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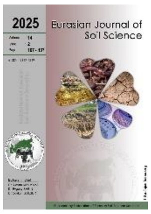

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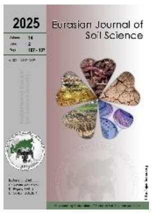

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Impact of Cadmium-contaminated water and irrigation levels on microbiological properties of soils with different textures

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Abstract

Cadmium (Cd) contamination poses a significant threat to soil health and agricultural productivity, particularly under varying water availability and soil textures. This study examines the effects of water levels (25%, 50%, 75%, and 100% field capacity) and soil textures (sandy clay loam, silty loam, and clay) on key microbiological properties, including basal soil respiration (BSR), microbial biomass carbon (Cmic), dehydrogenase activity (DHA), and catalase activity (CA), in Cd-contaminated soils. An incubation experiment was conducted under controlled conditions at 20 ± 0.5°C for 10 days. Microbiological properties were assessed using standard methods: alkali absorption for BSR, substrate-induced respiration for Cmic, spectrophotometric assays for DHA, and volumetric determination for CA. Optimal microbial activity across all parameters was observed at 75% field capacity, highlighting the importance of balanced soil moisture. Clay soils consistently exhibited the highest activity due to their superior organic matter content and buffering capacity, while sandy clay loam soils showed the lowest activity due to limited water retention and nutrient availability. Excessive moisture at 100% field capacity reduced oxygen diffusion, suppressing microbial activity, while insufficient moisture at 25% field capacity constrained microbial metabolism. These findings provide critical insights into the interplay between soil texture, water availability, and Cd contamination, offering valuable guidance for sustainable soil and water management practices to mitigate heavy metal toxicity in agricultural systems.

Keywords: Cadmium contamination, soil microbiology, water levels, soil texture, enzymatic activity.

Introduction

Soil health is critically important for both agricultural sustainability and ecological balance. Soil microorganisms play a vital role in fundamental biochemical processes such as carbon and nitrogen cycling, directly influencing the biological, chemical, and physical properties of soils (Vig et al., 2003; Chen et al., 2014; Kandziora-Ciupa et al., 2016; Li et al., 2018; Bayraklı and Dengiz, 2019). These microorganisms are essential for processes like organic matter decomposition, carbon sequestration, and the provision of bioavailable nitrogen to plants (Naidu et al., 2000; Effron et al., 2004).

Heavy metal contamination is one of the most significant threats to soil health. Cadmium (Cd) is a toxic heavy metal introduced into soils through anthropogenic activities such as industrial waste and mining, severely disrupting soil biochemical cycles by damaging microorganisms and enzymes (Moreno et al., 2001; Kızılkaya and Aşkın, 2002; Tan et al., 2014; Liu et al., 2021). Cadmium's characteristics of bioaccumulation and non-degradability make its environmental impacts severe and long-lasting (Chen et al., 2014; Liu et al., 2024).

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Microbiological parameters such as microbial biomass carbon (C_{mic}), basal soil respiration (BSR), dehydrogenase activity (DHA), and catalase activity (CA) are highly sensitive to environmental stressors, including cadmium contamination and water stress (Kızılkaya et al., 2004; Kızılkaya and Bayraklı, 2005; Li et al., 2018; Yeboah et al., 2021; Chebyshev et al., 2024; Zhang et al., 2024). These parameters are considered critical indicators for understanding how cadmium's toxic effects translate to disruptions in soil functions (Vig et al., 2003; Karaca et al., 2011; Kandziora-Ciupa et al., 2016).

However, there is limited knowledge about the interactions between cadmium contamination and varying water conditions on soil enzymatic activities. Specifically, the impacts of cadmium on microbial communities in soils with different textures remain underexplored (Naidu et al., 2000; Effron et al., 2004; Chen et al., 2014; Mandzhieva et al., 2014; Liu et al., 2024). This knowledge gap highlights the need for a better understanding of the complex interactions among soil texture, water levels, and heavy metal contamination.

The aim of this study is to comprehensively evaluate the effects of cadmium contamination and different water levels on microbiological properties and enzyme activities in soils with varying textures. This study seeks to provide novel insights into how optimal water management can mitigate the harmful effects of cadmium and improve soil health. Additionally, it aims to address existing gaps in the literature by elucidating the role of soil texture in cadmium bioavailability.

Material and Methods

Soil sampling, preparation and analysis

Soil samples representing three distinct textural classes were collected from agricultural fields in Azerbaijan, specifically from a depth of 0–20 cm. To ensure accuracy in the experiment, the samples were carefully cleaned to remove surface stones and plant debris. The prepared soil samples were then transported to the laboratory for detailed analysis.

Under controlled laboratory conditions, the samples underwent several preparatory steps. Initially, they were air-dried in a shaded, cool environment to prevent any chemical alterations that could occur from excessive heat or sunlight exposure. Once dried, the soil samples were ground to remove residual moisture and achieve uniformity, followed by sieving through a 2 mm mesh to ensure consistent particle size. This process resulted in finely homogenized samples, ready for subsequent analyses.

Standard scientific methods were employed to determine various soil parameters. Textural analysis was conducted using the hydrometer method (Bouyoucos, 1962). Soil pH and Electrical Conductivity (EC) were measured in a 1:1 (w/v) soil-to-distilled water ratio using a pH meter and EC meter, respectively, following the methods outlined by Peech (1965) and Bower and Wilcox (1965). The organic matter content was assessed through the wet oxidation method using $K_2Cr_2O_7$, as described by Walkley and Black (1934). Calcium carbonate ($CaCO_3$) levels were evaluated volumetrically using a Scheibler calcimeter (Rowell, 2010). Water retention properties, including field capacity, wilting point, and available water content, were determined based on procedures detailed by Klute (1965) and Peters (1965).

For heavy metal analysis, available forms of metals such as Fe, Cu, Zn, Mn, Cd, Pb, and Ni were extracted using the DTPA method, while total heavy metals were quantified through aqua regia and HF digestion, followed by measurement with Atomic Absorption Spectrophotometry (Lindsay and Norvell, 1978; EN 13656, 2002).

Water sampling, preparation and analysis

Water samples, including those collected from the Sugovushan Reservoir (40.323985 N, 46.743843 E) in Azerbaijan, were analyzed to assess their properties. Upon collection, the samples were promptly transported to the laboratory for processing and filtered using Whatman No. 41 filter paper to remove particulates.

The pH and electrical conductivity (EC) of the water samples were measured using a pH meter and an EC meter, respectively. Anion concentrations, including Cl^-, HCO_3^-, SO_4^{2-}, NO_2^-, NO_3^-, and PO_4^{3-}, as well as cations such as Ca^{2+}, Mg^{2+}, Na^++K^+, and NH_4^+, were determined following standardized procedures described by the US Salinity Laboratory Staff (1954). Heavy metal concentrations, including Fe, Cu, Zn, Mn, Cd, Pb, and Ni, were quantified using an Atomic Absorption Spectrophotometer to ensure precise measurements.

Soil incubation experiment

The experiment was carried out in a constant temperature incubator set at 20±0.5°C for 10 days. Soil water properties, including field capacity, wilting point, and available water content, were assessed following the methodologies described by Klute (1965) and Peters (1965). A randomized complete block design was employed, incorporating four water levels (%100, %75, %50, and %25 of field capacity) with three replications for each treatment.

Fifty grams of each soil sample were weighed and placed into 100 mL plastic beakers. Plant-available water sourced from the Cd-enriched Sugovushan Reservoir was applied to the soil at the designated water levels. After thorough mixing to ensure uniform distribution, the beakers were sealed with perforated parafilm, allowing adequate air exchange while minimizing water loss through evaporation.

The samples were maintained in the dark under controlled temperature conditions for the entire incubation period. On the 10th day, soil samples were retrieved, and selected microbiological properties were analyzed.

Microbiological properties

Basal soil respiration (BSR)

Basal soil respiration (BSR) at field capacity, measured as CO_2 production at 22°C without glucose addition, was determined following the method outlined by Anderson (1982). CO_2 released during a 24-hour incubation period was absorbed using a solution of $Ba(OH)_2·8H_2O$ and $BaCl_2$, and the residual OH^- was titrated with standardized hydrochloric acid, with phenolphthalein as an indicator. Each sample was tested in triplicate, and results were expressed as µg CO_2-C per gram of dry soil.

Microbial biomass carbon (C_{mic})

Microbial biomass carbon (C_{mic}) was assessed using the substrate-induced respiration (SIR) method described by Anderson and Domsch (1978). A moist soil sample equivalent to 100 g of oven-dry soil was mixed with 400 mg of glucose powder. The CO_2 evolution rate was recorded hourly for 4 hours, following the method of Anderson (1982). The maximum initial respiratory response was used to calculate C_{mic} as 40.04 mg CO_2 g^{-1} soil + 3.75. Each sample was analyzed in triplicate, with data expressed as mg CO_2-C per 100 g of dry soil per hour.

Dehydrogenase activity (DHA)

Dehydrogenase activity (DHA) was determined using the procedure described by Pepper et al. (1995). A mixture of 6 g of soil, 30 mg of glucose, 1 mL of 3% 2,3,5-triphenyltetrazolium chloride (TTC), and 2.5 mL of distilled water was prepared. The samples were incubated at 37°C for 24 hours. The formation of 1,3,5-triphenylformazan (TPF) was quantified spectrophotometrically at 485 nm. Results were expressed as µg TPF per gram of dry soil.

Catalase activity (CA)

Catalase activity (CA) was measured following the method of Beck (1971). Five grams of soil were combined with 10 mL of phosphate buffer (pH 7) and 5 mL of a 3% H_2O_2 substrate solution. The volume of oxygen released over 3 minutes at 20°C was recorded. Controls included the addition of 2 mL of 6.5% (w/v) NaN_3 to inhibit catalase activity. Each sample was tested in triplicate, and results were expressed as mL O_2 per gram of dry soil.

All determinations of Microbiological properties were performed in triplicate, and all values reported are averages of the three determinations expressed on an oven-dried soil basis (105 ^{0}C).

Results and Discussion

Soil Physico-chemical Properties

The characteristics of the soils used in the incubation experiment are summarized in Table 1. The results indicate significant differences in soil texture among the three samples analyzed. One soil is classified as 'Sandy Clay Loam' (SaCL), another as 'Silty Loam' (SiL), and the third as 'Clay' (C). All soils exhibit an alkaline pH and are calcareous in nature. While the SaCL soil is non-saline, the SiL and C soils are classified as saline. Additionally, the organic matter content across all samples is low, reflecting the typical characteristics of the sampled agricultural regions.

According to Kloke's (1980) classification, no heavy metal contamination was detected in the soils, as the heavy metal concentrations remained within the buffering capacity of the soils. Despite this, the total cadmium (Cd) content varied among the soils, with values of 1.75, 2.12, and 2.66 mg/kg for SaCL, SiL, and C soils, respectively. These differences align with findings by Holmgren et al. (1993), which suggest that Cd concentrations tend to increase with higher clay and organic matter content.

The threshold for Cd contamination in soils is generally considered to be 3 mg/kg or higher (Akbar et al., 2006). The Cd levels in the soils used for this study were below this threshold, indicating they are not significantly contaminated. Gradients in Cd concentration are commonly associated with proximity to industrial facilities, roadways, or urban areas (Page et al., 1987; Joimel et al., 2016). The absence of significant Cd contamination in these soils can be attributed to their origin from agricultural fields in Azerbaijan. These fields are typically fertilized with phosphorus-based chemical fertilizers to boost rice production (Islamzade et al., 2024), which could explain the moderate Cd levels. Although the Cd content is slightly elevated, it remains below the critical threshold of 3 mg/kg, indicating a non-contaminated status suitable for agricultural use.

Table 1. Characteristics of the Soils Used in the Incubation Experiment

	SaCL	SiL	C
Sampling point	38.6322740 N 48.8646310 E	40.2559770 N 47.6289990 E	40.5438710 N 47.2880790 E
Texture			
Sand, %	50,69	11,55	6,76
Silt, %	15,91	78,58	7,69
Clay, %	33,41	9,87	85,55
Class	Sandy Clay Loam	Silty Loam	Clay
Soil water properties			
Field Capacity, % Vol	32,30	30,90	44,90
Wilting point, % Vol	21,60	10,40	35,00
Available Water, % Vol	10,70	20,50	9,90
Bulk density, g cm^{-3}	1,48	1,37	1,19
Chemical properties			
pH	7,70	8,17	7,86
EC, dSm^{-1}	0,51	7,62	4,77
$CaCO_3$, %	12,93	15,06	6,69
Organic matter, %	1,61	0,88	2,47
Available heavy metals			
Fe, mg kg^{-1}	65,50	6,21	35,71
Cu, mg kg^{-1}	7,54	1,80	7,74
Zn, mg kg^{-1}	0,58	0,31	0,43
Mn, mg kg^{-1}	23,01	3,76	7,34
Cd, mg kg^{-1}	0,20	0,16	0,15
Pb, mg kg^{-1}	2,48	3,58	3,25
Ni, mg kg^{-1}	3,68	2,15	3,59
Total heavy metals			
Fe, %	3,12	3,81	5,39
Cu, mg kg^{-1}	84,82	75,36	95,15
Zn, mg kg^{-1}	191,17	185,58	296,61
Mn, mg kg^{-1}	0,18	0,13	0,25
Cd, mg kg^{-1}	1,75	2,12	2,66
Pb, mg kg^{-1}	86,85	93,19	95,85
Ni, mg kg^{-1}	75,69	65,48	86,15

Chemical Properties of the Water

The chemical composition of the water used in the incubation experiment is detailed in Table 2. The Sugovushan Reservoir, a vital water body in Azerbaijan, is primarily fed by the Terter River. The Terter River, the largest river in the Karabakh region, has historically supported the agricultural and domestic needs of over 400,000 residents. However, between 1994 and 2020, the river was heavily polluted due to gold mining activities during the period of occupation by Armenian forces (Babayeva et al., 2024). The absence of effective environmental regulations during this time led to the direct discharge of mining waste into the river system, severely affecting the ecosystem and contaminating both the Terter River and the Sugovushan Reservoir, with cadmium (Cd) being the most concerning pollutant.

The water used in the experiment exhibited an alkaline pH of 8.0 and an electrical conductivity (EC) of 3.10 dS m^{-1}, indicating salinity. The heavy metal analysis revealed that while most heavy metal concentrations were within acceptable limits for agricultural use, the Cd concentration was significantly elevated at 1.64 mg L^{-1}. This exceeds the upper threshold for Cd in irrigation water, which is set at 0.01 mg L^{-1} by FAO (1985) standards. Other heavy metals, such as Fe, Mn, and Pb, were present but did not pose a similar level of risk to agricultural activities.

The historical contamination of the Sugovushan Reservoir underscores the environmental consequences of unregulated industrial practices. While the water's chemical properties make it unsuitable for agricultural use without remediation, the data highlight the critical need for environmental restoration and sustainable water management in the region.

Table 2. Chemical properties of the water used in the incubation experiment

pH	8,00	**Anions**		**Heavy metals**	
EC, dSm^{-1}	3,10	Cl^-, mg L^{-1}	20,40	Fe, mg L^{-1}	210,00
Cations		HCO_3^-, mg L^{-1}	114,60	Cu, mg L^{-1}	<0,01
Ca^{2+}, mg L^{-1}	38,1	SO_4^{2-}, mg L^{-1}	67,90	Zn, mg L^{-1}	<0,01
Mg^{2+}, mg L^{-1}	9,90	NO_2^-, mg L^{-1}	0,01	Mn, mg L^{-1}	4,17
Na^++K^+, mg L^{-1}	64,80	NO_3^-, mg L^{-1}	1,43	Cd, mg L^{-1}	1,64
NH_4^+, mg L^{-1}	0,15	PO_4^3, mg L^{-1}	0,10	Pb, mg L^{-1}	9,82
Total cations	112,95	Total anions	204,44	Ni, mg L^{-1}	<0,01

Microbiological Properties of Soils

Basal Soil Respiration (BSR)

Basal Soil Respiration (BSR) serves as a crucial indicator of soil microbial metabolic activity, reflecting the processes of organic matter decomposition and the carbon cycle. In this study, BSR values showed significant variation across different irrigation levels and soil textures, emphasizing the interplay between moisture availability and soil composition (Table 3). The highest BSR values were observed at 75% field capacity in all soil textures, indicating that this moisture level provides an optimal environment for microbial activity by balancing oxygen diffusion and substrate availability.

Among the soil types, sandy clay loam soils exhibited the lowest BSR values, which can be attributed to their limited capacity to retain water and nutrients. In contrast, clay soils displayed the highest BSR values, likely due to their higher organic matter content and enhanced buffering capacity. Specifically, at 75% field capacity, clay soils recorded BSR values that were approximately 25% higher than those of sandy clay loam soils, demonstrating their superior ability to support microbial respiration under optimal moisture conditions.

At 25% field capacity, all soil textures experienced a notable decline in BSR values, reflecting the limitations imposed by insufficient water availability, which restricts microbial activity. Conversely, at 100% field capacity, excessive water inhibited oxygen diffusion, resulting in a suppression of BSR across all textures. These findings underscore the critical role of maintaining moderate moisture levels to optimize microbial activity and soil health.

Table 3. Basal soil respiration (BSR) (μg CO_2-C g^{-1} soil) across soil textures and water levels

Water Level (% Field Capacity)	Sandy Clay Loam	Silty Loam	Clay
25%	2.10 ± 0.25	2.80 ± 0.32	3.50 ± 0.41
50%	3.20 ± 0.30	3.90 ± 0.35	4.70 ± 0.48
75%	3.80 ± 0.45	4.60 ± 0.40	5.40 ± 0.52
100%	3.00 ± 0.20	3.60 ± 0.28	4.20 ± 0.30

Cadmium contamination has been shown to significantly suppress BSR due to its inhibitory effects on microbial communities. Chen et al. (2014) reported that increasing Cd concentrations reduced BSR values, although elevated CO2 levels partially mitigated these effects. Similarly, Vig et al. (2003) emphasized that the bioavailability of cadmium, rather than its total concentration, correlates more strongly with toxicity. The observed variations in BSR across soil textures align with this notion, as the clay soils' higher organic matter content and buffering capacity likely reduced cadmium bioavailability and mitigated its toxic effects. Additionally, the observed lower BSR in sandy soils highlights their susceptibility to cadmium due to reduced sorption capacity and nutrient availability (Vig et al., 2003; Naidu et al., 1997).

Microbial biomass carbon (C_{mic})

Microbial Biomass Carbon (Cmic) is a vital parameter that represents the active microbial community in soil, playing a key role in nutrient cycling and organic matter decomposition. In this study, Cmic values varied significantly across irrigation levels and soil textures, with the highest values observed at 75% field capacity across all soil types (Table 4). This suggests that moderate moisture conditions create an optimal environment for microbial growth and carbon sequestration.

Clay soils exhibited the highest Cmic values, attributed to their superior water retention capabilities and higher organic matter content, which together provide a stable and nutrient-rich environment for microbial communities. Specifically, at 75% field capacity, clay soils recorded Cmic values that were approximately 30% higher than those of sandy clay loam soils, highlighting their enhanced buffering capacity against cadmium toxicity. Silty loam soils followed, with moderately high Cmic values that reflect their balanced physical and chemical properties. Conversely, sandy clay loam soils consistently showed the lowest Cmic values, likely due to their limited organic matter content and poor capacity to retain water and nutrients.

At 25% field capacity, all soil textures displayed a marked reduction in Cmic values, indicative of water stress that restricts microbial activity. Meanwhile, at 100% field capacity, excessive moisture likely inhibited oxygen diffusion, reducing microbial biomass across all soil textures. These findings emphasize the critical role of maintaining adequate moisture levels to support microbial activity and enhance soil health.

Table 4. Microbial biomass carbon (C_{mic}) (mg C g^{-1} soil) across soil textures and water levels

Water Level (% Field Capacity)	Sandy Clay Loam	Silty Loam	Clay
25%	120.50 ± 6.50	140.20 ± 7.00	165.30 ± 7.50
50%	140.70 ± 7.20	160.50 ± 7.50	190.80 ± 8.00
75%	160.30 ± 8.00	185.40 ± 8.50	220.60 ± 9.00
100%	130.10 ± 6.80	150.30 ± 7.30	180.50 ± 7.80

Cadmium toxicity to microbial biomass is well-documented, with Chen et al. (2014), Vig et al. (2003), and Moreno et al. (2001) noting that soil properties such as organic matter content and pH play a critical role in modulating cadmium bioavailability. Higher organic matter and clay content in soils can reduce the free divalent cadmium ion, the most toxic species, thereby minimizing its adverse effects on microbial biomass (Naidu et al., 2000). Similarly, the findings of the study on water stress in rhizosphere soils suggest that microbial biomass is highly sensitive to changes in moisture availability, with significant reductions observed under both excessive and deficient water conditions (Zhang et al., 2024). These insights align with the observed trends in this study, where optimal moisture at 75% field capacity supported higher Cmic values, highlighting the importance of balanced water management.

Dehydrogenase activity (DHA)

Dehydrogenase activity (DHA) is a critical indicator of oxidative metabolism and overall microbial activity in soils. In this study, DHA values demonstrated significant variability across irrigation levels and soil textures, peaking at 75% field capacity for all soil types (Table 5). This peak reflects optimal conditions for microbial enzymatic functions, where oxygen diffusion and substrate availability are well-balanced.

Table 5. Dehydrogenase activity (DHA) (μg TPF g^{-1} soil $24h^{-1}$) across soil textures and water levels

Water Level (% Field Capacity)	Sandy Clay Loam	Silty Loam	Clay
25%	45.10 ± 2.50	52.40 ± 2.90	60.70 ± 3.10
50%	50.80 ± 2.70	58.60 ± 3.20	68.40 ± 3.60
75%	55.60 ± 2.90	65.10 ± 3.40	75.90 ± 3.80
100%	48.30 ± 2.60	55.20 ± 3.00	63.50 ± 3.40

Clay soils consistently exhibited the highest DHA values across all moisture levels, with a peak value of 75.90 μg TPF g^{-1} soil $24h^{-1}$ at 75% field capacity. This superior performance can be attributed to the higher organic matter content and buffering capacity of clay soils, which help mitigate the toxic effects of cadmium. Silty loam soils recorded intermediate DHA values, benefiting from their balanced texture and moderate organic matter content, while sandy clay loam soils consistently exhibited the lowest DHA values due to their limited organic matter and poor buffering capacity.

At 25% field capacity, the lowest DHA values were observed across all soil textures, reflecting the limitations of water stress on microbial metabolic activity. Conversely, excessive moisture at 100% field capacity likely restricted oxygen diffusion, leading to a significant reduction in DHA values. For instance, DHA values decreased by approximately 10% in clay soils and 14% in sandy clay loam soils compared to their respective peaks at 75% field capacity.

Previous studies support these findings. Vig et al. (2003), Naidu et al. (2003), and Chen et al. (2014) reported that cadmium contamination suppresses enzymatic activity by disrupting enzyme-substrate interactions and microbial metabolic pathways. Zhang et al. (2024) emphasized that such suppression is exacerbated under fluctuating moisture conditions, highlighting the importance of stable soil management practices. Moreover, Moreno et al. (2001) underscored the role of organic carbon in mitigating cadmium toxicity, with higher organic carbon levels correlating with reduced enzymatic inhibition. These observations align with the trends observed in this study, particularly the superior performance of clay soils under optimal moisture conditions.

Catalase Activity (CA)

Catalase activity (CA) is a vital enzymatic indicator of oxidative stress responses in soils, reflecting the ability of microbial communities to detoxify reactive oxygen species. In this study, CA values exhibited significant variation across irrigation levels and soil textures, with the highest values recorded at 75% field capacity for all soil types (Table 6). This peak underscores the importance of balanced moisture conditions for optimal microbial activity and effective stress management.

Table 6. Catalase activity (CA) (ml O_2 g^{-1} soil 3 min^{-1}) across soil textures and water levels

Water Level (% Field Capacity)	Sandy Clay Loam	Silty Loam	Clay
25%	4.30 ± 0.30	5.60 ± 0.40	6.40 ± 0.50
50%	5.10 ± 0.30	6.30 ± 0.40	7.30 ± 0.50
75%	5.80 ± 0.30	7.10 ± 0.50	8.10 ± 0.60
100%	4.90 ± 0.30	6.00 ± 0.40	7.00 ± 0.50

Among the soil textures, clay soils consistently exhibited the highest CA values, with a peak of 8.10 ml O_2 g^{-1} soil 3 min^{-1} at 75% field capacity. This superior performance is attributed to their higher organic matter content and buffering capacity, which mitigate the adverse effects of cadmium toxicity and provide a more stable environment for microbial communities. Silty loam soils recorded intermediate CA values, reflecting their moderate buffering capacity and organic matter content, while sandy clay loam soils exhibited the lowest CA values, highlighting their limited resilience to both moisture stress and cadmium toxicity.

At 25% field capacity, all soil textures experienced significant reductions in CA values due to water stress limiting microbial enzymatic responses. Similarly, excessive moisture at 100% field capacity likely restricted oxygen availability and increased cadmium bioavailability, leading to a notable decline in CA. For instance, CA values in clay soils decreased by approximately 13% compared to their peak at 75% field capacity.

These findings align with previous studies. Naidu et al. (2000), Vig et al. (2003), and Zhang et al. (2024) demonstrated that cadmium contamination significantly suppresses catalase activity by interfering with microbial enzymatic systems. Furthermore, Zhang et al. (2024) emphasized the sensitivity of catalase activity to water stress, with severe reductions observed under drought conditions. This highlights the necessity of optimal water regulation, particularly in cadmium-contaminated soils. Moreno et al. (2001) emphasized the role of higher organic carbon levels in reducing cadmium bioavailability, thereby mitigating its inhibitory effects on CA. The trends observed in this study reinforce the critical role of soil organic matter and texture in modulating oxidative stress responses under environmental stress conditions.

Conclusion

This study demonstrated that cadmium contamination and water levels significantly influence microbial properties and enzyme activities in soils with varying textures. Moderate moisture levels (75% field capacity) were shown to provide optimal conditions for microbial activity, while extreme moisture conditions (25% and 100% field capacity) suppressed enzymatic responses and microbial biomass. Clay soils exhibited the highest resilience to cadmium toxicity due to their superior organic matter content and buffering capacity, whereas sandy clay loam soils were the most vulnerable, showing the lowest microbial and enzymatic activity. These findings emphasize the necessity of maintaining optimal water levels to enhance microbial health and mitigate the adverse effects of cadmium in contaminated soils. Future studies should explore the long-term effects of cadmium on soil microbial dynamics under diverse environmental conditions and investigate the role of organic amendments in enhancing soil resilience. This research underscores the critical role of integrated water and soil management in addressing heavy metal contamination and improving agricultural sustainability.

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Development and adaptation of methods for PAHs extraction from bottom sediments using subcritical water extraction, saponification, and ultrasound extraction

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are persistent organic pollutants that pose significant environmental and health risks due to their widespread distribution and carcinogenic properties. Developing efficient and environmentally friendly extraction methods for PAHs from complex matrices like bottom sediments is essential for advancing pollution monitoring and mitigation efforts. The influence of temperature and time parameters of water in a subcritical state on the extraction of widespread, contrasting in physicochemical properties polycyclic aromatic hydrocarbons (PAHs) from bottom sediment samples of the Lena River with varying initial pollutant content was studied. It was shown that the optimal extraction parameters for naphthalene are 240°C for 20 minutes, for phenanthrene and fluoranthene – 240°C for 30 minutes, for benzo(a)pyrene – 250°C for 30 minutes, and for benzo(g,h,i)perylene – 260°C for 40 minutes. Under these conditions, the proportion of extracted PAHs varies from 76% to 85%. A comparison was conducted of widely used PAH extraction methods based on the use of toxic solvents from standard techniques. It was established that the efficiency of extraction methods can be ranked as follows: ultrasound extraction > subcritical extraction > saponification method. In this case, the value of the PAH extraction coefficient during subcritical extraction was 1.23-1.29, during saponification - 1.35 and 1.34, and during the ultrasonic extraction method - 1.10 and 1.08.

Keywords: Polycyclic aromatic hydrocarbons, organic pollutants, bottom sediments, pollution, naphthalene, phenanthrene, benzo(a)pyrene, extraction method, high-performance liquid chromatography.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of high-molecular compounds that exhibit carcinogenic and mutagenic properties (IARC, 2020; Fedorenko et al., 2021; Sushkova et al., 2021). These substances are part of hydrocarbon fossils, are formed during fires, and are products of incomplete combustion of carbon-containing materials (Tsibart and Gennadiev, 2013). Due to the wide variety of sources of their intake, PAHs are ubiquitous in the environment (Dudnikova et al., 2023a,b). From point sources of emission, PAHs in carrier particles can migrate for hundreds of kilometers, accumulating in natural environments of background territories (Chaplygin et al., 2023). At the same time, up to 98% of PAHs accumulate in depositing environments - soils and bottom sediments (Macdonald et al., 1996; Sojinu et al., 2010).

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To date, there is no unified concept for assessing the ecological state of depositing environments contaminated with PAHs. Despite the fact that the International Agency for Research on Cancer annually publishes a list of substances and factors that contribute to carcinogenesis. In this list, more than 30 PAHs are noted as substances that likely contribute to the formation of cancer (IARC, 2020). In world practice, when assessing and predicting the ecological state of soils, the content of 16 PAHs from the list of priority pollutants of the US Environmental Protection Agency is often determined (US EPA, 2020). In this regard, the use of reliable methods for analyzing and monitoring the content of PAHs in bottom sediments is required. However, at present, the quality of the assessment and forecast is limited by a number of factors, including the relevance of generally accepted methods for determining the mass fraction of pollutants in bottom sediments. Existing methods for extracting PAHs from natural environments are based on the use of various toxic organic solvents (Lau et al., 2010; Wu et al., 2019). The disadvantages of such methods include the duration of the sample preparation process, high consumption of organic solvents, which subsequently require specialized disposal (Song et al., 2002). An alternative extractant for organic pollutants from natural environments is subcritical water. Subcritical water is water in a liquid state at a temperature above 100°C and a pressure above the saturated vapor pressure (Zhang et al., 2020). By increasing the temperature of water in sealed conditions, it changes its physical and chemical properties, primarily the value of dialectical permeability, acquiring the characteristics of organic solvents. By varying the parameters of water temperature and time of exposure to the sample, it is possible to achieve conditions under which the yield of PAHs in the extract will be comparable to generally accepted methods of extracting pollutants (Sushkova 2014, 2024). In this regard, the aim of the study was to determine the optimal parameters of temperature and time of exposure to bottom sediment samples to determine the PAH content.

Material and Methods

The Lena River is located in Eastern Siberia, flowing across the entire territory of Russia. Its length reaches 4400 km, the basin area is 2.49 million km2, the average long-term flow is about 530.2 km^3/year. It flows into the Laptev Sea of the Arctic Ocean, forming the largest delta in the Arctic. Samples were collected from the bottom sediments of the Lena River delta using Van Veen Grab sampler in August 2024. Samples were packed in hermetically sealed bags and frozen at temperature -10 Celsius degree. Transportation to the laboratory was carried out under frozen conditions. To develop and adapt the method for PAH extraction using subcritical water, two samples of bottom sediments were selected, representing varying hydrological conditions at a distance from direct sources of pollutants with contrasting levels: d12 (72.572352°N; 126.435160°E) and d21 (72.563507°N; 126.452868°E). According to preliminary studies, the total content of priority PAHs in the bottom sediments of sample d12 was 169 ng g^{-1}, d21 – 407 ng g^{-1}. In general, the level of PAH content in the samples does not exceed international standards and concentrations established for bottom sediments from different regions of the world (Buchman, 2008; Rabodonirina et al., 2015; Marvin et al., 2021; Dudnikova et al. 2023a,b). However, the bottom sediment sample with the lowest PAH content (d12) was considered as an uncontaminated sample, d21 – as contaminated. The method was developed using the example of widespread pollutants included in the list of priority pollutants, contrasting in physicochemical properties and toxicity to representatives of the PAH group: 2-ring naphthalene (Sigma Aldrich, CAS: 91-20-3, 98%), 3-ring phenanthrene (Sigma Aldrich, CAS: 85-01-8, 98%), 4-ring -fluorene (Sigma Aldrich, CAS: 86-73-7, 98%), 5-ring benzo(a)pyrene (Sigma Aldrich, CAS: 50-32-8, 98%), 6-ring benzo(g,h,i)perylene (Sigma Aldrich, CAS: 191-24-2, 98%) (US EPA, 2020; IARC, 2020; ATSDR, 1995, 2020; Dudnikova et al., 2023a,b) (Table 1).

Table 1. Limits of detection (LOD) and limits of quantification (LOQ) of PAHs

№	PAHs	Formula	Number of benzene rings	Molar Mass, g/mol	Solubility in water, mg/l at 200˚C	LOD	LOQ	Retention time, min
1	Naphthalene	$C_{10}H_8$	2	128.2	30.00	0.11	0.05	5.2
2	Phenanthrene	$C_{14}H_{10}$	3	178.2	1.18	0.12	0.08	8.4
3	Fluoranthene	$C_{16}H_{10}$	4	202.3	0.28	0.09	0.05	10.5
4	Benzo(a)pyrene	$C_{16}H_{12}$	5	252.3	0.001	0.15	0.10	25.1
5	Benz(g,h,i)perylene	$C_{22}H_{12}$	6	276.3	0.000003	0.12	0.10	39.7

Methods

Selection of conditions for PAH extraction from bottom sediments using subcritical water

To select the conditions for PAH extraction in a subcritical water environment, we used the author's laboratory cartridge made of stainless steel with a metal thickness of 0.5 cm. The studied temperature parameters were in the range of 230-270°C, time - 10-50 minutes. These parameter ranges are most often encountered when studying the possibility of using subcritical technologies for the extraction of organic pollutants from bio-bone systems, plant and animal tissue (Latawiec and Reid, 2010; Sushkova, 2014, 2024; Yabalak et al., 2024). When determining the optimal conditions for PAH extraction, the analytical repetition in the experiment was ninefold. A 1 g sample of the analyzed substance from bottom sediments with a particle size of 1 mm, selected and prepared in accordance with GOST (MU 1424076, 1976; GOST 17.4.4.02-84, 1986) was placed in an extraction cartridge filled with broken Pyrex glass with a particle size of 1 mm and 0.5 mm of 0.5 g each. The cartridge was then connected to a laboratory setup, where the sample was heated to 230-270°C with a constant flow of bidistilled water passing through the cartridge under pressure. at a rate of 1 mL/min for 10-50 minutes. 5 mL of n-hexane (Sigma Aldrich, CAS 110-54-3, 97%) were added to the resulting aqueous extract and the mixture was shaken for 15 min. on a shaker at 135 rpm. The layers were separated in a 50 mL separatory funnel in three stages with the next portion of hexane. The hexane extracts were combined, passed through a funnel with anhydrous sodium sulfate (Sigma Aldrich, CAS 7757-82-6, 99%) into a clean dry rotary flask and evaporated to dryness on a rotary evaporator at a water bath temperature of 49°C. 1 mL of acetonitrile (Sigma Aldrich, CAS 75-05-8, 99%) was added to the dry residue and thoroughly mixed for 30 minutes. The scheme of the laboratory setup is shown in Figure 1.

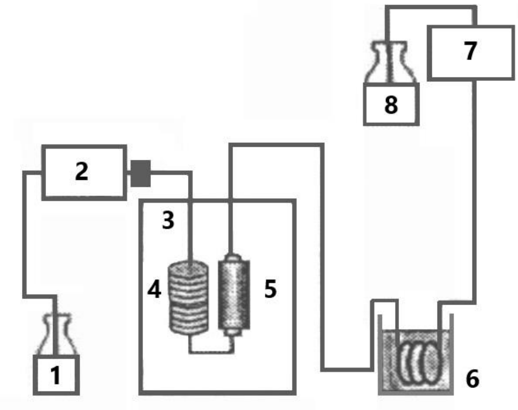

Figure 1. Schematic diagram of the setup for PAH extraction with subcritical water: 1. Tank for bidistilled water; 2. Pump "Elilex LABS, INC. MENLO PARK, CA" (model AA-100-S-2); 3. Thermostat (electric oven, model HRHK C-242 - 100/179/2150W, 230V); 4. Thermostat spiral (system communications L=3.5 m, folded in the form of a spiral L=12 cm); 5. Cartridge (stainless steel L=150 mm and internal diameter 4.5mm); 6. Ice bath; 7. Restrictor (pressure limiter); 8. Tank for collecting extracts

To establish the completeness of PAH extraction from river bottom sediments by the methods under consideration, a blank experiment was additionally conducted with the introduction of solutions of a given concentration into river bottom sediments (additive method). In this case, the additive is introduced as an acetonitrile solution of each PAH into a 1 g sample of river bottom sediments placed in a flask for a rotary evaporator. After evaporation of acetonitrile (at room temperature), the sample with the introduced additive is processed in accordance with the proposed method. The experiment is repeated nine times. The correction factor (k) was calculated using equations (1, 2):

$$k = \frac{C1}{C2} \quad (1)$$

$$C1 = Cst + \mathrm{C}p, \quad (2)$$

where C1 is the total concentration of each PAH in the river bottom sediment sample, ng g^{-1}; C2 is the concentration of each PAH in the river bottom sediment determined by the saponification method, ng g^{-1}. Cst is the concentration of each PAH in the river bottom sediment due to the addition of its standard solution, ng g^{-1}; Sp is the average concentration of each PAH in the river bottom sediment sample, ng g^{-1};

For the developed method for determining PAH in river bottom sediments, the random component of the measurement error was estimated, which for the concentration range of 1–500 ng g^{-1} was 0.5–1.5%.

To assess the efficiency of the method, the results obtained by means of PAH extraction in a subcritical water environment were compared with widely used methods based on the use of a large number of toxic organic solvents.

Extraction of PAHs using saponification method

The analysis was carried out in accordance with the requirements (RD 52.10.556-95), allowing the removal of interfering matrix components (Sushkova et al., 2024). This method is the most adapted to the analysis of bottom sediment components, therefore it was chosen as the main method for the analysis in this project.

Briefly. The selected bottom samples were crushed and sieved through a 1 mm diameter sieve. For further analysis, the interfering lipid fraction was removed by saponification of 1 g of bottom sediments with a 2% alcohol solution of KOH (Sigma Aldrich, CAS 1310-58-3, 99%) in a water bath in round-bottomed flasks attached to reflux water condensers for 3 hours. During boiling, saponification of lipids and resinous components and the aliphatic fraction of PAHs in bottom sediments occurred, which increased the recovery of PAHs and decreased the amount of extracted substances in the extract (Poole et al., 1990). The resulting percalate was poured into 100 mL conical flasks, 5 mL of distilled water and 15 mL of hexane were added. Then, extraction was carried out on a mechanical shaker for 10 minutes at a frequency of 135 shakes per minute in triplicate. The resulting hexane extracts were combined in a separatory funnel, transferred to a clean dry rotary flask, evaporated on a rotary evaporator with a water-jet pump at a water bath temperature of 49°C until a dry residue was obtained (Figure 4). 1 mL of acetonitrile was added to the resulting dry residue and left for 30 minutes.

Ultrasonic extraction

To determine the efficiency of the methods used in the work, an assessment of the conformity of the developed and adapted methods to the international level and standards was carried out. To solve the problem, PAH extraction from bottom sediments was carried out according to the international method of ultrasonic extraction US EPA 3550c (US EPA Method 3550C, 2007).

Briefly. A 1 g sample of a solid object is placed in a 50 cm3 conical flask with a ground glass stopper. 15 mL of a hexane / dichloromethane = 1/1 mixture are used as an extractant. The extraction procedure was carried out for 5 minutes on a Branson 5510 ultrasonic bath in triplicate. The resulting extract is placed in a glass column pre-filled with 2 g of aluminum oxide. After the precipitation of aluminum oxide, 0.5 g of anhydrous sodium sulfate was added. The column was then washed with 20 mL of hexane, discarding the eluate coming out of the column. Then the PAH was eluted with 30 mL of a mixture of dichloromethane/hexane = 1/4. The entire eluate was collected in a flask for evaporation and evaporated on a rotary evaporator at a temperature of 49°C. The dry residue was dissolved in 2 cm3 of acetonitrile.

Chromatographic analysis

The PAH content in the extracts obtained using different methods of samples was determined by high-performance liquid chromatography using an Agilent Technologies chromatograph (Santa Clara, CA, USA) with a fluorescence (FL-3000) detector. A mixture of acetonitrile and deionized water (4:1) was used as the mobile phase at a flow rate of 0.5 mL/min and a temperature of 20°C. The volume of the injected solution was 20 μl. The PAH content in the analyzed samples was calculated using the external standard method (absolute calibration). The detection limits (LOD) and quantification limits (LOQ) are given in Table 1.

The PAH content in river bottom sediments was calculated using the equation:

$$a = k\,SI \times C\text{ст} \times \frac{V}{S\text{ст} \times m}, \tag{3}$$

where a is the PAH content in river bottom sediments (ng g^{-1}); Sst and SI are the peak areas of the PAH standard solution and sample; Cst is the concentration of the PAH standard solution (ng/mL); k is the coefficient of PAH extraction from the sample; V is the volume of acetonitrile extract (mL); m is the mass of the sample (g).

Results and Discussion

It was found that for unpolluted soil, the highest yield of naphthalene in the extract is observed at 240°C for 20 minutes, phenanthrene and fluoranthene - at 240°C for 30 minutes, benzo(a)pyrene - at 250°C for 30 minutes and benzo(g,h,i)perylene - at 260°C for 40 minutes. With an increase in the extraction temperature and exposure time above the listed values, a significant decrease in the yield of PAHs in the extract is observed. The effect is enhanced with a decrease in the dimension and molecular weight of the substance, which is due to the degradation of PAH molecules in the subcritical water environment (Islam et al., 2015). For contaminated bottom sediments, the optimal conditions for PAH extraction are similar, but the proportion of extracted compounds is 2-6% higher compared to unpolluted bottom sediments (Figure 2).

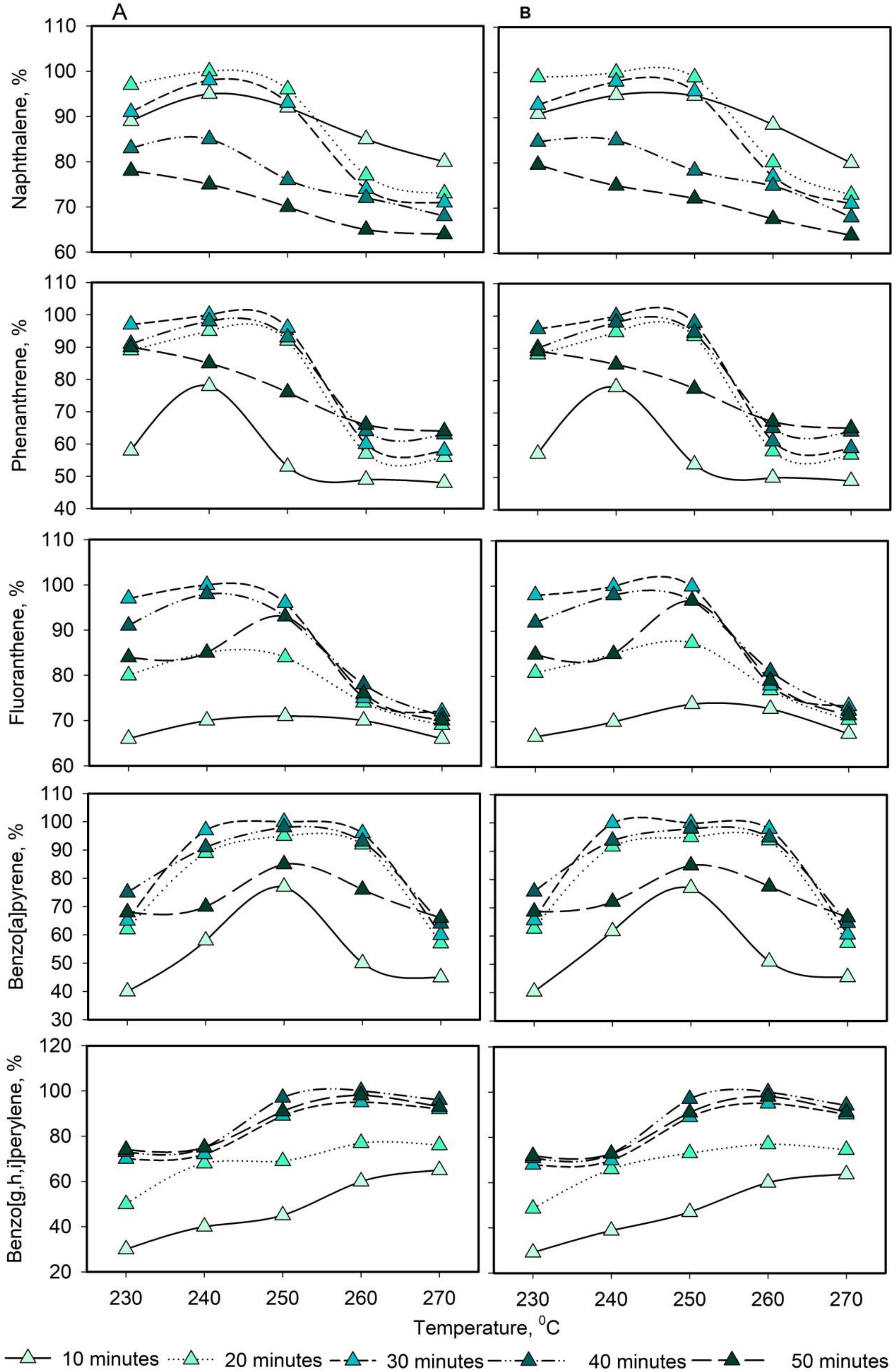

Figure 2. The proportion of PAH extraction from contaminated and uncontaminated bottom sediments depending on the temperature and time of extraction in a subcritical water environment

With increasing pollutant content in the deposition media, especially for low-carbon bio-based systems, PAH molecules are concentrated at low-affinity reaction sites (Xu et al., 2014). Therefore, the energy spent on pollutant desorption is lower, which leads to more complete extraction during subcritical extraction (Liang et al., 2016). In the unpolluted sample, PAH molecules are most likely concentrated in the pool of aromatic structures of organic matter and the energy spent on their extraction increases (Xu et al., 2014; Diagboya et al., 2018). Under optimal extraction conditions, the naphthalene content in the samples of uncontaminated and contaminated bottom sediments was 0.9 ng $g^{-1}$and 13.2 ng g^{-1}, phenanthrene – 72.6 ng $g^{-1}$and 233.2 ng g^{-1}, fluoranthene – 18.8 ng g^{-1} and 30.1 ng g^{-1}, benzo(a)pyrene 6.0 ng g^{-1} and 14.8 ng g^{-1}, benzo(g,h,i)perylene – 6.2 ng g^{-1} and 19.2 ng g^{-1}, respectively (Table 2).

Table 2. Extraction of PAHs from bottom sediment samples using subcritical water at different temperature and extraction time parameters

Not contaminated						Contaminated					
Temperature	Time					Temperature	Time				
	10	20	30	40	50		10	20	30	40	50
1	2	3	4	5	6	7	8	9	10	11	12
Naphthalene						Naphthalene					
230	0.84	0.92	0.86	0.79	0.74	230	12.10	13.19	12.37	11.29	10.61
240	0.90	0.95	0.93	0.81	0.71	240	12.66	13.33	13.06	11.33	9.99
250	0.87	0.91	0.88	0.72	0.66	250	12.64	13.18	12.77	10.44	9.61
260	0.81	0.73	0.70	0.68	0.62	260	11.79	10.68	10.26	9.99	9.02
270	0.76	0.69	0.67	0.65	0.61	270	10.66	9.73	9.46	9.06	8.53
Phenanthrene						Phenanthrene					
230	42.09	64.58	70.39	66.04	65.31	230	133.91	205.48	223.95	210.10	207.79
240	56.60	68.94	72.57	71.12	61.68	240	181.90	221.55	233.21	228.55	198.23
250	38.46	66.76	69.66	67.49	55.15	250	126.07	218.85	228.36	221.22	180.79
260	35.56	41.36	43.54	46.44	47.89	260	116.56	135.59	142.73	152.24	157.00
270	34.83	40.64	42.09	45.72	46.44	270	114.18	133.21	137.97	149.86	152.24
Fluoranthene						Fluoranthene					
230	12.42	15.05	18.25	17.12	15.81	230	20.05	24.31	29.47	27.65	25.52
240	13.17	15.99	18.82	18.44	15.99	240	21.05	25.57	30.08	29.48	25.57
250	13.36	15.81	18.06	17.50	17.50	250	22.23	26.30	30.05	29.11	29.11
260	13.17	13.92	14.11	14.68	14.30	260	21.91	23.17	23.48	24.42	23.79
270	12.42	12.98	13.55	13.36	13.17	270	20.26	21.18	22.10	21.79	21.48
Benzo(a)pyrene						Benzo(a)pyrene					
230	2.39	3.71	3.89	4.48	4.06	230	5.97	9.25	9.70	11.19	10.14
240	3.47	5.32	5.80	5.44	4.18	240	8.82	13.54	14.76	13.85	10.65
250	4.60	5.68	5.98	5.86	5.08	250	11.37	14.03	14.77	14.47	12.55
260	2.99	5.50	5.74	5.56	4.54	260	7.53	13.86	14.46	14.01	11.45
270	2.69	3.41	3.59	3.83	3.95	270	6.71	8.50	8.95	9.55	9.84
Benz(g,h,i)perylene						Benz(g,h,i)perylene					
230	1.87	3.12	4.37	4.56	4.62	230	5.58	9.29	13.01	13.57	13.76
240	2.50	4.24	4.49	4.68	4.68	240	7.44	12.64	13.38	13.94	13.94
250	2.81	4.31	5.55	6.05	5.68	250	8.62	13.22	17.05	18.58	17.43
260	3.74	4.80	5.93	6.24	6.11	260	11.49	14.75	18.20	19.16	18.77
270	4.06	4.74	5.74	5.99	5.80	270	12.21	14.27	17.28	18.03	17.46

During the experiment with pollutant additions to bottom sediment samples, the effect of PAH extraction methods on pollutant concentrations in extracts was compared. It was found that the extraction methods form a series in terms of efficiency: saponification method < subcritical extraction < ultrasonic extraction (Figure 3, 4). The differences between the methods are more pronounced when adding 132 ng g^{-1} PAH to the original bottom sediment samples, especially for naphthalene, which is easily desorbed compared to other PAHs, from a contaminated bottom sediment sample (Rhodes et al. 2012). On average, the proportion of naphthalene extracted from unpolluted and polluted bottom sediment samples during extraction in subcritical water is 77-78%, phenanthrene 78-81%, fluoranthene 78-82%, benzo(a)pyrene 79-81%, benzo(g,h,i)perylene 76-85%, during extraction by saponification method - 73-74%, 73-75%, 75-76% and 74-75%, during ultrasound extraction 94-95% 93-95%, 92-93%, 91-92% and 86-89%, respectively. The yield of PAHs in the extract can be described as desorption of pollutants from the biobone matrix, which occurs in several stages. Desorption of PAHs from soils and sediments often follows a biphasic profile, with an initial rapid desorption phase followed by slow and very slow-release phases. Slow or very slow desorption rates are due to the association of PAHs with the aromatic component of soils and can be bound or adsorbed in micropores, making them difficult to access for various extractants (Rhodes et al., 2012; Xu et al., 2014; Diagboya et al., 2018; Ren et al., 2018). Since the affinity of PAHs for organic matter in biosolids increases with increasing molecular weight of the pollutant, a consistent decrease in the efficiency of organic solvent-based extraction methods was observed in the recovery of target substances (Sushkova et al., 2015, 2024) (Figures 3, 4).

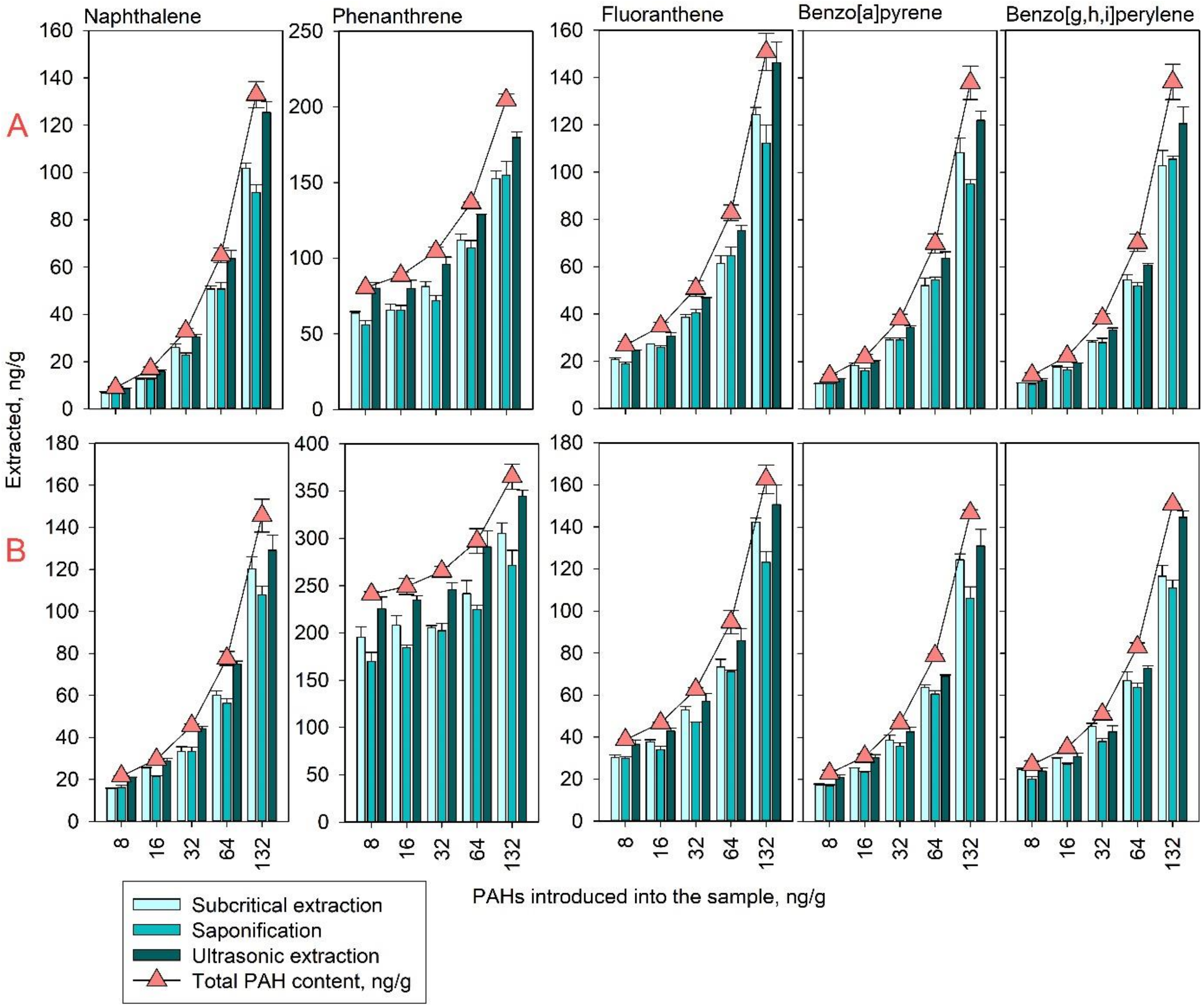

Figure 3. Extraction of PAHs from uncontaminated bottom sediment samples using subcritical water at different extraction temperature and time parameters

On the contrary, when the samples were exposed to water in a subcritical state, an increase in the extraction efficiency was observed for heavier molecular weight substances, such as 5-ring benzo(a)pyrene and 6-ring benzo(g,h,i)perylene, which is most likely due to some destruction of 2-4-ring compounds during the extraction process. The coefficients of PAH extraction from bottom sediments with different levels of contamination were determined. It was shown that the average coefficient of PAH extraction during subcritical extraction from uncontaminated bottom sediments was 1.29, from contaminated ones – 1.23, during extraction by the saponification method – 1.35 and 1.34, during ultrasonic extraction – 1.10 and 1.08, respectively (Figure 4).

Conclusion

It has been established that the extraction of PAHs from bottom sediments depends on the level of the initial content of pollutants in them, the type of pollutant, temperature and extraction, and the time of exposure to the sample. The optimal parameters for naphthalene extraction are 240°C for 20 minutes, phenanthrene and fluoranthene - 240°C for 30 minutes, benzo(a)pyrene - 250°C for 30 minutes, and benzo(g,h,i)perylene -

260°C for 40 minutes. The efficiency of PAH extraction methods decreases in the following order: ultrasonic extraction (86-95%) > subcritical extraction (76-85%) > extraction by saponification (73-76%). The extraction of pollutants from bottom sediments increases with an increase in the initial content of pollutants in them and a decrease in the molecular weight of the substance. The average recovery factor for subcritical extraction of naphthalene in uncontaminated and contaminated bottom sediment samples is 1.29 and 1.23, respectively.

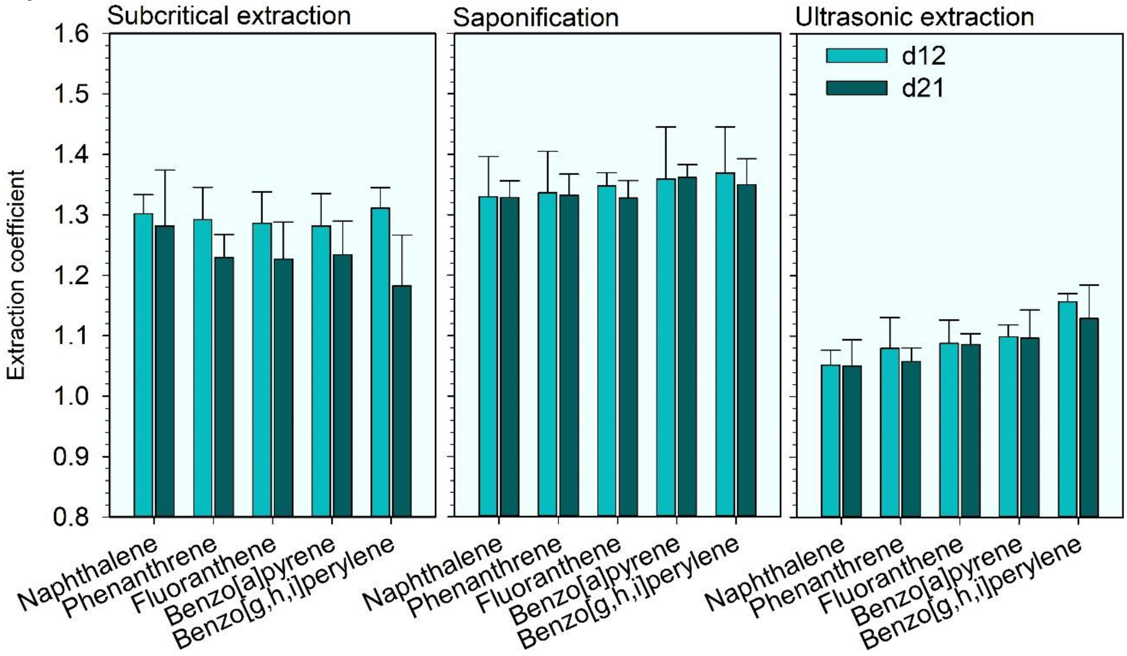

Figure 4. PAH extraction coefficient from unpolluted and polluted bottom sediments of the Lena River using different extraction methods

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Mitigation of earthworm behavior against lithium pollution using biochar

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Abstract

Application of lithium has been increased in recent years due to its use in various modern gazettes and forced to find new reserves and extraction through mining. The mining process and improper disposal of lithium containing gazettes significantly added this element to the surrounding areas, especially to the terrestrial and soil ecosystems. The increasing concentration of lithium affected the soil biodiversity and altered behavior was expected for macro-organisms. Present study aimed to investigate the different concentrations of lithium salt (Li_2CO_3) on the behavior of the species of earthworm (*Eisenia fetida*), according to ISO 17512-1:2008 standards. In recent years, researches on biochars are drastically increased due to its unique role in soil health improvement. Thus, the biochar has been included in this work as a conditioning material to study the mitigation effects of lithium on earthworm (*E. fetida*) behaviour. The findings suggested that lithium promoted the earthworm avoidance on dose dependent manner while 1% (w/w) addition of biochar in soil mitigated the avoidance behaviour. These mitigating effects were corelated to certain soil physio-chemical properties change, better soil's buffering capacity against stress by lithium in presence of biochar. The findings of present study may force new investigation to restore the soil health and earthworm behaviour near the mining areas.

Keywords: Biochar, Earthworm behaviour, Emerging contaminant, Lithium, Mitigation.

Introduction

The intensive lithium mining contributed to higher accumulation of this emerging contaminant in soil and linked to soil degradation, erosion, loss of biodiversity, and adverse impacts on local ecosystems (Bolan et al., 2021). Various chemicals are involves in lithium extraction which contribute contaminates to soil and water, with potential risks to human health and the environment (Aral and Vecchio-Sadus, 2011; Gao et al., 2021; Konstantinova et al., 2023). Compared to other soil cations, lithium is more mobile and prone to leaching, which can contaminate aquifers and be absorbed by plants, potentially causing toxicity at concentrations between 50 and 170 μg L^{-1} (Kszos and Stewart, 2003). Pollution resulting from lithium mining activities is not limited to extraction sites but also occurs during processing and improper disposal of waste, such as from lithium batteries (Gao et al., 2021).

To mitigate negative environmental impacts, effective remediation or mitigation strategies are necessary. Growing concerns on terrestrial ecosystems and human health caused by increasing lithium concentrations

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in soil at potentially toxic levels (Bolan et al., 2021; Shakoor et al., 2023). Among various strategies, the application of pyrogenic materials to soil, such as biochar, has been widely used (Myers et al., 2003). Biochar is a solid carbonaceous product obtained from biomass feedstock, such as agricultural, forestry, and urban waste etc (Yaashikaa et al., 2020). Biochar has garnered significant interest due to its ability to mitigate climate change effects, remove pollutants, and improve soil quality and health (Pathak et al., 2024; Rao et al., 2024), largely due to its unique properties, including high specific surface area, high porosity, functional groups, stability, and high cation exchange capacity (Burachevskaya et al., 2023). Biochar production is not only environmentally beneficial but also an efficient technology for waste reuse with low energy consumption (< 700 °C) (Myers et al., 2003), and can be used as a conditioning agent (Brar et al., 2024; Jha et al., 2023; Rajput et al., 2024). Bulk biochar, with particle sizes between 0.04 to 20 mm, is commonly used to improve soil quality and sustainability, both in agricultural and environmental practices (Brar et al., 2024).

The main objective of terrestrial ecotoxicology is to obtain information for contaminated matrices, such as soil, through laboratory tests using standardized species and conditions to assess associated risks (Loureiro et al., 2005). Such assays can help determine if lithium concentrations in soil are high enough to cause adverse effects in organisms used as biological models or bioindicators (Macedo et al., 2024). Soil plays a fundamental role by providing a range of ecosystem services critical for promoting environmental sustainability and food production (Telo da Gama, 2023). This assessment may follow a preliminary or prospective strategy, aiming to evaluate the potential risk of toxic elements in organisms through controlled laboratory tests using artificial or natural soil matrices at different concentrations (Albert and Bloem, 2023; de Santo et al., 2019). Biological indicators, due to their high sensitivity and notable soil biodiversity, allow the use of various organisms in these tests.

Earthworms are widely used as bioindicators due to their response to different soil uses and management and their high sensitivity to disturbances and contamination (Paoletti et al., 1998) . The species *Eisenia fetida* is used as a standard reference according to OECD (1984) guidelines, due to its high sensitivity, easy laboratory adaptation, and short generation time (OECD, 1984; Lowe and Butt, 2007; Paoletti et al., 1998). Considering the previously mentioned aspects regarding the potential effects of lithium on the soil's ability to provide ecosystem services and the potential of conditioning materials such as biochar. The main objectives of the current study are to evaluate: (i) the effect of different lithium concentrations (Li^+) in the form of lithium carbonate (Li_2CO_3) on the behavior of the species *E. fetida*, according to the standards required for such studies (ISO 17512-1:2008); and (ii) the effect of biochar application as a strategy to mitigate the potential effects of the studied lithium concentrations.

Material and Methods

An artificial soil (AS) was prepared according to the standards established for investigations based on the effect of chemical substances (OECD, 1984), serving as an alternative to natural soils. The artificial matrix consists in a mixture of 70 % fine sand, with particle sizes between 50 and 200 µm, 20 % kaolin, with a minimum of 30% kaolinite, and 10 % dry peat sieved and free of plant residues. A composite sample was collected, and the main physicochemical parameters were analyzed. The results for AS revealed a KCl 1M pH value of 4.7, which was corrected to a final value of 6.1 by adding $CaCO_3$, an electrical conductivity (EC) of 0.15 dS m^{-1}, organic matter content of 57.6 g kg^{-1}, cation exchange capacity of 6.2 $cmol_c$ kg^{-1} and a sandy loam texture class.

A commercial biochar, obtained from the pyrolysis of forest residues, at temperatures between 400-600 ºC, at limited oxygen availability, was used as a soil conditioner. Like the AS, a composite sample of biochar was collected for initial physicochemical characterization, with some parameters analyzed presented in Table 1.

Table 1. Physico-Chemical parameters of commercial biochar used in the present study.

parameter	d_{ap} [1] (g cm^{-3})	diameter (mm)	SS [2] (m^2 g^{-1})	H_2O [3] (g kg^{-1})	pH H_2O -	EC [4] (dS m^{-1})	VC [5] (g kg^{-1})
value	0.35	≤ 10	≥ 500	< 300	9.0	1.8	≤ 50

[1] Bulk density; [2] Specific surface; [3] Water content; [4] Electric conductivity; [5] Volatiles content.

For experimental design, five lithium treatments were considered, based on concentrations of 0, 10, 20, 40, and 80 mg Li kg^{-1}, in the form of lithium carbonate (Li_2CO_3). The selection of the lithium concentration range in the soil was based on values observed under edaphic stress conditions, resulting from lithium levels in soils of Portugal or pollution events, such as waste deposition sites from mining activities (Quina and Pinheiro, 2020). Based on the mitigation effects of lithium in the soil a second modality, with the same lithium concentrations, was considered with the biochar addition, at a dose of 1 % (w/w). The dose of applied biochar was selected as per previous studies where the dose of biochar was standardized for pollution toxicity (Li et al., 2023; Liberati et al., 2023; Rajput et al., 2023). For each treatment, a control treatment without lithium addition and with biochar addition was also included. Three repetitions were conducted for each treatment.

To study the effect of lithium concentration and biochar mitigation, an avoidance test was conducted following the guidelines for chemical substance studies (ISO 17512-1:2008), used the behavior of *E. fetida* biological model as the main endpoint. A total of 30 transparent PVC test-containers, with 19 cm*14 cm*14 cm dimensions, with similar perforated lids, were used according to the treatments and repetitions tested. Each test container was divided into two equal sections, each filled with 500 g of dried artificial soil (AS), adjusted to 60% of its maximum water-holding capacity (WHC). One section was treated with lithium (test-section) at the specified concentrations (Li10, Li20, Li40, and Li80) and was marked with a (+) sign. The other section, which served as the control soil, with no added lithium (Li0), was marked with a (–) sign. In accordance with the guidelines (ISO 17512-1:2008) (CF), a dual test was also conducted for validation purposes, where the respective sections of the control treatment were compared with each other. This procedure was also applied to treatments without lithium and with biochar application. A total of 10 adult *E. fetida* earthworms, acclimated to the artificial soil conditions and with developed clitellum and a fresh weight between 300 and 600 mg, were placed at the interface between the two soil sections in each test container (Figure 1).

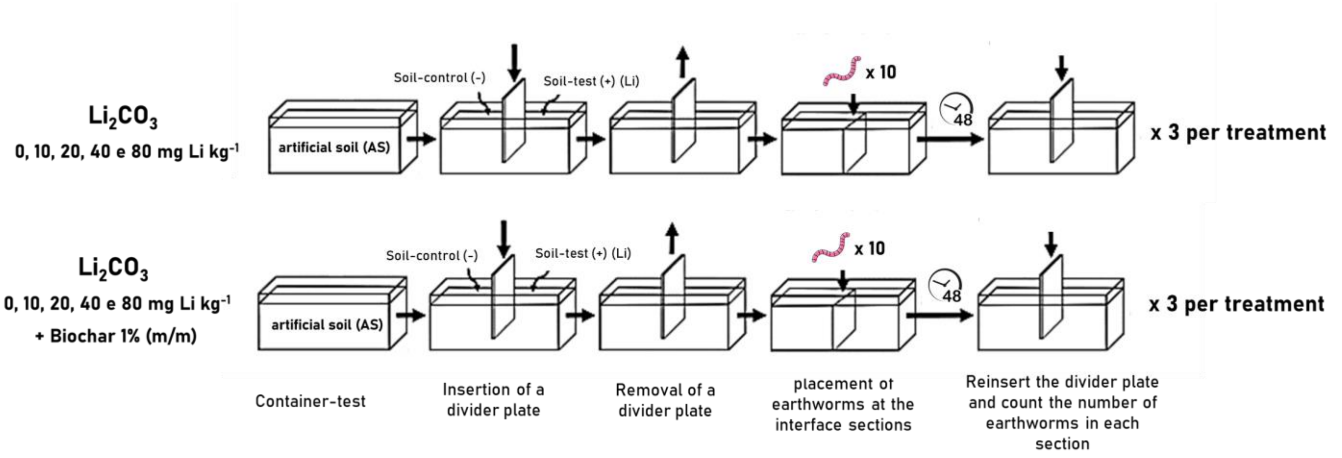

Figure 1. Avoidance test procedure applied to study lithium concentrations (0, 10, 20, 40, and 80 mg kg-1) in artificial soil (AS), with the addition of biochar at 1% (w/w).

The containers were sealed and placed under controlled laboratory conditions, with a temperature of 20 ± 2 ºC and a photoperiod of 16 hours of light and 8 hours of darkness, for a 48-hour exposure period, during which the earthworms were not fed. After the exposure period, the partition plate was repositioned at the interface between the compared sections in each test container, and the number of individuals in each section, control-soil (–) and test-soil (+), was counted for the different treatments and repetitions studied. Based on the number of individuals in each test section, the percentage of avoidance (%A) for each treatment was determined using the model described by Busch et al. (2012) ($\% A = [(n_c\text{-}n_t)/N] * 100$), where n_c, n_t, and N refer to the number of earthworms in the control-soil, test-soil, and total number of earthworms per test-container, respectively. According to the results, positive values (+) indicate avoidance, while negative values (–) suggest no response or attraction to the presence of lithium (ISO 17512-1:2008). After the exposure period, more dynamic physicochemical parameters of the soil, such as pH (KCl 1 M), EC and water content, were also determined to better understand the effect of lithium concentration and biochar presence on earthworm behavior.

The data were subjected to normality and homogeneity tests using Shapiro-Wilk and Bartlett tests, respectively, followed by a one-way analysis of variance (ANOVA) and a Least Significant Difference (LSD) test, with a 5% probability level.

Results and Discussion

For the dual or double control test (Figure 2), where the respective test-sections, correspond to the same treatment, are compared the earthworms do not show a significant preference, resulting in an average distribution between 40 and 60%, fulfilling the normative range for this type of treatment (OECD, 1984).

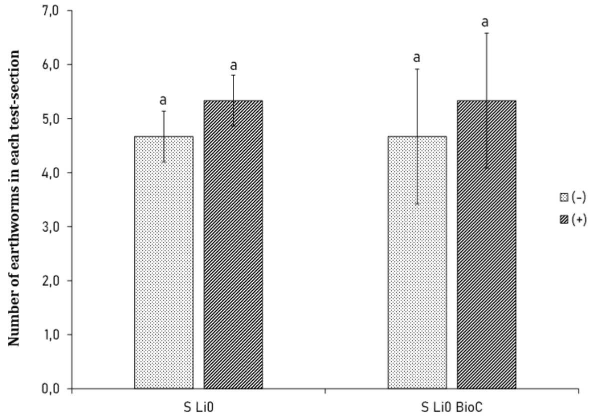

Figure 2. Mean number (n=3) of earthworms observed in the avoidance assay, for the respective sections, for the treatments without lithium (S Li0), and with biochar (S Li0 BioC), in the dual control test. (Bars relative to each treatment, followed by the same letter, do not differ significantly from each other, by the Student's t-test, for 5% probability; vertical bars indicate standard error (SE) (n=3).

In addition, the absence of mortality in all studied treatments reveal null interference of soil artificial matrix in earthworm's behavior, allowing the validation of the experimental test in accordance with the standards for this type of test (ISO 17512-1:2008). These results are supported by other authors (Loureiro et al., 2005), where observed a similar trend, demonstrating the innocuity of the artificial matrix on the behaviour of *E. fetida*. This is partly explained by a balance among the main soil factors, such as texture, organic matter, cation exchange capacity, and pH, according to the proportions of the constituent materials used (OECD, 1984). The results also demonstrate the absence of negative effects from the type and dose (1 %) of biochar used, with the results falling within the normative values, with the distribution of individuals between 40 to 60% observed between the test-sections for the control treatment with biochar (S Li0 BioC) (Figure 2). These results are related to the physicochemical characteristics of this type of material (Table 1), influenced by the source biomass (forest residues) and the pyrolysis conditions (T<700 ºC), which result in low levels of heavy metals and hydrocarbons (results not shown), thus not affecting the behaviour and distribution of the earthworms.

For lithium exposure, the avoidance results are presented in Figure 3. The negative avoidance results observed in the control treatment (Li0) (-6.7%) indicate a lack of response, suggesting an attraction or preference of the *E. fetida* for conditions in absence lithium in the soil.

In contrast, in the presence of lithium in the soil, the biological model exhibited avoidance behavior, which was concentration-dependent ($p<0.001$), with values ranging between 17% and 40% (figure 3). In an artificial matrix prepared according to OECD guidelines (OECD, 1984), using *E. fetida* and *E. andrei* as biological models, respectively, observed a similar trend, with avoidance behaviour dependent on the lithium presence and concentration in soil. Other studies, using different bioindicators or endpoints from those used in the current study, suggests a similar trend with a significant loss of soil habitability or an increase in oxidative stress (Xu et al., 2021), with increasing lithium concentration in the soil. Despite the avoidance behaviour observed in the presence of lithium in the soil, the recorded values for the highest concentration studied (80 mg kg^{-1}) remained below the soil habitability threshold of 60% avoidance, as defined by Loureiro et al. (2005).

In turn, in the treatments with the addition of biochar, negative avoidance values were observed at all tested lithium concentrations (Figure 3). In the case of the control with no lithium (Li0) and with biochar addition (Solo+BioC), the results showed a similar trend to the treatment without biochar (Solo), transcribe by a no response, reflected by negative avoidance values (-6.7%). These results indicate that the biochar applied not influence the behaviour of the earthworms, unlike other studies with *E. fetida*, where the application of biochar led to avoidance behaviour (Namoi et al., 2019; Wang et al., 2024). The type of biochar used, namely its characteristics related to the concentration of compounds such as heavy metals or polycyclic aromatic hydrocarbons, which are strongly influenced by both the source (de Resende et al., 2018) and the pyrolysis conditions (Burachevskaya et al., 2021), may can explain these differences. In the treatments with lithium, the addition of biochar promotes a non-response, indicating an attraction of the earthworms to the test-section containing the conditioning material. This trend became more pronounced with the increase of soil lithium concentration, with the avoidance values ranging between (-) 13% and (-) 33% (Figure 3).

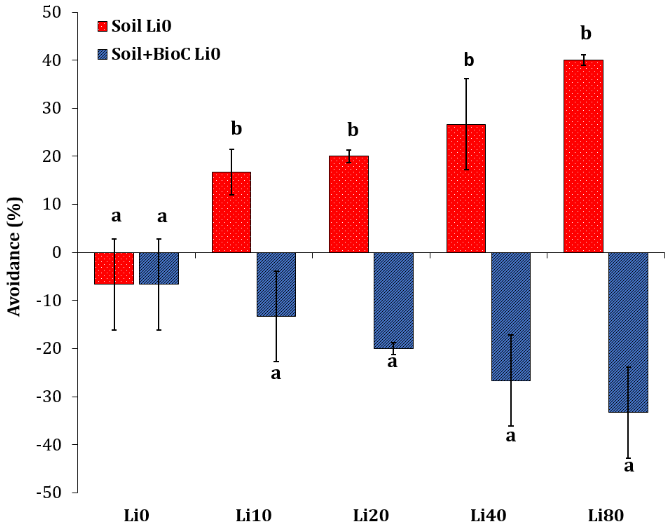

Figure 3. Percentage (%) of avoidance or preference of E. fetida in the lithium treatments with (Solo+BioC) and without (Solo) biochar application, for the different concentrations under studied. (for each concentration, bars relative to each treatment, followed by the same letter, do not differ significantly from each other, by the Student's t-test, for 5% probability; vertical bars indicate standard error (n=3).

The differences observed between treatments with and without biochar may be attributed to the influence of these conditioning materials on soil properties (Figure 4). These changes could impact soil habitability and subsequently alter the behaviour of *E. fetida*. The high adsorption capacity due to particle size reduction, increased surface area and electrical charges from the pyrolysis process, enhanced lithium reactivity through adsorption phenomena. This reduces its bioavailability, mitigates potential toxic effects on soil biology and improves soil habitability. At the level of soil physico-chemical properties, the removal of acidic functional groups during the pyrolysis process allows the carbonaceous material to exhibit alkaline characteristics. Biochar can be applied to the soil to raise pH levels due to its alkalizing effect, creating more favourable conditions for *E. fetida* (Wang et al., 2024). With the increase in lithium concentration in the soil, due to the alkalizing nature of the lithium salt used ($LiCO_3$), there is a corresponding rise in both soil pH and salinity in the treatments with lithium (figure 4). The application of biochar mitigates these effects creating less stressful conditions, explaining the higher preferences observed in treatments with higher lithium concentrations, with more negative avoidance values (Figure 3). The high adsorption capacity of the tested biochar material also contributes to increased water retention (Hu et al., 2023), creating more favourable conditions comparatively to treatments with null application (Figure 4). Unlike the treatments with biochar, the water content measured at the end of the exposure period are significantly below the optimal values, between 60% and 70% of the CMRA, influencing the behaviour of *E. fetida*, as observed by Wever et al. (2001) with juveniles of *Aporrectodea tuberculata*.

Other factors related to the fertilizing value of the biochar may also explain the observed results. These materials are rich in carbon, with some portions potentially being in more soluble forms, depending on the type of biomass and pyrolysis conditions (Burachevskaya et al., 2023), and can serve as a substrate for earthworms to obtain energy (Garbuz et al., 2020). In addition to high carbon content, these materials also contain significant levels of nutrients such as calcium and potassium, as well as nitrogen, phosphorus, and micronutrients (Garbuz et al., 2020). The improvement of soil chemical fertility resulting from biochar application (Singh Yadav et al., 2023), along with increased water retention and soil aeration, creates more favourable conditions for soil fauna growth and activity. This allows earthworms to develop resistance mechanisms against toxicity processes caused by the presence of elements such as lithium in the soil (Sun et al., 2022; Gudeta et al., 2023).

There are several mechanisms that can mitigate the toxic effects of lithium, involving physical, chemical, and biological processes. Biochar as a carbonaceous material promote efficient adsorption and mitigation of metals, including lithium (Kamran and Park, 2020). Lithium can be adsorbed through van der Waals forces or electrostatic interactions, which promote rapid adsorption of lithium on the surface and within the pores of biochar (Raji et al., 2023). The effect of this mechanism is quick, although its capacity is limited, depending on the lithium concentration and the type of biochar, particularly its surface area and micro- and mesoporous structure (Murtaza et al., 2022). A second mechanism of chemical origin is associated with the presence of hydroxyl (-OH), carboxylic (-COOH), and phenolic functional groups, which promote the complexation of lithium with oxygen or other atoms present in the biochar. This mechanism is stronger than physical adsorption, however, it has limitations related to its specificity and the availability of active functional groups. It was related by ion exchange phenomena, in which lithium ions present in the soil solution are exchanged with other cations in the biochar, such as sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}), or calcium (Ca^{2+}) (Kavanagh et al., 2018). This mechanism is strongly dependent on the initial composition of the biochar (Jagadeesh and Sundaram, 2023), which in turn is influenced by the feedstock materials and pyrolysis conditions (Qiu et al., 2022). In more stable carbonaceous materials, mitigation processes can occur through the reduction of lithium mobility via encapsulation, where lithium ions are trapped within the internal structure of the biochar, reducing their mobility and availability in solution (Murtaza et al., 2022).

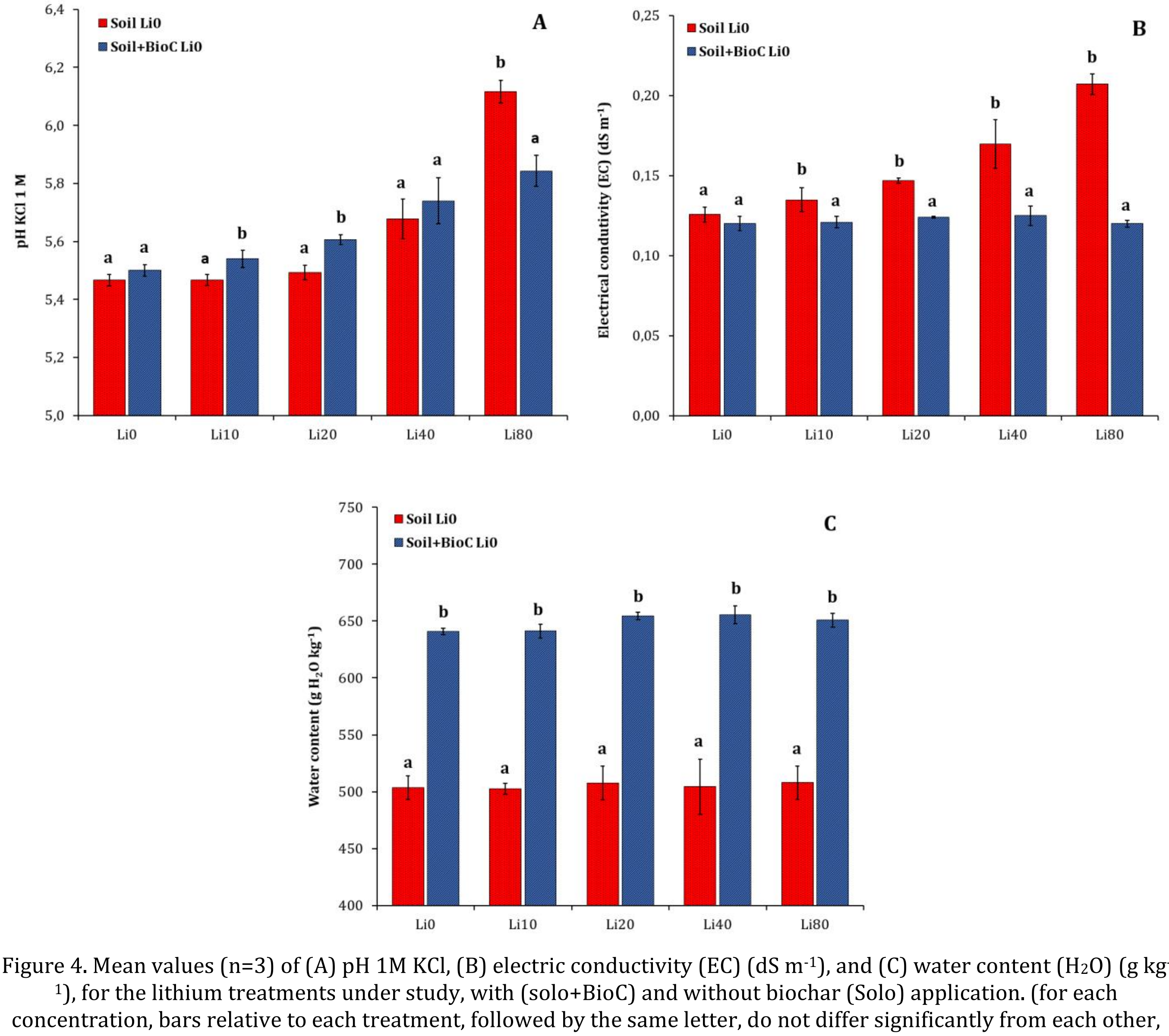

Figure 4. Mean values (n=3) of (A) pH 1M KCl, (B) electric conductivity (EC) (dS m^{-1}), and (C) water content (H_2O) (g kg^{-1}), for the lithium treatments under study, with (solo+BioC) and without biochar (Solo) application. (for each concentration, bars relative to each treatment, followed by the same letter, do not differ significantly from each other, by the Student's t-test, for 5% probability; vertical bars indicate standard error (n=3).

Conclusion

Based on the results obtained, it is possible to conclude that the presence of lithium in the soil promote earthworm's avoidance, with this behaviour depending on the concentration. The avoidance behaviour is mitigated by the application of biochar at doses equivalent to 1% (w/w), with the effects of this application becoming more pronounced for lithium soil highest concentration. These mitigating effects are related to the improvement of certain soil physico-chemical properties, which enhance the soil's buffering capacity against stress caused by the presence of lithium.

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Effect of foliar fertilization applied at different phenological stages on wheat (*Triticum aestivum* L.) yield and grain nutrient content under greenhouse conditions

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Abstract

This study aimed to evaluate the effects of foliar fertilization applied at different phenological stages on wheat (*Triticum aestivum* L.) yield, yield components, and grain nutrient content under controlled greenhouse conditions. The experiment was conducted using a randomized block design with four replications. Foliar fertilization treatments were applied at three key phenological stages: tillering (T), stem elongation (SE), and heading (H), along with their combinations (T+SE, T+H, SE+H, and T+SE+H). A control treatment without foliar fertilization was also included. Foliar fertilizers containing essential macro- and micronutrients were applied at a 0.5% concentration using a hand sprayer. The results demonstrated that foliar fertilization significantly improved wheat grain yield and nutrient composition compared to the control. The highest grain yield increase was observed in the T+SE and T+SE+H treatments, which enhanced yield by 71.01% and 73.45%, respectively, compared to the control. However, statistical analysis revealed no significant differences between these two treatments, suggesting that foliar fertilization at the tillering and stem elongation stages alone is sufficient to achieve maximum yield and nutrient uptake efficiency. Significant increases in nitrogen (N), phosphorus (P), potassium (K), and micronutrients such as iron (Fe), zinc (Zn), and manganese (Mn) were observed in response to foliar applications, while copper (Cu) content remained unchanged. These findings highlight the effectiveness of foliar fertilization in enhancing wheat productivity and nutrient content. Considering practical and economic aspects, the T+SE application is recommended as the most efficient approach. Nevertheless, further field trials are necessary to validate these results under real-world conditions and optimize foliar fertilization strategies for sustainable wheat production.

Keywords: Foliar fertilization, wheat yield, phenological stages, macro and micronutrients, greenhouse experiment.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important staple crops worldwide, providing a major source of calories and nutrients for human populations (Garg et al., 2021; Khalid et al., 2023). The demand for wheat continues to rise due to global population growth and changing dietary preferences, necessitating the adoption of advanced agricultural practices to optimize yield and grain quality (Pingali and Rosegrant, 1988; Awaad and Deshesh, 2019; Pandey and Mishra, 2024). One such practice is foliar fertilization, which has gained significant attention in recent years for its potential to enhance nutrient uptake efficiency, improve yield

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components, and increase the nutritional value of wheat grain (Gülser et al., 2019; Rossmann et al., 2019; McBeath et al., 2020; Andrade et al., 2024).

Foliar fertilization is a complementary strategy to traditional soil fertilization, offering several advantages, including rapid nutrient absorption, bypassing soil nutrient limitations, and targeted application at critical growth stages (Gülser et al., 2019; Bărdaş et al., 2024). Studies have shown that foliar nutrient applications can effectively enhance the uptake of macroelements such as nitrogen (N), phosphorus (P), and potassium (K), as well as essential micronutrients like iron (Fe), zinc (Zn), and manganese (Mn), thereby improving grain yield and quality parameters (McBeath et al., 2020; Abrol et al., 2021; Çolak Esetlili et al., 2024, Ferrari et al., 2025). Furthermore, foliar fertilization can play a crucial role in mitigating nutrient deficiencies and abiotic stress conditions, which are prevalent in wheat-growing regions (El-Hendawy et al., 2024; Shoormij et al., 2024).

The timing of foliar fertilizer application is critical to achieving optimal results (Peirce et al., 2019). Different phenological stages of wheat, such as tillering, stem elongation, and heading, exhibit varying nutrient demands, and applying fertilizers at these key growth stages can significantly influence plant development and final yield. Previous research has demonstrated that stage-specific foliar fertilization can enhance biomass accumulation, grain filling, and nutrient translocation within the plant (Abrol et al., 2021; Andrade et al., 2024; Bărdaş et al., 2024). However, there is limited consensus on the most effective phenological stage for foliar application to maximize wheat yield and quality under controlled conditions.

This study aims to evaluate the effects of foliar fertilization applied at different phenological stages on wheat yield, yield components, and grain nutrient content. The specific objectives are to (i) determine the impact of foliar fertilization timing on wheat yield and grain nutrient composition, (ii) identify the most effective stage for foliar application to achieve optimal agronomic benefits, and (iii) provide practical recommendations for improving wheat fertilization strategies.

Material and Methods

Experimental Materials

The wheat (Triticum aestivum L.) variety used in the experiment is a high-yielding local cultivar known for its suitability for the experiment. The seeds were obtained from a certified supplier.

The soil used in the experiment was collected from an agricultural field where wheat is commonly cultivated. The soil samples were air-dried in the shade, crushed with a wooden mallet, and passed through a 2 mm sieve to ensure homogeneity before being prepared for the experiment and subsequent analyses. To determine the physical and chemical properties of the soil, various standard methods were employed. Soil texture was analyzed using the hydrometer method (Bouyoucous, 1951). For chemical analyses, soil pH was measured in a 1:1 soil-to-water suspension using a pH meter (Peech, 1965), and electrical conductivity (EC) was measured in a 1:1 soil-to-water extract (Rowell, 1996). Organic matter content was determined by the Walkley-Black method (Walkley and Black, 1934), while total carbonate content was analyzed using the Scheibler calcimeter (Loeppert and Suarez, 1996). Total nitrogen (N) was measured using the Kjeldahl method (Bremner, 1965). Available phosphorus (P) was assessed using the Olsen method with a 0.5 M $NaHCO_3$ extraction (Olsen and Dean, 1965). Exchangeable cations (K, Ca, Mg, and Na) were extracted with 1 N ammonium acetate; K and Na were determined by flame photometry, while Ca and Mg were measured by EDTA titration (Pratt, 1965; Heald, 1965). Available micronutrients, including Fe, Cu, Zn, and Mn, were determined by DTPA extraction followed by Atomic Absorption Spectrophotometry (Lindsay and Norvell, 1978).

In the experiment, wheat seeds were sown with the application of diammonium phosphate (DAP) fertilizer containing 18% nitrogen (N) and 46% phosphorus pentoxide (P_2O_5). During the tillering and stem elongation stages, ammonium sulfate fertilizer (21% N) was applied. For foliar fertilization, Agrobigen D (17-17-17+micronutrients) was used during the tillering stage, and Agrobigen K (9-9-25+2MgO+ micronutrients) was applied during the stem elongation and heading stages. All fertilizers were procured from the market.

Experimental Design

This study was conducted under controlled greenhouse conditions to evaluate the effects of foliar fertilization applied at different phenological stages of wheat (Triticum aestivum L.) on yield and nutrient uptake. The experiment was designed using a randomized block design with four replications. For this purpose, 5 kg of soil (on a dry weight basis) was placed in each pot, and 30 wheat seeds were sown per pot. After the emergence of the first leaf, thinning was performed to leave 15 plants per pot. Basal fertilization was carried out by applying 0.5 g of diammonium phosphate (DAP) fertilizer per pot at the time of sowing, equivalent to 25 kg DAP per decare. Additionally, a total of 0.5 g of ammonium sulfate fertilizer (equivalent to 25 kg ammonium sulfate per decare) was applied to each pot, with half of the dose applied during the tillering stage and the other half during the stem elongation stage. To maintain the soil moisture content at 50% of field capacity, the pots were weighed daily, and the missing water amount was replenished accordingly.

For the foliar fertilization treatments, three main phenological stages (tillering, stem elongation, and heading) and all their combinations were considered. Eight different treatments were applied in the experiment:

- Control (C): No foliar fertilizer was applied.
- Tillering (T): Foliar fertilizer was applied only during the tillering stage.
- Stem elongation (SE): Foliar fertilizer was applied only during the stem elongation stage.
- Heading (H): Foliar fertilizer was applied only during the heading stage.
- Tillering + Stem elongation (T + SE): Foliar fertilizer was applied during both the tillering and stem elongation stages.
- Tillering + Heading (T + H): Foliar fertilizer was applied during the tillering and heading stages.
- Stem elongation + Heading (SE + H): Foliar fertilizer was applied during the stem elongation and heading stages.
- Tillering + Stem elongation + Heading (T + SE + H): Foliar fertilizer was applied at all phenological stages.

Foliar fertilizers were prepared as a 0.5% solution. The solution was applied uniformly to the plant surface using a hand sprayer in the early morning hours. In each application, 20 mL of fertilizer solution was sprayed onto each pot.

Harvest and Analysis

On the 85th day of the experiment, the wheat plants reached maturity and were harvested. The harvested plant materials were separated into grain and straw fractions, which were then dried in an oven at 65°C until a constant weight was achieved. The dried samples were subsequently weighed using a precision balance to determine the grain yields per pot. For nutrient analysis, the dried grain samples were ground to a fine powder and analyzed for their N, P, K, Mg, and micronutrient (Fe, Zn, Cu, and Mn) contents. The nitrogen content was determined using the Kjeldahl method, while phosphorus was analyzed using the molybdo-vanadate spectrophotometric method. Potassium content was measured with a flame photometer. The concentrations of Mg, Fe, Cu, Zn and Mn were determined by Atomic Absorption Spectrophotometry (AAS) following the methodology described by Jones (2001).

Statistical Analysis

The obtained data were analyzed using analysis of variance (ANOVA) to determine the effects of different phenological stages and their combinations. Treatment means were compared using the Least Significant Difference (LSD) test at a 5% significance level. Statistical analyses were performed using SPSS 25.0 software.

Results and Discussion

The soil used in the experiment consisted of 58% clay, 24% silt, and 18% sand, with a pH of 7.45 and an electrical conductivity (EC) of 0.85 dS/m. The organic matter content was 1.08%, lime content was 18.03%, total nitrogen (N) was 0.089%, available phosphorus (P) was 6.26 mg/kg, exchangeable potassium (K) was 0.983 meq/100 g, exchangeable calcium (Ca) was 21.311 meq/100 g, exchangeable magnesium (Mg) was 3.692 meq/100 g, and exchangeable sodium (Na) was 0.578 meq/100 g. Additionally, the available micronutrient contents of the experimental soil were determined as follows: iron (Fe) 4.56 mg/kg, copper (Cu) 0.95 mg/kg, zinc (Zn) 1.23 mg/kg, and manganese (Mn) 15.08 mg/kg. Based on these results, the experimental soil was classified as clay-loam in texture, slightly alkaline in reaction, calcareous, non-saline, with a low organic matter content, free from sodicity issues, and characterized by low total nitrogen and available phosphorus content. The levels of potassium, calcium, and magnesium were found to be moderate, while the micronutrient (Fe, Cu, Zn, and Mn) contents were at adequate levels.

Effects on Wheat Yield and Yield Components

The application of foliar fertilizers at different phenological stages significantly influenced wheat yield and yield components, including grain weight per pot, thousand grain weight, and the number of grains per spike (Figure 1). Treatments involving tillering and stem elongation stages (T+SE) and all stages combined

(T+SE+H) demonstrated the most pronounced yield improvements of 71.01% and 73.45%, respectively. These findings align with previous studies, which reported that foliar fertilization at key phenological stages enhances nutrient availability and translocation, resulting in improved biomass accumulation and grain filling (Bărdaş et al., 2024).

Several studies confirm the positive impact of foliar applications on wheat productivity. For instance, Gülser et al. (2019), McBeath et al. (2020) and Ferrari et al. (2025) highlighted that foliar-applied nitrogen and phosphorus fertilizers during the stem elongation stage significantly enhance grain yield and protein content. Similarly, Bărdaş et al. (2024) reported that a balanced foliar fertilization approach during tillering and stem elongation stages maximizes nutrient use efficiency and promotes grain development. The increased number of grains per spike and thousand grain weight in the current study suggests improved source-sink relationships, corroborating findings by Wu et al. (2022) and Wei et al. (2023), who emphasized that foliar fertilization during critical growth stages optimizes assimilate partitioning and photosynthetic efficiency.

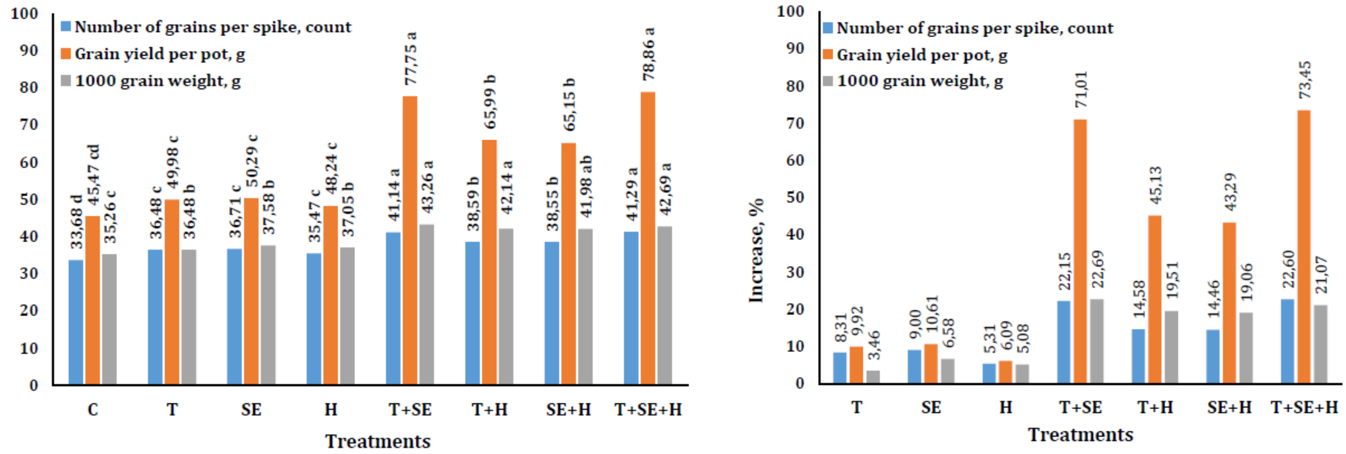

Figure 1. The effect of foliar fertilization applied at different phenological stages of wheat on grain yield, number of grains per spike, and thousand grain weight.

Effects on Macro Element Contents of Wheat Grain

The macroelement composition of wheat grains, including nitrogen (N), phosphorus (P), potassium (K), and magnesium (Mg), exhibited significant variations depending on the foliar fertilization regimen (Figure 2). Treatments T+SE and T+SE+H resulted in the highest increases in N, P, and K contents. Phosphorus showed the most notable increase (34.62%), emphasizing the importance of foliar P application during critical growth phases in enhancing its uptake and utilization (Bărdaş et al., 2024).

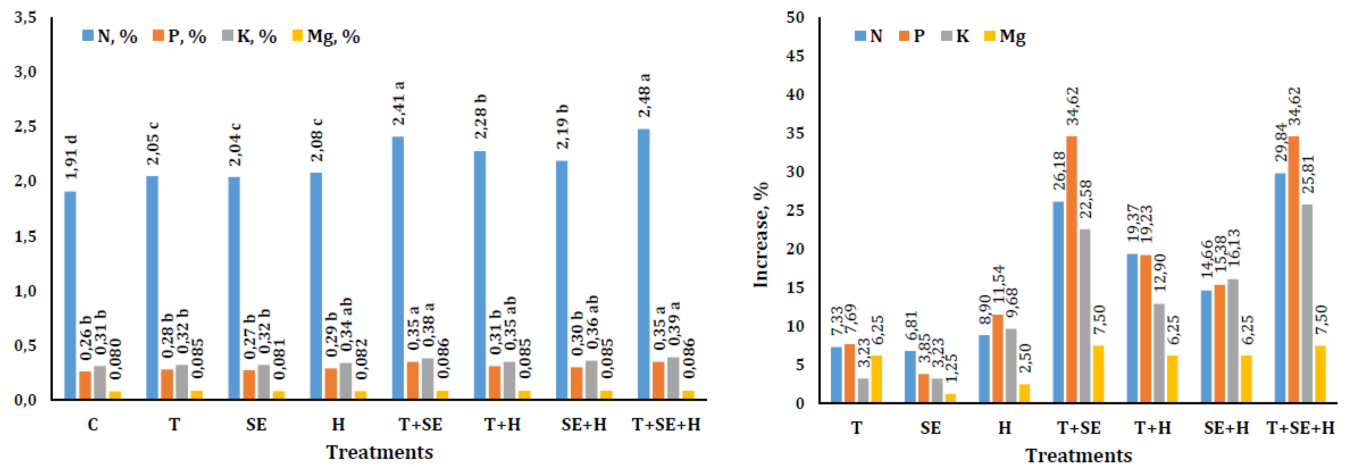

Figure 2. The effect of foliar fertilization applied at different phenological stages of wheat on the N, P, K, and Mg contents of the grain.

Shabnam et al. (2018) and Bourak et al (2023) reported similar trends, demonstrating that phosphorus fertilization at stem elongation enhances root development and energy transfer in wheat. The significant nitrogen content improvements align with findings by Rossmann et al. (2019), who found that foliar N application increases grain protein content and yield quality. Interestingly, the lack of significant changes in magnesium content in this study aligns with findings by Dölger et al. (2024), who suggested that Mg foliar applications are more effective under deficiency conditions rather than as a preventive measure.

Effects on Microelement Contents of Wheat Grain

Foliar fertilization significantly influenced the microelement composition of wheat grains, particularly iron (Fe), zinc (Zn), and manganese (Mn), whereas copper (Cu) did not show significant changes (Figure 3). The highest increases in Fe, Zn, and Mn contents were observed in the T+SE and T+SE+H treatments, highlighting the role of foliar applications in enhancing micronutrient bioavailability and uptake efficiency.

These results are in line with the findings of Wang et al. (2020), who reported that foliar Zn and Fe applications significantly improved wheat grain quality in rainfed conditions. Furthermore, recent studies indicate that foliar micronutrient supplementation can address hidden hunger in staple crops and improve human nutrition by increasing micronutrient density in grains (Saquee et al., 2023). The insignificant changes in Cu content suggest sufficient soil availability or potential antagonistic interactions with other nutrients, as discussed by Wairich et al. (2022), who recommended alternative application methods for better Cu uptake.

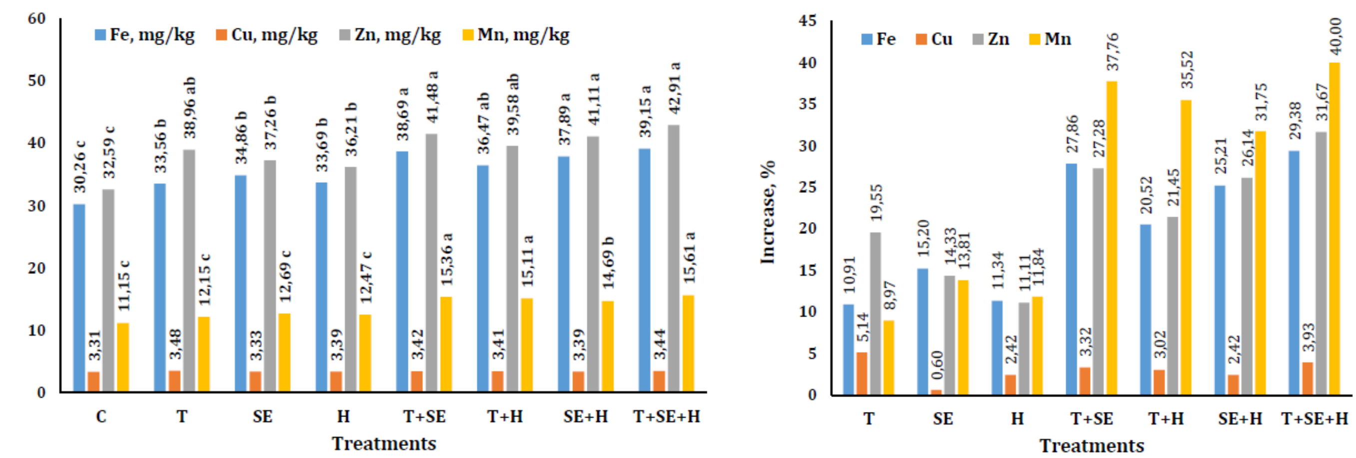

Figure 3. The effect of foliar fertilization applied at different phenological stages of wheat on the Fe, Cu, Zn, and Mn contents of the grain.

Conclusion

This greenhouse study demonstrates that foliar fertilization applied at different phenological stages significantly enhances wheat yield, yield components, and grain nutrient content. The findings indicate that foliar application at the tillering and stem elongation stages (T+SE) resulted in substantial improvements, comparable to the more comprehensive treatment including the heading stage (T+SE+H). However, considering economic feasibility and practical applicability, the T+SE treatment is recommended as an optimal strategy for improving wheat productivity.

Foliar fertilization has proven to be an effective agronomic practice for increasing nutrient availability, enhancing nutrient uptake efficiency, and ultimately improving grain yield and quality. By supplying essential nutrients directly to the plant during critical growth stages, foliar fertilization not only enhances yield components such as grain weight and number of grains per spike but also contributes to improved macro- and micronutrient concentrations in the grain. This highlights its importance in addressing nutrient deficiencies and optimizing plant growth.

Despite the promising results obtained under controlled greenhouse conditions, it is important to recognize that field environments present different challenges such as soil heterogeneity and climatic variability. Therefore, further field trials are essential to validate these findings and to determine the effectiveness of foliar fertilization under real-world conditions.

In conclusion, foliar fertilization is a valuable strategy for enhancing wheat productivity by improving both yield and nutritional quality. Future research should focus on field-scale implementation to confirm its practical benefits and to establish tailored fertilization strategies for sustainable wheat production.

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The enhancement of soil fertility and baby maize output by *Streptomyces panayensis* and vermicompost

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Abstract

Gradual reduction to chemical fertilizer application by adopting sustainable alternatives that naturally harness, nutritional sources from endophytic *actinobacteria processes* in combination with vermicompost (VP) is capable of improving the available nutrients of farmland and baby maize (BM) output. This field research observed the combined efficiency of *Streptomyces panayensis* (*S. panayensis)* inoculum and three VP rates on available nutrients and BM productivity. it was carried out by mean of two factors, consisting of factor 1: three VP levels (0, 4 and 8 t ha^{-1}) in a combination with factor 2 (supplementation and no supplementation of *S. panayensis*) on the BM variety "SG-7", utilizing a completely random block with six experimental plots with four replications. All plots of both *S. panayensis* and VP supplementation raised soil nutrients and ear number, weights of fresh ear and plant biomass compared to those with no *S. panayensis* and VP supplementation. The research emphasizes the supplementation of *S. panayensis* and VP application to increase availably nutritional concentrations in soil and augment BM productivity. The results of the research showed a 50% reduction in VP supplementation that could maintain productivity and soil fertility. These findings provide valuable insights for sustainable agriculture, presenting a promising approach to increase BM production, improve soil fertility, and protect the environment. The combination of endophytic actinobacteria inoculation and organic manure management in this integrated approach is proven to be a right pathway in modern agriculture, enhancing both soil health and biomass yields.

Keywords: Actinobacteria, addition, animal manures, cob yield, nutrition, output

Introduction

Exploring the innovative natural fertilizer options to replace chemical pesticides and inorganic fertilizers purposely is to obtain environmentally friendly agricultural practices to increase quality, yield, and crop protection. In agricultural cultivation, Actinobacteria (especially *Streptomyces* spp.) are the great option related to plant growth and protection (Kunova et al., 2016). Plant diseases coupled with nutrient deficiencies are the significantly contributing factors in the decrease of crop yields. Ameliorating this issue through the application of chemical fertilizers is not a sustainable or efficacious approach. Utilizing *Streptomyces* spp. in disease management, along with their contribution to the decomposition of organic matter into bioavailable nutrients for soil and plants, represents a necessary alternative strategy to address the challenges associated with chemical inputs. *Streptomyces*, a gram-positive saprophytic actinobacterium, exhibits a remarkable efficacy in combating phytopathogens that produce a diverse array of bioactive antimicrobial metabolites and enzymes that are capable of exterminating or inhibiting the proliferation of plant pathogens (Adhilakshmi et al., 2014). *Streptomyces* species are ubiquitous in natural environments, but are particularly abundant in the rhizosphere and endosphere. They can be employed as biocontrol agents to protect crops from diseases. Beyond their antagonistic potential against pathogens. *Streptomyces* can promote plant growth through various mechanisms. They synthesize plant growth-promoting substances,

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such as indole-3-acetic acid (IAA), cytokinins, and siderophores. Also, *Streptomyces* can suppress diseases via antibiosis, mycoparasitism, and nutrient competition. Furthermore, they are able to provide plants with essential minerals, including iron, copper, phosphorus, and sulfur. Therefore, it can be stated that *Streptomyces* spp. can serve as a viable alternative to chemical agents for controlling plant diseases (Al Hamad et al., 2021). The well known antibiotics have been produced by biology in which 80 % of the metabolites with biological activity in the total yield of actinomycetes are produced by *Streptomyces* genera (Barka et al., 2016). The scientists have identified up to 5,000 bioactive compounds created by the *Streptomyces* sp. *Streptomyces* isolated from the soil and plant, could inhibit the phytopathogen growth and degrade the soil organic matter (Kaur et al., 2019). *Streptomyces* genera that could promote plant growth and prevent root pathogens can also inhibit fungal pathogens. They produce degradative enzymes and antifungal agents to protect plant roots. Further, *Streptomyces* genera are capable of producing the siderophore or IAA to promote plant development playing a key role in the interactions of bacteria-actinobacteria and plant-microorganisms. These characteristics allow these microorganisms to be the promising agents as both biofertilizers and bio-pesticides (Bonaldi et al., 2014). Furthermore, several genera of *Streptomyces* could protect plant from diseased root fungus by producing degradative enzymes and antifungal agents, and soil organic matter (SOM) metabolites. They additionally can enhance available nutrients, and the ability of highly saline adaptation, drought and contaminated farmland. The positive efficiency of *Streptomyces* species has been proven for increasing plant output and decreasing the usage of inorganic fertilizers and pesticides, proven as an eco-friendly approach for agricultural cultivation (Jog et al., 2016; Tyc et al., 2017; Nazari et al., 2023). The application of 10t VP ha^{-1} or 50% N fertilizer combined with endophytic bacteria inoculation attained the maximum groundnut yield (Chuong, 2024, Chuong et al., 2024). Organic manure remarkably affected seed and stem yield. The amendment of animal manures brings the positive efficiency to raise the physical and chemical properties of soil, water holding ability, and soil microbial population in the climate change conditions (Chen et al., 2019; Bhanwaria et a., 2022). In dealing with the integrated nutrient management, it is suggested to use microbial inoculations in combination with organic manure addition to reduce the amount of chemical fertilizers applied. The prior researches have proven that the inefficiency of inorganic fertilizers could be decreased by amending endophytic microorganism in association with organic manure addition that are capable of enhancing availably nutrient uptake efficiency and plant growth promotion while decreasing inorganic fertilizer application by up to 50% without reducing any crop output loss compared to the fully applied control (Thuc et al., 2022; Chuong, 2023)

The BM (baby maize) contains high nutritional compositions. The application of endophytic microorganisms combined with organic manures can prevent pests and diseases for BM plants. This technology that has been used by biological methods for increasing the soil fertility and BM yield, is deemed critical (Kumar et al., 2014; Humaun et al., 2021). A study by Chuong (2024) showed that the inoculation of endophytic bacterium (K. quasipneumoniae) in BM seeds could reduce 50% nitrogen fertilizer application and increase total N concentration in soil and yield and cob quality traits. The BM cultivation has faced a number of serious diseases, generated by nematodes, fungi, bacteria, viruses, and insects. 110 different diseases are found to increasingly affect corn plants (Lv et al., 2020; Xu et al., 2021). Recently, endophytic *Streptomyces* spp. have been discovered for their disease resistance ability (Nazari et al., 2023). They filamentous allowing them to adapt during disadvantageous time. For this reason, they are able to adapt more effectively against other soil bacteria. *Streptomyces* spp. can create differently lytic enzymes, which can degrade organic matter, and break them to provide sucrose for being transformed and absorbed by plants (Vurukonda et al., 2018). The research aim is to assess the efficiency of S. panayensis with three vermicompost (VP) rates on soil fertility, and the output of BM cobs.

Material and Methods

Streptomyces panayensis selection

Streptomyces panayensis was firtly isolated from BM roots on CGA medium (Casein Glycerol Agar). Results described that organisms had the highest percent identity (99.78%) with sample sequence. The accession number in GenBank of *S. panayensis* species was MF462923.1 with sequence length at 1450 bp; closest type strain of *Streptomyces panayensis* strain 21; and identity at 99.78% to the previously known *S. panayensis*.

Experimental site and time

The field plots were carried out in the AGU Research Center from 1st May to 20th June, 2024. To determine the soil traits of the experimental location, initial farmland samples were taken from the depth of 0- 20 cm and certain physicochemical properties of the selected samples were determined using the methodology of

Carter and Gregoric (2007). The two factors included six treatments labeled BR0 (0.0t VP ha^{-1}+ no *S. panayensis* inoculation), BR1 (0.0t VP ha^{-1}+ *S. panayensis* inoculation), BR2 (4.0t VP ha^{-1}+ no *S. panayensis* inoculation), BR3 (4.0t VP ha^{-1}+ S. panayensis inoculation), BR4 (8.0t VP ha-1+ no *S. panayensis* inoculation), and BR5 (8.0t VP ha^{-1}+ *S. panayensis* inoculation), with four replications. All treatments were applied with a similar chemical fertilizer level (165 Urea – 370 P_2O_5 – 80 KCl kg per ha). The planting distances concluded the holes spaced at 30 cm x 20 cm, each of which was sowed with 02 seeds. The " SG-7" variety of BM was used during the experiment. Furthermore, the total research area covered 480 m2 (1 m x 20 m x 4 replicates x 6 treatments). The pure VP was collected and composted from local farms, containing 59.1% C, 2.30% N, 1.80% P_2O_5, 0.820 % K_2O, aerobic bacteria (2.0 x 10^8 CFU g^{-1}), phosphorus-solubilizing bacteria (10^7 CFU g^{-1}), and nitrogen fixing bacteria (10^7 CFU g^{-1}).

S. panayensis inoculation and seed sowing

The *S. panayensis* was increased to a density of 10^8 CFU mL^{-1} on CGA medium. The BM seeds of SG7 variety were collected from the Southern Breed Company, Vietnam, in which they had high-yielding, high-quality baby corn variety with disease resistance and able to be cultivated year-round. The *S. panayensis* was well sprayed on BM seeds and was amended to BM seeds for the treatments of BR1, BR3 and BR5 (except for BR0, BR2 and BR4).

Calculation and observation of growth and yield traits

The tassel removal became an important stage executed at 50 DAS. It helped a main process in rousing the BM growth to produce more BM ears and increase output and nutritional concentration. The agronomy characteristics, yield components, and edible corn cob yield were systematically evaluated from 15 DAS until harvest time. The growth traits consisting of height, leaf number per plant, weight of plant biomass, fresh pod, silk, husk, tassel, cob length and diameter, were determinated at harvest (45 DAS). After the BM matured, 10 uniformly grown plants were selected from each treatment for harvest and assessment of the number of marketable ears, yield, ear length, ear diameter, fresh ear weight, and yield calculation. Furthermore, all BM traits that were counted based upon the methodology as outlined by Jones (2001),

Soil physicochemical analysis

All soil samples were taken in the initial and the end of period of experiment (Exp) from all treatments to define the influences engendered by the amended plots on farmland physicochemical traits. A pH meter here was used to determine pH (soil/H_20:1/2.5) values. Meanwhile, mineral nitrogen (MN) concentration was determined by using Kjeldahl methodology. The soluble P (SP) utilized by the Bray II methodology, was used by extracting 0.1N HCl and 0.03N NH_4F with a ratio of 1 soil: 7 water (w/v). A spectrophotometer was utilized to determine SP (880 nm). The analysis method of SOM analyzed according to Sparks et al. (1996), used $K_2Cr_2O_7$ to oxidize organic compositions and 0.5 N $FeSO_4$ using the redundant $K_2Cr_2O_7$ concentration (Carter and Gregoric, 2007).

Statistical Data

To compare differences, the analysis of variance (ANOVA) was conducted, followed by Duncan's multiple range test at a 5% significance level, using Statgraphics software version XVIII. Here, data processing was carried out by the help of Microsoft Excel 2013.

Results

Effects of the association of VP addition and *S. panayensis* inoculation on the soil properties

Under the influences of both *S. panayensis* inoculation and VP addition, the concentrations of soil pH, SOM, MN, and SP in the initial experiment changed insufficient at the level of 5%. However, as shown in Table 1, both *S. panayensis* inoculation and VP addition presented changed sufficiently at the level of 5 and 1% in the Exp end (Except SP in factor B of the last experiment). The results in the initial and last soil pH of factor A and B ranged from 7.01 to 7.04 and from 6.75 to 7.296, respectively. Similarly, the influences of both *S. panayensis* inoculation and VP addition on soil nutrient properties, consisted of the SOM valued from 1.52 to 1.54 (initial Exp), 1.42 to 1.77% (Exp end), MN from 0.067 to 0.069% (initial Exp), 0.062 to 0.082% (Exp end), SP from 0.047 to 0.051% (initial Exp), 0.033 to 0.058% (Exp end), respectively. As shown in Table 1, all soil nutrient concentrations such as pH, SOM, MN and SP in the initial Exp had no significant differences in the plots of both *S. panayensis* inoculation and VP addition. In contrast, the treatments at harvest season with VP (4 or 8 t ha^{-1}) showed significantly higher pH, SOM, MN, and SP compared to the initially experimental soil and the control treatment (no inoculation or no VP application) at the end of Exp. The interactions (**$P<0.01$) between two factors led to significant differences for increasing the soil nutrition uptake such as SOM, MN and SP concentration

Table 1. Effects of Streptomyces and VP on soil chemical properties before and after the exp.

Factors		pH		SOM (%)		MN (%)		SP (%)	
		Initiation	Harvest	Initiation	Harvest	Initiation	Harvest	Initiation	Harvest
S.panayensis (A)	No	7.01 ±0.08	6.75±0.09b	1.52±0.02	1.45±0.02b	0.068±0.02	0.062±0.00c	0.048±0.0	0.033±0.0c
	Yes	7.05 ±0.08	7.29 ±0.09a	1.54±0.02	1.75±0.02a	0.069±0.02	0.075±0.00a	0.050±0.0	0.058±0.0a
VP (t ha^{-1}) (B)	0.0	7.01 ±0.08	6.93±0.09b	1.52±0.02	1.42±0.02b	0.069±0.02	0.047±0.00c	0.051±0.0	0.043±0.0
	4.0	7.04 ±0.08	7.17±0.09ab	1.53±0.02	1.75±0.02a	0.068±0.02	0.078±0.00b	0.049±0.0	0.043±0.0
	8.0	7.04 ±0.08	7.26 ±0.09a	1.54±0.02	1.77±0.02a	0.067±0.02	0.082±0.00a	0.047±0.0	0.045±0.0
F_{test}	F (A)	ns	**	ns	**	ns	**	ns	**
	F (B)	ns	*	ns	**	ns	**	ns	ns
	F (AxB)	ns	ns	ns	**	*	**	ns	**

Note: No: no *S. panayensis* inoculation; Yes: *S. panayensis* inoculation; ns: insufficient difference (P>0.05); *, ** = sufficient difference (P≤ 0.05 and ≤0.01, respectively); Sign (±): the standard deviation of 4 replications.

During the growth period of 15, 30 and 45 DAS, the plant height was significantly affected at 5% and 1% by the addition of VP levels and *S. panayensis*. However, the leaf number of BM was significantly affected at 5% (except for factor B at 15 DAS and 45 DAS). As shown in Table 2, plant height and the leaf number raised during the growth period of 15, 30 and 45 DAS that belonged to the following increase of VP levels. The plots at both factors with *S. panayensis* amendment and VP levels (4 or 8 t ha^{-1}) were significantly higher than those of the initial experiment for soil and the control treatment (no inoculation or no VP application) at the end of Exp. The interaction of two factors showed several significant differences at level of 5 and 1% (except for plant height at 30 DAS and leaf number at 45 DAS) (See Table 2)

Table 2. Effects of Streptomyces and VP on height and leaf number of BM plants

Factors		Plant heights			Leaf number (Leaves plant^{-1})		
		Days after sowing (DAS)					
		15	30	45	15	30	45
S. panayensis (A)	No	17.1±0.272b	68.5±1.592b	142±2.17b	5.0±0.035	8,4±0.148	11.9±0.095
	Yes	19.3±0.272a	73.4±1.592a	156±2.17a	5.0±0.035	8,8±0.148	12.1±0.095
VP (t ha^{-1}) (B)	0.0	16.2±0.333c	53.1±1.95b	130±2.658c	4.8±0.043b	8,4±0.181	11.5±0.116b
	4.0	17.7±0.333b	77.2±1.95a	150±2.658b	5.1±0.043a	8.8±0.181	12.1±0.116a
	8.0	20.8±0.333a	82.5±1.95a	168±2.658a	5.1±0.043a	8.6±0.,181	12.4±0.116a
F_{test}	F (A)	**	*	**	ns	ns	ns
	F (B)	**	**	**	**	ns	**
	F (AxB)	**	ns	**	*	*	ns

Note: No: no *S. panayensis* inoculation; Yes: *S. panayensis* inoculation; ns: insufficient difference (P>0.05); *,** = sufficient difference (P≤ 0.05 and ≤0.01, respectively); Sign (±) : the standard deviation of 4 replications.

As shown in Table 3, ear number per plant and fresh ear weight (ear, silk, husk and tassel) differed sufficiently at 5% and 1% under the influences of different VP rates and *S. panayensis* supplementation. At 50% and 100% addition of VP and *S. panayensis* inoculation, the ear number per plant and weight of ear, silk, husk and tassel were found more than those of no *S. panayensis* supplementation and no VP addition. The treatment added to 8.0 t VP ha^{-1}, which obtained the highest values of ear number per plant and fresh ear weight, followed by 4.0 tVP ha^{-1}, and the lowest results were no VP addition treatments. In the case of *S. panayensis* inoculation in seeds, the ear number per plant and fresh ear weight of BM, statistically was found higher than that of without the *S. panayensis*. There were no interactions between different VP application levels and *S. panayensis* inoculation (Except for ear number).

Table 3. Effects of Streptomyces and VP on on ear number, fresh ear weight and tassel of BM plants

Factors		Ear number (ears plant^{-1})	Fresh ear weight (t ha^{-1})			
			Ear	Silk	Husk	Tassel
S. panayensis (A)	No	2.6±0.021b	6.1±0.168b	1.0±0.039b	4.1±0.147b	4.3±0.113b
	Yes	2.8±0.021a	7.1±0.168a	1.2±0.039a	4.7±0.147a	4.8±0.113a
VP (t ha^{-1}) (B)	0.0	2.0±0.026b	5.1±0.206c	0.9±0.048b	3.3±0.180c	3,3±0,139c
	4.0	3.1±0.026a	6.9±0.206b	1.1±0.048a	4.7±0.180b	4,6±0,139b
	8.0	3.1±0.026a	7.8±0.206a	1.2±0.048a	5.3±0.180a	5,7±0,139a
F_{test}	F (A)	**	**	**	*	**
	F (B)	**	**	**	**	**
	F (AxB)	**	ns	ns	ns	ns

Note: No: no *S. panayensis* inoculation; Yes: *S. panayensis* inoculation; ns: insufficient difference (P>0.05); *, ** = sufficient difference (P≤ 0.05 and ≤0.01, respectively); Sign (±) : the standard deviation of 4 replications.

Table 4 shows that cob length, cob diameter, plant biomass and edible cobs differed sufficiently at 1% level under the influences of varying VP rates and *S. panayensis* amendment. At 4.0% and 8.0% of VP and *S. panayensis* inoculation, the values of cob length, cob diameter, plant biomass and edible cobs were found higher than those of no *S. panayensis* inoculation and no VP addition. While, the treatment fertilized to 4.0 t VP ha^{-1}, obtained the similar values of cob length, cob diameter, plant biomass and edible cobs compared to the addition of 8.0 tVP ha^{-1}, and the lowest results were found in the no VP addition treatments. Further, the cob output in the treatments with 100% VP addition was found insufficient differences with 50% VP in case of no *S. panayensis* inoculation. Productivity increased remarkably for the *S. panayensis* inoculation at these VP levels. This showed that potential output could have the higher plots of *S. panayensis* inoculation compared to chemical fertilizers alone. In brief, the inoculation of *S. panayensis* in combination with VP amendment was efficient in increasing soil fertility and BM yield. Here, there were no interactions between different VP application ratios and *S. panayensis* inoculation in the values of cob length, plant biomass and edible cobs (Except for cob diameter).

Table 4. Effects of Streptomyces and VP on plant biomass, edible cobs, the length and diameter of BM

Factors		Cob length (cm)	Cob diameter (cm)	Plant biomass (t ha^{-1})	Edible cobs (t ha^{-1})
S. panayensis (A)	No	47.7±0.817b	2.6±0.021b	47.7±0.817b	1.0±0.034b
	Yes	53.1±0.817a	2.8±0.021a	53.1±0.817a	1.2±0.034a
VP (t ha^{-1}) (B)	0.0	43.1±1.001b	2.0±0.026b	43.1±1.001b	0.9±0.041c
	4.0	52,6±1.001a	3.1±0.026a	52,6±1.001a	1.29±0.041a
	8.0	55,5±1.001a	3.1±0.026a	55,5±1.001a	1.30±0.041a
F_{test}	F (A)	**	**	**	**
	F (B)	**	**	**	**
	F (AxB)	ns	**	ns	ns

Note: No: no *S. panayensis* inoculation; Yes: *S. panayensis* inoculation; ns: insufficient difference (P>0.05); *, ** = sufficient difference (P≤ 0.05 and ≤0.01, respectively); Sign (±): the standard deviation of 4 replications.

Discussion

Endophytic microorganisms have been presenting in the plant roots and stems, protecting and promoting plant growth and yield to cope with many diseases and environmental stresses (Ahemad, 2014; Hayat, 2010). Furthermore, the long-term use of chemical fertilizers can lead to soil hardening, decreased soil fertility, water and soil pollution, and depletion of crucial soil nutrients and minerals, thereby posing a threat to the environment (Lin et al., 2019). Therefore, applying organic fertilizers in conjunction with *Streptomyces* sp. can enhance the availability of readily decomposable organic matter, transforming it into soluble nutrients for plants. *Streptomyces* sp. has a high adaption to survive in harsh environments such as salty and droughty conditions (Shaffique et al., 2022). They promotionally help the crop growth in combination with biocontrol ability (Sadeghi et al., 2017; Shaffique et al., 2022). High organic matter fertilization allows plants to thrive within a wider pH range. This promotes both the growth of Streptomyces and the formation of larger spores. From this enhanced population density in the nutrient-rich environment, they stimulate roots to exude more secretions to resist pathogens and secrete more H^+, leading to a decrease in soil pH after planting (Doolotkeldieva et al., 2015). A research demonstrated that *Streptomyces saraceticus* used as soil addition could decrease soil disease pathogens and raise plant yields. Moreover, this research discovered that *S. panayensis* that has an ability on biological control could be appropriate for organic cultivation (Wu et al., 2021). In repulsing soil-borne diseases and chemical fertilizer use in the reduction in organic farming, the application of animal manures combined with endophytic microorganisms could bring cost savings as well as and an increase for farmers' income (Nguyen, 2023; Chuong et al., 2024). The inorganic fertilizers could rapidly increase the crop output, but they harm soil health and agricultural product quality. In contrast, the use of endophytic microorganism inoculants has been a right option for a strategy to improve soil nutrients and plant yields (Vurukonda et al., 2018; Nguyen et al., 2024). *Streptomyces* addition attained the highest quality of cucumbers and reduced the nitrate and soluble sugar concentration in fruits. The quality compositions raised the antioxidant content and firm level compared to no *Streptomyces* addition. Streptomyces addition could reduce 25% of inorganic fertilizer during the plant period (Orouji et al., 2023). The inorganic fertilizers, could increase crop outputs; however, their excessive negative impacts on the environment, such as soil degradation and loss of nutrients, have been previously studied (Cai et al., 2015).

As shown in Tables 1, there were sufficient differences from soils, amended by *S. panayensis* and VP amendment treatments at differently added conditions at harvest season. The pH, SOM, MN and SP were positively determined by the soil fertility of experimental end. This was a cause of the addition of endophytic actinobacteria and animal manures by *S. panayensis and* VP, which could enhance nutrition from this combination. When added to the farmland, VP could raise the available nutrients and increase an availably nutrient uptake of plant (Mitter et al., 2021; Chuong, 2023; Sudaryati et al., 2024). Soil *Streptomyces* trains have an ability to survival and adapt in any harshly environmental conditions, and could cure themselves in case a damage or destructibility (Wang et al., 2019). St. supplementary is an enrichment process of N, P, K, and SOM in soils. When positive microorganisms are applied with a native soil microorganism, an ecological relationship will be formed through their competition (Haiming et al., 2020; Kamei-Ishikawa, 2020). The recent researches showed that animal manure amendment could increase the organically nutrient concentrations of microorganism such as biomass P, natural N and others (Manna et al., 2005). The addition of maize cob organic manure (12.5 t ha^{-1}) attained the maximum concentrations of total chlorophyll, root length, MN and SP uptake. The uptake of SP and EK is related to the biomass and root length of plants. Therefore, this studied results suggest that application of organic manure may increase the output and availably nutritional uptake of low nutrient soils (Budiastuti et al., 2023).

In the growth period of BM, both plant height and leaf number were positively affected by the added treatments *S. panayensis* and VP in which they obtained the higher values compared to control (no addition of *S. panayensis* and VP) (Table 2). The combination of newly discovered strains with organic fertilizers is increasingly being applied by researchers in limited areas within agriculture. Broadening their application in production through products such as biofertilizers and biological plant protection agents aims to directly or indirectly harness the positive effects of endophytic actinobacteria (Olanrewaju and Babalola, 2019; Asfaw, 2022; Chuong, 2024). The treatments of endophytic bacteria have significantly proven the same or higher MN and SP use efficiencies compared to full inorganic fertilizer application. These discoveries proved the reduction of 50% in the amounts of N and P fertilizers through the fertilization in combination with endophytic microorganisms (Thuc et al., 2022; Nguyen et al., 2024)

These endophytic microorganisms could form a positive relation with crop roots, raising the uptake of the micro-nutrients and micro-nutrients to promote the growth and yield of plants (Tang et al., 2020; Van and Tri, 2024).

As shown in Table 3 and 4, *S. panayensis* isolated and identified from BM roots, were completely tested both increased yield traits and promoted the edible cob yield compared to control. Treatments of *S. panayensis* addition reduced significantly on the deleterious diseases to plant growth. It is worth present that SP had a positive species on growth and yield of BM.

In this research, it was monitored that the supplementation of VP and/or *S. panayensis* at different levels to farmland, in conjunction with chemical fertilizers, increased either the pH, SOM, MN or SP concentration of the soil fertility and the productivity traits and productivity of BM (Table 1-4), alike prior discoveries (Samsami, 2016 Tran et al., 2021; Teka et al., 2024; Chuong, 2024). This was mainly due to separate SOM into its constituent parts in the combined supplementation of inorganic fertilizers, animal manures and endophytic microorganism inoculation, causing an increase in microbial density and an activity of endophytic microorganism (Abbasi et al., 2019; Tan et al., 2021; Chuong et al., 2024). The combined supplementation was more efficient in satisfying the nutrient demands of BM plants sufficiently, taking high yields. Furthermore, *S. panayensis* aids in SOM decomposition and diseased protection. From above results, the supplementation of VP and/or *S. panayensis* raised soil fertility and BM yield (Vergnes et al., 2020; Zhu et al., 2020; Chuong and Tri, 2024; Chuong, 2024).

Conclusion

The supplementation of *S. panayensis* co-ordinated with VP amendment had a positive efficacy at augmenting the fertility of highly sandy soils. Regarding the concentrations of SOM, MN, and SP, pH value significantly ameliorated soil structures, the physicochemical and biological properties of farmlands, and the bioavailability of solute nutrition in the crop soils. For the plant height and leaf number per plant, the productivity traits and output of BM rose in the plots amended with 4.0 and 8.0 t VP ha^{-1} P in a combination with *S. panayensis* addition compared to those in the treatments with no both *S. panayensis* inoculation and VP addition. Meanwhile, baby maize plants amended with 4.0 t ha $^{-1}$ of VP increased edible cob yield similar with the one in the plots applied with 8.0 t VP ha-1(1.29 and 1.3 t ha^{-1}of BM cob yield, respectively). The *S. panayensis* inoculation raised the higher yield (6.7%) compared to control treatment without any S. *panayensis* supplementation. The BM yield in the treatments supplemented with 4.0 tVP ha^{-1} and/or a S. *panayensis* inoculation increased the yield equivalently or even higher compared to the treatment with 8.0 t VP ha^{-1} and/or a *S. panayensis* inoculation. The new findings revealed a significant correlation between VP and *S. panayensis*. These substances demonstrated the remarkable effects on improving soil health, yield parameters, and the edible corm yield of BM. A 50% reduction in VP application in combination with *S. panayensis* resulted in a more increase by 16.7% compared to the control and no significant difference in the edible corm yield of BM compared to the recommended 100% VP application. This new discovery is a crucial point in sustainable agricultural cultivation, opening a new technology for producing safe food and protecting environment. Therefore, the research positions the combination of S. panayensis inoculation with VP reduction as a potential strategy in a new agricultural background.

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Effects of nitrogen application on potato (*Solanum tuberosum* L.) yield and soil nitrate dynamics in a sandy loam soil

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Abstract

Nitrogen (N) is a crucial nutrient for potato (Solanum tuberosum L.) production, but excessive application can lead to environmental degradation and reduced nitrogen use efficiency (NUE). This study evaluated the effects of different nitrogen application rates (0, 60, 120, 150, 180, 210, and 240 kg N/ha) on tuber yield, nitrogen uptake, and soil nitrate accumulation over two growing seasons. The results showed that the highest tuber yield (20.8 t/ha) was obtained at 150 kg N/ha, beyond which further increases in nitrogen application did not result in significant yield improvements ($P<0.05$). Nitrogen uptake increased with application rates but reached a saturation point beyond 150 kg N/ha, leading to declining NUE. Soil nitrate levels significantly increased at higher N rates, particularly in deeper soil layers (40–60 cm), posing a potential risk of nitrate leaching. Apparent nitrogen balance calculations indicated substantial nitrogen surpluses at rates above 180 kg N/ha, further emphasizing the risk of nitrogen losses to the environment. These findings suggest that applying nitrogen at 150 kg/ha optimizes potato yield while minimizing environmental risks. Precision nitrogen management strategies, including split applications and slow-release fertilizers, should be adopted to enhance NUE and reduce nitrate leaching. Further long-term studies are needed to refine nitrogen recommendations under varying soil and climatic conditions to ensure sustainable potato production.

Keywords: Nitrogen fertilization, Tuber yield, Soil nitrogen dynamics, NUE, Sandy loam soil, Fertilizer management.

Introduction

Potato (*Solanum tuberosum* L.) is one of the most widely cultivated and consumed crops worldwide, playing a crucial role in global food security and economic development. It is ranked as the fourth most important staple crop after wheat, rice, and maize, with its adaptability to diverse agro-ecological zones and high nutritional value contributing to its global significance (Birch et al., 2012; Kloosterman et al., 2013; Drewnowski and Rehm, 2013; Tokbergenova et al., 2017; Alimkhanov et al., 2021; Liu et al., 2021; Budanov, et al., 2023; Adilbayeva et al., 2024). However, achieving sustainable potato production is increasingly challenged by soil fertility constraints and inefficient nutrient management, particularly for nitrogen (N), a critical macronutrient for plant growth (Davenport et al., 2005; Koch et al., 2020).

Nitrogen is essential for the physiological and biochemical processes in potatoes, including photosynthesis, enzymatic activity, and protein synthesis (Ye at al., 2022). Optimal nitrogen management not only enhances tuber yield but also improves quality parameters such as starch content and dry matter (Sawicka et al., 2018; 2020). Conversely, suboptimal nitrogen application—whether excessive or insufficient—can lead to

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various agronomic and environmental challenges. Over-application of nitrogen often results in nitrate (NO_3-N) leaching, leading to groundwater contamination, greenhouse gas emissions, and soil acidification (Anas et al., 2010). On the other hand, nitrogen deficiency limits tuber growth and reduces marketable yield, affecting economic returns for farmers (Wilkinson et al., 2020).

Recent studies have underscored the importance of precise nitrogen management in potato cultivation. Solano et al. (2018) and Sawicka et al. (2020) highlighted that the apparent nitrogen balance—the difference between nitrogen inputs and crop uptake—is a critical metric for assessing nitrogen use efficiency (NUE). Positive nitrogen balances, indicative of excessive nitrogen application, are strongly associated with nitrate accumulation in the soil profile and increased risks of leaching (Bibi et al., 2016, Awaad and Deshesh, 2019). Moreover, the spatial distribution of nitrate within the soil profile is influenced by nitrogen application rates, soil type, and irrigation practices, necessitating site-specific management strategies to optimize nitrogen use (Barraclough et al., 1992; Bellido et al., 2013).

The relationship between nitrogen rates and potato yield is well-documented, with numerous studies demonstrating that yields increase with nitrogen application up to a certain threshold, beyond which no further benefits are observed (Tadesse et al., 2001; Jiao et al., 2013; Wang et al., 2020). Excess nitrogen often promotes excessive vegetative growth at the expense of tuber development, reducing NUE and leading to economic inefficiencies. This phenomenon underscores the need for determining optimal nitrogen rates that balance yield maximization with environmental sustainability.

In addition to yield optimization, nitrogen management has significant implications for soil health and environmental quality. Excess nitrate accumulation in the soil, particularly in deeper layers, increases the potential for leaching into groundwater, posing risks to water quality and human health (Wang et al., 2015; Bijay-Singh and Craswell, 2021). Furthermore, inefficient nitrogen use contributes to nitrous oxide (N_2O) emissions, a potent greenhouse gas that exacerbates climate change. Sustainable nitrogen management practices, including the use of slow-release fertilizers and nitrification inhibitors, have been proposed as potential solutions to enhance NUE and mitigate environmental risks (Millar et al., 2010; Liu et al., 2021).

This study aims to investigate the effects of different nitrogen application rates on potato tuber yield, nitrogen uptake, and soil nitrate dynamics under controlled field conditions. Specifically, the objectives are to: (i) Quantify the effects of nitrogen rates on tuber yield and nitrogen uptake, (ii) evaluate the apparent nitrogen balance and its relationship with soil nitrate stocks, and (iii) assess the distribution of nitrate nitrogen within the soil profile at varying nitrogen application rates.

Material and Methods

Study Site and Environmental Conditions

The field experiments were conducted over two consecutive growing seasons (2022 and 2023) at a research station with sandy loam soils (56% sand, 18% clay, and 26% silt), characterized by low organic matter content (1.2%), a neutral pH (7.12), non-saline conditions (electrical conductivity of 0.76 dS m^{-1}), and the absence of carbonate (3.65% $CaCO_3$). The total nitrogen and nitrate nitrogen contents were 0.138% and 13.25 mg kg^{-1}, respectively. According to the Köppen-Geiger classification, the climate of the experimental site is classified as Cfa (humid subtropical), with an average annual temperature of 13.1°C and an average annual precipitation of 936 mm.

Experimental Design

A randomized complete block design (RCBD) was employed to assess the effects of different nitrogen application rates on potato yield and soil nitrate dynamics. The experiment consisted of seven nitrogen treatments, each replicated three times. Individual plots measured 30 m^2 (5 m × 6 m) and were separated by buffer zones to minimize cross-contamination between treatments. The nitrogen treatments were as follows:

- N_0: 0 kg N ha^{-1} (control)
- N_{60}: 60 kg N ha^{-1}
- N_{120}: 120 kg N ha^{-1}
- N_{150}: 150 kg N ha^{-1}
- N_{180}: 180 kg N ha^{-1}
- N_{210}: 210 kg N ha^{-1}
- N_{240}: 240 kg N ha^{-1}

Nitrogen was applied in the form of urea (46% N), a widely used and cost-effective nitrogen source. To ensure that nitrogen was the only limiting factor, phosphorus (P_2O_5) and potassium (K_2O) fertilizers were applied uniformly across all treatments at 65 kg ha^{-1} and 115 kg ha^{-1}, respectively. The phosphorus source was calcium magnesium phosphate, while potassium chloride (KCl) was used as the potassium source.

Fertilizer Application

Nitrogen was applied in three split doses to align with key physiological stages of potato growth, enhancing nitrogen use efficiency (NUE) while minimizing losses due to leaching and volatilization. Before planting, 40% of the total nitrogen dose was incorporated into the topsoil (0–20 cm) along with the full phosphorus (P_2O_5) and potassium (K_2O) applications. At the vegetative growth stage (35 days after planting, DAP), an additional 30% of nitrogen was surface-applied to support shoot and foliage development. The remaining 30% was applied at the flowering stage (60 DAP) to promote tuber bulking and maximize final yield. This split application strategy ensured optimal nitrogen availability throughout the growing season, facilitating balanced nutrient uptake while mitigating the risk of nitrate leaching.

Crop Management

Certified mid-season potato seed tubers were planted using a ridge cultivation system. The ridges were 35 cm in height and spaced 60 cm apart, facilitating proper aeration, root development, and water drainage. The planting density was set at 4.200 plants ha^{-1} to maintain uniform growth and maximize yield potential. Standard agronomic practices, including pest and disease management, were employed throughout the growing season. Drip irrigation was used to maintain soil moisture at approximately 80% of field capacity, ensuring optimal water availability for tuber formation while minimizing water loss through deep percolation.

Soil and Plant Sampling

To evaluate soil nitrogen dynamics and crop response, soil and plant sampling was systematically conducted at critical growth stages. Soil samples were collected from three depth intervals (0–20 cm, 20–40 cm, and 40–60 cm) at planting, mid-season (vegetative stage), and harvest to monitor temporal variations in nitrate availability. Bulk density measurements were taken to facilitate the calculation of soil nitrate stocks. Additionally, soil samples were extracted using 2M KCl and analyzed for nitrate nitrogen (NO_3-N) using the Kjeldahl distillation method (Jones, 2001).

At harvest, plant biomass was separated into tubers and straw to assess yield and nutrient uptake. Tuber yield was determined by weighing the harvested tubers from each plot. Straw samples were initially dried at 105°C for 2 hours, followed by further drying at 85°C until a constant weight was achieved. The dried plant material was then ground using a micro-plant grinding machine and passed through a 0.5-mm mesh sieve for subsequent chemical analysis (Jones, 2001).

Measurements and Calculations

Tuber yield was determined by measuring both marketable (>30 mm diameter) and unmarketable tubers, with marketable yield expressed in tons per hectare (t ha^{-1}). To evaluate nitrogen uptake, the micro-Kjeldahl method was used to analyze nitrogen content in both tubers and straw. Apparent nitrogen uptake (N_{uptake}) was calculated using the equation 1:

$$N_{uptake} = N_{tuber} \times Y_{tuber} + N_{straw} \times Y_{straw} \times 0.001 \quad (1)$$

where N_{tuber} and N_{straw} represent the nitrogen content (g kg^{-1}) in tubers and straw, respectively, and Y_{tuber} and Y_{straw} correspond to the biomass yields (kg ha^{-1}) of these plant components.

Soil nitrate stocks were estimated to assess nitrogen dynamics in the soil. The nitrate nitrogen stock (NNS) was calculated using the equation 2:

$$NNS = NNC \times BD \times D \times 10 \quad (2)$$

where NNC represents the nitrate nitrogen content (mg kg^{-1}), BDB is the soil bulk density (g cm^{-3}), and DDD is the soil depth (cm). The total nitrate stock in the 0–60 cm soil profile was determined by summing the stocks at three depth intervals (0–20 cm, 20–40 cm, and 40–60 cm).

To evaluate nitrogen balance within the system, the apparent nitrogen balance ($N_{balance}$) was calculated as the difference between nitrogen input from fertilizers (N_{input}) and nitrogen uptake by the crop (N_{uptake}), following the equation 3:

$$N_{balance} = N_{input} - N_{uptake} \quad (3)$$

where $N_{balance}$ represents the nitrogen balance (kg ha^{-1}), N_{input} is the total nitrogen applied as fertilizer (kg ha^{-1}), and N_{uptake} is the total nitrogen absorbed by the plant. These calculations provided a comprehensive understanding of nitrogen efficiency, soil nitrate retention, and the potential for nitrogen losses in the experimental system.

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) to assess the effects of nitrogen treatments on tuber yield, nitrogen uptake, and soil nitrate stocks. Mean comparisons were performed using the least significant difference (LSD) test at a 5% significance level. Regression analysis was conducted to evaluate relationships between nitrogen application rates, apparent nitrogen balance, and soil nitrate dynamics.

Results and Discussion

Tuber yield response to nitrogen application

Nitrogen application significantly affected potato tuber yield across both growing seasons (Table 1). The lowest yield was observed in the control (N0) treatment, averaging 12.4 t ha^{-1} across both years. Yield increased significantly ($P<0.05$) with nitrogen rates up to 150 kg ha^{-1}, reaching a maximum of 20.8 t ha^{-1}. However, further increases in nitrogen application beyond this threshold did not result in significant yield improvements.

Table 1. Effect of nitrogen rates on tuber yield (t/ha) with standard deviations and statistical groupings.

Nitrogen Rate (kg/ha)	2022 Yield (t/ha)	2023 Yield (t/ha)	Mean Yield (t/ha)
N_0	12.1 ± 0.5 c	12.6 ± 0.4 c	12.4 ± 0.45 c
N_{60}	15.8 ± 0.6 b	16.3 ± 0.7 b	16.1 ± 0.65 b
N_{120}	19.2 ± 0.8 ab	19.8 ± 0.9 a	19.5 ± 0.85 a
N_{150}	20.4 ± 0.7 a	21.2 ± 0.8 a	20.8 ± 0.75 a
N_{180}	20.3 ± 0.7 a	21.0 ± 0.8 a	20.7 ± 0.75 a
N_{210}	19.9 ± 0.6 a	20.4 ± 0.7 a	20.2 ± 0.65 a
N_{240}	19.5 ± 0.6 a	20.0 ± 0.7 a	19.8 ± 0.65 a

Comparing the two years, yields were slightly higher in 2023 across all nitrogen treatments. For instance, the N_{150} treatment resulted in yields of 20.4 t ha^{-1} in 2022 and 21.2 t ha^{-1} in 2023. This slight variation may be attributed to differences in climatic conditions, particularly temperature and rainfall distribution, which influence nitrogen availability and uptake. Statistical analysis (LSD test, $P<0.05$) revealed significant differences among treatments. While nitrogen application up to 150 kg/ha led to statistically higher yields than the control, increasing nitrogen rates to 210 or 240 kg ha^{-1} did not result in further significant gains. Excess nitrogen at these levels likely promoted vegetative growth at the expense of tuber formation, a phenomenon commonly observed in potato cultivation under high nitrogen supply (Koch et al., 2020). Furthermore, when comparing treatments against one another, the yield at N_{180} (20.7 t ha^{-1}) was statistically similar to N_{150}, indicating no additional benefit from increasing nitrogen beyond 150 kg ha^{-1}. These results suggest that excessive nitrogen input does not translate into higher yield but may instead contribute to inefficiencies in nitrogen use.

The results of this study align with previous findings that demonstrate a diminishing return in yield response at high nitrogen application rates (Sawicka et al., 2018; Wang et al., 2020; Liu et al., 2021). The increase in yield with nitrogen application up to 150 kg/ha highlights the importance of adequate nitrogen availability for optimizing tuber formation. However, beyond this level, the lack of further yield increases suggests that the crop reaches a physiological limit where additional nitrogen does not enhance productivity.

From an agronomic perspective, these findings emphasize the need for optimizing nitrogen fertilization to balance productivity and environmental sustainability. Excessive nitrogen application not only leads to economic inefficiencies but also poses environmental risks such as nitrate leaching and increased greenhouse gas emissions (Wick et al., 2012; Bijay-Singh and Craswell, 2021). Therefore, precision nitrogen management strategies, such as split applications and the use of slow-release fertilizers, should be considered to maximize nitrogen use efficiency (NUE) while minimizing environmental impact.

In addition, inter-annual variations in yield response underscore the role of climatic conditions in nitrogen dynamics. Slightly higher yields in 2023 suggest that favorable weather conditions may have enhanced nitrogen uptake and utilization. Future research should consider long-term trials incorporating different climatic scenarios to develop more robust nitrogen management recommendations. Overall, the results support the recommendation of 150 kg ha^{-1} as the optimal nitrogen application rate for maximizing potato tuber yield in sandy loam soils while ensuring economic and environmental sustainability.

Nitrogen uptake and its efficiency across different application rates

Nitrogen uptake by potato plants exhibited a strong positive response to increasing nitrogen application rates across both years (Table 2). The lowest nitrogen uptake was recorded in the control (N_0) treatment, averaging 47.2 kg ha^{-1} across the two growing seasons. Uptake increased significantly ($P<0.05$) with nitrogen application up to 240 kg ha^{-1}, reaching a maximum value of 118.2 kg ha^{-1}. However, despite higher uptake at elevated nitrogen levels, nitrogen use efficiency (NUE) declined significantly beyond 150 kg ha^{-1}.

Table 2. Effect of nitrogen rates on nitrogen uptake (kg ha^{-1}) with standard deviations and statistical groupings

Nitrogen Rate (kg ha^{-1})	2022 Uptake (kg ha^{-1})	2023 Uptake (kg ha^{-1})	Mean Uptake (kg ha^{-1})
N_0	46.2 ± 3.2 d	48.1 ± 3.1 d	47.2 ± 3.15 d
N_{60}	78.5 ± 4.1 c	80.2 ± 4.2 c	79.4 ± 4.15 c
N_{120}	101.7 ± 5.2 b	103.5 ± 5.3 b	102.6 ± 5.25 b
N_{150}	118.3 ± 5.6 a	120.2 ± 5.8 a	119.2 ± 5.70 a
N_{180}	119.6 ± 5.7 a	121.4 ± 5.6 a	120.5 ± 5.65 a
N_{210}	118.9 ± 5.5 a	120.1 ± 5.4 a	119.5 ± 5.45 a
N_{240}	117.8 ± 5.3 a	118.5 ± 5.2 a	118.2 ± 5.25 a

Yearly comparisons indicate that nitrogen uptake was slightly higher in 2023 than in 2022 across all treatments. For instance, in the N_{150} treatment, uptake values were recorded as 118.3 kg ha^{-1} in 2022 and 120.2 kg ha^{-1} in 2023. This variation could be attributed to differences in soil moisture and mineralization rates, which affect nitrogen availability.

Statistical analysis (LSD test, $P<0.05$) showed significant differences among nitrogen treatments, particularly between low (N_0 and N_{60}) and high (N_{150}–N_{240}) nitrogen applications. However, uptake at N_{180}, N_{210}, and N_{240} was statistically similar, indicating that plants reached a nitrogen saturation threshold beyond which additional nitrogen did not significantly improve uptake efficiency. This is consistent with findings from Khangura et al. (2023), who observed that excessive nitrogen applications often lead to diminishing returns in nitrogen uptake.

While nitrogen uptake continued to increase with higher nitrogen rates, NUE decreased beyond 150 kg ha^{-1}, suggesting that plants were unable to fully utilize excess nitrogen. This highlights the importance of optimizing nitrogen inputs to maximize uptake efficiency while avoiding potential nitrogen losses through leaching or volatilization.

The results demonstrate that nitrogen uptake efficiency plays a critical role in determining the effectiveness of nitrogen fertilization strategies. While uptake increased with nitrogen rates, the declining NUE beyond 150 kg ha^{-1} suggests that excess nitrogen may not contribute to higher yield or efficiency, but rather lead to increased environmental losses. This is in agreement with previous studies highlighting the risks of excessive nitrogen fertilization (Wick et al., 2012; Bijay-Singh and Craswell, 2021).

From an agronomic perspective, applying nitrogen at rates beyond 150 kg ha^{-1} may not be economically viable due to diminishing uptake efficiency. Instead, targeted nitrogen management practices, such as split applications and the use of controlled-release fertilizers, could enhance NUE and minimize nitrogen losses. Additionally, integrating precision agriculture techniques, such as real-time nitrogen monitoring, could further improve nitrogen uptake efficiency. Inter-annual variations in nitrogen uptake emphasize the need for flexible fertilization strategies that account for climatic variability. Future research should focus on understanding the interactions between nitrogen application rates, soil properties, and weather conditions to develop site-specific fertilization recommendations. Overall, these findings highlight the necessity of balancing nitrogen inputs with plant uptake capacity to improve nitrogen use efficiency, optimize crop performance, and minimize environmental risks.

Soil nitrate accumulation and nitrogen balance under different application rates

Soil nitrate nitrogen accumulation exhibited a strong positive correlation with increasing nitrogen application rates (Table 3). The control (N_0) treatment had the lowest total nitrate stock, averaging 25.8 kg ha^{-1} in 2022 and 27.0 kg ha^{-1} in 2023. In contrast, the highest nitrogen treatment (N_{240}) resulted in significantly higher nitrate accumulation, exceeding 215 kg ha^{-1} in 2022 and 220 kg ha^{-1} in 2023.

Yearly comparisons indicate that soil nitrate levels were generally higher in 2023, likely due to increased soil moisture conditions facilitating nitrate retention. The LSD test ($P<0.05$) confirmed significant differences among treatments, particularly in deeper soil layers (40–60 cm), where nitrate accumulation increased at higher nitrogen rates.

At nitrogen application rates above 180 kg ha^{-1}, apparent nitrogen balance calculations revealed a substantial surplus of over 100 kg ha^{-1}, suggesting a high potential for nitrate leaching. This finding raises environmental concerns, as excessive nitrogen applications can contribute to groundwater contamination (Salo and Turtola, 2006). Therefore, optimizing nitrogen application rates is crucial for reducing the risks associated with nitrate leaching while maintaining high potato yields. These results underscore the necessity of adopting precision nitrogen management strategies, including split applications and controlled-release fertilizers, to mitigate nitrogen losses and enhance environmental sustainability.

Table 3. Soil nitrate stocks (kg ha^{-1}) at harvest with standard deviations and statistical groupings.

Year	Nitrogen Rate (kg ha^{-1})	mg NO_3-N kg^{-1} 0–20 cm	20–40 cm	40–60 cm	Total Nitrate Stock kg NO_3-N ha^{-1}
2022	N_0	12.4 ± 1.2 g	8.3 ± 1.1 g	5.1 ± 0.9 g	25.8 ± 1.5 g
	N_{60}	28.1 ± 2.5 f	17.6 ± 2.1 f	10.4 ± 1.7 f	56.1 ± 3.0 f
	N_{120}	47.3 ± 3.8 e	30.2 ± 3.2 e	19.7 ± 2.8 e	97.2 ± 4.5 e
	N_{150}	56.2 ± 4.0 d	37.9 ± 3.9 d	24.5 ± 3.2 d	118.6 ± 5.0 d
	N_{180}	68.3 ± 4.3 c	45.7 ± 4.2 c	30.1 ± 3.7 c	144.1 ± 5.3 c
	N_{210}	89.1 ± 4.7 b	59.4 ± 4.5 b	37.2 ± 4.0 b	185.7 ± 5.7 b
	N_{240}	102.4 ± 5.1 a	68.3 ± 4.8 a	45.1 ± 4.5 a	215.8 ± 6.0 a
2023	N_0	13.2 ± 1.3 d	9.5 ± 1.8 f	4.3 ± 0.8 e	27.0 ± 1.6 g
	N_{60}	29.0 ± 2.4 c	18.7 ± 2.3 e	10.3 ± 0.9 d	58.0 ± 3.1 f
	N_{120}	48.5 ± 3.9 b	32.5 ± 2.9 d	19.0 ± 1.9 c	100.0 ± 4.6 e
	N_{150}	57.0 ± 4.1 a	49.4 ± 4.2 c	13.6 ± 1.2 d	120.0 ± 5.1 d
	N_{180}	69.5 ± 4.4 a	61.4 ± 4.4 bc	17.1 ± 2.5 c	148.0 ± 5.4 c
	N_{210}	90.0 ± 4.8 a	65.8 ± 4.9 b	34.2 ± 3.2 b	190.0 ± 5.8 b
	N_{240}	104.0 ± 5.2 a	71.2 ± 5.6 a	44.8 ± 3.9 a	220.0 ± 6.1 a

Optimizing Nitrogen Management for Sustainable Potato Production

The findings of this study emphasize the importance of nitrogen management strategies that balance yield maximization with environmental sustainability. Applying nitrogen at 150 kg ha^{-1} was found to be the most effective rate for achieving high potato yields while minimizing the risks of nitrogen losses and nitrate accumulation in the soil.

Precision nitrogen management, including split applications and the use of slow-release fertilizers, should be considered to enhance nitrogen use efficiency (NUE). Additionally, incorporating real-time nitrogen monitoring techniques can help adjust fertilization rates according to plant demand, reducing excessive nitrogen application and environmental risks.

Future research should explore the integration of alternative nitrogen sources, such as nitrification inhibitors and organic amendments, to improve nitrogen retention in the soil while maintaining crop productivity. Moreover, long-term field studies under varying climatic conditions will be valuable for refining nitrogen management recommendations. Overall, this study underscores the necessity of a balanced nitrogen application approach to ensure sustainable potato production while mitigating environmental impacts. Policymakers and farmers should work together to implement best management practices that optimize nitrogen use, reduce nutrient losses, and promote agricultural sustainability.

Conclusion

This study provides valuable insights into the effects of nitrogen application rates on potato yield, nitrogen uptake, and soil nitrate accumulation. The results demonstrate that applying nitrogen at 150 kg ha^{-1} optimizes tuber yield while preventing excessive nitrogen surpluses. Although higher nitrogen rates (above 180 kg ha^{-1}) enhanced nitrogen uptake, the efficiency of nitrogen use declined, and nitrate accumulation in deeper soil layers increased significantly. The substantial nitrogen surpluses at excessive application rates raise concerns regarding nitrate leaching, which could pose environmental risks, especially in irrigated farming systems. These findings emphasize the need for efficient nitrogen management to sustain both crop productivity and environmental health.

Balancing nitrogen application rates is crucial for ensuring sustainable potato production. The implementation of split nitrogen applications and slow-release fertilizers can improve nitrogen use efficiency and reduce nitrogen losses. Moreover, integrating advanced fertilization techniques, such as nitrification inhibitors and organic amendments, can further enhance nitrogen retention and limit environmental risks. Long-term field experiments under varying soil types and climatic conditions are necessary to develop site-specific nitrogen management strategies. A holistic approach that incorporates precision nitrogen application, real-time monitoring, and sustainable soil fertility practices will be essential to achieving both high agricultural yields and environmental sustainability in potato farming.

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Enhancing of Early Seedling Vigour (ESV) parameters in Lentils through integrated priming with silicic and humic acid

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Abstract

Seed priming has emerged as an innovative and economical technique to elevate seed quality, fostering uniform, swift, and robust germination under both stress and non-stress conditions. This study endeavors to scrutinize the effects of organic (silicic acid, SA) and inorganic (humic acid, HA) acids, alongside their synergistic combinations, on seed quality parameters in three distinct lentil (Lens culinaris) genotypes: IPL-316 (tolerant), PSL-9, and PDL-1 (sensitive). Critical parameters assessed encompass germination percentage, root and shoot length, seed vigor indices I and II, and dry weight under meticulously controlled laboratory conditions. The priming agents were standardized across a spectrum of concentrations and durations. Sterilized seeds were immersed in silicic acid (1, 2, 3, 4, and 5 mM), humic acid (100, 200, 300, 400, 600, 800, and 1000 ppm), and their combinations over varying durations (2 to 18 hours), including control and hydropriming treatments. Following treatment, seeds were air-dried and subjected to growth assessments. The findings reveal that priming significantly bolsters early-stage plant growth across all three lentil genotypes, with the combined application of silicic and humic acids yielding remarkable enhancements in all seed quality parameters, intricately influenced by genotype and treatment combination.

Keywords: Seed priming, Silicic acid, Humic acid, Lentil (*Lens culinaris*), Germination percentage, Seed vigor indices, Root and shoot length, Dry weight, Integrated priming.

Introduction

Lentil (*Lens culinaris* Medik) is a cherished annual cool-season grain legume in India, cultivated across 1.45 million hectares, yielding 1.46 million tonnes—accounting for 26.5% of the world's annual production (GOI, 2021). The lower Gangetic plain (LGP) contributes significantly, encompassing 10.3% (0.16 million ha) of the nation's lentil growing expanse and 9.6% (0.15 million tonnes) of its annual yield. The rice fallows of this fertile region harbor approximately 150–200 mm of carryover soil moisture (Bandyopadhyay et al., 2018), offering an ideal environment for cultivating the water-efficient lentil. Yet, farmers typically plant lentils in the pre-winter months (late November to early December) post-harvest of long-duration puddled-transplanted rice, resulting in delayed sowing. Optimal growth requires temperatures between 18 to 30°C, with cooler conditions essential during early to mid-growth stages, while warm climes are vital for maturation (Sinsawat et al., 2004). Elevated temperatures exceeding 32/20°C (max/min) during flowering and pod-filling can devastate production potential (Bourgault et al., 2018).

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Priming seeds through controlled hydration induces mild stress, enhancing plants' resilience (Paparella et al., 2015). This eco-friendly method cultivates epigenetic memory, strengthens salt tolerance, promotes germination, plant development, and physiological adaptability, fortifying crops to withstand environmental adversities (Thakur et al., 2020; Guo et al., 2022). Exposure of seeds to mild stress imprints epigenetic memory in the primed seeds and strengthens plants to encounter future stresses (Marcos et al., 2018). This eco-friendly technique also activates signaling molecules to enhance the inherent salt tolerance potential in plants that helps in recovery from salt-induced damages (Guo et al., 2022; Khaitov et al. 2024). Seed priming facilitates seed germination and stand establishment of seedlings, boosts plant development, regulates physiological, biochemical, and molecular responses in plants, promotes nutrient uptake, and strengthens tolerance to stress factors.

Seed priming involves the gentle soaking of seeds in a solution characterized by low osmotic potential for a designated duration, followed by a return to their original humidity (Ceritoglu et al., 2023). This transformative process triggers a cascade of biochemical reactions within the seed, activating antioxidant defense systems while concurrently facilitating germination (Mauch-Mani et al., 2017). The overarching aims of seed priming encompass the enhancement of germination traits, the promotion of seedling vigor and nutrient absorption, the reduction of susceptibility to environmental stresses, and the augmentation of crop yields (Paul et al., 2022). Sirisuntornlak et al. (2021) found that treatment with 1 mM silicon significantly boosted nitrogen uptake in maize. Likewise, Özyazıcı et al. (2023) revealed that priming seeds with 0.25 mM salicylic acid improved both germination metrics and seedling development in switchgrass (*Panicum virgatum* L.). Raza et al. (2024) demonstrated that priming seeds with 6 mg L^{-1} selenium effectively enhanced quinoa yields under drought conditions. Notably, Mazhar et al. (2023) reported that priming with 75 ppm iron oxide nanoparticles elevated superoxide dismutase (SOD) and catalase (CAT) activities by 13% and 38%, respectively. Freitas and da Silva (2024) concluded that seed priming treatments bolster stress memory in plants.

Seed priming is a meticulous triadic process commencing with the imbibition of seeds in a carefully selected priming agent for a predetermined duration, a practice refined through the alchemy of trial and error. This initial phase paves the way for the activation phase, where a symphony of metabolic events unfurls at the cellular level spurring protein synthesis, catalyzing enzymes, fortifying the antioxidant machinery, and heralding the formation of new mitochondria alongside DNA repair. The final phase, known as rehydration, activates cell division and fuels the synthesis of nucleic acids and ATP, thereby amplifying cellular energy (Devika et al., 2021).

While research indicates that seedlings nurtured from primed seeds demonstrate enhancements in water content, improved regulation of the cell cycle, adept management of oxidative stress, and efficient reserve food mobilization, the success of seed priming remains intricately tied to both the plant species and the method employed (Raj and Raj, 2019; Johnson and Puthur, 2021). Notably, our findings underscore that the application of silicic and humic acids in lentils not only elevates seed quality parameters but also bolsters seed potential amid adverse conditions. This study is pioneering, illustrating the crucial role these agents play in fostering plant establishment and vigor during early growth phases.

Material and Methods

Seed priming

The current study has been conducted in the hallowed confines of the Seed Biochemistry Laboratory within the Division of Seed Science and Technology at the Indian Agricultural Research Institute (ICAR-IARI), New Delhi, India. Three distinct varieties of lentil seeds were procured from the Division of Genetics, hailing from the abiotic stress laboratory: IPL-316 (tolerant), PSL-9, and PDL-1 (sensitive). Two different priming agents, silicic and humic acid, were employed at varying concentrations and durations. Five concentrations of silicic acid1, 2, 3, 4, and 5 mM were utilized alongside control and hydropriming conditions. Additionally, six concentrations of humic acid 100, 200, 400, 600, 800, and 1000 ppm were also tested, complemented by control and hydropriming (Figure 1). A total of eleven combinations of humic and silicic acid were meticulously standardized across differing durations and concentrations (Table 1). Initially, the seeds were sterilized using a 1% sodium hypochlorite solution, after which they were immersed in the priming agent solution (1:1 w/v) at 20°C, tailored for specific durations prior to achieving 2 mm of radical emergence.

Table 1. Experiment design and standardization

Silicic acid	Humic acid	Combination of humic and silicic acids
Durations = 2, 4, 6, 8, 10, 12, 15, 16, 18 hours		
T1 = SA @ 1 mM	T1 = HA @ 100 ppm	T_1 = HA + SA @ 100 ppm+1 mM
T2 = SA @ 2 mM	T2 = HA @ 200 ppm	T_2 = HA + SA @ 200 ppm+2 mM
T3 = SA @ 3 mM	T3 = HA @ 400 ppm	T_3 = HA + SA @ 300 ppm + 3 mM
T4 = SA @ 4 mM	T4 = HA @ 600 ppm	T_4 = HA + SA @ 400 ppm + 4 mM
T5 = SA @ 5 mM	T5 = HA @ 800 ppm	T_5 = HA + SA @ 600 ppm + 5 mM
T6 = Control	T6 = HA @ 1000 ppm	T_6 = HA + SA @ 100 ppm + 3 mM
T7 = Hydropriming	T7 = Control	T_7 = HA + SA @ 200 ppm + 3 mM
-	T8 = Hydropriming	T_8 = HA + SA @ 400 ppm + 3 mM
-	-	T_9 = HA + SA @ 600 ppm + 1 mM
-	-	T_{10} = HA + SA @ 600 ppm + 2 mM
-	-	T_{11} = HA + SA @ 600 ppm + 3 mM
-	-	T_{12} = HA + SA @ 600 ppm + 4 mM
-	-	T_{13} = Control
-	-	T_{14} = Hydropriming
After standardization		
T1 = Control		
T2 = SA @ 3 mM for 18 hr		
T3 = HA @ 600 ppm for 18 hr		
T4 = HA+SA @100 ppm + 1 mM for 16 hr		
T5 = Hydropriming @ 18 hr		

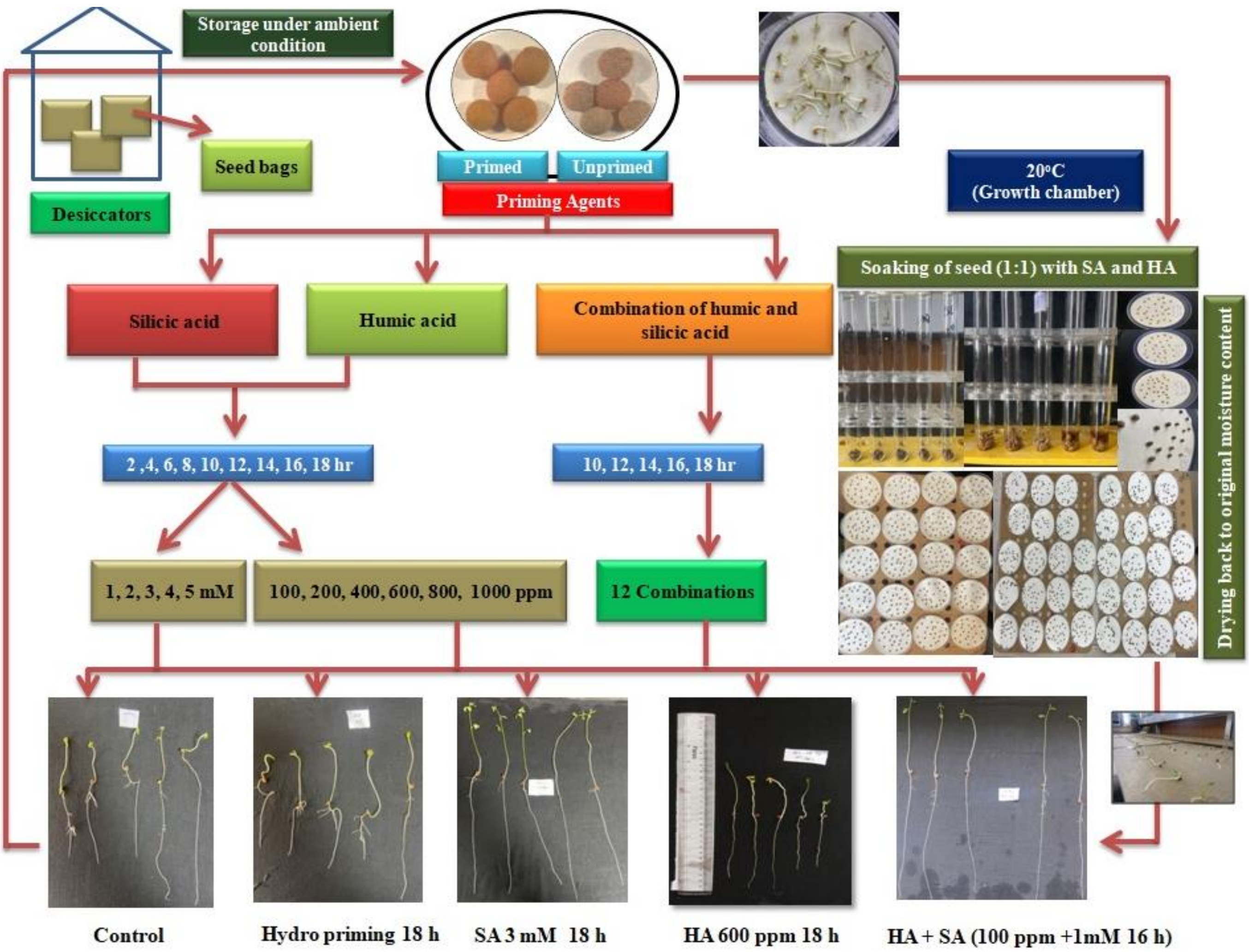

Figure 1. The experimental methodology

Germination

In the experiment, a total of 50 seeds per three replicates were utilized. Following the priming process, the seeds were gently dried to restore their original moisture content. Germination commenced using the top-paper method. After the initial count, the seeds were then transitioned to the between-paper method for further development (ISTA, 2022).

The culmination of the study was marked by a comprehensive evaluation of the seedlings, conducted on the designated final count day. This assessment encompassed the classification of the seedlings into categories: normal, abnormal, hard, dead, and those exhibiting signs of disease. Each seedling's fate was meticulously recorded, reflecting the intricate interplay of environmental influences and inherent genetic potential. Through this careful observation, the experiment sought to unveil the delicate balance between life and adversity, illuminating the nuances of seed germination and seedling development. Ultimately, the outcomes would contribute valuable insights to the realm of botany and agricultural science.

Root and Shoot length (cm)

On the final day of measurement, the lengths of the root and shoot were meticulously recorded using a calibrated metal scale. A selection of ten seedlings was made based on their morphological characteristics. A vibrant red cloth was employed to gently cradle the seedlings, ensuring the fabric remained moist and free from drying. Each seedling's length was measured by hand with precision, capturing the essence of their growth. Following this careful measurement, the selected seedlings were placed in an oven, prepared to undergo the process of drying after recording their fresh weight (ISTA, 2022).

Dry weight (gm)

The chosen seedling, having undergone a gentle drying process in the oven at 42°C for three days, was carefully extracted from the beaker. The dry weight was meticulously measured using a precise weighing scale. According to ISTA (2022), a greater dry weight serves as a clear and direct indicator of superior seed quality.

Seed Vigour Index

The seed vigour index is meticulously calculated following the methodologies established by Abdul-Baki and Anderson (1973). Specifically, Seed Vigour Index-I emerges from the product of the germination percentage and the total length of seedlings measured in centimeters. Meanwhile, Seed Vigour Index-II is derived by multiplying the germination percentage by the dry weight of the seedlings, expressed in grams, in accordance with the guidelines set forth by ISTA (2022).

Root Scanning

The seedling underwent meticulous scrutiny by the root scanning machine (REGENT LA2400 Scanner expertly calibrated for image analysis). From the final count, five carefully selected seedlings were gathered for observation. The process of root scanning encompassed a multitude of parameters, including area, width, length, surface area, primary area, volume, the count of tips, forks, crosses, and nodules. Each parameter revealed the intricate tapestry of the seedling's underground architecture, laying bare the delicate balance between nature's design and the relentless pursuit of growth. In this harmonious convergence of technology and biology, the seedlings emerged not merely as botanical entities, but as testaments to the resilience and complexity of life itself, grasping at the soil from which they sprang, imbued with the promise of flourishing above the earth.

Results

Germination energy

The findings unveil a remarkable journey of germination, demonstrating that the pinnacle of success an astounding 97% germination rate was attained through the treatment with silicic acid (T_3) at a concentration of 3mM for an enduring 18 hours. Following closely, T_5 at 5mM produced a commendable 91%. In stark contrast, the control and hydropriming treatments languished at significantly lower rates of 89% and 87% respectively before standardization (Figure 2). Similarly enchanting was the outcome in the realm of humic acid, where T_4 at 600 ppm achieved an immaculate 100%. The T_2 treatment at 200 ppm, after 18 hours, yielded a satisfying 95%, while control and hydropriming treatments were again reminiscent of lower achievements at 95% and 97% (Figure 2). In the interplay of combined treatments, T_1 (HA+SA at 100ppm+1mM for 16 hours) reached a noteworthy 96%, trailed by T_2 at 94% (200ppm+2mM for 16 hours). Yet again, control and hydropriming resulted in lesser performances at 92% and 88%. Post-standardization revealed T4 with HA+SA leading at 94%, with T_3 and T_2 both sustaining robust 92% rates. The least favorable result, a modest 88%, was noted in the control treatment. The impacts of salinity stress were addressed in prior research (Figure 2).

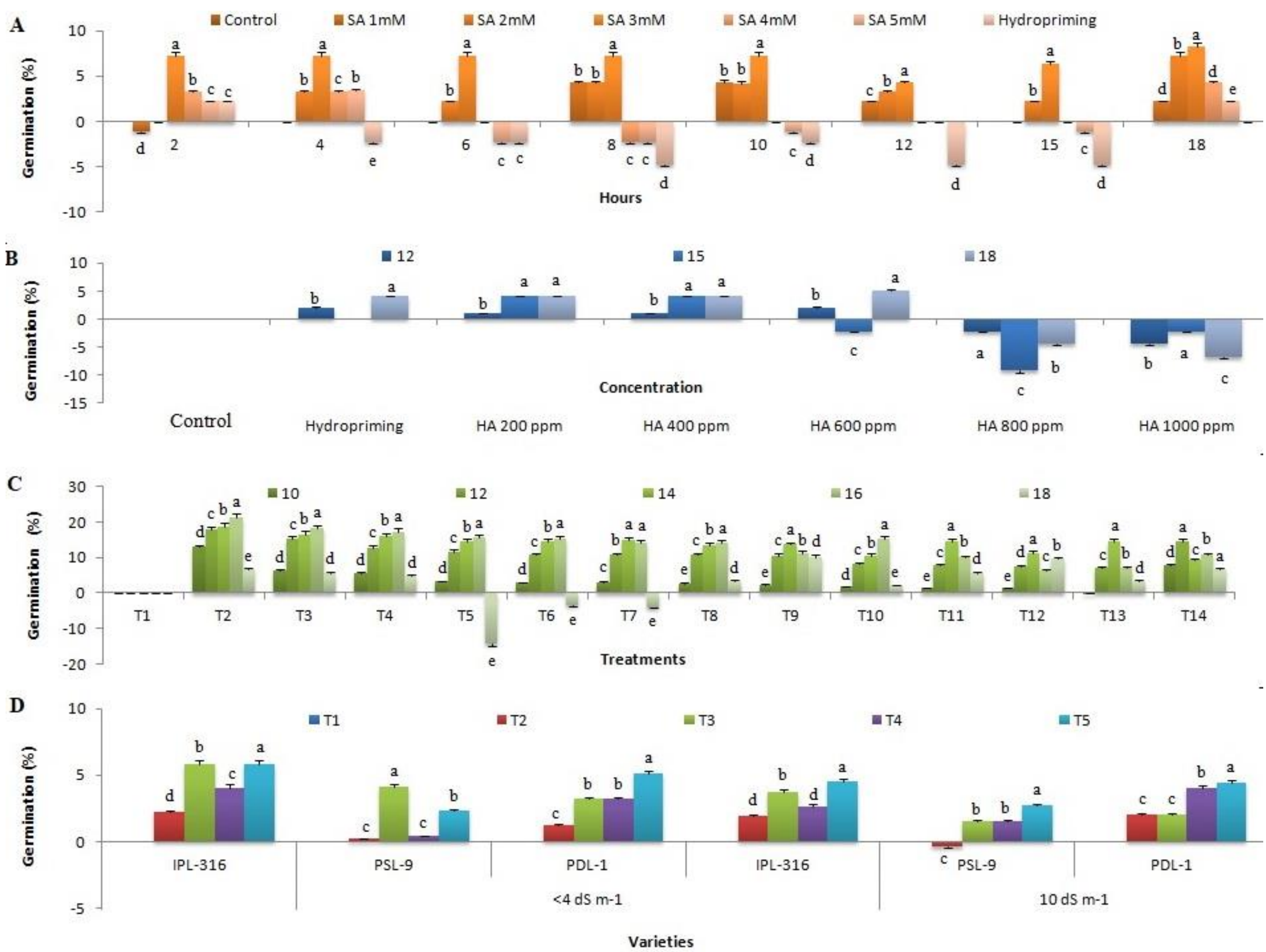

Figure 2. Effect of different treatments on the germination percentage at different durations and concentrations (A); Different concentrations and durations of silicic acid. (B); Different concentrations and durations of humic acids. (C): Combination of humic and silicic acid. (D);After standardization under normal and salinity stress condition.

Seed vigour index-I

Among the various treatments of silicic acid, the apex of seed vigour index-I was recorded in T_3 (SA @ 3mM for 18 hours) with a remarkable value of 3596. Close behind was T_2 (SA @ 2mM for 18 hours), boasting an index of 3491. In stark contrast, the lowest indices emerged from the control group (2821 and 3063) and various hydropriming treatments (Figure 3). Turning to humic acid, T_4 (HA @ 600 ppm for 18 hours) yielded the highest vigour index of 1891, followed closely by T_3 (HA @ 600 ppm for 18 hours) at 1767. The control and hydropriming treatments languished at the bottom, recording 1571 and 1474, respectively (Figure 3). In a harmonious blend of humic and silicic acids, T_1 (HA+SA @ 100 ppm + 1mM for 16 hours) reached the zenith with an impressive vigour index of 3852, followed by T_4 (HA+SA @ 400 ppm + 4mM for 16 hours) at 3681. The control and hydropriming conditions again trailed, resulting in 2866 and 2945. Post-standardization, T_4 claimed the highest vigour index of 2787, with T_3 and T_2 at 2561 and 2479, while the control and hydropriming treatments persisted in their lower realm, recording 2345 and 2446 (Figure 3).

Seed Vigour Index-II

The pinnacle of seed vigor index-II was attained with treatment T_3, wherein silicic acid (SA) was administered at 3 mM for an 18-hour duration, yielding a score of 6.23. This success was closely followed by treatment T_2, utilizing 2 mM of SA for the same period. Meanwhile, the control and hydropriming treatments languished with the lowest indices of 4.91 and 5.47, respectively (Figure 4). In the realm of humic acid application, treatment T_3 emerged triumphant, employing humic acid (HA) at 600 ppm for 18 hours, succeeded by treatment T_2 with HA at 400 ppm. The control and hydropriming treatments, once again, displayed the least vigor, with indices of 0.85 and 0.96. Within the realm of combined treatments, prominence was observed in treatment T_1, which melded HA and SA at 100 ppm and 1 mM for 16 hours, reaching a value of 7.19. Following closely, treatment T_2 combined HA and SA at 200 ppm and 2 mM (Figure 4).

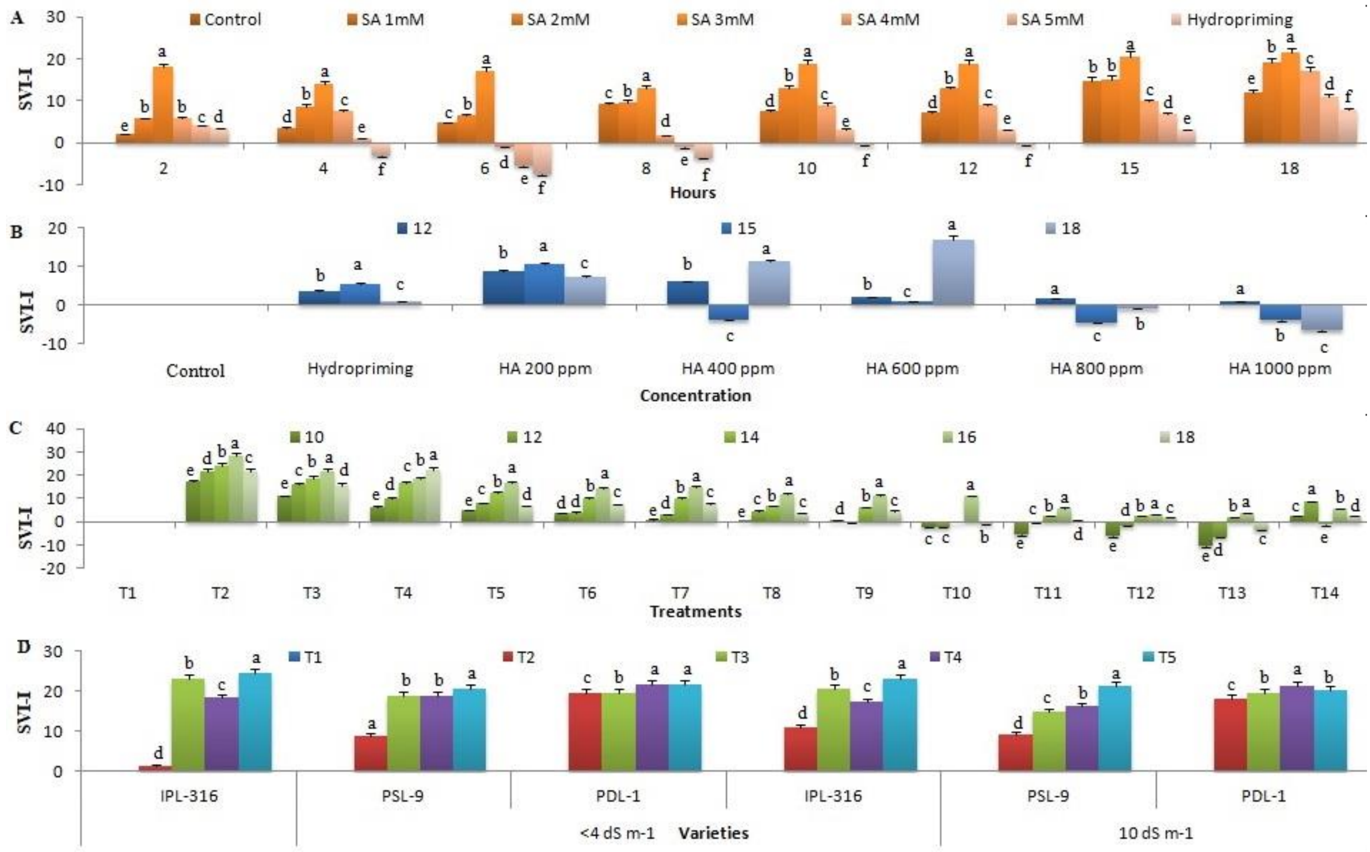

Figure 3. Effect of different treatments on the seed vigour index-I at different durations and concentrations (A); Different concentrations and durations of silicic acid. (B); Different concentrations and durations of humic acids. (C): Combination of humic and silicic acid. (D);After standardization under normal and salinity stress condition.

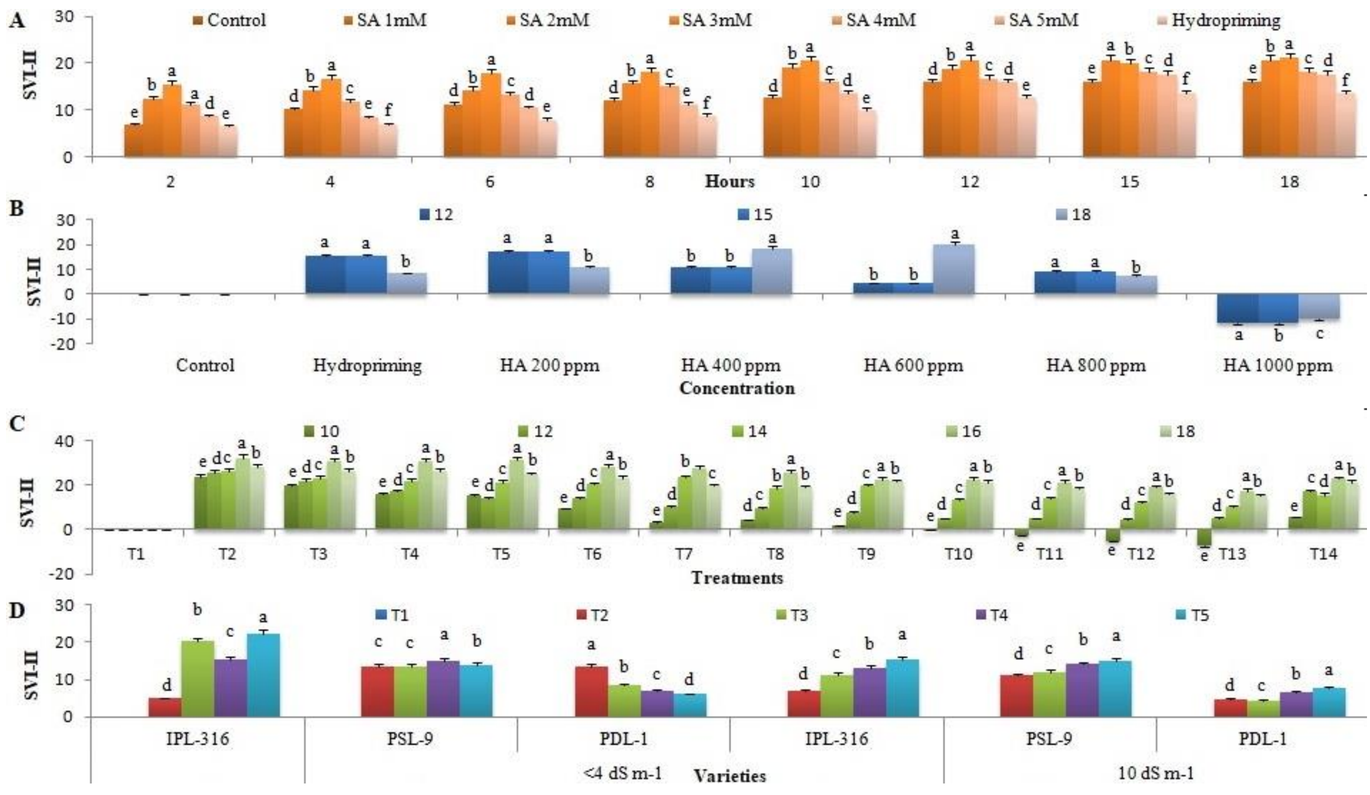

Figure 4. Effect of different treatments on the seed vigour index-II at different durations and concentrations (A); Different concentrations and durations of silicic acid. (B); Different concentrations and durations of humic acids. (C): Combination of humic and silicic acid. (D);After standardization under normal and salinity stress condition.

The control and hydropriming methods continued to show the lowest vigor, with scores of 4.89 and 6.33. Ultimately, the zenith of seed vigor index-II, across all trials, was 6.94, achieved through the blend of HA and SA at 100 ppm and 1 mM for 16 hours in treatment T_3. Treatments T_3 and T_2 followed this benchmark. The least vigorous indices, yet again, arose from the control and hydropriming endeavors, with values of 6.05 and 6.46 (Figure 4).

Root scanning

The study reveals that the treatment (T_4), which combined humic and silicic acid at 100ppm and 1mM for 16 hours, showed the most significantly different root scanning parameters, followed by treatments T_3 and T_2 (Table 2). The lowest significant results were observed in the control treatment (T_1) and hydropriming (T_5). Similarly, the correlation (Figure 5) and heat map (Figure 6) studies indicated positive correlations among all the parameters. The highest significant results for all root scanning parameters, including length, surface area, projected area, volume, average diameter, number of tips, forks, and crossings, were observed in treatment (T_4), followed by the other treatments (Figure 7).

Table 2. Effect of the seed priming on the different root scanning parameters

	Root scanning							
	Length (cm)	Surface area (cm)	Projected area (cm)	Volume (cm^2)	Avg. Diameter (mm)	No. of Tips	No. of forks	No. of cross
T1	118.123 ^{d}	25.397 ^{d}	8.084 ^{d}	0.436 ^{d}	0.684 ^{d}	325 ^{d}	140 ^{d}	7 ^{d}
T2	140.081 ^{bc}	28.538 ^{bc}	9.084 ^{bc}	0.463 ^{bc}	0.648 ^{ab}	376 ^{ab}	147 ^{bc}	18 ^{ab}
T3	146.587 ^{ab}	29.973 ^{ab}	9.541 ^{ab}	0.488 ^{ab}	0.651 ^{ab}	361 ^{ab}	157 ^{ab}	17 ^{ab}
T4	170.523 ^{a}	35.131 ^{a}	11.183 ^{a}	0.656 ^{a}	0.656 ^{a}	425 ^{a}	270 ^{a}	24 ^{a}
T5	107.587 ^{cd}	29.639 ^{bc}	9.434 ^{cd}	0.417 ^{d}	0.563 ^{cd}	345 ^{c}	114 ^{cd}	15 ^{cd}
	P=0.05							
CD (Treatment)				0.561				
CD (Parameter)				0.754				
CD (T*P)				0.985				

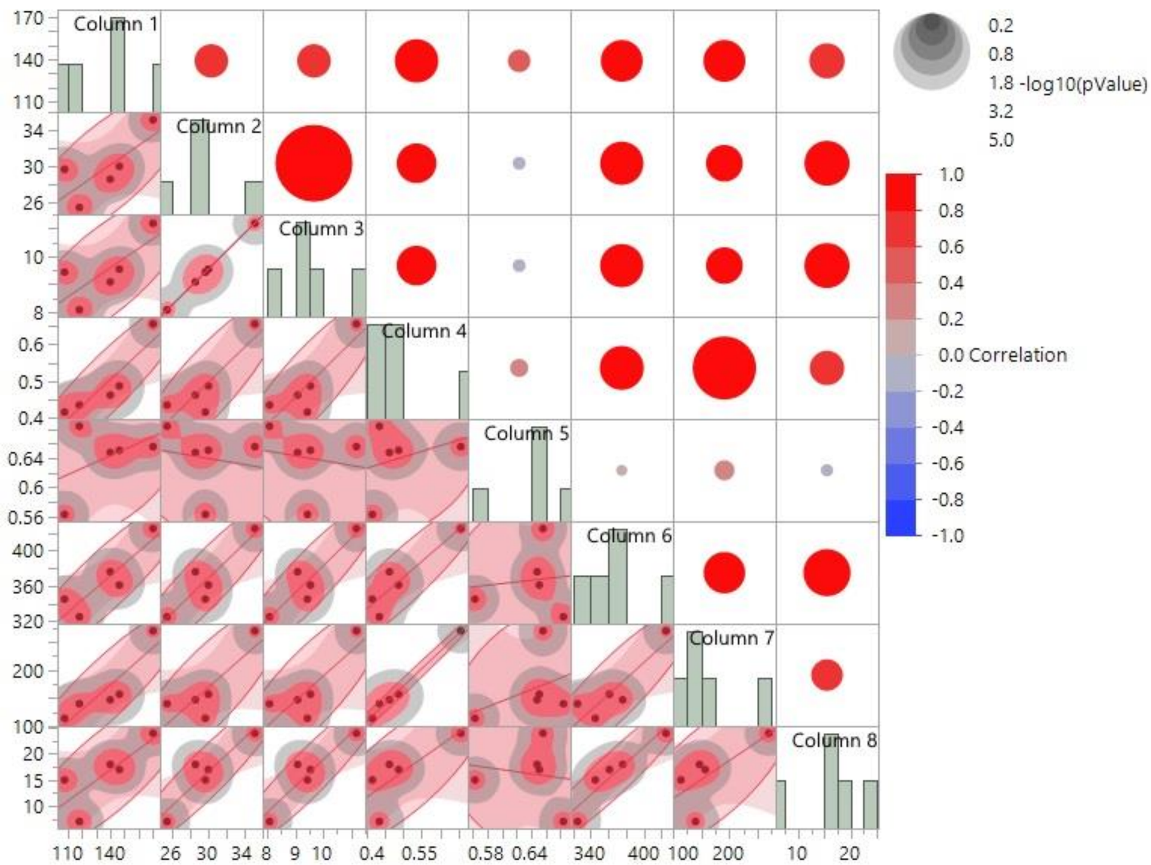

Figure 5. The correlation studies among all the root scanning parameters whereas, the column 1= Length (cm), column 2= surface area (cm2), column 3= projected area (cm), column 4=volume (cm2), column 5= average diameter (mm), column 6= number of tips, column 7= number of forks, column 8= number of cross.

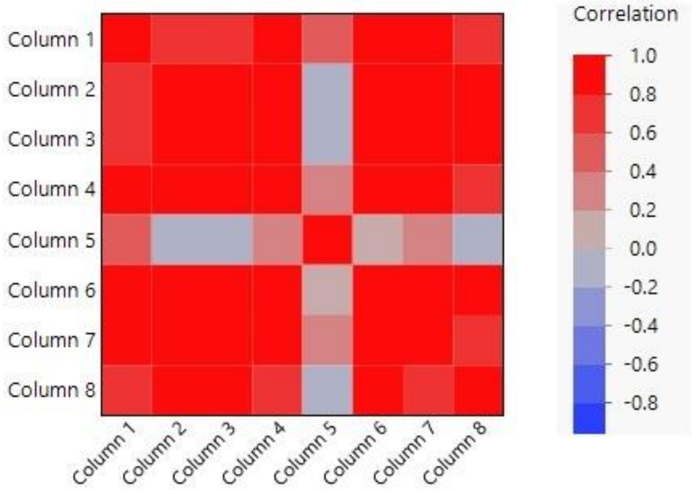

Figure 6. The heat map among all the root scanning parameters whereas, the column 1= Length (cm), column 2= surface area (cm2), column 3= projected area (cm), column 4=volume (cm2), column 5= average diameter (mm), column 6= number of tips, column 7= number of forks, column 8= number of cross.

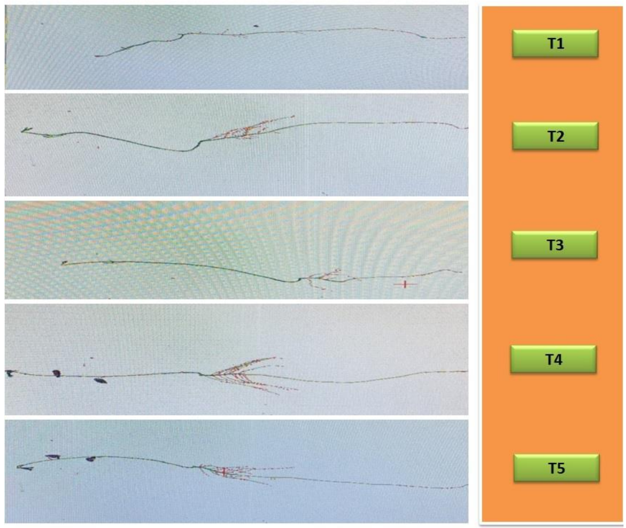

Figure 7. The scanned images of all the treatments whereas, T1= control, T2= SA @ 3mM for 18 hr, T3= HA@ 600ppm for 18 hr, T4= Combination of HA+SA (100pp+1mM for 16 hr) and T5= Hydropriming @ 18 hr.

Discussion

Seeds of pulses swiftly succumb to a decline in vigor and viability when stored under ambient conditions, especially in tropical climes where humidity reigns. For instance, lentil seeds, freshly harvested with an initial germination rate of 75%, may dwindle to a mere 50-60% within a year's storage at ambient temperatures, accompanied by a marked deterioration in seed quality measures, namely seed vigor indices I and II. Beyond the mere loss of viability, seed vigor diminishes significantly, leading to lethargic germination, stunted seedling growth, and reduced dry weight in seedlings. This degradation in seed quality wreaks havoc on field establishment and is exacerbated under conditions of water stress. To counteract these deleterious effects, seed priming emerges as a proposed panacea, mitigating the adversities of seed aging on both germination and seedling development. Priming treatments are believed to awaken enzymes, stimulate protein synthesis, mend cellular membranes, and bolster antioxidant defenses (Mohamed et al., 2018; Karim et al., 2020).

The present study that priming with humic acid (HA) and silicic acid (SA) profoundly enhanced crucial physiological traits, such as germination percentage, seed vigor indices, and root scanning parameters, thereby invigorating both germination and growth in moderately aged seeds. Notably, hydro priming failed to elicit a comparable boost in physiological traits, underscoring the critical role of reactivating antioxidant defenses for the rejuvenation of aged lentil seeds. The results from HA and SA priming underscore the pivotal influence of priming duration in modulating germination and vigor attributes by adjusting seed moisture content to allow only the preliminary resumption of metabolic activities vital for seed repair. Yet, excessively prolonged priming may inflict seed damage due to the advancement of germination beyond the repair phase, potentially impairing seeds during subsequent drying (Aghamir et al., 2016). These findings highlight the necessity of optimizing the priming duration to maximize the efficacy of seed priming techniques in aged seeds.

Research into lentil responses to salt stress reveals that seed priming with silicic and humic acids enhances seed quality parameters and invigorates seedlings (Ruan et al., 2002). This study delved into the impact of such priming on germination percentage (GP), seed vigor index-I (SVI-I), seed vigor index-II (SVI-II), and root scanning across various lentil varieties. The findings demonstrate that seed priming significantly improves these parameters for all lentil types under salinity stress, while also mitigating antioxidant damage induced by the stress. Seed priming is renowned for mending membrane damage caused by seed storage or abiotic stress (Asgedom and Becker, 2001). Prior studies indicate that seed priming induces biochemical transformations, such as activating enzymes linked to cellular metabolism, halting inhibition of metabolism, ending dormancy, and ensuring water uptake, thereby facilitating germination (Ajouri et al., 2004; Bahrani and Pourreza, 2012).

Seed priming, a preparatory technique before sowing, involves immersing seeds in various substances to enhance germination and the initial growth of seedlings. Among these substances, silicic acid and humic acid, along with their amalgamations, have become esteemed as priming agents celebrated for their beneficial impact on plant growth and development (Ghosh et al., 2024). Soluble silicic acid, a mineral element integral to plant growth, has consistently demonstrated its ability to increase seed germination, promote plant development, and fortify resilience against biotic and abiotic stressors. Illustrative studies indicate that priming with silicic acid enhanced seedling growth and germination rates in wheat and lentil plants under saline conditions (Chourasiya et al., 2021; Rao et al., 2023). Similarly, silicic acid priming has been reported to improve early growth and salt stress resilience in pea, wheat, and rice seedlings (Dhiman et al., 2021).

Humic acid, an intricate organic substance born from the natural decay of plant and animal remnants, serves extensively as a transformative soil enhancer and fertilizer. Among its manifold benefits are the enrichment of soil structure, the augmentation of nutrient accessibility, and the fostering of plant growth. Notably, the application of humic acid through seed priming has shown to elevate germination rates and bolster seedling development in maize plants enduring drought conditions (Hussain et al., 2023). Likewise, reports indicate that humic acid priming not only augments seed germination but also supports early growth in lentil seedlings (Poomani et al., 2023). The synergy of humic acid and silicic acid as seed priming agents has proven to advance germination rates, amplify seedling development, and enhance nutrient absorption in wheat cultivated under salty environments (Rao et al., 2024). Additionally, it was revealed that the combined use of humic and silicic acids significantly promotes seed germination and early development in cucumber seedlings (Richmond and Sussman 2003).

The present study unveiled significant variations in seedling characteristics across different priming practices. Among the diverse applications and control plants, hydropriming and priming with 3 mM silicic acid and 600 ppm humic acid emerged as the most effective. Chemical priming accelerated germination by promoting rapid water absorption by the seeds. These priming practices initiate germination by activating the seed's biochemical machinery, fostering enzyme production, cell wall expansion, and breaking dormancy. Silicic acid, a vital bioactive element, plays an essential role in enhancing leaf morphology, root penetration, stress tolerance, plant growth, resistance to pathogens, and nutrient uptake. It also creates silicic acid deposits in the roots, diminishing apo plastic flow and absorption of toxic minerals, thereby reducing water loss through transpiration. The promotion of lateral root formation by priming applications is a significant outcome for seedling development. Lateral roots form critical components of the plant's comprehensive root system, inclusive of all underground organs, and play a vital role in water and nutrient absorption.

Conclusion

In essence, the practice of priming with silicic and humic acids is paramount in elevating seed quality characteristics. Research reveals that these priming techniques bolster germination rates, nurture seedling growth, and enhance root development. Moreover, the application of silicic and humic acids, whether alone or in tandem, amplifies antioxidant enzyme activity, thereby mitigating oxidative harm and lipid peroxidation. Their combined use reveals a synergistic effect, significantly improving seed and seedling resilience under saline stress conditions. However, further exploration, especially at the molecular level, is essential to unravel the biochemical dynamics at varying degrees of salinity stress. Long-term field trials are also advocated to evaluate the sustainability and practical efficacy of these priming methods in agriculture. Ultimately, these techniques emerge as promising and sustainable strategies for advancing seed quality and plant vigor in saline environments. Continued research into their deployment across diverse crops and ecological settings will be crucial in refining their agricultural applications.

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A comparative study of fresh and residual biochar effects on wheat growth and yield metrics

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Abstract

Biochar is a highly stable carbon compound produced through pyrolysis, and it has been widely studied for its potential to enhance soil fertility and carbon sequestration. However, the impact of fresh and residual biochar is not thoroughly explored. Therefore, a comparative study on fresh and residual biochar were conducted at filed conditions on wheat cultivation, using a randomized block design. A fresh biochar (S1), residual biochar of previous season crop (S2) and two season old residual biochar (S3) with nine different treatments using varied amounts of rice husk and rice straw biochar along with the fertilizers (recommended doses of N, P, K) were considered in triplicate. Result clearly indicates that biochar application significantly improved plant height, leaf area, fresh and dry biomass of plant, internodal length, node & internode diameter, as well as biological yield, grain and straw yield of wheat crop. S1 had the most significant impact on plant growth and yield-attributing characteristics compared to S2 and S3, even at higher doses. In S1, the most significant results were observed at a biochar application rate of 5 tons/ha, while S2 showed maximum impact at 10 tons/ha. In S3, the highest impact was recorded at the highest biochar dose of 15 tons/ha. The present findings conclusively showed the efficiency of fresh biochar to enhance soil fertility for agricultural production as well as the residual impact of biochar in succeeding crop.

Keywords: Biochar application, Soil amendment, Residual effects, Wheat productivity.

Introduction

Human activities and agricultural residue burning continue to raise atmospheric CO_2 concentrations, this leads to global warming, which poses considerable risks to human health, food security, and biodiversity (Muluneh, 2021). To diminish environmental impacts and enhance the sustainable practices, it is essential to implement climate change mitigation strategies that focus on carbon sequestration and decline in GHG emission (Rao et al., 2024). The most critical soil concerns for agronomic systems are the decrease in soil fertility and production caused by organic matter loss (Fawzy et al., 2020). It is necessary to manage soil by incorporating organic matter to maintain its fertility (Singh et al., 2024). Soil quality is critical for enhanced agricultural output, and testing during drought circumstances might indicate areas of concern for long-term production and soil fertility (Tripathy et al., 2023).

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Soil productivity refers to the optimal setting for plant development, this encompasses an adequate supply of nutrients and a suitable growth medium. Soil quality is also linked to various factors and can be assessed through a range of indicators, such as chemical, biological, and physical properties. Enhancing soil quality can lead to increased crop yields (Nunes et al., 2022). Cation exchange capacity is often utilized to analyze soil texture, while the biochemical properties of the soil also play a crucial role in determining its texture and structure (Zheng et al., 2018). Under this situation, the application of biochar can be a beneficial for long-term to increase soil carbon sequestration, soil fertility, crop productivity and reducing GHG emission (Vijay et al., 2021; Sushkova et al., 2021).

European Biochar Certificate describes biochar as "a charcoal-like material produced from sustainable feedstocks through pyrolysis under controlled conditions, intended for uses other than rapid mineralization to CO_2." Biochar enriches soil by supplying essential nutrients and improving water retention, while its porous structure promotes beneficial microorganisms, enhancing nutrient availability (Brar et al., 2024; Faizan et al., 2024). Biochar also alters the physical and chemical properties in the soil including changed pH, redox, CEC and surface reactivity/sorption (Ismail and Man, 2024). Physical and chemical alterations to the soil environment cause changed biological activity including nutrient destiny and functioning of the microbial population (Birol and Günal, 2024; Devendrapandi et al. 2024).

Currently, the impact of adding biochar on crop productivity are equivocal. These effects can depend on the type and properties of the biochar, as well as experimental conditions such as soil type, crop species, application timing, and environmental factors. Therefore, it is essential to further investigate the influence of biochar on crop yields in specific site conditions (Abhishek et al., 2022; Long and Dung, 2023). It necessary to explore how the addition of biochar affects critical factors that influence crop growth and on availability represents. Wheat (*Triticum aestivum* L.) is considered in current research as it is one of the most cultivated crops globally, yields over 650 million tonnes annually, ranks as the third most produced cereal and known for its excellent nutritional qualities. The goal of this study was to investigate the influence of fresh and residual biochar on durum wheat development and yield matrices.

Material and Methods

Experimental site and treatment details

A two-year study was conducted on the PBW 824 variety of wheat at the agricultural research farm of Lovely Professional University in Jalandhar, Punjab, India from 2022 to 2024. The coordinates for the location are latitude 31°14'30.5''N and longitude 75°41'52.1" E. Soil properties were analysed before sowing as shown in Table 1. A total of 9 treatments were executed using a Randomised Block Design (RBD), with three replications, i.e., T1-Absolute control (without fertilizers), T2- 100% Recommended Doses of Fertilizers (RDF) (N:P:K 120:60:60 kg/ha), T3- 100% of recommended doses of N and P without K, T4- T3+ rice husk biochar @5 tons/ha, T5- T3+ rice husk biochar @10 tons/ha, T6- T3+ rice husk biochar @15 tons/ha, T7- T3+ rice straw biochar @5 tons/ha, T8- T3+ rice straw biochar @10 tons/ha and T9- T3+ rice straw biochar @15 tons/ha. The plot size was 5m×5m (25 m^2) with a row to row spacing of 22.5 cm. The drone layout of the research trial is shown in Figure 1. Total four irrigation were applied during Crown root initiation (CRI), tillering stage, flowering stage and milking stage and no additional fertilizers were added except above combination. The first wheat trial was carried out during the Rabi season in 2022, followed by a pigeon pea crop in the Kharif season 2023.To evaluate the residual impacts of biochar, a new plot was built parallel to the previous one, where fresh biochar was applied before seeding pigeon pea in two distinct plots: one with fresh biochar and the other with biochar residual effect. The following wheat trial conducted in Rabi season 2023, using a similar strategy with a new plot constructed alongside the previous two, and fresh biochar was added to the new plot. Wheat was seeded in three plots to conduct a comparative investigation of the impact of biochar on crop performance. The weather data for the experimental site is shown in Figure 2.

Table 1. Different properties of soil, rice husk and straw biochar before application.

Properties	Soil	Rice Husk Biochar	Rice Straw Biochar
pH	7.4	10.4	10.2
EC (dSm^{-1})	0.17	0.16	0.13
Carbon	0.49 %	71.2 %	64.6%
Nitrogen	176 kg/ha	0.9%	1.1%
Phosphorus	8.95 mg/kg	4.7%	5.1%
Potassium	45 mg/kg	1.7 %	2.1%

Figure 1. Drone image of the experimental area

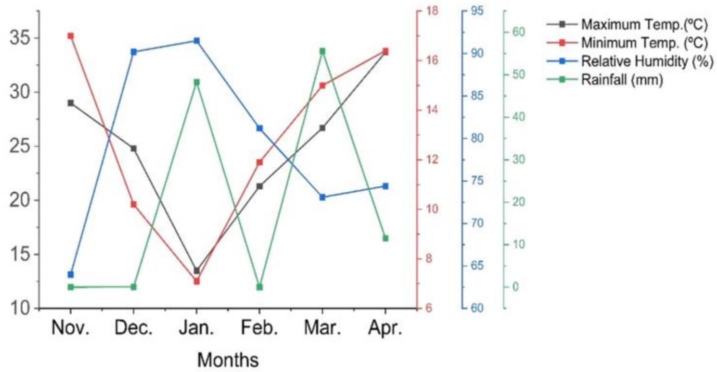

Figure 2. Presents the weather, and monthly average data of temperature, relative humidity and rainfall, collected

Biochar preparation and analysis

Biochar was fabricated by thoroughly dried rice straw and husk,. The rice straw and husk were colonized over an open fire in a stainless-steel container measuring 48 cm tall and 142 cm in circumference to prepare biochar. The open flame is an auto-thermal procedure that burns a portion of the feedstock to heat the remaining material and produce char. The feedstock was placed inside the open-burn tank and fired. Carbonisation of feedstocks happened below the flames, where oxygen is non-existent, as the flames devour all of it, producing a pyrolysis zone. Due to a lack of oxygen, biomass smoulders but does not produce fumes or smoke. Instead, much of it is converted into carbon-rich charcoal, oil, and gas. Rice straw was pyrolysed at 400-600 ºC and recorded using a heat sensor thermometer. Feedstocks were constantly added until the tub until it was not full, after which it was quenched with water. On a dry weight basis, the biochar output ranged between 45 and 50%. Biochar was air-dried and put to the field. The properties of produced biochar are shown in Table 1.

Plant assessment

In this study, major agronomic characteristics of wheat across experimental plots were examined. Plant height was measured from the basal node to the apical meristem of 10 randomly selected plants with a calibrated measuring tape to ensure precision in vertical growth measurement. Wheat plants were harvested at a standardised length of one meter and leaf area was measured in square centimetres using a digital leaf area meter, providing exact foliar surface area measurements for evaluating photosynthetic efficiency. A computerised Vernier calliper was used to measure node and internode diameters, internode distances, and other morphological parameters.

Chlorophyll was extracted from a 100 mg sample using 20 ml of 80% acetone. After centrifugation for 10 minutes at 5000 rpm, the supernatant was transferred to a volumetric flask, and the extraction was repeated until the residue became colourless. The extract's absorbance was measured at 645 and 663 nm wavelength using a spectrophotometer, and the chlorophyll content was calculated using a formula (Arnon, 1949).

The plants of wheat were harvested at a standardised length of one metre, and fresh weight was measured with an analytical balance. The samples were then dried in an oven at 55 °C until they reached a constant weight, showing that all moisture had been removed. The dry biomass was determined by weighing the dried samples using an analytical balance. The biological yield was calculated by harvesting a one-meter piece of evenly matured wheat and weighing the whole biomass. The grain yield was then determined by drying the collected biomass and physically threshing it to separate the grains, resulting in precise estimation of the economic output. Finally, straw yield was estimated by subtracting grain yield from biological yield.

Statiscal analysis

The data in this study was analysed using R Studio software (version 4.2.2) using ANOVA at a significance level of $p<0.05$ indicating significant differences between group means. Following the ANOVA, Duncan's Multiple Range Test (DMRT) was used in post-hoc analysis to discover particular group differences. Additionally, Origin Pro software (Origin 2024b) was also used to construct graphical visualisations of the data, allowing for a clear and effective assessment of the results. This thorough strategy ensured a strong statistical analysis and improved the interpretability of our results.

Results and Discussion

A significant ($p<0.05$) improvement in plant height was observed by the application of biochar (Figure 3). In S1, as relative to control (T2), a significant increase in plant height was recorded in T4 (15.8%). In S2, this increase was recorded maximum in T5 (5.3%) while in S3, the maximum increase was recorded in T6 (1.9%). The results clearly indicates that fresh biochar, even at lower doses (5 tons/ha), had a significant impact on plant height compared to the long term residual impact of higher doses of biochar i.e. 10 tons/ha and 15 tons/ha. Fresh biochar can provide immediate nutrient availability due to its inherent nutrient content and the ability to retain moisture and nutrients in the soil. In contrast, residual biochar may not offer the same level of nutrient availability as it ages and loses its initial nutrient content through leaching or microbial consumption that's why residual biochar is less effective (Cong et al., 2023; Premalatha et al., 2023; Muema et al., 2024).

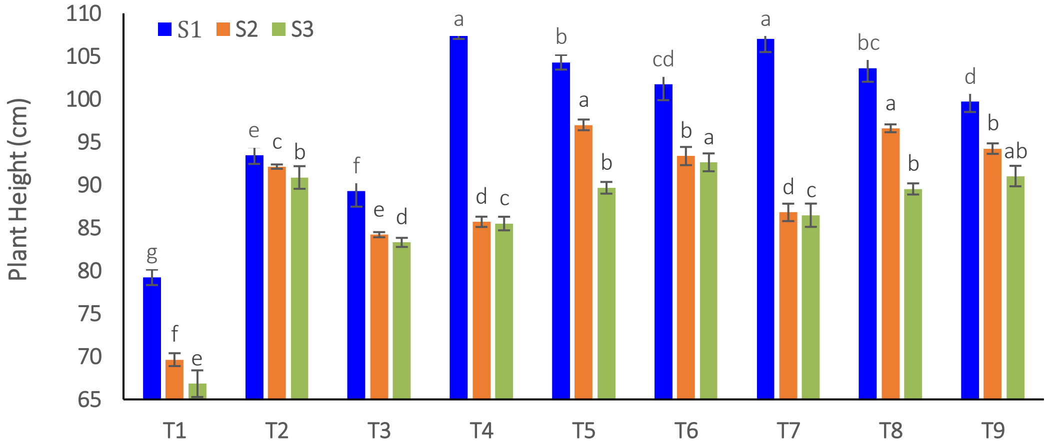

Figure 3. Effect of biochar application on plant height. S1 (Fresh biochar), S2 (Previous season residual biochar) and S3 (two seasons old residual biochar). Different lower cases indicate significant differences between treatments ($P < 0.05$).

The internodal distance of plant was also influenced by biochar application (Figure 4). The improvement in internodal distance persisted throughout the plant vegetative phase. In S1, compared to the T2, a significant improvement of 36.5% was recorded in T7. In S2, this improvement was recorded maximum in T6 (upto 13.0%) while in S3, the maximum improvement was recorded in T9 (2.6%). Fresh and residual biochar also altered the diameter of node and internode of the plant (Figure 5 and 6). In S1, compared to the T2, a significant increase of node diameter was recorded in T7 i.e. 34.7%, 21 % and 16.4 % at 60, 90 and 120 DAS respectively while in S2, it was recorded maximum in T9 i.e. 14.1%, 7.9% and 9.1% at 60, 90 and 120 DAS respectively. In S3, the maximum increase in node diameter was recorded in T6 (9%, 3.8% and 7.6% at 60, 90 and 120 DAS respectively). Similar trend was observed for internode diameter of plants as well. In S1, the maximum increase of internode diameter was recorded in T7 (42.4%, 27.8% and 19.5% at 60, 90 and 120 DAS respectively). In S2, the maximum increase in internodal diameter was recorded in T9 (16.5%, 12.2% and 6.2% at 60, 90 and 120 DAS respectively) while in S3, treatment T9 (14.4 % at 60 DAS) showed a significant increase at the initial phase but in the later phase, T6 (9.1%, 3.2% at 90 and 120 DAS respectively) had the maximum impact. Overall, the impact of fresh biochar application was more prominent than that of residual biochar on internodal distance, node and internode diameter of the plant. The inclusion of biochar significantly improves the xylem and phloem areas of the main vascular bundle, as well as stem thickness and wall density which allows the co-deposition of silica, hemicellulose, and lignin in the cell walls, that contributes to improved lodging resistance and crop yield (Meng et al. 2021; Miao et al. 2023).

The fresh and residual characteristics of biochar had a considerable influence on plant leaf area (Figure 7). In S1, as relative to T2, the maximum improvement in leaf area was recorded in T4 (40.7%). In S2, the maximum increase was recorded in T9 (16%) while in S3, the maximum increases of 16% was recorded in T9. Improved stomatal conductance and transpiration rates associated with biochar application further support increased leaf area by optimizing photosynthesis and nutrient transport within the plant. Furthermore, biochar reduce oxidative stress in plants by enhancing antioxidant enzyme activities, which help maintain cellular health and promote growth under stress conditions (Blanco-Canqui, 2017). Biochar also enhances root system expansion and plant nutrient uptake, promoting leaf area and production of the crop. However, ageing of biochar may impact on its physical and chemical properties, reducing its ability to retain and release nutrients effectively (Muema et al., 2024).

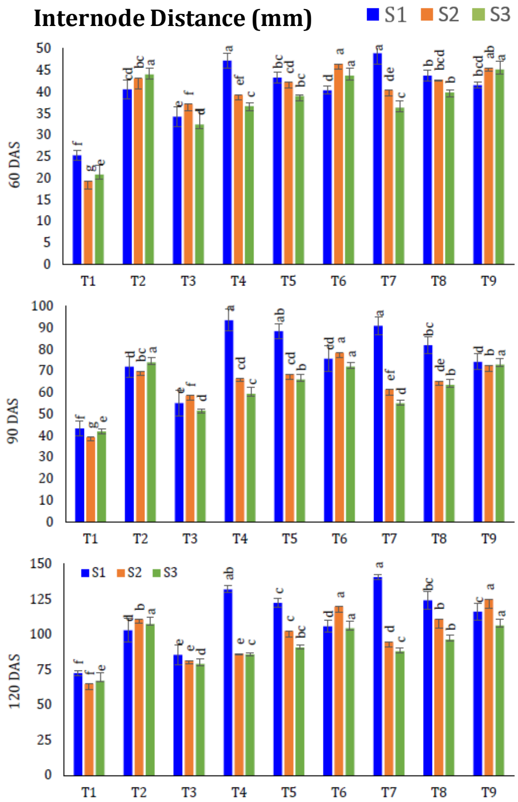

Figure 4. The impact of biochar application on internode distance at 60, 90 and 120 DAS. S1 (Fresh biochar), S2 (Previous season residual biochar) and S3 (two seasons old residual biochar). Different lower cases indicate significant differences between treatments (P < 0.05).

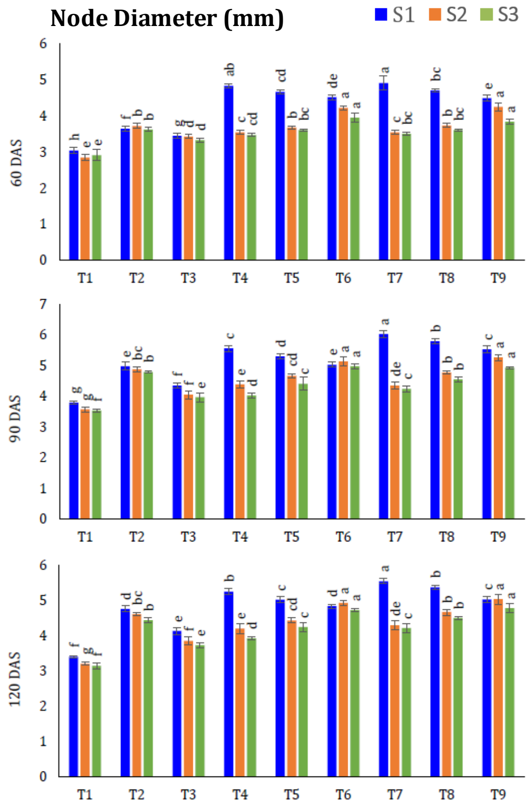

Figure 5. The impact of biochar application on node diameter at 30, 60 and 90 DAS. S1 (Fresh biochar), S2 (Previous season residual biochar) and S3 (two seasons old residual biochar). Different lower cases indicate significant differences between treatments (P < 0.05).

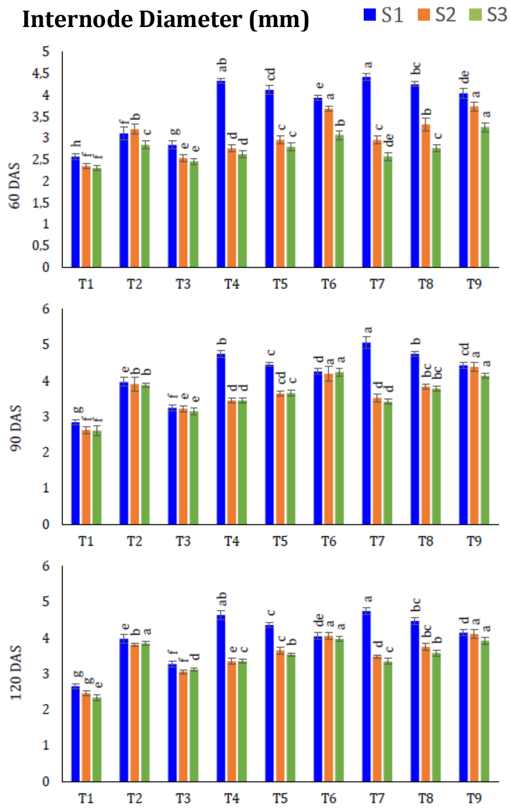

Figure 6. The impact of biochar application on internode diameter at 30, 60 and 90 DAS. S1 (Fresh biochar), S2 (Previous season residual biochar) and S3 (two seasons old residual biochar). Different lower cases indicate significant differences between treatments (P < 0.05).

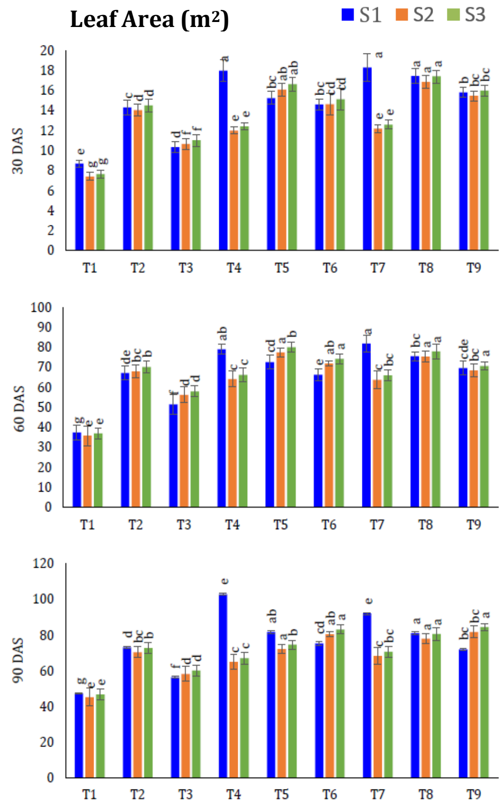

Figure 7. The impact of biochar application on leaf area (m^2) at 30, 60 and 90 DAS. S1 (Fresh biochar), S2 (Previous season residual biochar) and S3 (two seasons old residual biochar). Different lower cases indicate significant differences between treatments (P < 0.05).

A significant improvement in plant fresh and dry biomass was recorded with the inclusion of biochar (Figure 8). In S1, compared to the T2, a significant increase of fresh biomass was recorded in T7 (54% at 30 DAS) and T4 (30.3%, 17.5 % at 60 and 90 DAS respectively). In S2, during the initial growth stage (30 DAS), the maximum increase was recorded in T9 (34.7 %), while in the later growth stages it was recorded maximum in T6 (33.9%, 19.5% at 60 and 90 DAS respectively). Similarly, in S3, the maximum increase was recorded in T6 (14.7%) during the initial phase (30 DAS). In the mid-growth stage (60 DAS), the maximum increase was observed in T9 (6.3%), while in the later phase (90 DAS), the maximum increase was recorded again in T6 (12.2%). A similar trend was also observed for dry biomass (Figure 9). In S1, as compared to T2, the maximum increase was recorded in T7 (59.1%) at the initial phase (30 DAS). In the mid-growth stage (60 DAS) the maximum increase was observed in T4 (21.2%), while in the later phase (90 DAS) T4 & T7 both have same impact i.e. 21% increase. In S2, the maximum increase was recorded in T5 and T8 (30% at 30 DAS) and 15.7% during the mid-growth stage (60 DAS). In the later phase (90 DAS), the maximum increase was again recorded in T8 (13.1%). In S3, the maximum dry biomass increase was recorded in T6 (43.8%, 29.3% and 19.9% at 30, 60 and 90 DAS respectively). Biochar inclusion increases biomass accumulation due to its high potassium content, which acts as a catalyst, speeding up the process and accumulation of more plant biomass (Guo et al., 2019; Wan et al., 2024; Fachini et al., 2024). Fresh biochar improves soil structure, porosity, and water retention immediately after application, the long-term benefits may not persist as effectively with residual biochar. Soil properties can change over time due to various factors such as compaction or organic matter decomposition, which may limit the residual biochar's ability to enhance crop growth (Alkharabsheh et al., 2021)

Both fresh biochar and its residual effect had a significant effect on chlorophyll content. Analysis of variance ($p<0.05$) revealed that maximum chlorophyll content denotes during the anthesis stage as compared to grain filling stages. In S1, as relative to T2, the maximum increase of chlorophyll a content was recorded in T7 (23.9%). In S2, it was recorded maximum for T9 (11.9%) while in S3, there were no significant impact on chlorophyll a content (Figure 10). A similar trend was observed for chlorophyll b. In S1, the maximum increase was recorded in T7 (61.7%). In S2, the maximum increase was recorded in T7 (61.9%) while in S3, it was recorded maximum for T6 (4.1%) (Figure 11). Compared to the control, the total chlorophyll content in S1 showed maximum increases of 36.6% for T7. In S2, the maximum increase was recorded in T9 (8.4%) while in S3 there were no significant difference recorded among the treatments (Figure 12). The chlorophyll content is the main aspect that defines plant health and growth. Biochar significantly impacts on physiological attributes of the plant including chlorophyll content (Laird et al., 2010, Agegnehu et al., 2015, Trupiano et al., 2017; Farouk et al., 2023; Murtaza et al., 2024). The use of biochar boosts plant photosynthesis, the quantity of chlorophyll, and transpiration rate. Biochar can help mitigate the effects of environmental stresses, such as drought and salinity, which can negatively impact chlorophyll levels. By improving water use efficiency and reducing oxidative stress in plants, biochar application helps maintain higher chlorophyll content even under stressful conditions. The previous research findings reveals that fresh biochar application significantly increases the chlorophyll content compared to residual biochar application. This enhancement is due to improved nutrient availability, better soil properties, and mitigation of environmental stressors (Khan et al., 2021; Murtaza et al., 2024).

There was a significant impact of biochar application on plant yield attribute viz. biological yield, straw yield and grain yield (Figure 13). When comparing S1, S2 and S3, it was observed that S1 had the greatest impact on yield attributing characters followed by S2 and then S3. As compared to T2, in S1, the maximum increase in biological yield was recorded in T7 (19%). In S2, it was recorded maximum in T8 (9.7%) while in S3, treatment T6 (4.8%) had the most significant impact on improved biological yield. The improved biological yield also influenced the grain yields. S1 showed the highest gain yield followed by S2 and S3. In S1, compared to the T2, the grain yield showed a maximum increases of 30% in T7. In S2, the maximum increase in grain yield was recorded in T8 (15.6%), while in S3, the maximum increase was recorded in T6 (9.4%). A similar trend was observed for straw yield. In S1, the maximum increase was recorded in T7 (11.4%) followed by T4 (10.4%). In S2, it was recorded maximum for T8 (4.9%) followed by T6 (5.4%) while in S3, the maximum increase for straw yield was recorded in T6 and T9 (2.1%). The significant impact of biochar on crop yield attributes is primarily due to its ability to enhance soil fertility by improving nutrient retention and availability, particularly for essential nutrients like nitrogen, phosphorus, and potassium. These nutrients support better spike development and grain filling (Alkharabsheh et al., 2021; Khan et al., 2021). Collectively, these benefits contribute to higher grain yield attributes of crop. Several studies suggested that biochar improves plant growth, increases shoot dry matter, and boosts the yield of various crops (Major et al., 2010; Khan et al., 2022; Wan et al., 2023).

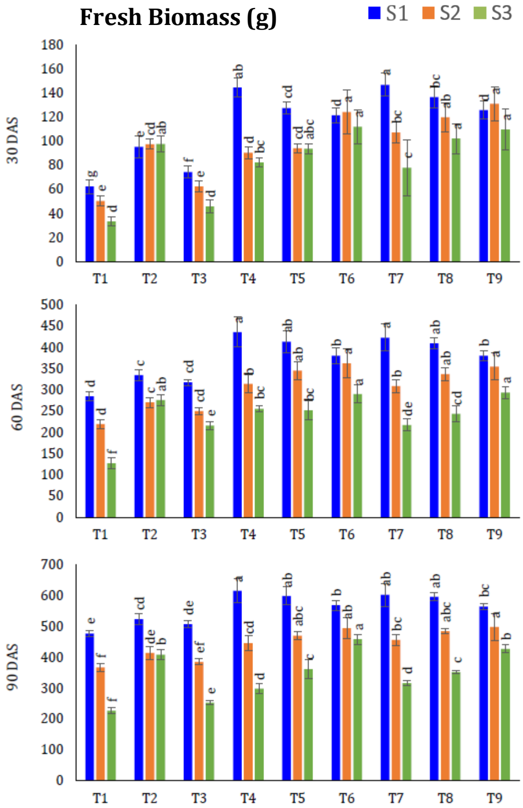

Figure 8. The impact of biochar application on fresh biomass at 30, 60 and 90 DAS, S1 (Fresh biochar), S2 (Previous season residual biochar) and S3 (two seasons old residual biochar). Different lower cases indicate significant differences between treatments (P < 0.05).

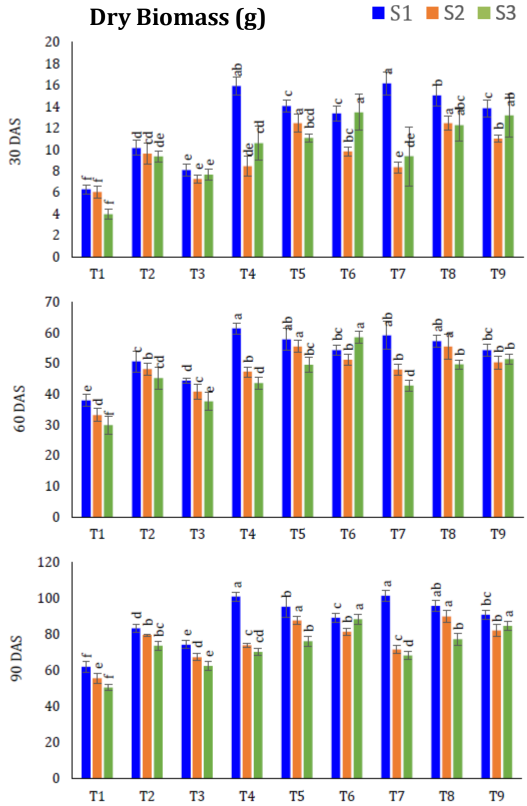

Figure 9. The impact of biochar application on dry biomass at 30, 60 and 90 DAS, S1 (Fresh biochar), S2 (Previous season residual biochar) and S3 (two seasons old residual biochar). Different lower cases indicate significant differences between treatments (P < 0.05).

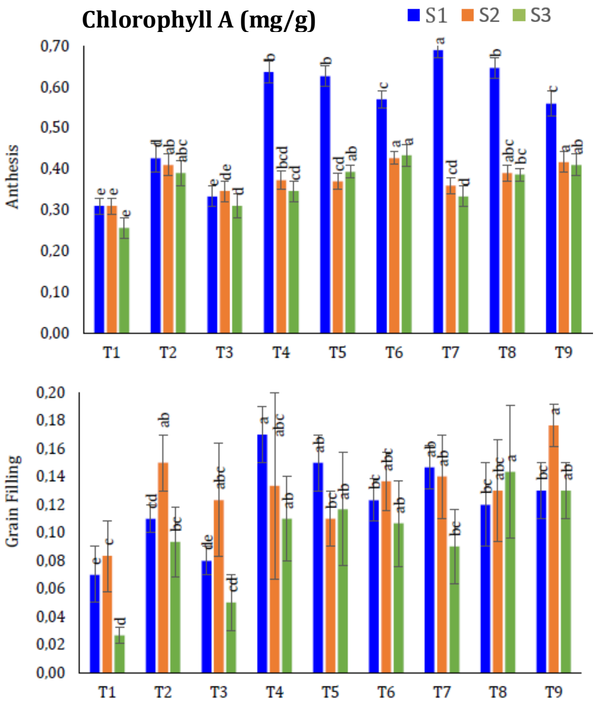

Figure 10. Effect of biochar application on total chlorophyll content (mg/g) at anthesis and grain filling stage. S1 (Fresh biochar), S2 (Previous season residual biochar) and S3 (two seasons old residual biochar). Different lower cases indicate significant differences between treatments (P < 0.05).

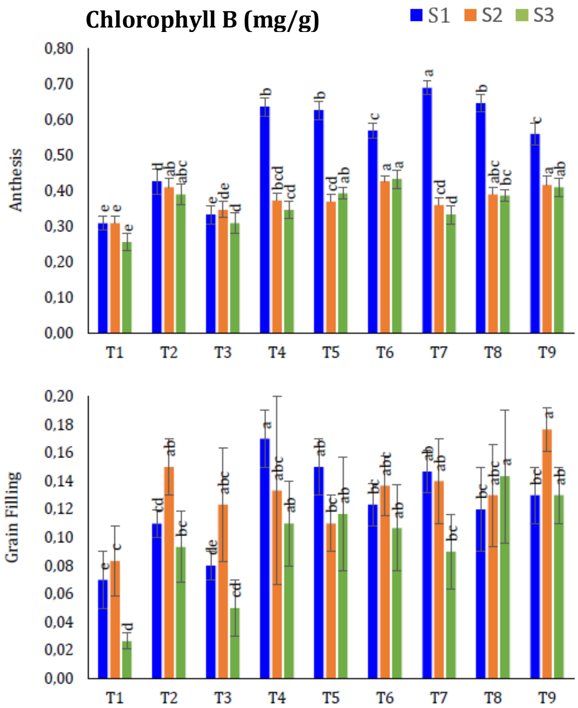

Figure 11. Effect of biochar application on total chlorophyll content (mg/g) at anthesis and grain filling stage. S1 (Fresh biochar), S2 (Previous season residual biochar) and S3 (two seasons old residual biochar). Different lower cases indicate significant differences between treatments (P < 0.05).

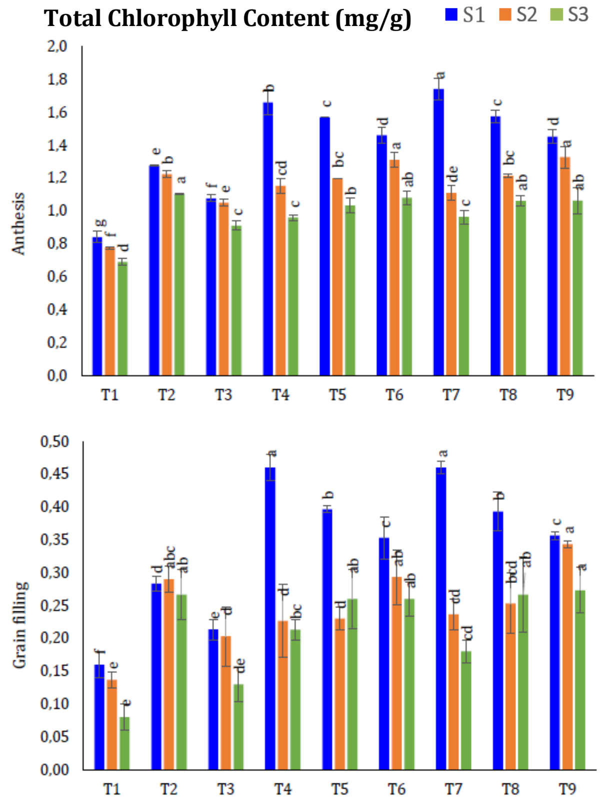

Figure 12. Effect of biochar application on total chlorophyll content (mg/g) at anthesis and grain filling stage. S1 (Fresh biochar), S2 (Previous season residual biochar) and S3 (two seasons old residual biochar). Different lower cases indicate significant differences between treatments (P < 0.05).

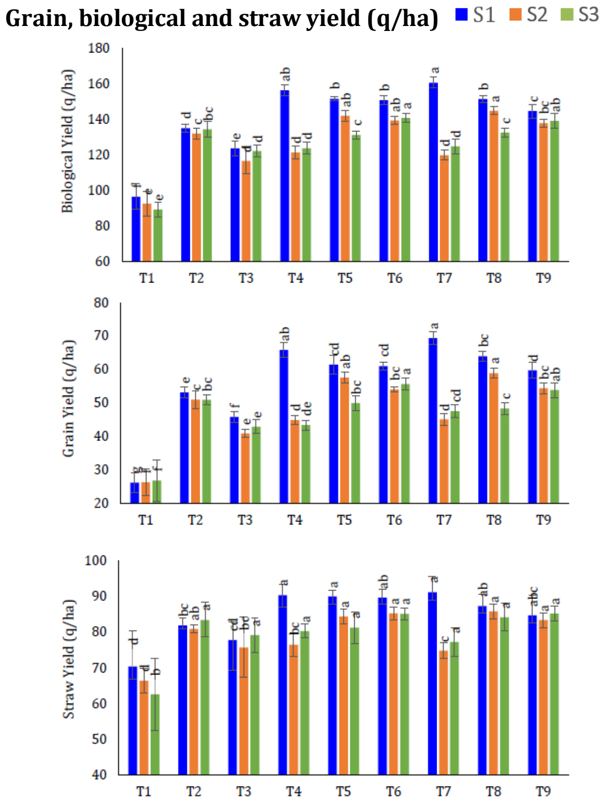

Figure 13. Effect of biochar application on grain yield, biological yield and straw yield (q/ha). S1 (Fresh biochar), S2 (Previous season residual biochar) and S3 (two seasons old residual biochar). Different lower cases indicate significant differences between treatments (P < 0.05).

Conclusion

Biochar is rich in nutrients and facilitates the transport of essential nutrients that improves growth and yield attributing characters of the wheat crop. In the present study, the most significant results were observed with a fresh biochar application at a dose of 5 tons/ha. Meanwhile, a 10 tons/ha biochar application significantly enhanced growth in one-season-old biochar, whereas the optimum result for two-season-old biochar was achieved at a dose of 15 tons/ha. When comparing fresh biochar application with residual biochar, fresh biochar had the greatest impact on plant growth and yield attributes. These findings highlight that the fresh application of biochar at an optimal dose has the greatest potential to improve plant health and productivity of wheat.

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Seasonal effects on growth and reproduction of *Eisenia fetida* and *Eudrilus eugeniae*

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Abstract

An experiment was conducted during the winter and summer seasons at Sylhet Agricultural University (SAU), Sylhet, Bangladesh, to examine the seasonal variations in the life cycle, growth, and reproduction of two epigeic earthworm species, *Eisenia fetida* and *Eudrilus eugeniae*. Earthworm species were reared in plastic containers filled with cow dung as the feeding medium, maintaining a moisture level of 60%–80%. Growth and reproductive characteristics were recorded at various stages. The results indicated that *Eisenia fetida* exhibited a longer incubation period (24±4.69 and 23.0±4.16 days), a higher number of hatchlings per cocoon (2.4±1.19 and 2.7±0.96), and greater hatching success rates (82.5% and 87.5%) during both winter and summer seasons, respectively. In contrast, *Eudrilus eugeniae* attained the greatest body length (12.98±0.69 cm and 13.09±0.54 cm per worm) and the highest weight (775.67±66.40 mg and 703.5±55.56 mg per worm) in winter and summer, respectively. Both species reached sexual maturity relatively earlier in winter. Additionally, *E. fetida* produced a higher number of cocoons per worm per week (2.35 ± 0.30 in winter and 3.00 ± 1.35 in summer). Cocoon production per worm per week in *E. fetida* showed a significant positive correlation with temperature (r=0.61**) during winter.

Keywords: Seasonal effects, life cycle, growth, reproduction, *Eisenia fetida, Eudrilus eugeniae.*

Introduction

Earthworms are important soil organisms, usually found in different ecosystems (tropical and temperate regions) around the world (Byambas et al., 2019; Kahneh et al., 2022). They have a significant role in changing the structure of the soil, influencing soil properties, hastening organic matter degradation, promoting soil ecosystems, and adding value to nutrient cycling in soil-plant systems (Hättenschwiler and Gasser, 2005; Baker, 2007; Blouin et al. 2013; Radaei and Izadi, 2016; Lavelle et al. 2016; Yahyaabadi et al., 2018). There are more than 8300 species of earthworms within the class Oligochaeta (Reynolds and Wetzel, 2004) and among them, more than 4,000 species have been described and many are still unknown (Fragoso, 2001). Earthworms are categorized into three ecological types based on their morphological characteristics, habits, and soil location: epigeic, endogeic, and anecic (Bouche, 1977), and grouped into 13 families (Byambas et al. 2019). Epigeic earthworms consume and decompose organic waste materials and live near the soil surface or surface litter. *Eisenia fetida* and *Eudrilus eugineae* are two common epigeic earthworms found in vermiculture and vermicomposting facilities around the world. *E. fetida* is generally referred to as redworm or red wiggler worm, although it is also known as brandling worm, panfish worm, trout worm, tiger worm, and other names. The adult worms have a length of 7-9 cm, a diameter of 3-5 mm, and individual weight of 500-600 mg (Venter and Reinecke, 1988). This worm is captivated by rotting vegetation and is used in both domestic and industrial vermicomposting. *E. eugeniae* is popularly known as the 'African Night Crawler,' is a reddish- brown large worm that grows rapidly and reasonably prolifically at temperatures ranges from 25°C to 30°C (Viljoen and Reinecke, 1992; Segun, 1998). Vermiculture and vermicomposting is

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getting popular day by day at farmers' level in Bangladesh. *Eisenia fetida* and *Eudrilus eugeniae,* these two earthworms species are the most commonly rearing species. Understanding the influence of seasonal variations on the biological parameters of these two epigeic earthworms is crucial for optimizing their commercial culture and ensuring sustainable vermiculture practices. Temperature and moisture fluctuate across seasons, significantly affecting earthworm growth rates, reproductive efficiency, and overall survival. A comprehensive evaluation of these factors can provide valuable insights into species-specific adaptations and resilience, ultimately aiding in the development of strategies to maximize biomass production, cocoon viability and vermiculture business. Moreover, such studies can contribute to refining organic waste decomposition efficiency and soil health improvement through vermicomposting under varying environmental conditions. Few studies have comprehensively evaluated the seasonal impact on the life cycle, growth and reproduction of epigeic earthworms in tropical climates. Said research gaps are needed to be addressed to boost up vermicomposting process. Hence, the current study was undertaken to see the seasonal variations (winter and summer seasons) on life cycle, growth, and reproduction of the two vermicomposting earthworms species (*Eisenia fetida* and *Eudrilus eugeniae*).

Material and Methods

The experiment was conducted over two seasons at the vermicompost production shed and the laboratory of the Department of Soil Science in Sylhet Agricultural University, Sylhet. The experiment was started in the winter season from November 2021 and ended up April 2022. Later it was repeated in summer season, from March 2022 to August 2022.

Collection of earthworms and feeding material

Eisenia fetida and *Eudrilus eugeeniae* were used from the stock in the vermicompost production shed. The earthworm species were reared in plastic containers filled with cow dung as the feeding medium, with moisture levels maintained at 60%–80%. During winter, the ambient temperature in the vermicompost shed varied between 16°C and 25°C, while in summer, it ranged from 17°C to 36°C. The ambient humidity in the vermicompost shed ranged from 40% to 60% during the winter season and from 60% to 80% during the summer. Cowdung was pre-decomposed for 30 days and loosened properly before being used as a nutritive medium for the earthworms.

Treatments of the experiment

The experiment was designated with the following two species of earthworm - T_1: *Eisenia fetida*, T_2: *Eudrilus eugeniae*

Experimental design

The experiment was conducted following a completely randomized design (CRD) with four replications. Initially, petri-dishes were used for keeping cocoons during the determination of the incubation period. Later, plastic containers were also used to rear the hatchlings of both earthworm species. Height of the plastic containers was 20 cm with 28 cm in diameter.

Experimental details

The experiment was carried out in four distinct steps. The first step was intended to determine the duration of cocoon incubation. Initially, ten newly laid cocoons of each earthworm species were collected from the stock. Collected cocoons were placed on petri-dishes (ten cocoons per petri-dish) to clearly observe the hatching process and emergence of hatchlings. The number of hatchlings cocoon^{-1} as well as their length and weight were also recorded.

The hatching success (%) was computed as follows:

$$\text{Hatching success (\%)} = \frac{\text{Number of cocoons hatched}}{\text{Number of cocoons incubated}} \times 100$$

The second step included the measurement of the length and weight of earthworms at seven-day intervals. Firstly, ten new-born hatchlings of each earthworm species were inoculated on each replication containing 100 g of cowdung after their length and weight had been determined individually. After four weeks, when they had reached juvenile status, they were transferred to new plastic bowls containing cowdung based feed medium (1kg). Thereafter, length and weight of both earthworm species were recorded at seven-day intervals till the end of the experiment. The temperature and moisture content of cowdung of each plastic bowl was also recorded at the same time. The moisture content of 60%–80% was maintained by spraying water as per requirement. During collecting weekly data, the approximate timing of clitellum development was also observed. Hand sorting was used to separate the earthworms from the feeding material after the cowdung was removed from the plastic bowl. After separating them, the development of clitellum, commencement of cocoon production in each earthworm species was monitored with naked eyes at seven-day intervals.

The third step was aimed to investigate the cocoon production of two earthworm species. Number of cocoons was recorded weekly basis by hand sorted process. Thus, the rate of cocoons production worm^{-1} day^{-1} was calculated as follows and expressed numerically.

$$\text{Rate of cocoon production (cocoon/worm/day)} = \frac{W2 - W1}{T2 - T1}$$

Where, W1= Initial number of cocoons at the first day of cocoon production; W2= Total cocoons number at the end of the experiment; T1= Age of the earthworms at the first day of cocoon production (in days), and T2= Age of the earthworms at the end of the experiment (in days)

Following collection, cocoons were initially kept on a small plastic bowl containing water. The fourth step was included to observe the morphological characteristics of cocoons using a magnifying glass. Before measuring the length and weight, the cocoons were lightly washed with water and dried with tissue paper to remove any debris that had adhered to the sticky hull. The duration of life cycle was measured for both earthworms species from cocoon to cocoon production. Data on different parameters were statistically analyzed using the computer-based statistical program R software version 4.2.1 in accordance with the basic principles outlined by Gomez and Gomez (1984) whereas means were adjudged by LSD test.

Results

Incubation period and hatching performance of cocoons in winter and summer

During winter and summer, incubation period, hatchlings number cocoon^{-1}, and hatching success (%) had not varied significantly for two earthworm species (Table 1).

Table 1. Incubation period and hatching performance of cocoons in winter and summer season

Earthworm species	Incubation period (days)	Hatchlings number cocoon^{-1}	Hatching success (%)
	Winter season		
E. fetida	24 ± 4.69	2.4 ± 1.19	82.5
E. eugeniae	21.5 ± 4.00	2.0 ± 1.31	75
LS	NS	NS	-
CV (%)	21.30	64.52	-
	Summer season		
E. fetida	23.0 ± 4.16	2.7 ± 0.96	87.5
E. eugeniae	19.75± 4.57	2.2 ± 0.67	80
LS	NS	NS	-
CV (%)	20.45	46.28	-

All values were introduced as the mean ± SD (standard deviation), LS = Level of significance, CV = Co-efficient of variance, NS = Non significant

The transformation process of cocoon to hatchling has shown in Figure 1. During incubation period, the cocoons were hardened gradually after emergence and turned brownish. The brown color became deeper over time, eventually turned dark brown just before hatching.

Weekly change in the length of two earthworm species during winter and summer

Significantly greater length was recorded in *Eudrilus eugeniae* compared to *Eisenia fetida* across all measurement dates during both seasons (Figure 2). In winter, *E. fetida* reached its longest length (10.09 ± 0.42 cm worm^{-1}) at the 13th week, while *E. eugeniae* achieved its longest length (12.98 ± 0.69 cm worm^{-1}) at the 16th week. In summer, *E. fetida* reached its longest length (10.05 ± 0.69 cm worm^{-1}) at the 15th week, and *E. eugeniae* attained its longest length (13.09 ± 0.54 cm worm^{-1}) at the 16th week. After reaching their maximum length, the growth of both earthworm species slowed down, remaining relatively stable till the 22nd week.

Weekly change in the weight of two earthworm species during winter and summer

Eudrilus eugeniae consistently attained significantly higher weight than *Eisenia fetida* at all measurement dates during both seasons (Figure 3). In winter, the highest weight for *E. fetida* was recorded at the 14th week (565.2 ± 17.32 mg worm^{-1}), while *E. eugeniae* reached its highest weight of 775.67 ± 66.40 mg worm^{-1} at the 15th week. In summer, *E. fetida* attained its highest weight of 516.45 ± 19.89 mg worm^{-1} at the 15th week, whereas *E. eugeniae* achieved its highest weight of 703.5 ± 55.56 mg worm^{-1} at the 16th week. After reaching their peak weight, both earthworm species exhibited a gradual decline in weight till the 22nd week in both seasons.

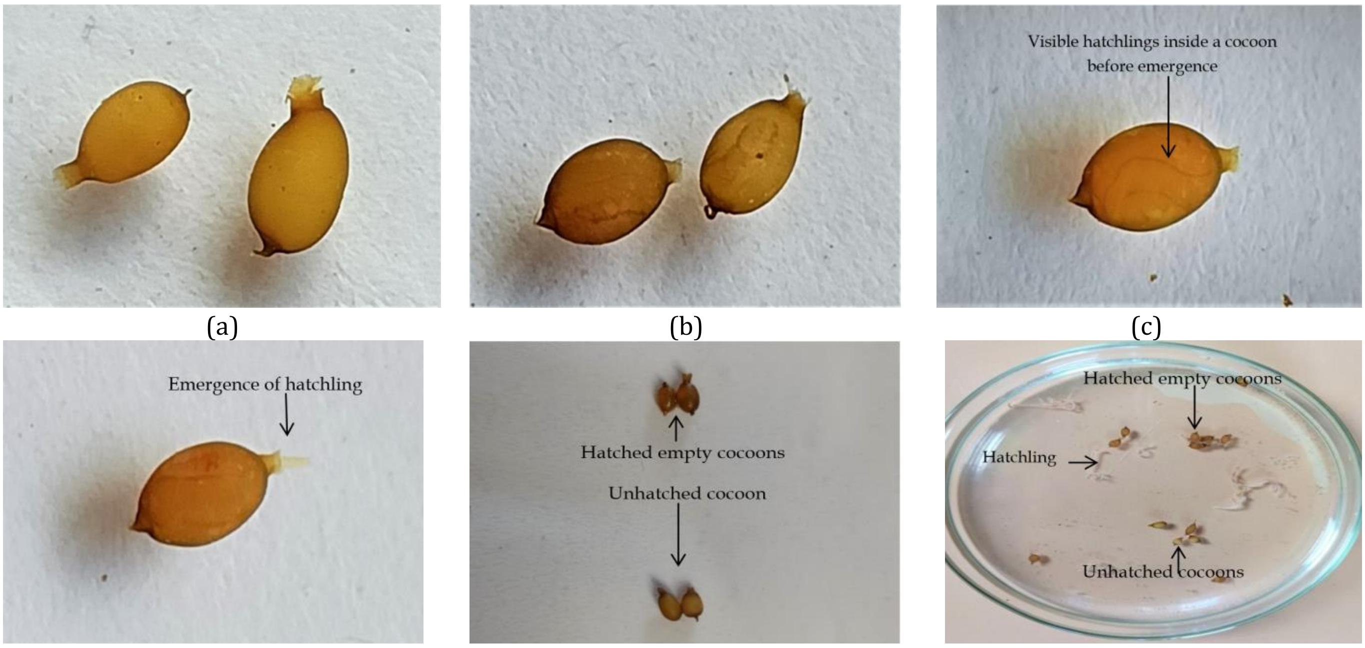


Figure 1. (a) Cocoons become deeper in colour over time (b) Cocoons turned dark brown just before hatching (c) Cocoon just before emergence of cocoon , (d) Emergence of hatchling from cocoon (e) Hatched and unhatched cocoons (f) Hatched cocoons, unhatched cocoons and hatchlings in a petri-dish

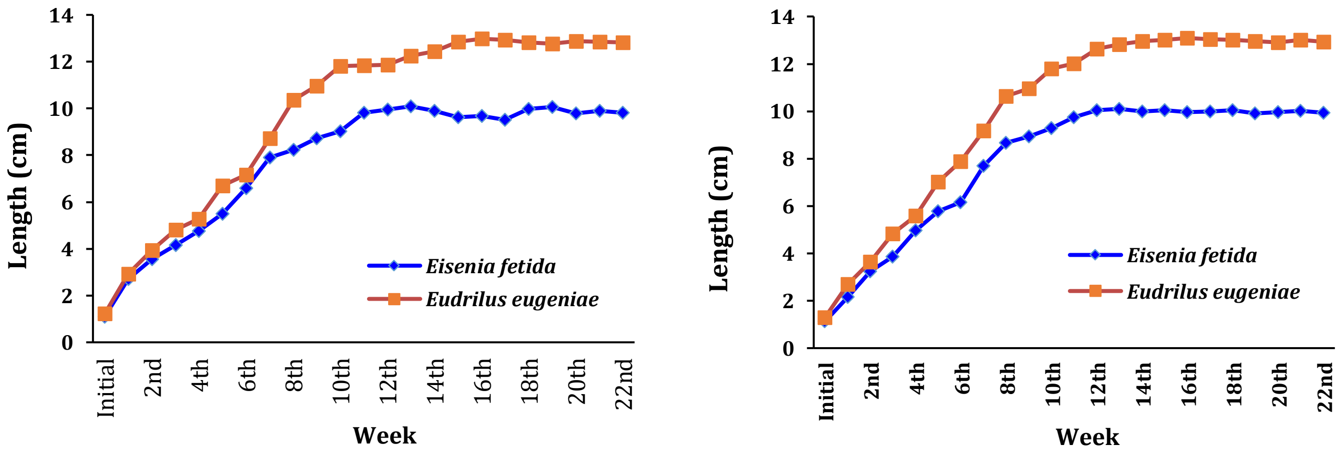


Figure 2. Weekly change in the length of two earthworm species in (a) winter and (b) summer

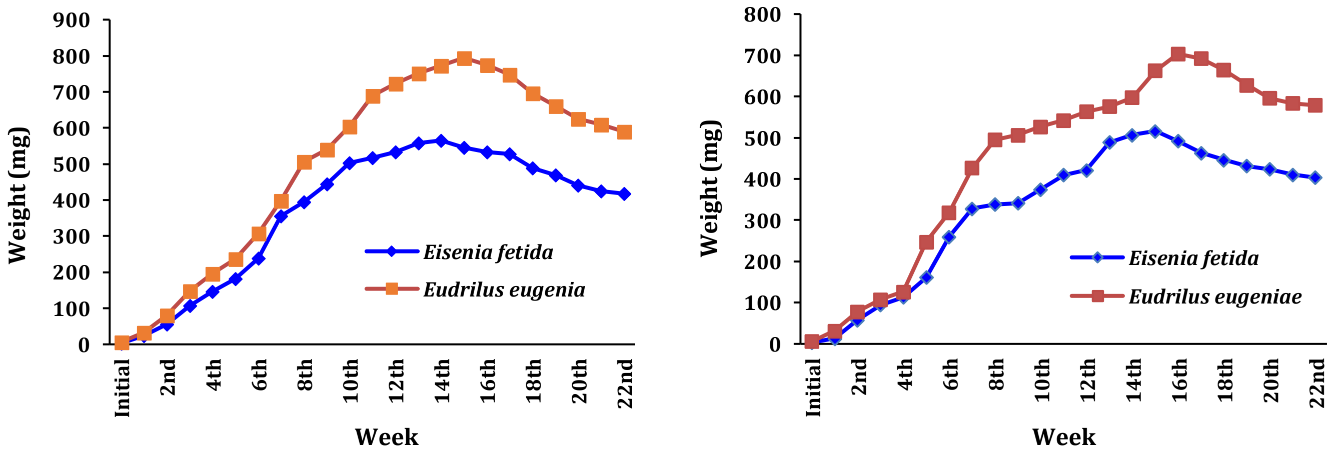


Figure 3. Weekly change in the length of two earthworm species in (a) winter and (b) summer

Percentage of clitellum development at different week during winter and summer

A number of worms of *E. fetida* and *E. eugeniae* started to develop clitellum from 7th week and completed (100%) within 10th and 12th week, respectively during winter (Table 2). In summer, a number of *E. fetida* worms (27.5%) began to develop clitellum as early as the 6th week, and completed (100%) within 7th week. In case of *E. eugeniae*, a few numbers of worms (17.5%) began to develop clitellum at 6th week, and completed (100%) within 8th week.

Table 2. Percentage of clittelum development in two earthworms species during winter and summer season

Earthworm species	Weeks 6th	7th	8th	9th (%)	10th	11th	12th
Winter season							
Eisenia fetida	-	40	70	92.5	100	100	100
Eudrilus eugeniae	-	35	55	62.5	70	75	100
Summer season							
Eisenia fetida	27.5	100	100	-	-	-	
Eudrilus eugeniae	17.5	95	100	-	-	-	

Weekly cocoon production by two earthworm species in winter and summer

The number of cocoons worm^{-1} week^{-1} was recorded significantly higher in *E. fetida* over *E. eugeniae* at all the dates in both seasons (Figure 4). During winter, cocoon production worm^{-1} week^{-1} by *E. fetida* and *E. eugeniae* was initially followed an increasing trend and then a zigzag trend till 22nd week. The highest number of cocoon worm^{-1} week^{-1} in *E. fetida* (2.35 ± 0.30) and *E. eugeniae* (1.85 ± 1.73) had laid on 14th week. The total number of cocoons worm^{-1} (19.22 ± 2.83) and rate of cocoon production (0.18 ± 0.02 cocoon worm^{-1} day^{-1}) was observed significantly higher in *E. fetida* over *E. eugeniae* (Table 3).

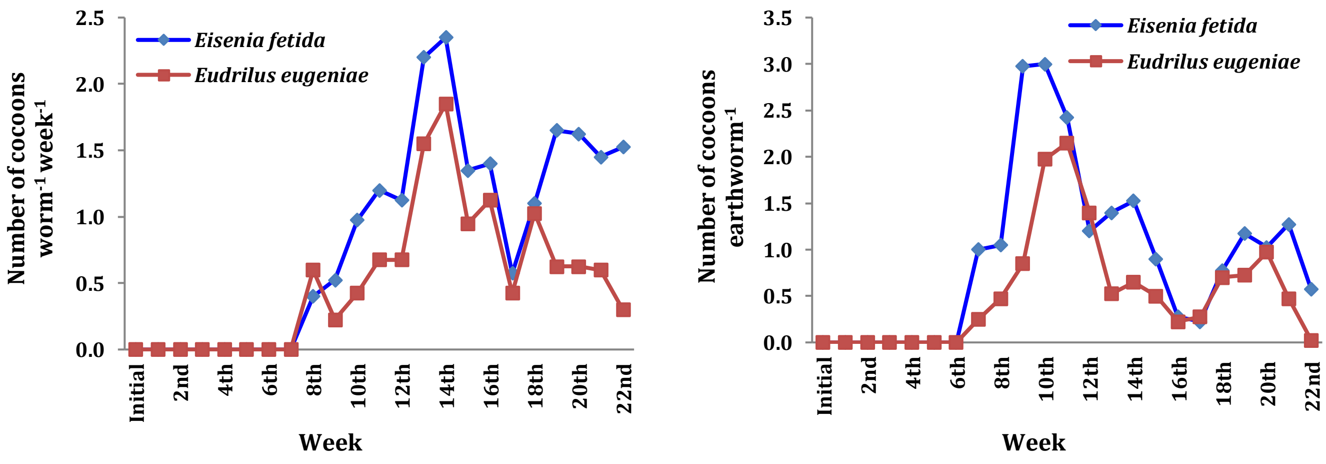


Figure 4. Weekly cocoon production by two earthworm species in (a) winter and (b) summer

During summer, higher number of cocoons was significantly produced in *E. fetida over E. eugeniae* at most of the dates (Figure 4). Cocoon production worm^{-1} week^{-1} by *E. fetida* and *E. eugeniae* was initially followed an increasing trend and then a zigzag trend till 22nd week. In *E. fetida,* the highest number of cocoon worm^{-1} week^{-1} of 3.0 ±1.35 was recorded at 10th week whereas *E. eugeniae* laid the highest number of cocoon worm^{-1} week^{-1} of 2.15 ± 0.54 at 11th week. Table 3 shows that *E. fetida* had significantly a higher total number of cocoons worm^{-1} (20.80 ± 2.88) and rate of cocoon production (0.19±0.02 cocoon worm^{-1} day^{-1}) than *E. eugeniae*. After completing mating process, both earthworms species were laid cocoons within feeding materials and then cocoons were separated through hand sorting (Figure 5).

Effect of temperature on cocoon production during the winter and summer

During winter, both earthworm species had a significant positive correlation between temperature and the number of cocoon production worm^{-1} week^{-1} (Figure 6). Cocoon production in *E. fetida* showed higher positive correlation with temperature (r = 0.61**) than in *E. eugeniae* (r = 0.55**).

During summer, both earthworm species showed non-significant positive correlation between temperature and the number of cocoons produced worm^{-1} week^{-1} (Figure 6). Numerically, cocoon production in *E. fetida* relatively had a higher positive correlation with temperature (r = 0.31) than *E. eugeniae* (r = 0.27).

Table 3. Cocoon production by two earthworm species in the winter and summer

Earthworm species	Total no. of cocoons produced worm^{-1}	Highest no. of cocoon produced worm^{-1} week^{-1}	Rate of cocoon production (cocoon worm^{-1} day^{-1})
Winter season			
Eisenia fetida	19.22 ± 2.83	2.35 ± 0.30	0.18 ± 0.02
Eudrilus eugeniae	11.83 ± 1.29	1.85 ± 0.17	0.12 ± 0.01
LS	**$p<0.01$	*$p<0.05$	**$p<0.01$
CV (%)	15.52	11.66	14.90
Summer season			
Eisenia fetida	20.80 ± 2.88	3.00 ± 1.35	0.19 ± 0.02
Eudrilus eugeniae	11.98 ± 1.03	2.15 ± 0.54	0.12 ± 0.01
LS	**$p<0.01$	*$p<0.05$	**$p<0.01$
CV (%)	13.21	12.36	13.91

All values were introduced as the mean ± SD (standard deviation), LS = Level of significance, CV = Co-efficient of variance, NS = Non significant, '*' = Significant at 5% level of probability, '**' = Significant at 1% level of probability

Table 4. Morphological features of cocoons of two earthworms species in winter season

Parameters	*E. fetida*	*E. eugeniae*
Shape	Oval to irregular oval	Oval to irregular oval
Ornamentation	Bristle at one end and other end pointed	Bristle at one end and other end pointed
Colour	Light yellowish	Light straw
Frequency of cocoon production	Continuous	Continuous
Winter season		
Length range (mm)	3.0-4.7	3.1-4.5
Mean length (mm)	4.02 ± 0.45	3.93 ± 0.39
Fresh weight (mg cocoon^{-1})	12.67 ± 0.62	12.30 ± 0.67
Summer season		
Length range (mm)	3.2-5.3	3.0-4.9
Mean length (mm)	4.08 ± 0.69	4.02 ± 0.37
Fresh weight (mg cocoon^{-1})	12.70 ± 0.29	12.25 ± 0.31

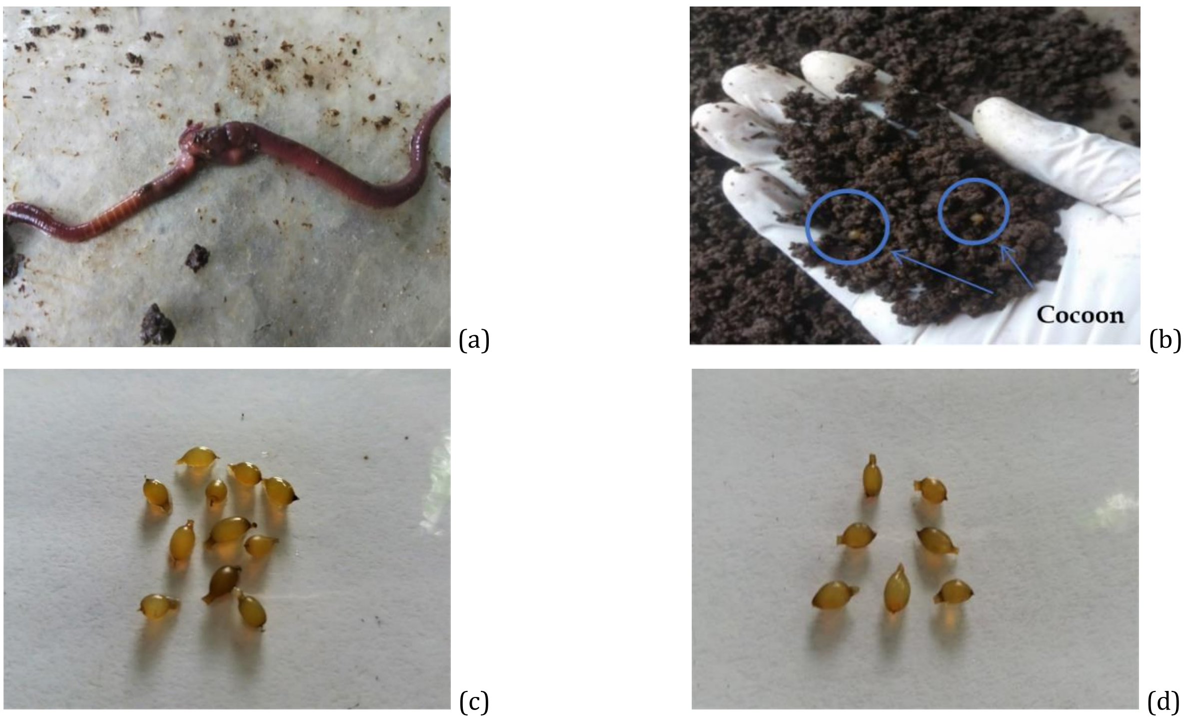
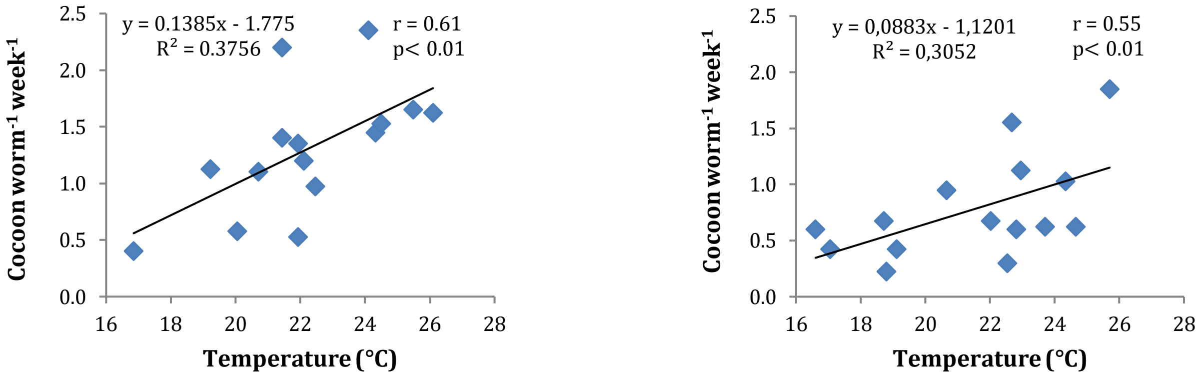
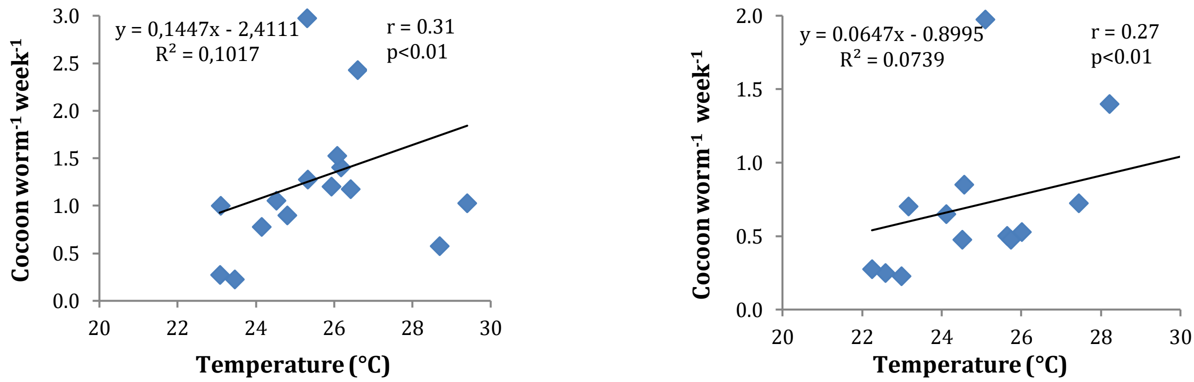


Figure 5. (a) Mating of earthworms (*E. fetida*), (b) Separating cocoons from cowdung by hand sorting, (c) Cocoons of *E. fetida*, (d) Cocoons of *E. eugeniae*



(a) (b)

Figure 6. Correlation between temperature and number of cocoon production worm^{-1} week^{-1} for (a) *E. fetida* and (b) *E. eugeniae* in winter



(a) (b)

Figure 7. Correlation between temperature and number of cocoon production worm^{-1} week^{-1} for (a) *E. fetida* and (b) *E. eugeniae* in summer

Life cycle of *Eisenia fetida* and *Eudrilus eugeniae*

The life cycle of *Eisenia fetida* and *Eudrilus eugeniae* is illustrated in Figures 8 and 9. The incubation period for cocoons was longer in *E. fetida*. Hatchlings of both species transitioned to the juvenile stage within four weeks, reaching sexual maturity within 7–10 weeks in winter and 6–7 weeks in summer. Once sexually mature, the earthworms developed a clitellum and initiated mating. Cocoon production began one week after clitellum formation in mature earthworms.

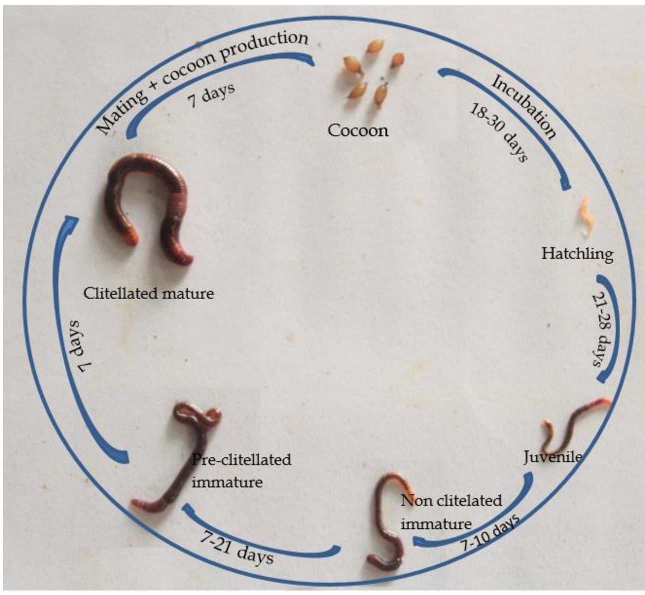


Figure 8. Life cycle of *E. fetida*

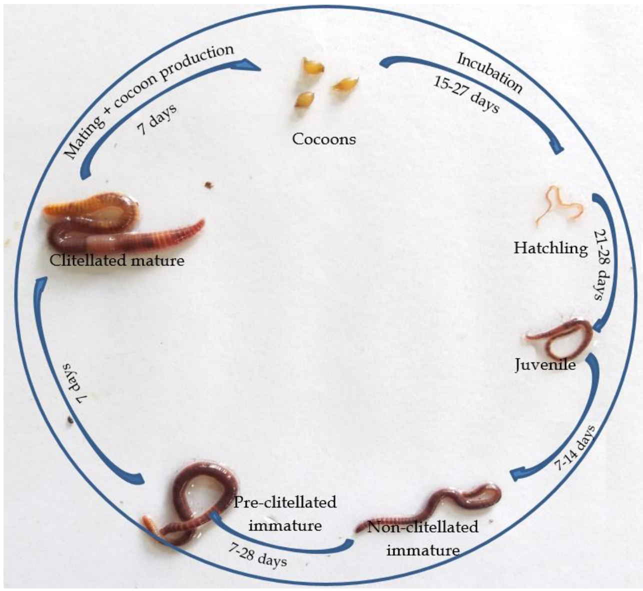


Figure 9. Life cycle of *E. eugeniae*

Life cycle of *Eudrilus eugeniae*

Figure 9 depicts the life cycle of *E. fetida*. Incubation time for *E. eugeniae* cocoons ranges from 21.5 ± 5.00 and 19.75 ± 4.57 days in winter and summer, respectively. Hatchlings become juvenile within 4 weeks and attain sexual maturity within 6 to 12 weeks in winter and within 6-8 weeks in summer. Then, with the development of clitellum, sexually matured earthworms began to mate. Cocoon formation starts within one week after clitellum development in mature earthworms.

Discussion

Incubation period, hatching success, and hatchlings number

The present study revealed that *Eisenia fetida* had a longer incubation period and a higher cocoon hatching success rate than *Eudrilus eugeniae* in both seasons. The hatching success of cocoons for both species was greater in summer than in winter, likely due to the higher temperatures (20–30°C) in summer, which were more favorable compared to the winter temperatures (15–26°C). The incubation period observed for *E. fetida* was consistent with the findings of Bondhare and Desai (2019), while the incubation period for *E. eugeniae* closely aligned with the results reported by Parthasarathi (2007).

The results indicated that temperature influenced the number of hatchlings per cocoon. In *Eisenia fetida*, the number of hatchlings per cocoon was recorded as 2.4 ± 0.96 in winter and 2.7 ± 1.19 in summer. Venter and Reinecke (1988) reported a similar hatchling count of 2.7 for *E. fetida*, whereas higher values of 3.5 and 3.8 were observed by Graff (1982) and Loehr et al. (1985), respectively. For *Eudrilus eugeniae*, the average number of hatchlings per cocoon was 1.9 ± 1.31 in winter and 2.2 ± 1.19 in summer. Previous studies reported hatchling numbers of 2.2 (Graff, 1982), 2.5 (Loehr et al., 1985), and 2.7 (Knieriemen, 1984) for *E. eugeniae*, all of which closely align with the findings of the present study.

Growth

Eudrilus eugeniae exhibited significantly greater length and weight than *Eisenia fetida* in both seasons. The length of both species increased progressively during the initial weeks, followed by a slower growth rate. This trend is likely due to the natural growth pattern of living organisms, where size increases over time until reaching full maturity. In terms of weight, both species experienced a rapid increase in the early weeks, followed by a slower growth phase in the middle weeks and a gradual weight decline thereafter. During winter, the highest weight gain occurred between the 5th and 11th weeks, whereas in summer, peak weight gain was observed between the 5th and 7th weeks. This pattern may be attributed to the transfer of earthworms to a fresh feeding medium, which provided optimal conditions for growth and increased energy availability. These findings align with the observations of Venter and Reinecke (1988), Mba (1983), and Knieriemen (1984).

During winter, the weight gain of *Eisenia fetida* and *Eudrilus eugeniae* slowed down in the middle weeks (10th to 17th and 11th to 15th, respectively), although biomass continued to increase. A similar pattern was observed in the summer season, with *E. fetida* and *E. eugeniae* showing slower growth between the 7th to 15th and 8th to 14th weeks, respectively. This slower growth was likely due to the onset of cocoon production and the shift to the reproductive phase, where energy was allocated for cocoon formation in addition to growth. A slower growth rate during the reproductive phase has also been reported by Viljoen and Reinecke (1994) and Graff (1982).

The weight of *Eisenia fetida* and *Eudrilus eugeniae* began to decline gradually after the middle weeks in both seasons. This weight loss could be attributed to the reduced availability of feeding materials, as no new feeding materials were added during the later weeks of the experimental period. The limited supply of food likely acted as a constraint on the growth of the earthworms. These findings align with the work of Bhat et al. (2015). Tiwari (1993) noted that adding organic matter increases worm population density and biomass. The availability of food is a key factor influencing both biomass and reproductive rates in earthworms (Garg et al., 2005; Sangwan et al., 2008). Overall, *E. eugeniae* demonstrated a greater growth advantage compared to *E. fetida*, consistent with the results reported by Emperor et al. (2016) and Venter and Reinecke (1988).

Maturation

The findings of the current study clearly indicated that *Eisenia fetida* reached sexual maturity faster than *Eudrilus eugeniae* during both seasons. Both species required less time to mature in the summer, likely due to the favorable temperature range of 20–30°C. This is consistent with the findings of Dominguez et al. (2001), who reported that worms reach sexual maturity most quickly when temperatures range from 25 to 30°C, regardless of population density. The time required for reproduction to begin can vary even among individuals of the same species, potentially due to environmental factors (Podolak et al., 2020). Previous studies have reported varying timeframes for sexual maturity in *E. fetida*, such as 28 to 30 days (Dominguez and Edwards, 2011), 30 days (Lofs-Holmin, 1985), six to eight weeks (Edwards, 1988), four to six weeks for 50% of individuals and ten weeks for all worms (Neuhauser et al., 1979), and 60 days (Venter and Reinecke, 1988). In the case of *E. eugeniae*, Viljoen and Reinecke (1989) reported sexual maturity within 45 days after hatching, while Edwards (1988) stated it took approximately five weeks.

Cocoon production

During both seasons, the total number of cocoons produced worm^{-1} week^{-1} and the rate of cocoon production (cocoon worm^{-1} per^{-1}) were significantly greater in *E. fetida*. This could be species specific adaptations to the experimental conditions. Cocoon production was found to be higher in summer for both earthworm species while temperature ranged from 20-30°C. A similar finding was reported by Thirumagal and Deivanayaki (2017). During winter, a lower number of cocoon worm^{-1} week^{-1} was recorded in *E. eugeniae* over *E. fetida*. It is possibly due to *E. eugeniae* is very sensitive to low temperature and has a narrow tolerance range for temperature. Temperature ranges from 22-28 °C is favourable for higher growth, maturation rate, cocoon production and fecundity of *E. eugeniae* (Shagoti et al. 2001; Viljoen and Reinecke, 1992). There were a lot of variations in cocoon production in different weeks. Results of this study showed that cocoon production worm^{-1} week^{-1} was increased along with increase in temperature during winter. Cocoon production in *E. fetida* increases linearly as temperature rises from 10 to 25°C (Reinecke and Kriel 1981). The findings indicated that both earthworm species were sensitive to extreme temperatures, making regions with excessively high or low temperatures unsuitable for vermiculture. Further research is needed to explore this aspect. The small sample size and limited seasonal duration were constraints in this study. To gain a deeper understanding of these earthworm species and vermiculture practices, future research should involve a larger sample size and year-round studies on growth and reproduction.

Morphology of cocoons

Initially, the freshly laid cocoon of *Eisenia fetida* was light straw in color, while the cocoon of *Eudrilus eugeniae* appeared light yellowish. As the vascularization of the pre-emergent hatchling increases, the cocoon gradually hardens and turns brownish after emergence (Debnath and Chowdhury, 2020). Over time, the brown color deepens, eventually becoming dark brown just before hatching. Different earthworm species produce cocoons of varying shapes and sizes (Dominguez and Edwards, 2011; Edwards and Arancon, 2022).

Life-cycle

Both earthworm species required comparatively less time to complete egg to egg cycle during summer whereas *E. eugeniae* took less time than *E. fetida*. *E. eugeniae* exhibited a short life span and a high reproduction rate when raised on cattle manure (Viljoen and Reinecke, 1988; Parthasarathi, 2007).

It is clear from the findings that, growth of both earthworms species were comparatively higher in winter, whereas the reproductive activity of both species was lower. It is possibly due to lower winter temperatures reduced reproductive activity in both earthworm species, which could result in weight gain due to a reduction in reproductive costs and a reallocation of resources toward growth. Similar finding was reported by Kawecki and Stearns, (1993). Dissimilar result was found by Seenappa (2011) and Biradar et al. (2003), who reported that growth and reproduction of *E. fetida* and *E. eugeniae* were significantly more in winter and monsoon than in summer.

Conclusion

The study found that *Eudrilus eugeniae* exhibited greater growth advantages, while *Eisenia fetida* demonstrated better reproductive performance across both seasons. The growth of both earthworm species was higher in winter, whereas reproductive activity peaked in summer. Cocoon production worm^{-1} week^{-1} showed a positive correlation with temperature for both species during winter. New farmers in Bangladesh could benefit economically from vermiculture with *Eisenia fetida*, as it has a higher reproductive rate than *Eudrilus eugeniae* in both seasons.

Acknowledgements

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Effect of foliar-applied humic acid-based fertilizers on potato (*Solanum tuberosum* L.) yield, tuber quality, and nutrient uptake efficiency, with implications for sustainable fertilization

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Abstract

This study investigated the effects of foliar-applied humic acid-based fertilizers on potato (Solanum tuberosum L.) yield, tuber quality, and nutrient uptake efficiency under irrigated conditions in Western Kazakhstan. A three-year field experiment (2021–2023) was conducted using the Silvana potato variety, a medium-early cultivar with high yield potential. The randomized complete block design included five treatments: (1) Control (no fertilizers), (2) Reasil Micro Hydro Mix, (3) Reasil Micro Hydro Mix + Reasil Forte Carb-Nitrogen-Humic, (4) Potassium Humate, and (5) Potassium Humate + Reasil Forte Carb-Nitrogen-Humic. All fertilizers were applied as foliar sprays at three critical growth stages: stem formation, bud appearance, and tuber formation. The humic acid-based fertilizers used in the study were produced by LLC "Life Force Group". Potassium Humate is an 80% alkaline extract of humic and fulvic acids from leonardite. Reasil Micro Hydro Mix contains various essential micronutrients, including N, Mg, B, Fe, Zn, and amino acids. Reasil Forte Carb-Nitrogen-Humic is rich in N (20%, including 18% amide-N) and also contains humic acids (6.2%), hydroxycarboxylic acids (6.2%), and amino acids (6%). Results showed that foliar humic acid application significantly increased potato yield and improved tuber quality. The highest average marketable yield (28.79 t/ha) was obtained with Potassium Humate + Reasil Forte Carb-Nitrogen-Humic, reflecting a 20% increase over the control. Starch content was also highest in this treatment (16.9%), while vitamin C content was better maintained in treated plots under stress conditions. Additionally, nitrate accumulation in tubers was reduced, improving food safety. Nutrient uptake efficiency was significantly enhanced by humic acid-based foliar treatments. The Potassium Humate + Reasil Forte Carb-Nitrogen-Humic treatment recorded the highest N, P, and K absorption levels, confirming the role of foliar humic applications in optimizing nutrient translocation. These findings demonstrate that humic acid-based foliar fertilization is an effective strategy for increasing potato productivity while reducing reliance on conventional fertilizers. These findings highlight the potential of foliar-applied humic substances as a sustainable alternative to conventional fertilization, particularly in semi-arid agricultural systems.

Keywords: Foliar fertilization, humic substances, potato yield, nutrient uptake, starch content, sustainable agriculture.

Introduction

Potato (*Solanum tuberosum* L.) is one of the most widely cultivated crops globally, playing a critical role in food security and economic sustainability. It serves as a staple food in many countries, providing essential

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nutrients such as carbohydrates, vitamins, and minerals. Also, potatoes are an important crop in the Central Asian Republics (Loebenstein and Manadilova, 2003). They are the second most important crop in Kazakhstan, after wheat, grown on about 205.000 ha and its yields average 19.5 t ha^{-1} (Alimkhanov et al., 2021).However, achieving high potato yields and quality is often constrained by environmental stress, nutrient availability, and soil fertility. To address these challenges, sustainable fertilization strategies have been explored, with increasing interest in humic substances as a plant growth enhancer (Mora et al., 2010; Chen et al., 2017; Akladious and Mohamed, 2018; Mukhametov et al., 2024).

Humic substances, including humic acid and fulvic acid, are naturally occurring organic molecules formed during the decomposition of plant and microbial residues. They have been widely studied for their role in improving plant growth, increasing stress resistance, and enhancing nutrient absorption (de Moura et al., 2023). While traditionally applied to the soil to improve structure and nutrient retention, recent research has demonstrated that humic substances can be highly effective when applied as foliar sprays, directly benefiting plant metabolism and nutrient uptake (Zhou et al., 2019; Bayat et al., 2021). Foliar application allows plants to absorb humic substances through leaf tissues, improving nutrient availability, photosynthetic efficiency, and enzymatic activity (Li et al., 2019; Tang et al., 2021).

Unlike soil applications that primarily modify soil fertility, foliar-applied humic substances enhance nutrient absorption directly through the leaves and facilitate internal nutrient translocation within the plant (Muminova et al., 2022; Tastanbekova et al., 2024). Studies have demonstrated that humic substances applied via foliar spraying improve nitrogen assimilation, increase enzymatic activity related to photosynthesis, and regulate hormonal balance, thereby enhancing plant growth and productivity (Zhou et al., 2019; Bayat et al., 2021). These effects are particularly valuable in potato cultivation, where efficient nutrient uptake directly impacts tuber development, starch accumulation, and overall yield quality.

The benefits of foliar-applied humic substances are particularly evident in crops like potatoes, where efficient nutrient uptake is crucial for tuber formation and quality. Studies have shown that foliar humic applications enhance nitrogen (N), phosphorus (P), and potassium (K) uptake, increasing translocation efficiency and improving overall plant health (Lumactud et al., 2022). Furthermore, humic substances have been reported to stimulate hormonal activities such as cytokinin and auxin production, leading to improved root and shoot development, stress tolerance, and yield stability (Akram et al., 2009; Kanai et al., 2011). These effects are particularly important under variable environmental conditions, where nutrient absorption from the soil may be limited.

Another advantage of foliar-applied humic substances is their ability to reduce dependence on conventional fertilizers while maintaining or even improving crop productivity. Research indicates that humic acids can enhance plant growth by increasing nitrate uptake, stimulating polyamine metabolism, and improving photosynthetic efficiency (Nardi et al., 2002; Mora et al., 2010). These mechanisms contribute to better tuber starch accumulation, improved vitamin C content, and higher marketability of potatoes (Chen et al., 2004; Suh et al., 2014).

Despite the growing evidence supporting the effectiveness of foliar humic substances, further research is needed to evaluate their performance under different climatic conditions and application rates (de Moura et al., 2023). While previous studies have examined their effects on various crops, there is limited data specifically on potatoes grown under Western Kazakhstan's agro-climatic conditions (Muminova et al., 2022; Tastanbekova et al., 2024). Understanding how foliar-applied humic substances influence tuber yield, quality, and nutrient uptake in these soils is essential for optimizing fertilization strategies.

This study aims to evaluate the impact of foliar-applied humic acid-based fertilizers on potato yield, tuber quality, and nutrient uptake efficiency under irrigated dark chestnut soil conditions in Western Kazakhstan. Specifically, the study will (i) Assess the effects of different humic acid formulations on potato yield and biomass production, (ii) Investigate changes in nutrient absorption and translocation efficiency following foliar humic acid application.

Material and Methods

Experimental Site and Climatic Conditions

The field experiments were conducted from 2021 to 2023 at the KH "Arystanov" farm in the Baiterek district, West Kazakhstan region. The experimental site is located at 52°16'06" N latitude and 51°02'44" E longitude. The soil in this region is classified as medium-depth dark chestnut soil with a heavy loamy texture. The humus content in the 0–30 cm soil layer ranged from 2.8% to 3.3%, and the soil pH was slightly alkaline (7.2–7.3). The pH of the aqueous extract was measured using electrodes.

The climate in the study area is sharply continental, with an average annual precipitation of 320 mm and an average annual temperature of +2.8°C. The frost-free period lasts 135–150 days, and the hydrothermal coefficient (HTC) varies between 0.7 and 0.8, indicating semi-arid conditions.

Experimental Design

The study was designed to evaluate the effects of foliar-applied humic acid-based fertilizers on potato (Solanum tuberosum L.) yield, tuber quality, and nutrient uptake efficiency. The trials were conducted using a randomized complete block design with five treatments and four replications, totaling 336 m^2. Each experimental plot measured 16.8 m^2 (2.1 m × 8 m). The distance between plots was not explicitly stated.

Plant Material and Fertilizer Treatments

The potato variety used in the study was Silvana, a medium-early, table variety known for its high marketability and adaptability. The plants are tall, semi-erect, with medium-sized, open leaves of light green to green color. Tubers are round with medium-depth eyes, smooth yellow skin, and yellow flesh. The variety is characterized by a marketable tuber weight of 92–148 g, starch content of 13.6–15.3%, and marketable yield ranging from 17 to 37.4 t/ha. The number of tubers per plant varies between 7 and 14, and its storage quality is rated at 91%.

The humic acid-based foliar fertilizers used in the experiment were sourced from LLC "Life Force Group" (Saratov, Russia) and included:

- Potassium Humate: An 80% alkaline extract of humic and fulvic acids derived from the leonardite mineral.
- Reasil Micro Hydro Mix: A micronutrient complex containing hydroxycarboxylic acids (18%) as a chelating agent, total nitrogen (12%), magnesium (4%), boron (2%), cobalt (0.1%), copper (0.8%), iron (5%), manganese (2.5%), molybdenum (0.25%), zinc (3%), and amino acids (8%), with a pH of 8.5.
- Reasil Forte Carb-Nitrogen-Humic: A nitrogen-rich formulation containing total nitrogen (20%), amide nitrogen (18%), humic acids (6.2%), hydroxycarboxylic acids (6.2%), and amino acids (6%).

All fertilizers were applied as foliar sprays at specific growth stages to ensure optimal nutrient absorption through the leaves. The treatments included:

- Control (T1): No fertilizer application.
- Reasil Micro Hydro Mix (T2): Applied three times at 1.0 L/ha per treatment.
- Reasil Micro Hydro Mix + Reasil Forte Carb-Nitrogen-Humic (T3): 1.0 L/ha Reasil Micro Hydro Mix in the first treatment, followed by 2.0 L/ha Reasil Forte Carb-Nitrogen-Humic in the second and third treatments.
- Potassium Humate (T4): Applied three times at 1.0 L/ha per treatment.
- Potassium Humate + Reasil Forte Carb-Nitrogen-Humic (T5): 1.0 L/ha Potassium Humate in the first treatment, followed by 2.0 L/ha Reasil Forte Carb-Nitrogen-Humic in the second and third treatments.

All fertilizers were dissolved in 200 L of water per hectare and applied using a manual sprayer with a working pressure of 0.3 atm.

Agronomic Practices

Soil preparation involved moldboard plowing to a depth of 22–25 cm, followed by pre-plant cultivation to 8–9 cm. Potatoes were planted using a potato planter at a row spacing of 0.7 m, with three inter-row cultivations conducted during the growing season.

Sowing and Harvesting Dates:

- 2021: Sowing on April 30, harvest on August 12 (104-day growth period).
- 2022: Sowing on May 3, harvest on August 15 (105-day growth period).
- 2023: Sowing on May 5, harvest on August 24 (112-day growth period).

No pre-germination of tubers was conducted before planting.

Irrigation and Fertilization

The experimental field was irrigated using sprinkler irrigation. Specific irrigation rates and seasonal water norms are detailed in the results section.

No basal (soil-applied) fertilization was performed. Only foliar-applied humic fertilizers were used.

Pest and Disease Management

Pest and disease development was not systematically studied during the experiment. However, visual inspections indicated that the crops remained healthy throughout the study period.

Data Collection and Analytical Methods

Tuber and vegetative mass samples were collected before harvesting. The following measurements and analyses were conducted acorrding to Kalra (1998), Jones (2001) and Singh (2024):

- Yield Determination: Harvesting was performed manually, and total tuber yield was recorded for each treatment.
- Nutrient Content Analysis: Nitrogen, phosphorus, and potassium concentrations in tubers and vegetative biomass were determined using wet ashing with concentrated sulfuric acid.
- Starch Content: Measured using a polarimeter after acid hydrolysis, following the Evers method.
- Vitamin C (Ascorbic Acid) Content: Extracted using a hydrochloric and metaphosphoric acid mixture and analyzed using the Murray method.
- Total Sugar Content: Determined after hydrolysis with a 10% hydrochloric acid solution.
- Nitrate Content: Measured using a standard ion-selective electrode.

Results and Discussion

Yield Performance of Potato under Humic Acid-Based Foliar Fertilization

The application of humic acid-based foliar fertilizers significantly influenced the yield performance of potato tubers throughout the three-year experimental period (2021–2023). The highest average yield (28.79 t/ha) was obtained from the Potassium Humate + Reasil Forte Carb-Nitrogen-Humic treatment (T5), reflecting a 20% increase over the control. The combination of humic substances with chelated micronutrients (Reasil Micro Hydro Mix + Reasil Forte-T3) also demonstrated a notable yield increase of 16% compared to the control, further supporting the role of humic acids in improving plant nutrient uptake and biomass production (Chen et al., 2017; Akladious and Mohamed, 2018; Zhou et al., 2019; Bayat et al., 2021).

Yield performance varied across the three years due to environmental conditions (Table 1). In 2021, favorable climatic conditions contributed to higher overall yields. The Potassium Humate + Reasil Forte treatment (T5) recorded the highest yield at 32.13 t/ha, while the control plot (T1) produced only 26.45 t/ha. However, 2022 was marked by adverse weather conditions, including high temperatures and low rainfall, leading to a decrease in yields across all treatments. The control treatment (T1) suffered the most significant reduction, yielding only 16.49 t/ha (a 38% decrease compared to 2021). In contrast, humic acid-treated plots showed improved resilience, with Potassium Humate + Reasil Forte Carb-Nitrogen-Humic (T5) maintaining 77% of its multi-year average, demonstrating the potential of humic substances to enhance stress tolerance in potato crops (Akladious and Mohamed, 2018; de Moura et al., 2023). The yield performance rebounded in 2023 with improved hydrothermal conditions. The Potassium Humate + Reasil Forte Carb-Nitrogen-Humic treatment (T5) once again exhibited the highest yield stability (31.99 t/ha), highlighting the long-term benefits of humic acid foliar applications under varying climatic conditions.

Table 1. Yield of Marketable Silvana Potato Tubers Under Irrigation Conditions (t/ha)

Treatments	2021	2022	2023	Average	Increase (t)	% Increase	Marketability, %
T1	26.45	16.49	29.20	24.05	-	100	91
T2	28.69	19.55	31.12	26.45	2.40	110	94
T3	30.18	20.50	33.15	27.94	3.89	116	95
T4	29.55	18.49	30.32	26.12	2.07	109	95
T5	32.13	22.25	31.99	28.79	4.74	120	96

T1: Control (No Fertilizer); T2 : Reasil Micro Hydro Mix; T3 : Reasil Micro Hydro Mix + Reasil Forte Carb-Nitrogen-Humic; T4 : Potassium Humate; T5 : Potassium Humate + Reasil Forte Carb-Nitrogen-Humic

Table 1 presents the yield data for different fertilizer treatments. The results indicate that three-time foliar application of humic acid-based fertilizers consistently increased potato yields compared to the control (T1). The data confirms that the highest marketable yield was consistently achieved with the combined application of Potassium Humate + Reasil Forte Carb-Nitrogen-Humic (T5). These findings align with previous studies that demonstrated humic acid's role in enhancing soil structure, increasing water retention, and improving plant tolerance to abiotic stress (Akram et al., 2009; Kanai et al., 2011).

Research suggests that humic acids contribute to improved soil microbial activity, which enhances nutrient availability even under challenging conditions. The ability of humic substances to stimulate root growth and increase water and nutrient absorption likely played a role in the observed yield stability, particularly in the dry 2022 season. Additionally, the increased uptake of essential nutrients such as nitrogen, phosphorus, and potassium in fertilized plots contributed to overall yield improvements (Suh et al., 2014; Akladious and

Mohamed, 2018; Li et al., 2019; Tang et al., 2021; Lumactud et al., 2022). The results of this study reinforce the benefits of incorporating humic acid-based fertilizers into potato cultivation strategies, particularly in regions with variable climatic conditions. By improving both yield and marketability, these fertilizers offer a sustainable approach to enhancing potato production in Western Kazakhstan.

Influence on Tuber Number and Size

Humic acid-based foliar fertilization significantly influenced tuber number and size distribution. The highest number of tubers per plant was recorded in the Potassium Humate + Reasil Forte Carb-Nitrogen-Humic treatment (T5), followed closely by Reasil Micro Hydro Mix + Reasil Forte Carb-Nitrogen-Humic (T3), both of which significantly outperformed the control (T1). Tuber formation showed noticeable variations across the years (Table 2). In 2021, the number of tubers per m^2 was highest in the Potassium Humate + Reasil Forte Carb-Nitrogen-Humic (T5, 33.5), while the control (T1) recorded only 21.1. The following year, environmental stress conditions led to an overall decline in tuber numbers, yet the fertilized treatments maintained significantly higher values than the control. By 2023, tuber numbers rebounded, with humic acid-treated plots demonstrating a consistent trend of improvement, reaffirming the role of foliar-applied humic substances in enhancing tuber formation and plant resilience.

Table 2. Structure of the Biological Yield of Marketable Silvana Potato Tubers

Treatments	Tubers per m^2				Tubers per plant				Tubers (kg) per bush			
	2021	2022	2023	Avg.	2021	2022	2023	Avg.	2021	2022	2023	Avg.
T1	26,0	15,1	20,7	20,6	4,8	4,1	4,9	4,6	0,46	0,41	0,45	0,44
T2	36,6	22,0	23,9	27,5	6,1	4,9	6,4	5,8	0,59	0,55	0,51	0,55
T3	35,5	28,3	36,1	33,3	6,9	6,0	7,8	6,9	0,69	0,48	0,60	0,59
T4	30,2	27,7	26,1	28,0	6,3	6,0	5,4	5,9	0,67	0,58	0,55	0,60
T5	34,4	29,0	35,3	32,9	7,2	7,6	5,9	6.9	0,74	0,65	0,62	0,67

T1: Control (No Fertilizer); T2 : Reasil Micro Hydro Mix; T3 : Reasil Micro Hydro Mix + Reasil Forte Carb-Nitrogen-Humic; T4 : Potassium Humate; T5 : Potassium Humate + Reasil Forte Carb-Nitrogen-Humic

The observed increase in tuber number and weight aligns with previous research findings that attributed these benefits to enhanced root development, improved soil aggregation, and increased nutrient availability (Butler and Muir, 2006; Liu et al., 2014; Zhao et al., 2020; Li et al., 2022; Budanov et al., 2023). Humic acids also stimulate hormonal activity, further promoting tuber formation (Rathor et al., 2024). The ability of humic substances to regulate osmotic stress is another key factor in their positive impact on tuber formation (Jindo et al., 2020). The results of this study reinforce the benefits of incorporating humic acid-based fertilizers into potato cultivation strategies, particularly in regions with variable climatic conditions. By improving both yield and marketability, these fertilizers offer a sustainable approach to enhancing potato production in Western Kazakhstan.

Effect on Starch, Vitamin C and Nitrate Content

Humic acid-based foliar fertilization significantly influenced the starch content in potato tubers (Table 3). In 2021, the highest starch concentration was observed in the Potassium Humate + Reasil Forte Carb-Nitrogen-Humic treatment (T5, 17.2%), while the control (T1) had the lowest (15.2%). However, in 2022, due to adverse environmental conditions, the starch content decreased across all treatments, with the Potassium Humate + Reasil Forte treatment dropping slightly to 15.7% and the control falling to 13.8%. By 2023, starch levels recovered, reaching a peak of 16.9% in the Potassium Humate + Reasil Forte treatment, while the control remained relatively lower at 14.8%. This pattern suggests that humic substances play a key role in stabilizing starch accumulation despite environmental stressors.

The vitamin C (ascorbic acid) content in potato tubers followed a relatively stable pattern across the years (Table 3). In 2021, the highest vitamin C concentration was recorded in the Potassium Humate + Reasil Forte treatment (23.8 mg/100g), while the control contained 23.9 mg/100g. In 2022, vitamin C levels dropped slightly due to environmental stress, but humic acid treatments helped maintain higher levels compared to the control. By 2023, vitamin C levels increased again, with the Potassium Humate + Reasil Forte Carb-Nitrogen-Humic treatment (T5) reaching 23.9 mg/100g. These results suggest that foliar-applied humic substances contribute to maintaining the nutritional quality of potatoes even under stress conditions.

Nitrate accumulation in potato tubers was significantly reduced in all humic acid-treated variants compared to the control (Table 3). In 2021, the highest nitrate content was recorded in the Potassium Humate + Reasil Forte Carb-Nitrogen-Humic (T5, 57 mg/kg raw mass), while the control (T1) had 49 mg/kg. In 2022, nitrate levels dropped across all treatments, with the lowest value of 39 mg/kg in the Potassium Humate (T4) treatment, compared to 36 mg/kg in the control (T1). By 2023, nitrate content further decreased, with the

Reasil Micro Hydro Mix + Reasil Forte Carb-Nitrogen-Humic (T3) treatment achieving the lowest recorded nitrate level of 24 mg/kg, highlighting humic substances' role in regulating nitrogen metabolism in plants and reducing excessive nitrate accumulation.

Table 3. Starch and Nutrient Content of Marketable Silvana Potato Tubers

Treatments	Starch (%)				Vitamin C (mg/100g)				NO_3 (mg/kg raw mass)			
	2021	2022	2023	Avg.	2021	2022	2023	Avg.	2021	2022	2023	Avg.
T1	15,2	13,8	14,8	14,6	23,9	20,8	23,1	22,6	49	36	35	40
T2	16,5	14,9	16,3	15,9	23,2	21,4	22,0	22,2	50	40	39	43
T3	17,3	15,9	16,0	16,4	23,6	21,8	23,0	22,8	55	41	24	40
T4	17,0	16,8	14,2	16,0	23,9	20,9	22,4	22,4	52	39	44	45
T5	17,2	15,7	16,9	16,6	23,8	21,6	23,9	23,1	57	42	33	44

T1: Control (No Fertilizer); T2 : Reasil Micro Hydro Mix; T3 : Reasil Micro Hydro Mix + Reasil Forte Carb-Nitrogen-Humic; T4 : Potassium Humate; T5 : Potassium Humate + Reasil Forte Carb-Nitrogen-Humic

These findings align with studies that reported humic acid's ability to stimulate starch synthesis enzymes such as AGPase, UGPase, and SSS, leading to increased starch accumulation in tubers (Tiessen et al., 2002; Li et al., 2019). Additionally, humic acids contributed to an increase in total sugars and dry matter content, making them a valuable tool for improving potato quality (de Moura et al., 2023). The results of this study reinforce the benefits of incorporating humic acid-based fertilizers into potato cultivation strategies, particularly in regions with variable climatic conditions. By improving both yield and marketability, these fertilizers offer a sustainable approach to enhancing potato production in Western Kazakhstan.

Nutrient Uptake Efficiency

Nutrient uptake fluctuated across the years (Table 4), largely influenced by soil moisture availability. In 2021, nutrient uptake was highest across all treatments due to optimal environmental conditions. However, in 2022, a decline in soil moisture led to lower nutrient absorption, but Humic acid-based foliar fertilizer treated plots still performed significantly better than the control. In 2023, nutrient uptake recovered, with Humic acid-based foliar fertilizer treated plants exhibiting sustained high levels of nitrogen and potassium absorption, emphasizing the long-term benefits of humic substances in improving plant nutrition. Since the treatments were applied as foliar sprays, nutrient uptake efficiency in potato plants was assessed based on their ability to absorb and utilize nitrogen (N), phosphorus (P), and potassium (K) from the applied solutions rather than soil sources. The highest nutrient uptake was recorded in the Potassium Humate + Reasil Forte Carb-Nitrogen-Humic treatment (T5), followed by Reasil Micro Hydro Mix + Reasil Forte Carb-Nitrogen-Humic (T3).

Nitrogen uptake varied significantly across the years, largely due to environmental conditions affecting foliar absorption. In 2021, nitrogen uptake was highest in all treatments, with the P Potassium Humate + Reasil Forte Carb-Nitrogen-Humic treatment (T5) reaching 161.4 kg/ha, compared to 111.6 kg/ha in the control (T1). However, in 2022, limited atmospheric moisture reduced nitrogen absorption efficiency, with the control dropping to 69.6 kg/ha and even the best-performing treatment 5 (Potassium Humate + Reasil Forte Carb-Nitrogen-Humic treatment) decreasing to 111.8 kg/ha. By 2023, nitrogen uptake rebounded, reaffirming the role of humic substances in improving nutrient bioavailability and translocation within plants.

Phosphorus uptake followed a similar trend, with the highest uptake in 2021, a decline in 2022, and recovery in 2023. In 2021, the Potassium Humate + Reasil Forte Carb-Nitrogen-Humic treatment (T5) recorded the highest phosphorus uptake at 74.1 kg/ha, whereas the control (T1) had the lowest at 51.1 kg/ha. The drought stress in 2022 reduced phosphorus absorption across all treatments, with the control dropping to 31.9 kg/ha and the Potassium Humate + Reasil Forte Carb-Nitrogen-Humic treatment (T5) decreasing to 51.3 kg/ha. By 2023, phosphorus uptake improved again, with humic acid-treated plants maintaining significantly higher levels than the control (T1).

Potassium uptake was also significantly affected by humic acid applications. In 2021, the Potassium Humate + Reasil Forte Carb-Nitrogen-Humic treatment (T5) exhibited the highest uptake at 208.5 kg/ha, while the control (T1) only absorbed 147.6 kg/ha. The drought conditions of 2022 negatively impacted potassium uptake, with the control declining to 92.0 kg/ha and the Potassium Humate + Reasil Forte Carb-Nitrogen-Humic treatment (T5) dropping to 144.4 kg/ha. In 2023, potassium absorption improved again, reinforcing the role of humic substances in enhancing potassium availability.

Table 4. Nutrient Uptake by Silvana Potatoes from Soil (kg/ha)

Treatments	Nitrogen (N)				Phosphorus (P_2O_5)				Potassium (K_2O)			
	2021	2022	2023	Avg.	2021	2022	2023	Avg.	2021	2022	2023	Avg.
T1	111,6	69,6	123,2	101,5	51,1	31,9	56,5	46,5	147,6	92,0	162,9	134,2
T2	122,1	83,2	132,5	112,6	56,0	38,1	60,7	51,6	161,6	110,1	175,3	149,0
T3	128,2	87,1	140,8	118,7	58,8	39,9	64,5	54,4	170,2	115,6	187,0	157,6
T4	147,6	92,4	151,5	130,5	66,6	41,7	68,4	58,9	167,3	104,7	171,7	147,9
T5	161,4	111,8	160,7	144,6	74,1	51,3	73,8	66,4	208,5	144,4	207,6	186,8

T1: Control (No Fertilizer); T2 : Reasil Micro Hydro Mix; T3 : Reasil Micro Hydro Mix + Reasil Forte Carb-Nitrogen-Humic; T4 : Potassium Humate; T5 : Potassium Humate + Reasil Forte Carb-Nitrogen-Humic

One of the primary benefits of foliar-applied humic substances is their ability to optimize nutrient use efficiency by enhancing translocation within the plant. Humic acids facilitate the uptake and redistribution of essential nutrients such as nitrogen (N), phosphorus (P), and potassium (K), reducing nutrient losses and ensuring a steady supply of nutrients during critical growth stages. Recent studies have also highlighted that foliar-applied humic substances can increase photosynthetic efficiency and enzymatic activity, leading to improved nutrient assimilation (Nikbakht et al., 2008; Verlinden et al., 2009; Xiong et al., 2023; Santi et al., 2024). This mechanism plays a crucial role in ensuring stable productivity under varying environmental conditions. These findings align with studies showing that foliar-applied humic substances enhance nutrient absorption, increase plant tolerance to stress, and improve metabolic functions. The results of this study reinforce the benefits of using humic acid-based foliar fertilizers as an effective strategy for increasing potato yield and quality under varying climatic conditions.

Conclusion

This study demonstrated that foliar-applied humic acid-based fertilizers significantly enhance potato yield, tuber quality, and nutrient uptake efficiency. The findings highlight the advantages of humic substances in improving plant metabolism, nutrient translocation, and stress tolerance, ultimately leading to higher productivity and better tuber quality.

The highest potato yield and starch content were consistently observed in the Potassium Humate + Reasil Forte treatment, indicating that humic acid application plays a crucial role in optimizing carbohydrate metabolism and biomass accumulation. Additionally, humic acid-treated plants exhibited improved vitamin C content and reduced nitrate accumulation, suggesting a positive impact on overall tuber nutritional quality.

One of the key benefits of foliar humic application is its ability to enhance nutrient uptake efficiency by facilitating the absorption and redistribution of essential macronutrients such as nitrogen, phosphorus, and potassium. The study confirmed that foliar humic acid application improves enzymatic activity and photosynthetic performance, contributing to more efficient nutrient utilization and plant growth.

Furthermore, the results suggest that foliar humic acid application is a sustainable fertilization strategy that can reduce reliance on excessive chemical fertilizers while maintaining high crop productivity. Given the environmental and economic benefits, humic acid-based foliar fertilization presents a promising approach for enhancing potato production under varying agro-climatic conditions, particularly in regions with limited soil fertility and water availability. Future research should focus on optimizing application rates and timing to maximize the efficiency of foliar-applied humic substances. Additionally, further studies on their interactions with different nutrient management strategies and their long-term impact on soil and plant health will be essential for developing comprehensive, sustainable fertilization programs.

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