its richness in macro and micronutrients, as well as organic matter, elements often lacking in conventional fertilization, SS can be effectively used in combination with MF, even at a rate as low as 30%, to boost biomass production under low-input conditions and contribute to preserve soil health, thus supporting sustainable agriculture practices. However, further studies involving the direct measurement of mineralized are necessary to enable a more accurate evaluation of N availability, and help establish a clearer link between the N actually released from SS and barley performance, thereby improving the assessment of SS efficiency as a fertilizer. In addition, long-term studies are required to evaluate the agronomic value of SS and to validate its potential as a sustainable alternative to synthetic fertilizers. In particular, long-term and continuous field trials are recommended to assess the cumulative impacts of repeated SS applications on the plant-soil system. While a single application of SS with low heavy metal content, as in the present study, may not pose immediate risks, repeated use could potentially lead to soil and crop contamination. Therefore, continuous monitoring of heavy metal accumulation and other potential pollutants is essential to ensure the safe and sustainable agriculture reuse of SS.

Supplementary information ↑

Funding sources

The authors received no specific funding for this work.

Supplementary material

Not applicable.

Data availability

Not applicable.

Acknowledgements

Not applicable.

Authorship contribution statement

Rajia Kchaou: Conceptualization, Methodology, Data curation Formal analysis, Supervision, Validation, Visualization, Writing – original, draft Writing – review & editing.

Abir Rahmani: Methodology, Data curation.

Nidhal Mazougui: Data curation, Formal analysis.

Salah BenYoussef and Mohamed Hachicha: Methodology, Resources.

Competing interests

The authors have declared that no competing interests exist.

Statement on the use of Artificial Intelligence

Not applicable.

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