THE INFLUENCE OF ACTIVATED SLUDGE ON GERMINATION, GROWTH DEVELOPMENT AND BIOCHEMICAL PARAMETERS OF TOMATO SEEDLINGS *(Lycopersicon esculentum Mill.)*

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RESEARCH ARTICLE

Abstract

This study evaluated the effects of activated sludge on germination, early growth, photosynthetic pigments, and antioxidant capacity in Lycopersicon esculentum. Four treatments were applied: Control, LC, MC, and HC. Activated sludge markedly enhanced the germination index, with the strongest response in the HC group, likely due to its nutrient-rich composition and microbial activity. Biometric measurements showed improved plant height, root length, and leaf number in MC and HC treatments, indicating stimulated early growth. In contrast, chlorophyll a and b concentrations decreased with increasing sludge levels, suggesting a potential stress response or treatment-specific factors affecting pigment synthesis. Antioxidant assessments revealed substantial increases in total phenolic content and DPPH radical-scavenging activity in MC and HC groups, reflecting an enhanced antioxidant defense. The activated sludge shows promise as a sustainable organic amendment that improves germination and stress-related antioxidant capacity. Further research is needed to clarify the mechanisms underlying pigment reduction and to optimize application rates for agricultural use.

Keywords: active sludge, sustainability, sustainable agriculture
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INTRODUCTION

Lycopersicon esculentum (tomato) is one of the most important horticultural plants worldwide, with significant economic importance due to its extensive use in food consumption and the food processing industry. Tomatoes are widely cultivated in various regions globally and are valued for their rich content of vitamins, minerals, and antioxidants, such as lycopene.

The economic significance of tomatoes stems from the constant global demand, both for fresh consumption and for processed products such as sauces, tomato paste, and canned goods.

In the current context, agriculture faces multiple challenges, including climate change and drought. In this framework, the use of alternative resources, such as activated sludge derived from wastewater treatment, represents an innovative solution for improving seed germination and plant growth. Activated sludge is a byproduct of the wastewater treatment process and contains essential elements, including nutrients and beneficial microorganisms, which can stimulate plant growth and enhance soil fertility. Compared to regular water, activated sludge provides additional advantages, such as a high content of organic matter and macro- and micronutrients (nitrogen, phosphorus, potassium, calcium, magnesium, etc.).

Previous studies have highlighted the beneficial effects of dried sludge on plant growth, but limited research has explored the use of liquid activated sludge. For example, a study conducted by Garcia, (2021) demonstrated that applying dried sludge to soil improved wheat seed germination and plant vigour by enriching the soil with essential nutrients. However, applying liquid activated sludge may offer additional advantages, such as faster nutrient absorption and increased activity of beneficial microorganisms in the soil (Seleiman et al., 2024).

This study makes a significant contribution to the scientific literature by investigating the effects of liquid activated sludge on tomato seed germination and growth, a relatively unexplored area. The use of liquid activated sludge presents an innovative approach that could provide viable solutions for farmers facing soil fertility issues and limited water availability.

The primary objective of this study was to evaluate the effects of activated sludge on germination and germination indicators in

tomato plants (*Lycopersicon esculentum).* Specifically, this research aims to determine:

- *Germination rate*: comparing the percentage of germinated seeds under activated sludge treatment versus regular water conditions.
- *Germination speed*: analysing the germination rate in both treatment conditions.
- *Plant vigour*: assessing plant vigour through growth measurements (plant height, biomass) and overall plant health.

This topic was chosen to highlight the potential benefits that activated sludge can bring to plant growth and productivity. The motivation for research arises from the need to find sustainable agricultural solutions, particularly in the context of drought and limited water availability. Additionally, this study contributes to expanding existing knowledge by offering new insights into the use of activated sludge in agriculture, contrasting with previous research that has primarily focused on dried sludge.

MATERIALS AND METHODS

1. *Plant Material*

This study used tomato seeds (*Lycopersicon esculentum),* purchased from a local supermarket (Auchan). The seeds were selected to ensure uniformity and quality for germination and growth experiments.

2. *Experimental Design*

The experiment consisted of four treatment groups:

- Control (C): seeds were watered with regular water for 14 days.
- Low Concentration (LC): seeds were treated with a 2.5% activated sludge solution (2.5 ml sludge in 100 ml water).
- Medium Concentration (MC): seeds were treated with a 5% activated sludge solution (5 ml sludge in 100 ml water).
- High Concentration (HC): seeds were treated with a 10% activated sludge solution (10 ml sludge in 100 ml water).

The seeds were placed in four trays (15 x 10 cm) lined with cotton and filter paper. Each tray contained 24 selected seeds and was kept at room temperature (19-20^{0}C) under natural light.

The experiment lasted 14 days. At the end of the experiment, the following analyses were performed:

- Germination analysis: germination rate and speed were recorded daily.
- Biometric measurements: plant height and biomass were assessed to evaluate plant vigour.
- Post-experimental processing: after measurements, plants were dried at 40^{0}C for 9 hours, and prepared for further biochemical analyses:
- Chlorophyll and carotenoid content analysis
- DPPH assay: Antioxidant capacity evaluation using the DPPH method (2,2-diphenyl-1-picrylhydrazyl).
- Total polyphenolic content by Folin-Ciocalteu assay

BIOMETRIC MEASUREMENTS

To evaluate plant growth and development, biometric measurements were conducted at the end of the 14-day period. The recorded parameters included:

- Plant height: Measured from the base of the stem to the tip of the highest leaf using a metric ruler.
- Root length: Measured from the root-stem junction to the longest root tip, assessing root system development.
- Stem length: The segment between the root insertion point and the first major branching or fully developed leaf.
- Leaf count per plant: The number of fully developed leaves, serving as an indicator of photosynthetic activity and plant health.

All measurements were taken under consistent conditions to minimize variability. The same operator conducted all assessments to ensure methodological accuracy.

PHOTOSYNTHETIC PIGMENT DETERMINATION

Dried plan samples were ground, and 0.1 g of the powered material was extracted with 3 ml of 70% ethanol. The mixture was centrifuged at 10.000 rpm for 15 minutes, and the supernatant was analysed to

determine chlorophyll a, chlorophyll b, and total carotenoid content. Spectrophotometric measurements were performed using a UV-VIS spectrophotometer (UV mini-1240, Shimadzu) following the protocol described by Nayek S. et al., 2014.

DETERMINATION OF TOTAL PHENOLIC COMPOUNDS - FOLIN CIOCALTEU METHOD

The total phenolic content was determined using the Folin-Ciocalteu method, with slight modifications based on Singleton et al., 1999 and Vicas et al., 2019. The calibration curve was established using gallic acid standards in the range of 0.05-0.25 mg/ml, and results were expressed as mg gallic acid equivalents (GAE)/g of dry weight (dw). The calibration curve followed the regression equation=2.2793x+0.0862(R^2=0.3584).

DETERMINATION OF ANTIOXIDANT CAPACITY BY THE DPPH METHOD

The antioxidant capacity of the extracts was determined using the DPPH radical scavenging assay, following the protocol described by Miliauskas et al., 2003. For each sample, 0.1 mL of extract was mixed with 2.9 mL of 80μM DPPH solution and incubated in darkness for 30 minutes. Absorbance was measured at 517 nm using a Shimadzu UV-mini-1240 UV-Vis spectrophotometer. The calibration curve was established using Trolox as a standard, and results were expressed as the percentage of DPPH radical inhibition, calculated using the formula: Percent of inhibition of DPPH(%) $= \frac{(A_0 - A_s) \times 100}{A_0}$

Where:

- A_0= absorbance at 517 nm for the blank sample
- A_s= absorbance at 517 nm for the extract samples.

The regression equation for the calibration curve was: $y = 0.218x + 97.125$ and $R^2 = 0.2043$ and the results were expressed as mol Trolox Equivalents (TE)/g dw.

RESULTS

1. Germination Index

Figure 1 shows the Germination Index (GI) for the four experimental groups, an indicator reflecting both the proportion of germinated seeds and the speed of germination. All treatments with activated sludge (LC, MC, and HC) resulted in significantly higher GI values compared with the control ($p < 0.05$). The HC group exhibited the highest GI, statistically exceeding both LC and MC, while the latter two did not differ significantly from each other. The control group recorded the lowest GI, indicating reduced germination performance relative to all treated groups. These results demonstrate a clear, concentration-dependent increase in the Germination Index across the activated-sludge treatments.

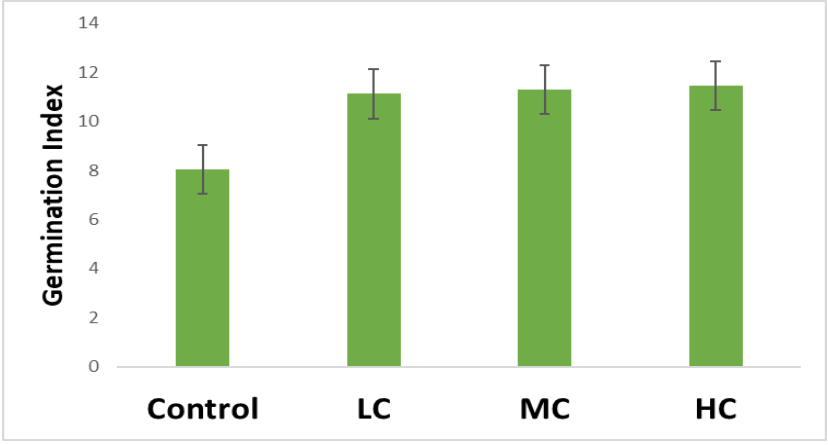

Figure 1 **Germination Index (GI) of Lycopersicon esculentum seeds across the four experimental groups: Control, LC, MC, and HC. Bars represent mean ± standard deviation.**

2. *Photosynthetic Pigment Analysis*

Figure 2 shows the concentrations of chlorophyll a, chlorophyll b, and carotenoids across the four treatment groups. Chlorophyll *a* levels were highest in the control group (6.81 µg/mL) and progressively decreased with increasing sludge concentration, reaching the lowest value in the HC group (5.79 µg/mL). A similar pattern was observed for chlorophyll b: the control group displayed the highest concentration (7.00 µg/mL), while the HC treatment recorded the lowest (6.32 µg/mL). Carotenoid content showed a slightly different trend. The highest carotenoid concentration occurred in the MC group (0.87 µg/mL), followed by HC (0.81 µg/mL), whereas the control and LC groups exhibited comparable values (0.78–0.79 µg/mL). Overall, these results demonstrate that activated sludge treatments modified the pigment profile of Lycopersicon esculentum seedlings. Increasing sludge concentration was associated with a gradual reduction in chlorophyll a and b levels, while carotenoid content showed a moderate increase at intermediate and high treatments.

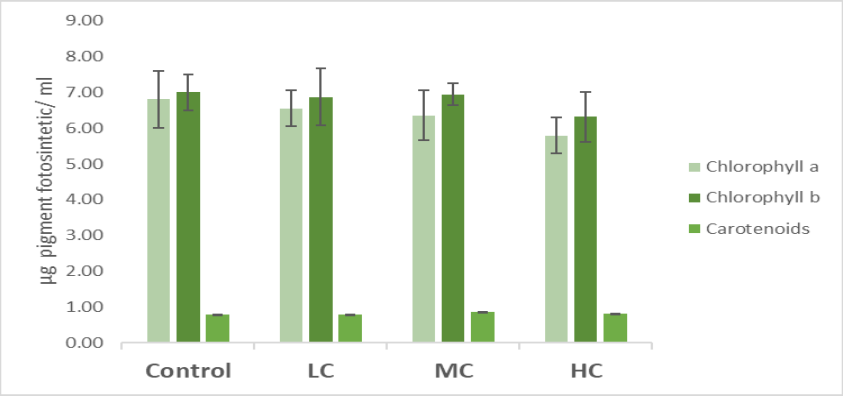

Figure 2 **Concentrations of chlorophyll a, chlorophyll b, and carotenoids in Lycopersicon esculentum seedlings subjected to Control, LC, MC, and HC treatments. Values are presented as mean ± standard deviation**

3. *Determination of total phenolic compounds - Folin Ciocalteu Method*

Figure 3 presents the total phenolic content (TPC) of Lycopersicon esculentum seedlings across the four treatment groups. The MC group exhibited the highest TPC value (5.32 mg GAE/g dw), followed by the HC group (3.28 mg GAE/g dw). In contrast, the Control and LC groups showed markedly lower and comparable TPC values (1.07 and 1.11 mg GAE/g dw, respectively). These results indicate that medium and high concentrations of activated sludge led to a pronounced increase in phenolic compound accumulation, whereas low-concentration treatment produced no substantial change relative to the untreated control.

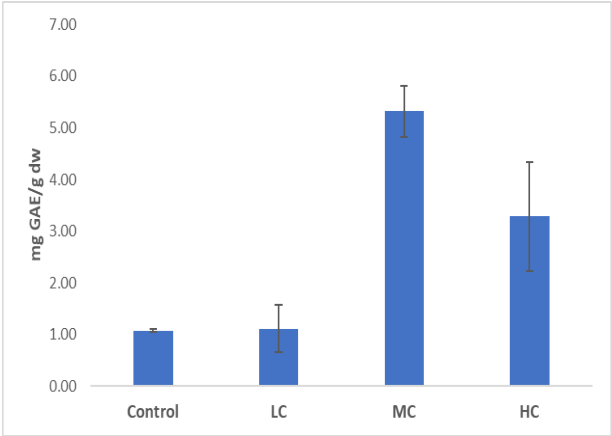

Figure 3 **Total phenolic content (TPC) of *Lycopersicon esculentum* seedlings subjected to Control, LC, MC, and HC treatments. Values are expressed as mg gallic acid equivalents/gram dry weight (mg GAE/g dw) and presented as mean ± standard deviation.**

4. *Determination of antioxidant capacity using the DPPH method*

Figure 4 shows the antioxidant capacity of *Lycopersicon esculentum* seedlings, expressed as DPPH radical-scavenging activity. The lowest antioxidant activity was observed in the LC group (0.95 mol/g dw), followed by the Control group (2.40 mol/g dw). In contrast, the MC treatment produced a marked increase in antioxidant capacity (4.97 mol/g dw), and the highest value was recorded in the HC group (6.59 mol/g dw). These results indicate a clear dose-dependent enhancement of antioxidant activity, with medium and high sludge concentrations substantially increasing the plant's radical-scavenging potential.

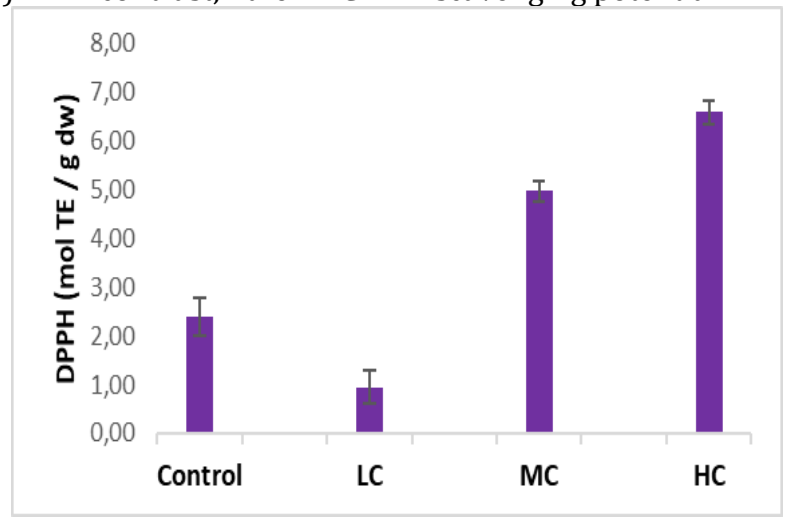

Figure 4 **Antioxidant capacity of Lycopersicon esculentum seedlings determined by the DPPH radical-scavenging assay across the Control, LC, MC, and HC treatments. Values are expressed as mol DPPH scavenged per gram dry weight (mol/g dw) and presented as mean ± SD.**

DISCUSSIONS

Germination Analysis

The results of this study show that activated sludge treatments significantly enhanced the germination index of Lycopersicon esculentum seeds, with the most pronounced effect observed in the HC treatment. This stimulation contrasts with some previous findings, where organic amendments such as compost teas or wastewater by-products either had no significant influence or negatively affected germination due to phytotoxic compounds (Coria et al., 2022; Smith, 2020). The discrepancy may be attributed to differences in the physicochemical properties of the organic materials used. Activated sludge contains elevated levels of

nitrogen, micronutrients, and beneficial microbial communities that may accelerate enzymatic processes linked to early germination and radicle emergence (Seleiman et al., 2019).

Furthermore, sludge-derived microorganisms may contribute to the breakdown of inhibitory seed coat components or support early root development, resulting in improved germination performance compared with the control. These findings highlight the importance of sludge composition, stabilization process, and application rate in determining its biological impact on seed germination.

Photosynthetic Pigment Determination

Unlike several previous studies reporting increases in chlorophyll a and b following the application of organic fertilizers or compost extracts (Mthiyane et al., 2024; Carrascosa et al., 2023), the present study revealed a reduction in chlorophyll content as sludge concentration increased. This decline may indicate a mild stress response, potentially linked to salinity, heavy metal traces, or an imbalance in nutrient availability commonly associated with certain types of wastewater-derived amendments (Irin et al., 2024).

Chlorophyll reduction is a well-documented physiological marker of plant stress, often associated with disruptions in chlorophyll biosynthesis, enhanced degradation, or impaired nutrient uptake (Li et al., 2015). It is possible that exceeding a threshold concentration of particularly in MC and HC treatments—resulted in conditions unfavorable for pigment stability and photosynthetic efficiency.

These results emphasize the need to optimize sludge dosage to balance its fertilizing benefits with potential physiological constraints on photosynthetic machinery.

Antioxidant Capacity Determination

The antioxidant assays demonstrated a strong dose-dependent increase in radical-scavenging activity, with the HC treatment showing the highest DPPH values. This pattern differs from some previous reports where organic amendments triggered neutral or reduced antioxidant responses (Rusli et al., 2022). However, increased antioxidant activity under sludge treatment is consistent with studies indicating that moderate abiotic stress caused by organic residues, salinity, or micronutrient accumulation, can stimulate the biosynthesis of phenolic compounds as part of the plant's protective mechanism (Kaczur et al.,2025; Ray et al., 2024).

The elevated total phenolic content observed in MC and HC groups supports this interpretation, suggesting that activated sludge induced a metabolic adjustment aimed at counteracting oxidative stress. Phenolics and related secondary metabolites function as radical scavengers, metal chelators, and modulators of stress signaling, which together contribute to the increased DPPH capacity recorded in this study. Differences from earlier findings likely stem from variations in sludge composition, extraction solvents, and antioxidant assessment methods, all of which are known to influence phenolic quantification and radical scavenging results.

CONCLUSIONS

This study evaluated the effects of activated sludge on germination, early vegetative development, photosynthetic pigments, and antioxidant capacity in *Lycopersicon esculentum*. The results highlight the potential of activated sludge as a sustainable organic amendment with measurable benefits for plant performance.

Activated sludge markedly enhanced seed germination, with the highest concentration producing the greatest improvement. This stimulatory effect is likely associated with its enriched nutrient profile and beneficial microbial communities, contrasting with reports in the literature where organic treatments have shown neutral or inhibitory effects. Biometric parameters including plant height, root length, and leaf

number, also increased in response to sludge application, particularly at medium and high concentrations, indicating a positive influence on early plant growth.
In terms of physiological traits, a decline in chlorophyll a and b levels was observed as sludge concentration increased. This finding diverges from previous studies reporting enhanced pigment synthesis following organic amendments and may reflect treatment-specific differences in sludge composition or associated stress factors.

Conversely, antioxidant capacity increased substantially under sludge treatments, with the highest values recorded at higher concentrations. The elevated DPPH radical-scavenging activity and the corresponding rise in total phenolic content suggest that activated sludge may induce protective metabolic responses that strengthen plant resilience to oxidative stress.

Overall, the findings demonstrate that activated sludge has promising applications as an organic soil amendment capable of improving seed germination, growth, and antioxidant potential in tomato plants. Nonetheless, further investigations are needed to elucidate the underlying biochemical mechanisms, assess long-term soil and plant impacts, and determine optimal application rates to ensure safe and effective use in agricultural systems.

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