The North African Journal of Scientific Publishing (NAJSP)
مجلة شمال إفريقيا للنشر العلمي (NAJSP)
E-ISSN: 2959-4820
Volume 3, Issue 1, January - March 2025
Page No: 97-110
Website: https://najsp.com/index.php/home/index
SJIFactor 2024: 5.49 **0.71 :2024 (AIF) معامل التأثير العربي** **ISI 2024: 0.696**

Assessment of the Physiological and Biochemical Effects of *Phragmites australis* L. Aqueous Extracts from Juliana Lake on the Germination, Growth, and Metabolic Activities of *Triticum aestivum* L.

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تقييم التأثيرات الفسيولوجية والكيميائية الحيوية للمستخلصات المائية لـنبات *Phragmites australis* L. من بحيرة جليانة على إنبات ونمو والأنشطة الأيضية لنبات لـ *Triticum aestivum* L

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Received: November 07, 2024	Accepted: January 21, 2025	Published: February 16, 2025

Abstract:
This study investigates the physiological and biochemical effects of aqueous extracts from *Phragmites australis*. *P. australis* was chosen for its ecological and economic value, providing habitat, stabilising soil, filtering pollutants, and thriving under extreme conditions. Its impact on other plants in saline environments aids in managing resource competition. Due to its importance, samples of leaves and stems were collected from Libya's Juliana Lake in Benghazi in April 2024, a key site with coastal salt marshes that support salt-tolerant organisms and protect against coastal erosion. To study the effect of this invasive plant, wheat (*Triticum aestivum* L.), an adaptable and significant cereal crop in Libya, was used. The findings revealed that higher concentrations of leaf extract had a stronger inhibitory effect on wheat germination compared to stem extracts. The study also examined the impact of these extracts on seedling growth, where lower concentrations of stem extract increased seedling length, while higher concentrations reduced it. Fresh weight varied depending on the extract concentrations, and higher concentrations significantly reduced protein synthesis compared to the control group's levels. These findings suggest potential applications for *Phragmites australis* extracts as natural herbicides at higher concentrations to prevent weed growth, while lower concentrations might stimulate germination and growth under controlled conditions. The dual role of these extracts highlights their complexity and practical utility in ecological management and agricultural practices within saline wetland environments.

Keywords: Allelopathy, *Triticum aestivum*, Natural herbicide, phytotoxicity.

الملخص
تدرس هذه الدراسة التأثيرات الفسيولوجية والكيميائية الحيوية للمستخلصات المائية لنبات *Phragmites australis*. تم اختيار *P. australis* لقيمته البيئية والاقتصادية، حيث يوفر موئلًا للكائنات الحية، ويساهم في تثبيت التربة وتنقية الملوثات، ويزدهر في ظروف قاسية. يسهم تأثيره على النباتات الأخرى في البيئات المالحة في إدارة تنافس الموارد. بسبب أهميته، تم جمع عينات من الأوراق والسيقان من بحيرة جليانة في بنغازي، ليبيا في أبريل 2024، وهو موقع رئيسي يحتوي على أراضي ملحية ساحلية تدعم الكائنات المتحملة للملوحة وتحمي من تآكل الشواطئ. لدراسة تأثير هذا النبات الغازي، تم استخدام القمح(*Triticum aestivum* L.) ، وهو محصول حبوب مهم وقابل للتكيف في ليبيا. كشفت النتائج أن التركيزات الأعلى لمستخلص الأوراق لها تأثير مثبط أقوى على إنبات القمح مقارنة بمستخلص السيقان. كما فحصت الدراسة تأثير هذه المستخلصات على نمو الشتلات، حيث زادت التركيزات المنخفضة لمستخلص السيقان من طول الشتلات بينما قللته التركيزات العالية. تراوح الوزن الطازج حسب تركيزات المستخلصات، وقللت التركيزات العالية بشكل كبير من تخليق البروتين مقارنة بمستويات مجموعة التحكم. تشير هذه النتائج إلى تطبيقات محتملة لمستخلصات *Phragmites australis* كمبيدات أعشاب طبيعية عند التركيزات العالية لمنع نمو الأعشاب، بينما قد تحفز التركيزات المنخفضة الإنبات والنمو في ظروف محكمة. يبرز الدور المزدوج لهذه المستخلصات تعقيدها وفائدتها العملية في الإدارة البيئية والممارسات الزراعية داخل البيئات الرطبة المالحة.

الكلمات المفتاحية: الأليلوباثي، *Triticum aestivum*، مبيد أعشاب طبيعي، السمية النباتية.

Introduction:

Researchers in various scientific fields, including physiology, ecology, geography, and geology, are highly concerned about the biotic and abiotic factors that influence the growth of wild plant species. Some abiotic factors affecting plant species include the availability of food and water resources, pollutants, and harsh environmental conditions such as high temperatures or salinity (Dixit, Sivalingam, Baskaran, Senthil-Kumar, & Ghosh, 2024).

Among the biotic factors, competition between plant populations plays a significant role, involving the capacity of one or more species to encroach. Various explanations exist for the success of invasive plant species in new areas, including life-history traits, physiological attributes, rapid genetic changes, and escape from natural enemies. The ability of invasive species to acquire resources and suppress the vigour of their neighbouring species through chemical interference, known as allelopathy, is a crucial factor that facilitates their successful invasion and establishment, especially in regions where they produce novel biochemical weapons. Invasive species often thrive by producing unique allelopathic chemicals that inhibit native plants, although the full impact of these chemicals is not entirely understood. Enhanced allelochemical production provides invasive plants with a competitive edge. Factors such as resource availability and propagule pressure heavily influence invasiveness, often outweighing species traits. Additionally, extended residence time increases the likelihood of successful invasion and dominance in plant communities (Gioria, Hulme, Richardson, & Pyšek, 2023).

Many plant species produce allelopathic compounds, which can either stimulate or inhibit depending on the identified action type. Various plant organs release these chemicals into the environment, including roots, leaves, and stems. Allelopathy, the process by which these chemicals inhibit the germination, growth, and development of neighbouring plants, can affect even economically or scientifically significant invasive species. These allelopathic compounds, known as secondary metabolites, vary in composition, types, and production reasons according to plant species and their natural habitats. Additionally, environmental stress conditions significantly contribute to their production as a defence mechanism. These compounds enhance the plant's ability to endure harsh environmental conditions, such as salinity in wetlands (marshes) (Hussain, 2020).

Numerous studies have been conducted to determine the causes behind the detrimental effects of certain dominant plants. Wetlands are among the most biodiverse landscapes and are vital natural resources. These ecosystems are often referred to as "nature's kidneys" and "biological gene banks" due to their role in environmental regulation and the conservation of genetic diversity. Moreover, extensive research has identified *Phragmites australis*, an invasive member of the Poaceae family and the genus *Phragmites*, as one of the most pervasive invasive species within wetland environments (Park & Blossey, 2008). The vigorous growth of *Phragmites australis* can quickly establish dense monospecific stands, effectively outcompeting and displacing native plant species. One of the primary factors contributing to the successful invasion of *Phragmites australis* is its strong competitive ability and the production of allelopathic compounds. In addition to rapid clonal propagation, efficient nutrient acquisition, and high salt tolerance, the potential allelopathic impacts on indigenous plant species may also play a crucial role in its invasive success (Md Nazim Uddin & Robinson, 2017).

The aqueous extract of *Phragmites australis* has been demonstrated to effectively inhibit the germination and early growth of certain plant species (T. Rudrappa, Bonsall, Gallagher, Seliskar, & Bais, 2007) (Md N Uddin, Caridi, & Robinson, 2012). However, there is limited understanding of how the extract of Phragmites australis influences the metabolism and development of specific herbal plants

(Md N Uddin et al., 2012; Md N Uddin, Robinson, & Caridi, 2014). By investigating the phytotoxic effects of *Phragmites australis* extract on selected herbal plants, the current study aims to provide experimental evidence to support the hypothesis that the allelopathic chemicals produced by *Phragmites australis* may have inhibitory effects on the recipient plants (Gao *et al.*2022; Uddin and Robinson 2017; Rudrappa *et al.*2007).

Phragmites australis (common reed) is a perennial and belongs to the Poaceae family it can grow up to three meters tall and up to ten feet. The stems in this structure are commonly hollow and often jointed. The root structure of *Phragmites australis* often allows for the vegetative spread of the plant by sending out rhizomes, which reproduce mainly through rhizomes and by seeds but at a low rate. It thrives in temperate regions globally, with a significant presence in North America and numerous European countries (Meyerson, Cronin, & Pyšek, 2016; Packer, Meyerson, Skálová, Pyšek, & Kueffer, 2017; Wang et al., 2023).

Phragmites australis holds significant ecological and economic value. As a typical wetland plant, *Phragmites* plays a crucial role by providing shelter and foraging grounds for various animal species. It contributes to soil stabilisation in wetlands and along water bodies, filters pollutants and excess nutrients, reduces nitrogen loads, and supplies oxygen to the rhizosphere (Wahman, Sauvêtre, Schröder, Moser, & Letzel, 2020).

Extensive research has focused on mitigating environmental contamination, with the common reed (*Phragmites australis*) emerging as a key species due to its resilience in extreme conditions such as high temperatures, elevated CO_2 levels, and high salinity. These traits stem from its adaptive mechanisms, making it a preferred choice in ecological engineering for improving wastewater quality in challenging ecosystems. Salt marshes, known for their unique soil properties and population distribution, play a vital role in ecological protection, particularly in controlling shoreline erosion. Recent efforts have prioritised the creation and restoration of wetlands to address these challenges, with a focus on managing dominant vegetation like *P. australis*, which thrives in diverse ecological systems and plant communities. As one of the most extensively studied species globally, particularly in its invasive ranges, *P. australis* serves as an excellent model organism for studying plant invasions due to its dominance in globally significant wetland habitats. Our understanding of managing this species, especially along the edges of Great Lakes wetlands, continues to evolve (Bowe, Simek, Dávalos, & Blossey, 2024; Lee, Chapman, Mozdzer, Eller, & Langley, 2024).

Salt marshes are essential ecosystems that contain high amounts of seawater and support organisms tolerant to salt. These marshes provide a critical barrier between land and sea, protecting the land from coastal erosion and hosting a diverse range of plants and habitats for various bird species throughout the year. Libya's coastline spans approximately 1975 km, featuring extensive salt marshes. During the summer, high salinity and evaporation leave many of these marshes largely uninhabited by plants, resulting in a thick salt layer on the marsh floor. Vegetation around these marshes is sparse, mainly consisting of halophytes and desert plants due to coarse sandy soils and semiarid conditions. These salt marshes play a significant role in the ecosystem, offering resources like salt and chemicals, and are important to local communities and wildlife. Benghazi's urban area (NE Libya) retains some of the few remaining natural habitats, including karst lakes and coastal salt marshes (locally known as Sebkhas), with Juliana Lake being a prominent example (Blizzard, 2023; Elbabour & Abdulsamad, 2018).

This scientific paper aims to clarify the significance of *Phragmites australis*, a prominent plant species in Sebkha, and its potential benefits in colonizing marshes and wetlands, enhancing soil quality and purifying pollutants. It is essential to study the impact of this species on the germination and development of seedlings of other economically important plants that can thrive in saline marshy environments. Understanding these interactions aids in managing resource competition among plant populations, which determines the ecological structure at various scales.

Coastal wetlands host a wide range of competitive *Phragmites australis* that thrive in harsh conditions due to their high adaptability. Despite its allelopathic effects, the influence of *P. australis* on adjacent plant growth remains understudied in precise physiological experiments. Soil salinization has become a global issue, making the study of plant salt tolerance crucial for enhancing crop productivity, developing salt-tolerant plants, and utilizing saline land effectively. Given the high salinity in marsh environments, alongside the stress from dominant species like reed plants, it is vital to select economically important species that have demonstrated salinity tolerance(Gao et al., 2022).

Wheat (*Triticum aestivum* L.) is a highly adaptable species, capable of growing in diverse environments and being sown in both winter and spring, which contributes to its global distribution and high consumption. Wheat can be classified based on type (winter or spring), hardness (hard or soft), colour (red or white), and protein content. It is a crucial cereal crop in Libya, germinating at temperatures between 4°C and 37°C, with an optimal range of 12°C to 25°C. Wheat is a staple food, rich in minerals,

vitamins, amino acids, beneficial metabolites, and dietary fibres (Filip, Woronko, Stępień, & Czarniecka, 2023).
Ongoing research aims to evaluate wheat's ability to withstand various stress conditions, both biological (such as exposure to allelopathy and other wild plants) and abiotic (such as resistance to pests and harsh environmental conditions like salinity, drought, or high temperatures). This research also investigates the effects of chemical pollutants, such as heavy metals, on wheat. The objective is to produce plant varieties that can tolerate harsh environmental conditions, thereby improving soil properties in those areas. One of the study's goals is to cultivate plant varieties that are economically viable in conditions of drought, salinity, and competition with other plants (Chaichi et al., 2022).
To understand the response of seed germination and early seedling growth of *Triticum* aestivum L. (Poaceae) to extracts from various organs of *P. australis* at different concentrations, germination experiments were conducted using various methods. The study sought to answer whether *P. australis* extracts affect the germination and growth of oat seeds, which plant part has the most significant influence, and what the effective concentrations are.

Material and methods:

1. Study site description:

"Juliana Lake, also known as Benghazi Lake, was one of the most significant Karst lakes and Sebkha formations in the region. It is located in the southwest of Benghazi city, at the southwestern end of Lake 23rd July, which is connected to Benghazi Harbor. Its geographical coordinates are approximately 32°05'19.68" N, 20°03'18.81" E."

2. Seeds Collection:

The seeds of *Triticum aestivum* L. were from local seeds and were collected from the local market in the city of Benghazi / the eastern part of Libya.

3. Sample collection and aqueous extracts of *Phragmites australis*:

In April 2024, leaves and stems of *Phragmites australis* were collected from Juliana Lake. Individual organs (leaves and stems) were dried at 40°C in an oven until a constant weight was achieved. The oven-dried plant samples were then ground and sieved (0.5 mm) for further experiments.

Figure 1: Geographical features and location chart of Juliana Lake, Benghazi City.
https://maps.app.goo.gl/991j4uRTmdziGvd48

To prepare the extracts, 20 g of dry ground material from each organ was submerged in 100 mL of distilled water (20% extract) and agitated on a shaker (Bibby Stuart Platform Rocker STR6) at 70 rpm for 24 hours at room temperature. The plant extracts were filtered first through medical gauze sheets and then through Whatman No. 9 filter paper.
The pH of the stock solution of the aquatic plant extract was determined using a pH meter (TRACER PockeTester™ pH/Salt/TDS/Conductivity/Temp). The pH of the 20% aquatic extract of leaves was 5.12 at 23°C, and the pH of the 20% aquatic extract of stems was 4.31 at 23°C(Md N Uddin et al., 2014)

In addition to using distilled water as a control (0%), five concentrations of the extracts were prepared (2.5%, 5.0%, 10.0%, 15.0%, and 20.0%) from both the leaf and stem extracts. These were stored in labeled bottles in a refrigerator at 5°C."

4. Germination Count and Growth Parameters:

Purified seeds of the above-mentioned plant species were selected of similar size to avoid any morphological variations. They are sterilized with 3 % sodium hypochlorite (Clorox) which is a chemical compound with the formula (NaOCl). Sodium hypochlorite solution is frequently used as a disinfectant. Seeds were thoroughly washed with distilled water many times, and twice with their tested solutions (Yamaguchi *et al*., 1998).

Seeds were germinated in a 9 cm diameter petri dish containing two layers of filter paper with 5 ml of test solution. Ten selected *Triticum aestivum* L. and *Ricinus communis* seeds of full and uniform size were placed evenly on the filter paper after the filter paper had been completely soaked with test solution or water as control, and three replicates of Petri dishes were set up for each treatment.

They were incubated at room temperature. Distilled water and tested solutions were added whenever were needed. Seeds were allowed to germinate for one week. Germination Parameters were calculated by using the following formula:

- First Day of Germination (FDG) (day): -

  FDG=Day on which the first germination event occurred
- Last Day of Germination (LDG) (day): -

  LDG=Day on which the last germination event occurred
- Time Spread of Germination (TSG) (day): -

  TSG=The time in days between the first and last germination events occurring in a seed lot (Kader, 2005)
- Final Germination Percentage (FGP) (%)

  FGP= Final no. of seeds germinated in a seed lot × 100

The higher the FGP value, the greater the germination of a seed population. (Martínez-Peralta, Altamirano-Vázquez, Rojas-Aréchiga, Mandujano, & Golubov, 2024; Seid, Wondimu, Degu, & Assefa, 2023)

- Mean germination time (MGT) (day): -

$$MGT = \sum n \times d / \sum n$$

n=Seeds germinated on day d

The lower the MGT, the faster a population of seeds has germinated. (Martínez-Peralta et al., 2024)

- Mean daily germination (MDG): -

$$MDG = FGP/D$$

FGP is the final germination per cent, D is a day of maximum germination (experiment period) (Seid et al., 2023; Xu & Du, 2023)

- Coefficient of Velocity of Germination (CVG): -

$$CVG = \frac{\sum(\frac{G}{T})}{\sum G} \times 100$$

Where:

G is the number of seeds germinated each day. - T is the number of days from the start of the germination period. - ∑G is the total number of seeds germinated. (Lazim & Ramadhan, 2019)

- Germination Rate Index (GRI) (%/day): -

$$GRI = \frac{G1}{T1} + \frac{G2}{T2} + \cdots + \frac{Gn}{Tn}$$

G= is the number of seeds germinated on a specific day.

T= is the number of days from the start of the germination period.(Ullah et al., 2022)

- Peak Value (PV): -

  PV = Highest seed germinated/ Number of days

(Asinwa, Kazeem-Ibrahim, Olaifa, & Asabia, 2019; Prasad, 2012)

- Germination Value (GV): -

$$GV = PV \times MDG$$

PV = Peak Value, MDG= Mean daily germination (Asinwa et al., 2019)

5. Early seedling growth:

The germinated seeds of both species under different types of extracts were allowed to grow for some time to develop into seedlings by keeping them under the same conditions for another two weeks after the first week of germination. The seedlings were harvested and the following measurements were carried out, these include: shoot and root lengths (cm), and fresh weight of seedlings (g) .Seedlings were separated, whenever it is possible, into their seedlings and were dried in an oven at 85 °C for

seventy-two hours, and then weighed in grams using four – decimals balance. Seedling Parameters were calculated by using the following formula:

1. Seedling lengths of shoots and roots (cm)
2. Fresh and dry weight of seedlings (g)
3. Seedling vigor index (SVI): -

$$\text{SVI} = \text{Seedling length (cm)} \times \text{Germination percentage}$$

(Rashi & Kaushik, 2024)

4. Tolerance index (TI) (%): -

$$\text{TI} = \frac{\text{Length of seedling in treatment}}{\text{Length of seedling in control}} \times 100$$

(Hakmaoui, Ater *et* al. 2007)

5. The tissue water content (TWC) (%): -

$$\text{TWC} = \frac{\text{Freshweight} - \text{Dry weight}}{\text{Freshweight}} \times 100$$

(Aghamir, Bahrami, Malakouti, Eshghi, & Sharifi, 2016)

6. Protein contents and quantification

- Sample Preparation:
  - Approximately 1 gram of plant sample.
- **Digestion**
  - The sample is hydrolyzed with 15 mL of concentrated sulfuric acid (H_2SO_4) containing two copper catalyst tablets.
  - The mixture is heated in a heat block (Kjeltec system 2020 digestor, Tecator Inc., Herndon, VA, USA) at 420 °C for 2 hours.
- **Cooling and Addition**
  - After digestion, the sample is allowed to cool.
  - Water is added to the hydrolysates.
- **Neutralization and Titration**
  - The mixture is neutralized.
  - Titration is performed to determine the amount of total nitrogen.
- **Protein Content Calculation**
  - The total nitrogen content in the raw materials is multiplied by the traditional conversion factor of 6.25.
  - Species-specific conversion factors are also used to determine the total protein content.

$$\text{Total Protein} = \text{N} \times \text{Conversion Factor}$$ (Cunniff & Washington, 1997)

    - N represents the total nitrogen content measured in the sample.
    - 6.25 (Conversion Factor): This factor is used to convert the amount of nitrogen in the sample to the equivalent amount of protein. The factor 6.25 assumes that proteins typically contain 16% nitrogen by weight (1/0.16 = 6.25).
  - Convert Total Protein to Percentage (Horwitz, 2000):

$$\text{Protein Percentage} = \left(\frac{\text{Total Protein}}{\text{Weight of the Sample}}\right) \times 100$$

Statistical analysis:

The data of all experiments were statistically analyzed using the computer program Minitab (Professional Version 19). One–way analysis of variance was used for the determination of the significance within the treatment, and Tukey's *pairwise* comparisons test to determine the significant differences between the means. (Arminian, Karimi, Rodrigues, & Ozgur, 2019; Metz, 2008)

Results and discussion

1. Germination Parameters of *Triticum aestivum* L.

This study evaluated the effects of various concentrations of leaf and stem extracts from *Phragmites australis* on the final germination percentage (FGP) of *Triticum aestivum* (wheat) seeds. The control group treated with distilled water had an FGP of 100.0% ± 0.00, providing a baseline for comparison. For the stem extract, a 2.5% concentration maintained an FGP of 100.0% ± 0.00, similar to the control. However, higher concentrations significantly reduced the FGP: 70.0% ± 5.77 at 5.0%, 60.0% ± 5.77 at 10.0%, 53.3% ± 3.33 at 15.0%, and 33.3% ± 3.33 at 20.0%. The one-way ANOVA for the stem extract showed a highly significant impact on FGP ($F_{(5, 12)} = 47.5$, $P < 0.001$). This decrease suggests that phytochemicals in the stem extract might interfere with water uptake or enzymatic activities essential for germination, leading to inhibition. Similarly, the leaf extract demonstrated a concentration-

dependent inhibitory effect on FGP. At a 2.5% concentration, the FGP was 80.0% ± 5.77, but it significantly decreased with higher concentrations: 66.7% ± 3.33 at 5.0%, 46.6% ± 3.33 at 10.0%, 13.3% ± 3.33 at 15.0%, and 6.67% ± 3.33 at 20.0%. The one-way ANOVA for the leaf extract indicated significant differences among concentrations ($F_{(5, 12)} = 106.2$, $P < 0.001$), suggesting a stronger inhibitory effect than the stem extract. This could be due to higher concentrations of allelopathic compounds in the leaves, known to suppress seed germination and growth. Previous studies support these findings, highlighting the allelopathic effects of *Phragmites australis*. For example, research by (Batish, 2007) found that *Phragmites australis* leaf extracts inhibit the germination and growth of various crops. Similarly, studies by (T. Rudrappa, Bonsall, J., Gallagher, J.L., Seliskar, D.M., & Bais, H.P. , 2007) showed that root exudates from *Phragmites australis* have significant allelopathic effects on neighbouring plant species. In summary, both stem and leaf extracts of *Phragmites australis* exhibit a concentration-dependent inhibitory effect on the germination of *Triticum aestivum* seeds. The leaf extract, however, demonstrates a more pronounced inhibitory effect at lower concentrations compared to the stem extract, likely due to higher levels of allelopathic compounds. These findings suggest potential applications for *Phragmites australis* extracts as natural herbicides in agricultural practices. (Figure 1 B)

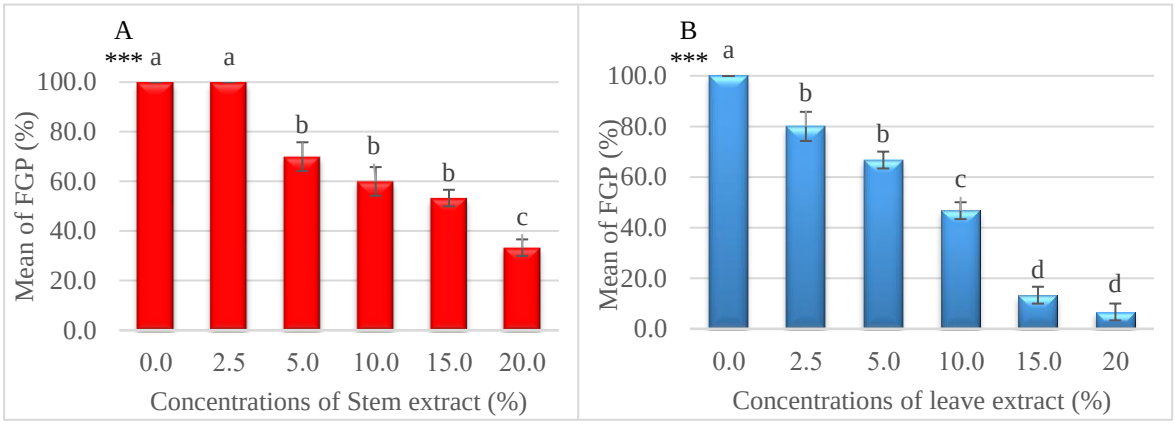


Figure 2: Effect of different concentrations of *Phragmites australis* (A) stem extract (B) leaves extract on final germination percentage (FGP) of *Triticum aestivum* L. (Wheat) seeds.
*** = *P*< 0.0001. Similar letters = Not significant. Different letters = significant. Bar = SEMean.

Similar to the final germination percentage, in Tables 1 & 2, this study evaluated the effects of varying concentrations of leaf and stem extracts from Phragmites australis on the germination of *Triticum aestivum* (wheat) seeds, analyzing the first day of germination (FDG), the last day of germination (LDG), the time spread of germination (TSG), mean time germination (MTG), mean daily germination (MDG), coefficient velocity of germination (CVG), germination rate index (GRI), peak value (PV), and germination value (GV).

"The effects of various concentrations of leaf and stem extracts from *Phragmites australis* on the germination of *Triticum aestivum* (wheat) seeds were evaluated using descriptive statistics, one-way ANOVA, and Tukey's test."
The first day of germination (FDG) in the control group was 1.00 ± 0.00, establishing a baseline. For the stem extract, concentrations of 2.5%, 5.0%, and 10.0% did not affect the FDG (1.00 ± 0.00). However, concentrations of 15.0% and 20.0% delayed germination (FDG of 2.00 ± 0.00 and 3.33 ± 0.33, respectively), with a significant effect indicated by the ANOVA ($F_{(5, 12)} = 49.6$, $P < 0.01$). This delay suggests that higher concentrations of the stem extract contain phytochemicals that inhibit the initial germination process. For the leaf extract, FDG remained unaffected at lower concentrations (2.5% and 5.0%), but higher concentrations of 10.0%, 15.0%, and 20.0% resulted in delayed germination (FDG of 1.33 ± 0.33, 5.00 ± 1.15, and 4.67 ± 1.20, respectively), with the ANOVA showing significant differences ($F_{(5, 12)} = 7.85$, $P < 0.01$). The more pronounced delay with leaf extract at higher concentrations might be due to higher levels of allelopathic compounds known to impede seed germination.

The last day of germination (LDG)

LDG for the control group was 1.00 ± 0.88. For the stem extract, concentrations of 2.5%, 5.0%, 10.0%, 15.0%, and 20.0% showed varying LDGs, with no significant effect indicated by ANOVA ($F_{(5, 12)} = 3.74$,

$P < 0.05$), suggesting that the stem extract's impact on LDG is less consistent. In contrast, the leaf extract had higher LDGs at all concentrations, with significant differences only between certain groups ($F_{(5, 12)} = 2.11$, $P = 0.134$), implying that leaf extract may prolong the germination period more variably than stem extract.

The time spread of germination (TSG)

TSG in the control group was 0.00 ± 0.00. Stem extract concentrations showed no significant differences in TSG ($F_{(5, 12)} = 2.06$, $P = 0.142$), while leaf extract concentrations also did not significantly affect TSG ($F_{(5, 12)} = 1.94$, $P = 0.161$), indicating minimal impact on the overall spread of the germination process.

Mean Time Germination (MTG)

MTG showed that the control group had an MTG of 1.00 ± 0.00. For the stem extract, MTG increased significantly at 15.0% and 20.0% concentrations (3.34 ± 0.43 and 3.80 ± 0.29, respectively), with ANOVA indicating a highly significant effect ($F_{(5, 12)} = 26.1$, $P < 0.001$). Similarly, the leaf extract caused significant delays at higher concentrations, particularly at 15.0% and 20.0% (5.66 ± 0.66 and 2.33 ± 1.20), with ANOVA confirming significant effects ($F_{(5, 12)} = 12.61$, $P < 0.01$). The increased MTG indicates that higher concentrations of both extracts delay germination, likely due to phytochemicals interfering with enzymatic activities essential for seed germination.

Table 1: Effect of different concentrations of *Phragmites australis* stem extract on first day of germination (FDG), last day of germination (LDG), time spread of germination (TSG), mean time germination (MTG), mean daily germination (MDG), coefficient velocity of germination (CVG), germination rate index (GRI) peak value (PV) and germination value (GV) of *Triticum* aestivum L. (Wheat) seeds.

Conc. (%)	FDG	LDG	TSG	MGT	MDG	CVG	GRI	PV	GV
0.00	** $1.00^{a} \pm 0.00$	* $1.00^{a} \pm 0.88$	+ $0.00^{a} \pm 0.00$	*** $1.00^{a} \pm 0.00$	*** $14.29^{a} \pm 0.00$	*** $100.0^{a} \pm 0.00$	*** $10.0^{a} \pm 0.00$	*** $1.43^{a} \pm 0.00$	*** $20.4^{a} \pm 0.00$
2.5	$1.00^{a} \pm 0.00$	$2.66^{a} \pm 0.88$	$1.66^{a} \pm 0.88$	$1.16^{a} \pm 0.08$	$14.28^{a} \pm 0.00$	$75.1^{ab} \pm 12.5$	$9.19^{a} \pm 0.10$	$1.38^{a} \pm 0.04$	$19.7^{a} \pm 0.68$
5.0	$1.00^{a} \pm 0.00$	$4.00^{a} \pm 1.53$	$3.00^{a} \pm 1.53$	$1.61^{a} \pm 0.23$	$10.0^{b} \pm 0.82$	$59.2^{bc} \pm 7.00$	$5.77^{b} \pm 0.80$	$1.00^{a} \pm 0.08$	$10.1^{b} \pm 1.65$
10.0	$1.00^{a} \pm 0.00$	$1.66^{a} \pm 0.33$	$0.66^{a} \pm 0.33$	$1.32^{a} \pm 0.06$	$8.57^{b} \pm 0.83$	$66.5^{b} \pm 4.61$	$5.11^{b} \pm 0.38$	$0.85^{a} \pm 0.08$	$7.48^{b} \pm 1.42$
15.0	$2.00^{b} \pm 0.00$	$5.00^{a} \pm 1.00$	$3.00^{a} \pm 1.00$	$3.34^{b} \pm 043$	$7.61^{b} \pm 0.47$	$30.5^{cd} \pm 1.72$	$1.90^{c} \pm 0.18$	$0.76^{ab} \pm 0.04$	$5.85^{bc} \pm 0.74$
20.0	$3.33^{c} \pm 0.33$	$4.66^{a} \pm 0.33$	$1.33^{a} \pm 0.33$	$3.80^{c} \pm 0.29$	$4.76^{c} \pm 0.48$	$24.9^{d} \pm 1.84$	$0.91^{c} \pm 0.12$	$0.47^{b} \pm 0.04$	$2.31^{c} \pm 0.47$

*** = $P < 0.0001 / 0.001$. ** = $P < 0.01$. * = $P < 0.05$. + = $P > 0.05$.
Similar letters = Not significant. Different letters = Significant. ± = SEMean.

Table 2: Effect of different concentrations of *Phragmites australis* leave extract on first day of germination (FDG), last day of germination (LDG), time spread of germination (TSG), mean time germination (MTG), mean daily germination (MDG), coefficient velocity of germination (CVG), germination rate index (GRI) peak value (PV) and germination value (GV) of *Triticum* aestivum L. (Wheat) seeds.

Conc. (%)	FDG	LDG	TSG	MGT	MDG	CVG	GRI	PV	GV
0.00	** $1.00^{a} \pm 0.00$	+ $1.00^{a} \pm 0.00$	+ $0.00^{a} \pm 0.00$	** $1.00^{a} \pm 0.00$	*** $14.2^{a} \pm 0.00$	*** $100.0^{a} \pm 0.00$	*** $10.0^{a} \pm 0.00$	*** $1.42^{a} \pm 0.00$	*** $20.4^{a} \pm 0.00$
2.5	$1.00^{a} \pm 0.00$	$4.67^{a} \pm 1.86$	$3.67^{a} \pm 1.86$	$1.68^{ab} \pm 0.38$	$11.4^{b} \pm 0.82$	$67.6^{ab} \pm 16.4$	$6.83^{ab} \pm 0.58$	$1.14^{b} \pm 0.08$	$13.2^{b} \pm 1.89$
5.0	$1.00^{a} \pm 0.00$	$5.00^{a} \pm 2.00$	$4.00^{a} \pm 2.00$	$2.00^{ab} \pm 0.63$	$9.52^{b} \pm 0.47$	$66.3^{ab} \pm 17.0$	$5.64^{bc} \pm 0.96$	$0.95^{b} \pm 0.05$	$9.12^{b} \pm 0.88$
10.0	$1.33^{a} \pm 0.33$	$3.33^{a} \pm 0.33$	$2.66^{a} \pm 0.66$	$2.36^{ab} \pm 0.56$	$6.66^{c} \pm 0.50$	$43.8^{b} \pm 7.67$	$2.71^{c} \pm 0.61$	$0.66^{c} \pm 0.05$	$4.49^{c} \pm 0.61$
15.0	$5.00^{b} \pm 1.15$	$6.33^{a} \pm 0.66$	$1.33^{a} \pm 1.33$	$5.66^{bc} \pm 0.66$	$1.90^{d} \pm 0.47$	$18.3^{b} \pm 2.02$	$0.27^{d} \pm 0.10$	$0.19^{d} \pm 0.05$	$0.40^{cd} \pm 0.21$
20.0	$4.67^{b} \pm 1.20$	$4.67^{a} \pm 1.20$	$0.00^{a} \pm 0.00$	$2.33^{c} \pm 1.20$	$0.95^{d} \pm 0.47$	$19.4^{b} \pm 10.0$	$0.19^{d} \pm 0.10$	$0.09^{d} \pm 0.04$	$0.13^{d} \pm 0.06$

*** = P< 0.0001 / 0.001. ** = P< 0.01. * = P< 0.05. + = P> 0.05.
Similar letters = Not significant. Different letters = Significant. ± = SEMean.

The mean Daily Germination (MDG)

MDG in the control was 14.29 ± 0.00. Stem extract at 5.0%, 10.0%, 15.0%, and 20.0% significantly reduced MDG, with 20.0% showing the lowest MDG (4.76 ± 0.48), and ANOVA revealing significant effects ($F_{(5, 12)} = 47.5$, $P < 0.001$). For the leaf extract, higher concentrations drastically decreased MDG, particularly at 15.0% and 20.0% (1.90 ± 0.47 and 0.95 ± 0.47), with ANOVA indicating strong significance ($F_{(5, 12)} = 106.1$, $P < 0.001$). These results suggest both extracts hinder germination rates, with leaf extract having a more substantial impact.

The coefficient Velocity of Germination (CVG)

CVG for the control group was 100.0 ± 0.00. The stem extract significantly decreased CVG at higher concentrations, with 20.0% showing the lowest CVG (24.9 ± 1.84), as supported by ANOVA ($F_{(5, 12)} = 20.4$, $P < 0.001$). The leaf extract also decreased CVG, especially at 15.0% and 20.0% concentrations (18.3 ± 2.02 and 19.4 ± 10.0), confirmed by ANOVA ($F_{(5, 12)} = 8.34$, $P < 0.001$). This indicates both extracts slow down the speed of germination, with leaf extract showing a more pronounced effect at lower concentrations.

The Germination Rate Index (GRI)

GRI was 10.0 ± 0.00 in the control group. The stem extract significantly reduced GRI at all tested concentrations, especially at 20.0% (0.91 ± 0.12), with ANOVA showing strong significance ($F_{(5, 12)} = 95.70$, $P < 0.001$). The leaf extract had an even more significant inhibitory effect, drastically reducing GRI at 15.0% and 20.0% (0.27 ± 0.10 and 0.19 ± 0.10), with ANOVA revealing highly significant differences ($F_{(5, 12)} = 51.8$, $P < 0.001$). This suggests both extracts severely inhibit the overall germination rate, with leaf extract having a more substantial impact.

Peak Value (PV)

PV for the control was 1.43 ± 0.00. The stem extract caused significant reductions in PV at 15.0% and 20.0% (0.76 ± 0.04 and 0.47 ± 0.04), supported by ANOVA ($F_{(5, 12)} = 39.9$, $P < 0.001$). The leaf extract had an even more significant effect, with PV dropping drastically at 15.0% and 20.0% (0.19 ± 0.05 and 0.09 ± 0.04), as confirmed by ANOVA ($F_{(5, 12)} = 106.17$, $P < 0.001$). These results indicate that higher concentrations of both extracts significantly reduce the peak germination rate.

Germination Value (GV)

GV in the control group was 20.4 ± 0.00. The stem extract significantly decreased GV at all concentrations, particularly at 20.0% (2.31 ± 0.47), with ANOVA revealing significant effects ($F_{(5, 12)} = 56.1$, $P < 0.001$). The leaf extract also significantly reduced GV, especially at higher concentrations (0.40 ± 0.21 and 0.13 ± 0.06 for 15.0% and 20.0%), with ANOVA showing highly significant differences ($F_{(5, 12)} = 79.13$, $P < 0.001$). This suggests that both extracts strongly inhibit overall germination potential, with leaf extract exhibiting a stronger inhibitory effect.

Previous research aligns with these findings, such as the studies by (Batish, 2007) and (T. Rudrappa, Bonsall, J., Gallagher, J.L., Seliskar, D.M., & Bais, H.P. , 2007), which showed that *Phragmites australis* extracts inhibit the germination and growth of various plants due to their allelopathic properties. The significant delays in the first day of germination (FDG) inconsistent effects on the last day of germination (LDG) and time spread of germination (TSG) observed in this study suggest that both leaf and stem extracts of *Phragmites australis* have potent inhibitory effects on wheat germination, likely due to the presence of allelopathic compounds. These strong inhibitory effects on other germination metrics such as mean time germination (MTG), mean daily germination (MDG), coefficient velocity of germination (CVG), germination rate index (GRI), peak value (PV), and germination value (GV) further support the conclusion that these extracts significantly hinder the germination process.

The results indicate that the leaf extract generally had a more substantial inhibitory effect on germination compared to the stem extract. For instance, the leaf extract significantly reduced MDG at higher concentrations, with 15.0% and 20.0% concentrations showing drastic reductions (1.90 ± 0.47 and 0.95 ± 0.47, respectively), whereas the stem extract showed less dramatic but still significant reductions. Similarly, the leaf extract had stronger inhibitory effects on SVI and TI metrics. However, interestingly, at lower concentrations (2.5%), both extracts appeared to stimulate germination to some extent, with the stem extract.

These findings suggest that while higher concentrations of both extracts inhibit germination due to their allelopathic compounds, lower concentrations might stimulate germination. This highlights the complex nature of allelopathy, where the same compounds can have different effects based on their concentration. This dual effect underlines the potential of *Phragmites australis* extracts not only as natural herbicides at higher concentrations but also possibly as growth stimulators at lower concentrations.

2. Seedling growth of *Triticum aestivum* L.

The treatments with different extracts of *P. australis* significantly affected the growth, development and bio-physiological parameters of wheat seeds (Tabes 3 & 4).

In this study, we examined the effects of different concentrations of stem and leaf extracts from *Phragmites australis* on the development of *Triticum aestivum* (wheat) seedlings, using a range of metrics including seedling length, fresh weight, dry weight, seedling vigour index (SVI), tolerance index (TI), and tissue water content (TWC).

Seedling Length

The control group had a seedling length of 18.8 cm ± 1.34. For the stem extract, lengths at 2.5% concentration (23.6 cm ± 1.44) exceeded the control, but higher concentrations showed significant reductions, especially at 20.0% (3.33 cm ± 0.96), with ANOVA indicating a significant effect ($F_{(5, 12)} = 13.54$, $P < 0.001$). Similarly, the leaf extract led to significant reductions at higher concentrations, with 10.0%, 15.0%, and 20.0% concentrations resulting in lengths of 8.80 cm ± 2.21, 0.833 cm ± 0.59, and 1.167 cm ± 0.82 respectively ($F_{(5, 173)} = 27.05$, $P < 0.001$). The shorter lengths suggest phytochemicals in the extracts inhibit growth.

Fresh Weight

For fresh weight, the control group was 0.211 g ± 0.015. The stem extract showed the highest fresh weight at 2.5% (0.288 g ± 0.016), but this declined significantly at higher concentrations, particularly 20.0% (0.076 g ± 0.024), indicated by ANOVA ($F_{(5, 173)} = 7.25$, $P < 0.001$). Similarly, leaf extract significantly reduced fresh weight at higher concentrations, notably 15.0% and 20.0% (0.016 g ± 0.011 and 0.007 g ± 0.005 respectively) ($F_{(5, 174)} = 24.92$, $P < 0.001$).

Dry Weight

The control group's dry weight was 0.023 g ± 0.002. The stem extract increased dry weight at 2.5% (0.029 g ± 0.003) but reduced it significantly at higher concentrations, particularly 20.0% (0.013 g ± 0.004) ($F_{(5, 174)} = 3.68$, $P < 0.001$). The leaf extract showed a similar pattern, with dry weight significantly reduced at 15.0% and 20.0% (0.002 g ± 0.001 and 0.003 g ± 0.002) ($F_{(5, 174)} = 6.45$, $P < 0.001$).

Seedling Vigor Index (SVI)

The control group had an SVI of 1883 ± 343. The stem extract increased SVI at 2.5% (2360 ± 100) but drastically reduced it at higher concentrations, particularly 20.0% (110.7 ± 16.3) ($F_{(5, 12)} = 25.48$, $P < 0.001$). The leaf extract significantly reduced SVI at higher concentrations, particularly at 15.0% and 20.0% (16.7 ± 16.7 and 11.7 ± 11.7) ($F_{(5, 12)} = 11.90$, $P < 0.001$).

Tolerance Index (TI)

The control TI was 0.93 ± 0.046. The stem extract decreased TI significantly at 15.0% and 20.0% concentrations (0.65 ± 0.142 and 0.21 ± 0.074) ($F_{(5, 174)} = 6.15$, $P < 0.01$). The leaf extract showed even more significant reductions at higher concentrations, particularly 15.0% and 20.0% (0.490 ± 0.342 and 0.445 ± 0.347) ($F_{(5, 174)} = 13.63$, $P < 0.001$).

Tissue Water Content (TWC)

The control TWC was 82.7% ± 4.14. The stem extract significantly reduced TWC at higher concentrations, particularly 20.0% (21.7% ± 7.87) ($F_{(5, 174)} = 11.50$, $P < 0.001$). The leaf extract showed an even greater reduction in TWC at 15.0% and 20.0% (5.66% ± 3.93 and 3.82% ± 2.66) ($F_{(5, 174)} = 28.06$, $P < 0.001$).

The results indicate that while both extracts at lower concentrations (2.5%) can have positive effects on certain metrics such as seedling length and fresh weight**,** seedling vigour index (SVI)**,** tolerance index (TI) and tissue water content (TWC), generally have negative impacts at higher concentrations. The leaf extract generally had a more substantial inhibitory effect on germination and seedling development compared to the stem extract. For example, the stem extract at 2.5% concentration increased seedling length to 23.6 cm ± 1.44 and fresh weight to 0.288 g ± 0.016, demonstrating a positive physiological effect. Conversely, higher concentrations of the stem extract, such as 20.0%, reduced seedling length to 3.33 cm ± 0.96 and fresh weight to 0.076 g ± 0.024.

Table 3: Effect of different concentrations of *Phragmites australis* stem extract on seedlings of *Triticum aestivum* L. (Wheat) seedlings.

Conc. (%)	Seedling length(cm)	Fresh weight (g)	Dry weight (g)	SVI	TI	TWC
0.00	*** 18.8^{ab} ± 1.34	*** 0.211^{ab} ± 0.015	** 0.023^{ab} ± 0.002	*** 1883^{a} ± 343	** 0.93^{a} ± 0.04	*** 82.7^{a} ± 4.14
2.5	23.6^{a} ± 1.44	0.288^{a} ± 0.016	0.029^{a} ± 0.003	2360^{a} ± 100	1.12^{a} ± 0.10	83.5^{a} ± 4.25
5.0	18.0^{ab} ± 2.48	0.201^{ab} ± 0.028	0.018^{ab} ± 0.003	1229^{ab} ± 105	1.01^{a} ± 0.19	60.6^{ab} ± 7.97
10.0	17.7^{ab} ± 2.65	0.208^{ab} ± 0.035	0.019^{ab} ± 0.003	1072^{ab} ± 128	1.02^{a} ± 0.18	56.5^{b} ± 8.05
15.0	11.4^{b} ± 2.11	0.156^{bc} ± 0.031	0.020^{ab} ± 0.004	605.7^{bc} ± 59.3	0.65^{ab} ± 0.14	44.1^{bc} ± 8.02
20.0	3.33^{c} ± 0.96	0.076^{c} ± 0.024	0.013^{b} ± 0.004	110.7^{c} ± 16.3	0.21^{b} ± 0.07	21.7^{c} ± 7.87

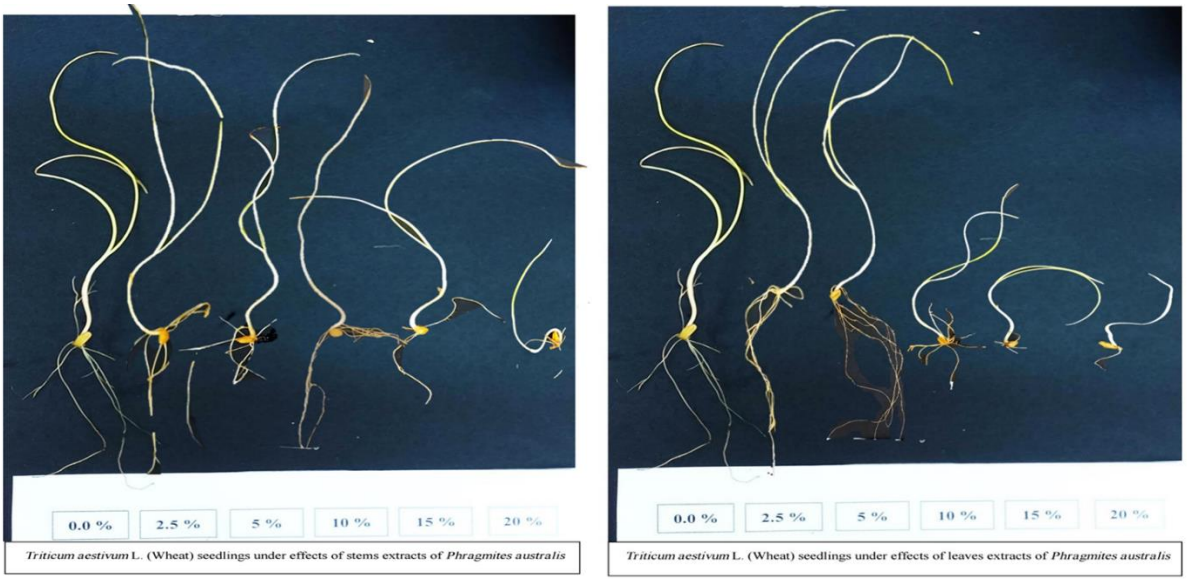
*** = *P*< 0.001. ** = *P*< 0.01. Similar letters = Not significant.
Different letters = Significant. ± = SEMean. SVI = seedling vigor index.
TI = tolerance index TWC= The tissue water content

Table 4: Effect of different concentrations of *Phragmites australis* leaves extract on seedlings of *Triticum aestivum* L. (Wheat) seedlings.

Conc. (%)	Seedling length (cm)	Fresh weight (g)	Dry weight (g)	SVI	TI	TWC
0.00	*** 18.83^{a} ± 1.34	*** 0.211^{a} ± 0.014	*** 0.023^{a} ± 0.001	*** 1883^{a} ± 343	*** 3.055^{a} ± 0.263	*** 82.73^{a} ± 4.14
2.5	21.17^{a} ± 2.35	0.248^{a} ± 0.029	0.037^{ab} ± 0.012	1703^{a} ± 189	2.525^{ab} ± 0.278	63.15^{a} ± 8.29
5.0	20.27^{a} ± 2.60	0.246^{a} ± 0.031	0.024^{ab} ± 0.003	1378^{b} ± 420	1.852^{bc} ± 0.239	62.9^{a} ± 7.66
10.0	8.80^{b} ± 2.21	0.112^{b} ± 0.027	0.012^{b} ± 0.003	433^{b} ± 188	1.098^{cd} ± 0.272	32.8^{b} ± 8.01
15.0	0.833^{c} ± 0.59	0.016^{c} ± 0.011	0.002^{b} ± 0.001	16.7^{b} ± 16.7	0.490^{d} ± 0.342	5.66^{c} ± 3.93
20.0	1.167^{c} ± 0.82	0.007^{c} ± 0.005	0.003^{b} ± 0.002	11.7^{b} ± 11.7	0.445^{d} ± 0.347	3.82^{c} ± 2.66

*** = *P*< 0.001. ** = *P*< 0.01. Similar letters = Not significant.
Different letters = Significant. ± = SEMean. SVI = seedling vigor index.
TI = tolerance index

These findings suggest that *Phragmites australis* extracts could potentially be used as natural herbicides at higher concentrations to inhibit unwanted plant growth, while at lower concentrations, they might stimulate growth in a controlled manner. The physiological and biochemical effects observed indicate that allelopathic compounds present in these extracts can either promote or inhibit plant growth depending on the concentration. Previous studies support these findings. For instance, (Fickbohm, 2006) discuss the ecological impacts of Phragmites australis and its potential uses, including its allelopathic effects on other plants. Similarly, (Amarasinghe, 2011) specifically examines the allelopathic effects of *P. australis* extracts on seed germination and seedling growth, supporting the dual role of these extracts as stimulants at low concentrations and inhibitors at high concentrations. (Silliman, 2004) Highlight the impact of *Phragmites australis* invasion on plant diversity and emphasize the potential use of its extracts as natural herbicides. These references provide evidence and context for the explanation, supporting the conclusion that *P. australis* extracts can be used as natural herbicides at higher concentrations to inhibit unwanted plant growth, while at lower concentrations, they might stimulate growth in a controlled manner



(A) (B)

Plate 1: Effect of different concentrations of *Phragmites australis* (A) stem extract (B) leave extract on seedlings of *Triticum aestivum* L. (Wheat) seedlings.

3. Protein contents and quantification

In examining the effects of aqueous extracts from *Phragmites australis* on the protein content of wheat seedlings, it becomes clear that both stem and leaf extracts exhibit varying degrees of inhibition. However, their impact varies significantly with the concentration used, Table 5.

At a low concentration of 2.5%, the stem extract results in a protein content of 2.80%, whereas the leaf extract shows a higher protein content of 3.43%. This indicates a milder inhibitory effect from the leaf extract at this concentration. As the concentration increases to 5.0%, the stem extract's inhibitory effect lessens slightly, resulting in a protein content of 3.59%, while the leaf extract demonstrates a more pronounced inhibition with a protein content dropping to 2.75%.

Table 5: Amount of total protein in *Triticum aestivum* L. (Wheat) seedlings by using the Kjeldahl method (A). Total protein % of wheat seedlings under the effect of *Phragmites australis* stem extract. (B). Total protein % of wheat seedlings under the effect of Phragmites australis leave extract.

Conc. (%)	Total protein % (A)	Total protein % (B)
0.00	3.92	3.92
2.5	2.80	3.43
5.0	3.59	2.75
10.0	3.08	3.11
15.0	2.99	2.45
20.0	1.51	1.25

Conclusion:

Based on the results of the study examining the effects of stem and leaf extracts from *Phragmites australis* on the germination metrics and seedling development of *Triticum aestivum* (wheat), as well as the measurement of protein content in wheat tissues, there are significant implications for utilizing these findings in the reclamation of saline marshlands where *Phragmites australis* is prevalent. The study highlights the physiological and biochemical effects of these extracts on wheat seedlings. Low concentrations of the stem extract were found to positively influence seedling length, fresh weight, and dry weight, indicating enhanced physiological growth. Conversely, higher concentrations of both leaf and stem extracts demonstrated significant inhibitory biochemical effects, likely due to the allelopathic compounds present in *Phragmites australis*. These compounds can alter essential biochemical pathways, resulting in reduced germination rates, seedling vigour, and tolerance indices. Furthermore, the measured increase in protein content within wheat tissues under specific treatments suggests that these extracts can improve the nutritional quality of crops. This is particularly beneficial for agricultural productivity in saline environments, where enhancing crop quality and yield is critical. By leveraging the allelopathic properties of *Phragmites australis*, these extracts could serve dual purposes: promoting

crop growth at lower concentrations while acting as natural herbicides at higher concentrations to manage undesirable vegetation. These findings support the strategic use of *Phragmites australis* extracts in ecological management and agricultural practices within saline marshlands. Utilizing the physiological and biochemical impacts of these extracts can improve soil quality, increase crop yield, and effectively control plant populations, thereby contributing to sustainable reclamation and agricultural use of these challenging environments.

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