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ALLELOPATHIC EFFECTS OF AQUEOUS EXTRACT OF *PHRAGMITES AUSTRALIS* ON GERMINATION AND GROWTH OF *BRASSICA NIGRA*

IKRAMULLAH KHAN[1], MEHWISH SHAH[1], ABUR RAUF[1], SAFIA GUL[2], ABDUL BASIT[3], FARKHANDA BIBI[*1]

[1]*Department of Botany, Abdul Wali Khan University Mardan, Garden Campus*
[2]*University College for Women, Abdul Wali Khan University Mardan*
[3]*Department of Botany, Islamia College Peshawar, Pakistan*

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Abstract

Brassica nigra was most negatively affected by the allelopathic effects of *Phragmites australis* aqueous extracts, especially at higher extract concentrations. Higher quantities, particularly of stem extracts, consistently hindered biomass production, root and shoot elongation, and germination, while low doses of leaf extracts marginally promoted germination and growth parameters. Furthermore, biochemical tests showed that whereas leaf extracts at lower concentrations encouraged the formation of flavonoids, high concentrations of stem extracts decreased levels of chlorophyll, carotenoids, and IAA, suggesting stress and repression of metabolic activities. These results support the idea that allelopathy is a mechanism for the invasive success of *P. australis*, as demonstrated by the considerable inhibitory (phytotoxic) impact of leaf and stem extracts on the germination, growth, and physiological characteristics of nearby plants. Allelochemicals from *P. australis* had a detrimental overall effect on *B. nigra*, particularly at higher extract concentrations, underscoring the invasive species' potential danger to agricultural production and plant community structure.

Key Points: Allelochemical, Biochemical Alterations, Germination Response, Growth Inhibition, Phenolic Compounds

INTRODUCTION

Allelopathy is a fascinating biological phenomenon in which one organism releases chemicals that influence the growth, survival, and reproduction of neighboring species. This interaction often involves secondary metabolites, which are compounds produced by plants that do not participate in basic metabolic processes but have significant ecological roles (Hierro and Callaway, 2021). Among the vast array of allelopathic interactions, the release of allelochemicals from

Phragmites australis (common reed) has attracted considerable attention for its potential applications in agriculture and ecological management. Historically, the concept of allelopathy has been known for centuries, with early records of allelopathic effects found in ancient texts. Theophrastus, in 300 BC, noted that certain weeds suppressed the growth of crops, while Chinese texts from the first century AD documented plants with pesticidal and allelopathic properties (Xu *et al*., 2023) . Modern research has expanded upon these observations, emphasizing the role of allelopathy in natural ecosystems and agriculture. The chemical interactions between plants, particularly those involving allelochemicals, have been shown to significantly affect plant growth, survival, and reproduction, thereby influencing plant coexistence and community dynamics (Canning and Death, 2021) .

Brassica nigra (black mustard) is acknowledged for its allelopathic properties and is significant in agriculture as an oilseed crop and a source of green manure (Oduor *et al*., 2020; Wang *et al*., 2020) . The seeds contain a high oil content, utilized for both culinary and industrial applications,

while the plant biomass can enhance soil fertility and naturally inhibit weed growth. *B. nigra* is recognized for its contribution to sustainable agriculture, as its allelochemicals-primarily glucosinolates and their hydrolysis products-can assist in managing weed populations, decreasing dependence on synthetic herbicides, and enhancing crop rotation systems. Thus, *B. nigra* enhances sustainable and integrated weed management strategies in contemporary agricultural systems through its economic value and ecological benefits (Pavlacky Jr *et al*., 2022; Uddin, 2014) .

Furthermore, the use of allelopathic plants such as *P. australis* in controlling the growth of invasive weeds can have profound ecological implications. Invasive species, by disrupting local plant communities, can alter nutrient cycling and ecosystem stability. Allelopathic interactions, such as those between *Phragmites* and neighboring plant species, can significantly influence the success of invasions by suppressing the establishment of native plants. This creates a competitive advantage for the invader, allowing it to dominate and alter the structure of the ecosystem (White et al., 2018) . In this way, understanding the allelopathic properties of *P. australis* becomes critical in developing strategies to mitigate its spread and protect native plant biodiversity (Britz *et al*., 2021; Wato, 2020) .

Despite the well-documented allelopathic effects of *P. australis*, the specific mechanisms by which its allelochemicals affect agriculturally important species such as *B. nigra* remain insufficiently understood. Therefore, this study aims to investigate the allelopathic effects of *P. australis* on the germination and growth of *B. nigra*, using laboratory and field trials to assess the impact of *P. australis* extracts at varying concentrations on *B. nigra* seedlings.

MATERIALS AND METHODS

Plant Collection

The plant species *P. australis* was chosen as the donor plant for the study. The plant samples were collected from the indigenous community of Takkar in Mardan District. Following collection, the plant material was thoroughly washed to eliminate any external contaminants, after which it was sliced into fragments and air-dried at ambient temperature for approximately two weeks (Huang, 2021) . The dried plant material was then ground into a fine powder using a mechanical grinder to facilitate the extraction of the bioactive compounds. The seeds of *B. nigra*, commonly known as black mustard, were obtained from a local market in Mardan for use in bioassay experiments.

Preparation of plant extract

For the preparation of the plant extract, four different concentrations were used: 0%, 2%, 4%, and 6%. Specifically, *P. australis* powder was accurately weighed and suspended in distilled water to achieve the desired concentrations. To prepare a 2% solution, 2 g of the powdered material was dissolved in enough distilled water to make a final volume of 100 mL. Similarly, a 4% solution was prepared by dissolving 4 g of powder in distilled water and making up to 100 mL, and a 6% solution by dissolving 6 g and making up to 100 mL. Each concentration was prepared in quadruplicate. After thorough mixing, the solutions were filtered through Whatman filter paper to remove any particulate matter. The filtered solutions were then used immediately for the experiment to minimize the risk of contamination.

Bioassay for Growth

The experimental design followed a Completely Randomized Design (CRD) with four treatments (0%, 2%, 4%, and 6%) and three replicates for each treatment. The control treatment

consisted of distilled water only. Each treatment group received a 3 mL aliquot of the respective extract solution. For each replicate, the appropriate concentration of the plant extract (0%, 2%, 4%, or 6%) was prepared as described. A 3 mL volume of the extract solution was carefully pipetted onto the surface of the agar media in each Petri dish, ensuring even distribution. Seeds (10–15 per Petri dish) were then placed directly onto the agar surface, ensuring contact between the seeds and the applied extract. The control group received only distilled water, applied in the same manner. The CRD was selected to minimize bias and ensure the observed effects were solely attributable to the treatments rather than external variables (Lee *et al*., 2024). The experiment was conducted independently in triplicate to ensure the reliability and consistency of the results

Field Experiment

For the field trial, the substrate was prepared by mixing one-part manure with three parts soil. *B. nigra* seeds were pretreated by immersing them in distilled water for 24 hours to promote uniform germination by breaking dormancy and ensuring synchronized seedling emergence. This was followed by sterilization using a 70% ethanol solution for 1 minute to eliminate surface-borne pathogens and reduce the risk of microbial contamination. Each pot was seeded with 10–15 treated seeds, and the experiment was arranged in a Randomized Complete Block Design (RCBD) with three replicates per treatment (Kaltenbach, 2021). In each pot, two seeds were planted in five evenly spaced holes (1 cm deep), irrigated daily with tap water, and exposed to natural sunlight. After germination, seedlings were treated with *P. australis* extract solutions at concentrations of 0%, 2%, 4%, and 6%, applying 10 mL of the respective solution to each pot, while the control group received tap water only. This pretreatment ensured that observed effects were due to the experimental treatments rather than variations in seed quality or contamination (Casler, 2018).

Data Collection Parameters

The following parameters were measured to assess plant growth and development.

Root Length

To assess root development, the plants were carefully removed from the growth medium, and the roots were washed gently to remove any residual soil (Schlüter *et al*., 2018). The root length was measured from the base of the stem to the tip of the longest root for each plant. The mean root length for each treatment was calculated to ensure a consistent method of measurement across replicates.

Shoot Length

Shoot length was measured after carefully removing the seedlings to avoid damage. Using a ruler, the distance from the base of the stem to the apex of the shoot was recorded. This was done for five seedlings per experimental unit to ensure a representative measure of shoot growth (Tabatabaei *et al*., 2019).

Shoot Fresh Weight

After harvesting, the shoot material was thoroughly washed to remove any contaminants, and the fresh weight was measured using an analytical balance. This procedure was repeated for each seedling within the treatment group to determine the average shoot biomass (Tilly *et al*., 2014) .

Root Fresh Weight

The root system was carefully separated from the aerial parts, washed, and weighed using an analytical balance to determine the root fresh weight. This process was repeated for all treatment groups to obtain the mean fresh root weight per treatment (Zhao *et al*., 2019) .

Dry Weight

After measuring fresh weights, the plant material (shoots and roots) was placed in an oven to dry at

60°C for 48 hours. Once dried, the material was weighed again to determine the dry weight, which was used to estimate the plant's biomass under the different treatment conditions (Catteau *et al*., 2020) .

Number of Roots. The total number of roots per plant was counted after the roots were separated and cleaned. This data allowed for an analysis of root development across the different treatments (Delory *et al*., 2017).

Biochemical assessment

Chlorophyll content was determined spectrophotometrically according to the method described by Arnon (1949). Leaf samples (0.5 g) were ground in 80% acetone, and the homogenate was centrifuged. The supernatant was analyzed at 663 nm (chlorophyll a), 645 nm (chlorophyll b), and the total chlorophyll content was calculated by adding the individual chlorophyll a and b values (Unuofin et al., 2024) (21). While for the analysis of Carotenoid contents (Li et al., 2021) (22). The absorbance of the extract was measured at 470 nm using a spectrophotometer. Similarly, phenolic and flavonoid contents were estimated using the Folin-Ciocalteu reagent method and aluminum chloride method, respectively (Arnon, 1949; Lichtenthaler, 1987). Leaf samples (0.2 g) were homogenized in methanol and incubated with the Folin-Ciocalteu reagent. The absorbance was measured at 765 nm, and phenolic content was calculated using a standard gallic acid curve. Briefly, 0.5 g of ground leaves was extracted with methanol, and the flavonoid content was quantified by measuring the absorbance at 415 nm. To analyze the level of indole acetic acid and sugar contents of *B. nigra* the method of (Chang *et al*., 2002; Singleton and Rossi, 1965) was followed. For IAA leaf samples were homogenized in a phosphate buffer, and the optical density was determined using a Spectrophotometer to determine IAA concentration. While Leaf samples (0.1 g) were extracted with hot ethanol, and after evaporation, the residue was dissolved in distilled water. The sugar content was determined by measuring absorbance at 620 nm (Yemm and Willis, 1954; Sauter *et al*., 2002).

Statistical Analysis

All data were analyzed using SPSS statistical software.

RESULTS

Experimental Bioassay

Impact of *Phragmites australis* Aqueous Extracts (Leaf and Stem) on Germination Percentage

The allelopathic effects of *P. australis* aqueous extracts (leaf and stem) on the germination percentage of *B. nigra* were observed to vary with increasing concentration (0%, 2%, 4%, 6%). (Figure 1). By observing the germination percentage of *B. nigra* the control group (0%) exhibited a germination rate of 55%, while a slight increase in germination was observed with higher concentrations, reaching up to 65% in the 6% extract of the prescribes species (Figure 1). Notably, the leaf extract demonstrated a marginal increase in germination percentage from 56% to 64% as concentration increased, while the stem extract showed a decline in germination, starting at 55% and remaining relatively unchanged across increasing concentrations (Figure 1).

Table1: Analysis of Variance Table for gemination

Source	DF	SS	MS	F	P	
replication	2		9.7500	4.87500		
factor	3		5.8333	1.94444	0.82	0.5019
Error	18		42.9167	2.38426		
Total	23		58.5000			
Grand Mean 10.250		**CV 15.06**				

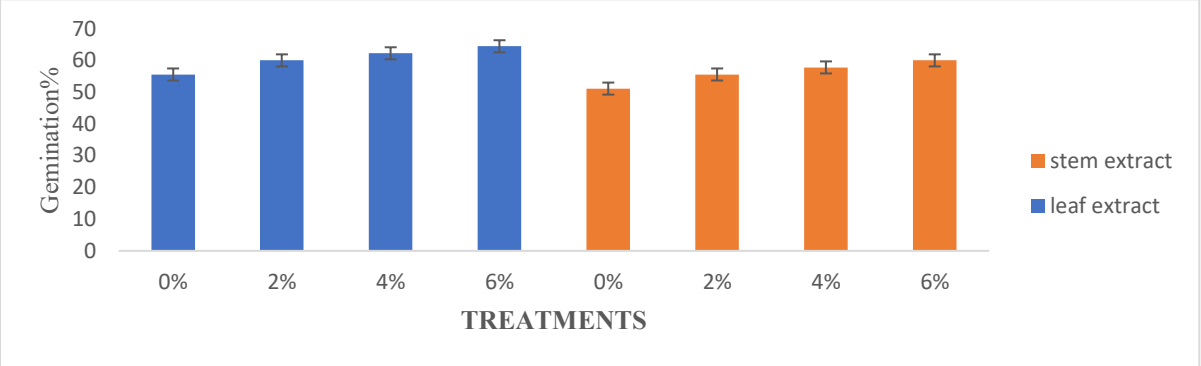

Figure 1: Impact of various concentration of aqueous extracts *P. australis* (leaf and stem) on germination percentage of *B. nigra*.

Number of Leaves

The impact of *P. australis* aqueous extracts on the number of leaves produced by *B. nigra* was observed at varying concentrations (0%, 2%, 4%, and 6%). The leaf extract significantly promoted leaf growth, with the number of leaves increasing progressively to approximately 3.5 leaves at the 6% concentration (Figure 3). In comparison, the stem extract showed a gradual increase in leaf number, reaching a maximum of about 3 leaves at 6%. Although the stem extract also promoted leaf development, the leaf extract demonstrated a stronger effect on leaf production.

Table 2: Analysis of Variance Table for number of leaves

Source	DF	SS	MS	F	P	
replication	2		2.5833	1.29167		
factor	3		9.3333	3.11111	10.34	0.0004
Error	18		5.4167	0.30093		
Total	23		17.3333			
Grand Mean 3.1667		**CV 17.32**				

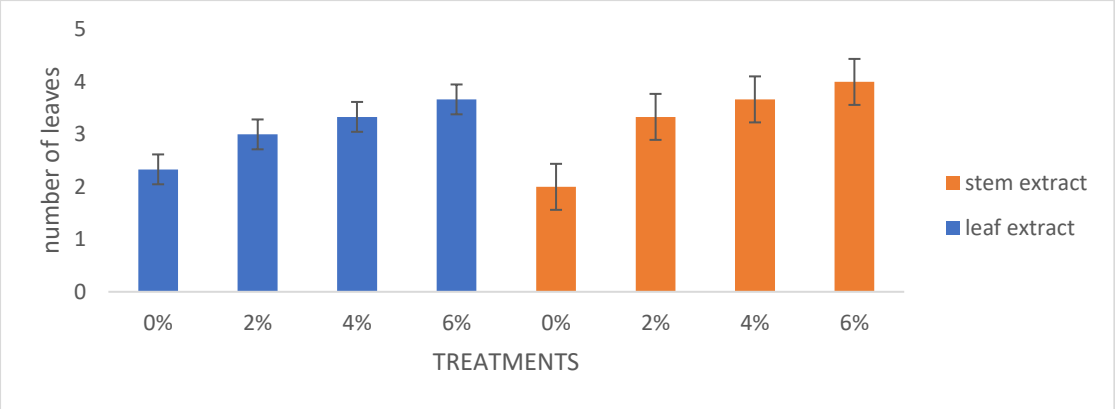

Figure 2: Effect of *P. australis* Aqueous Extracts (Leaf and Stem) on *B. nigra* number of leaves.

Shoot Length

The effect of *P. australis* aqueous extracts on the shoot length of *Brassica nigra* was analyzed at concentrations of 0%, 2%, 4%, and 6%. Both leaf and stem extract positively affected shoot length, with the stem extract having a more significant influence, especially at higher concentrations (Figure 2). The shoot length of plants treated with stem extract grew steadily, reaching approximately 10 cm at the 6% concentration by the 6-week period. On the other hand, the leaf extract maintained a steady shoot length between 7–8 cm across all concentrations. These results indicate that the stem extract promotes greater shoot elongation compared to the leaf extract (Figure 2).

Table 3: Analysis of Variance Table for shoot length

Source	DF	SS	MS	F	P
replication	2		6.7408	3.37042	
factor	3		4.5900	1.53000	0.77 0.5261
Error	18		35.8025	1.98903	
Total	23		47.1333		
Grand Mean 8.3333		**CV 16.92**			

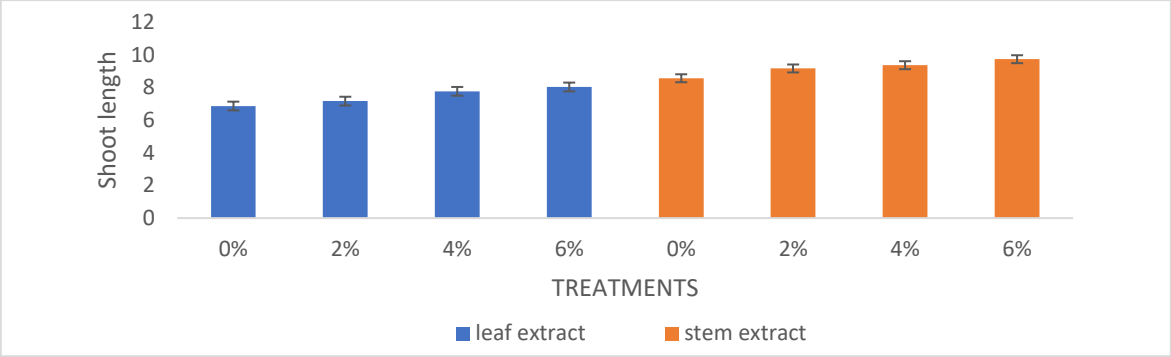

Figure 3: Impact of various concentration of aqueous extracts *P. australis* (leaf and stem) on *B. nigra* shoot length.

Shoot Fresh Weight

The effect of *P. australis* aqueous extracts on the fresh weight of *B. nigra* shoots was assessed at concentrations of 0%, 2%, 4%, and 6%. The leaf extract caused a steady increase in shoot fresh weight, from approximately 0.166 g at 0% to 0.35 g at 6% (Figure 4a). The stem extract exhibited a similar trend but with a more modest increase, from just below 0.3 g at 0% to about 0.33 g at 6%. While both extracts promoted shoot growth, the leaf extract resulted in slightly higher shoot fresh weight at each concentration level, indicating a stronger effect on shoot growth than the stem extract (Figure 4a).

Shoot Dry Weight

The effect of *P. australis* aqueous extracts on shoot dry weight was analyzed across concentrations of 0%, 2%, 4%, and 6%. The leaf extract showed a significant positive impact on shoot dry weight, with an increase from 0.16 g at 0% to 0.63 g at 6%. In comparison, the stem extract resulted in a more modest increase in dry weight, reaching only 0.16 g at 6%. These findings indicate that the leaf extract is more effective in promoting shoot biomass accumulation compared to the stem extract, particularly at higher concentrations (Figure 4b).

Table 3: Analysis of Variance Table for shoot fresh weight

Source	DF	SS	MS	F	P
replication	2		0.00263	0.00132	
factor	3		0.00385	0.00128	1.65	0.2141
Error	18		0.01402	0.00078	
Total	23		0.02050		
Grand Mean 0.3129		**CV 8.92**			

Table 4: Analysis of Variance Table for shoot dry weight

Source	DF	SS	MS	F	P
replication	2		0.00303	0.00152	
factor	3		0.00877	0.00292	1.57	0.2316
Error	18		0.03353	0.00186	
Total	23		0.04533		
Grand Mean 0.1367		**CV 31.58**			

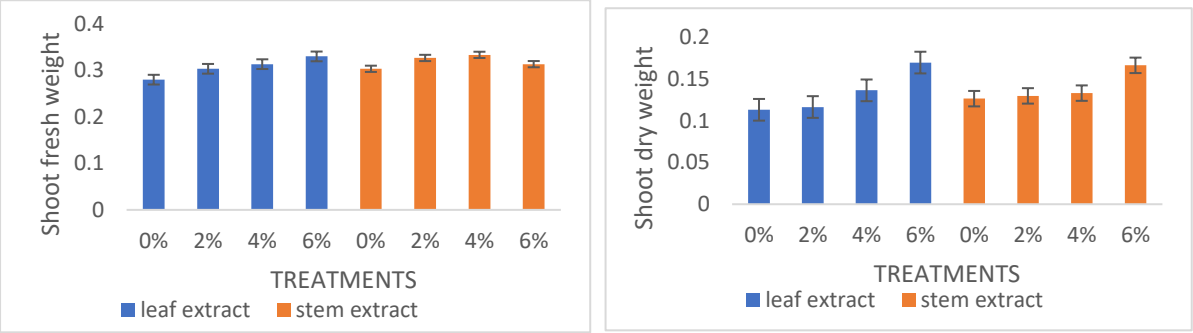

Figure 4: Effect of *P. australis* aqueous extracts *B. nigra* Shoot Fresh and Dry Weight under variable concentration of leaf and stem extract. a) fresh weight b) dry weight

Roots Growth Parameters

The effect of *P. australis* aqueous extracts on root length was assessed at concentrations of 0%, 2%, 4%, and 6%. Both extracts resulted in increased root elongation, with the leaf extract showing a more pronounced effect. At the 6% concentration, root length reached approximately 2.5 cm for the leaf extract, while the stem extract showed relatively stable root lengths close to 2 cm across all concentrations (Figure 5a). This suggests that the leaf extract has a greater impact on root elongation compared to the stem extract. Similarly, the root fresh weight was evaluated at 0%, 2%, 4%, and 6% concentrations. Both leaf and stem extracts promoted an increase in root fresh weight. The leaf extract caused a gradual increase, from 0.05 g at 0% to 0.15 g at 6%. While the stem extract showed a more pronounced increase, starting at 0.03 g at 0% and reaching over 0.2 g at 6%, particularly at higher concentrations (Figure 5b).

By determining the roots' dry weight, the resultant data demonstrates that the stem extract has a more substantial allelopathic effect on root dry mass compared to the leaf extract. Both extracts showed positive effects on root dry mass, with the stem extract consistently promoting higher root dry weight than the leaf extract. At 6% concentration, the stem extract resulted in approximately 0.14 g of root dry weight, while the leaf extract reached 0.12 g, respectively (Figure 5c).

Table 5: Analysis of Variance Table for root length

Source	DF	SS	MS	F	P
replication	2	1.20801	0.60400		
factor	3	1.67385	0.55795	2.49	0.0933
Error	18	4.03574	0.22421		
Total	23	6.91760			
Grand Mean 2.2046		**CV 21.48**			

Table 6: Analysis of Variance Table for root dry weight

Source	DF	SS	MS	F	P
replication	2	0.00070	3.500E-04		
factor	3	0.00091	3.042E-04	3.13	0.0514
Error	18	0.00175	9.722E-05		
Total	23	0.00336			
Grand Mean 0.0338		**CV 29.22**			

Table 7: Analysis of Variance Table for root fresh weight

Source	DF	SS	MS	F	P
replication	2	0.00008	0.00004		
factor	3	0.03405	0.01135	1.68	0.2064
Error	18	0.12144	0.00675		
Total	23	0.15556			
Grand Mean 0.1738		**CV 47.27**			

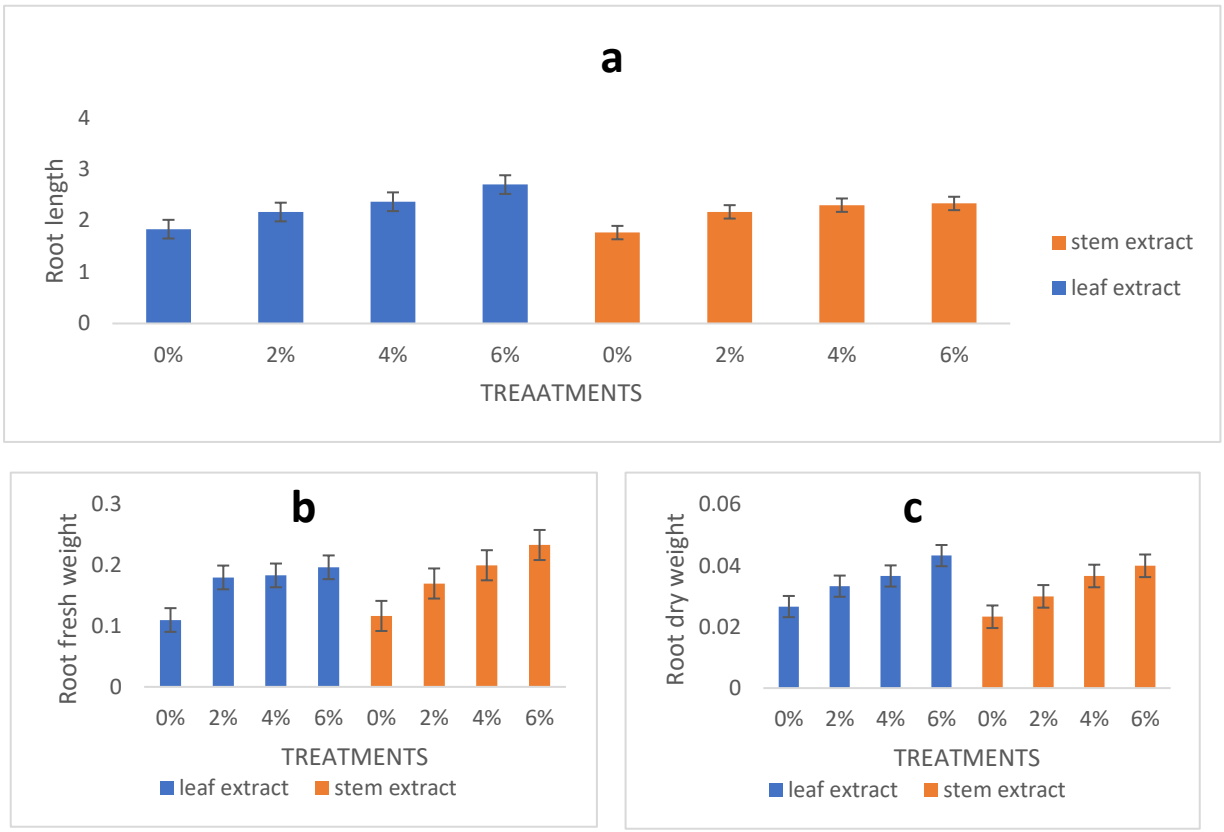

Figure 5: effect of *P. australis* aqueous extracts on root Growth Parameters a) root length b) fresh weight and c) root dry weight

Biochemical Analysis of *B. nigra* Under the Effect of *P. australis* Aqueous Extracts of Leaf and Stem

This study investigates the biochemical impacts of *P. australis* aqueous extracts on various biochemical markers in *B. nigra*, such as chlorophyll content, carotenoids, flavonoids, phenols, sugars, and plant hormones like indole acetic acid (IAA). The analysis evaluates how different concentrations (0%, 2%, 4%, and 6%) of leaf and stem extracts affect the plant's metabolic and physiological processes. Below are the key findings regarding specific compounds and their interactions with extract concentration.

Effect of *P. australis* Aqueous Extracts on Chlorophyll a, b, and Total Chlorophyll of *B. nigra*

Chlorophyll a: Chlorophyll a content in *B. nigra* showed varying responses to *P. australis* leaf and stem aqueous extracts. At lower concentrations (0%), the leaf extract did not significantly affect chlorophyll a level, maintaining relatively stable concentrations. However, the stem extract exhibited a pronounced inhibitory effect, with the chlorophyll a content decreasing as the concentration of the stem extract increased, especially at 2% and higher concentrations. The lowest chlorophyll a content was observed at the highest concentration (6%) of the stem extract (Figure 6a). the resultant data revealed that *P. australis* contains allelopathic compounds that inhibit chlorophyll a synthesis more

potently than the leaf extract, particularly at higher concentrations.

Chlorophyll b: Similarly, chlorophyll b content in *B. nigra* also exhibited differential responses to the extracts. The leaf extract consistently showed a modest increase in chlorophyll b levels, peaking at the highest concentration (6%). Conversely, the stem extract exhibited a decline in chlorophyll b content as the concentration increased, with the highest levels occurring at 0% and 2%, and a marked decrease at 4% and 6% (Figure 6b). This suggests that higher concentrations of stem extract may inhibit chlorophyll b synthesis in *B. nigra*, with the effect becoming more pronounced as the concentration increases.

Total Chlorophyll: The total chlorophyll content, which is a combined measure of chlorophyll a and b, revealed similar trends. The leaf extract demonstrated a gradual increase in total chlorophyll content, reaching its highest value at 6%. On the other hand, the stem extract resulted in a significant decline in total chlorophyll content at higher concentrations (4% and 6%), indicating that the inhibitory effect of the stem extract on chlorophyll synthesis is more pronounced than that of the leaf extract (Figure 6c).

Table 8: Analysis of Variance Table for Chlorophyll a

Source	DF	SS	MS	F	P		
replication	2		5.333	2.6667			
factor	3		157.500	52.5000	2.82	0.0682	
Error	18		335.000	18.6111			
Total	23		497.833				
Grand Mean 14.917		**CV 28.92**					

Table 9: Analysis of Variance Table for Chlorophyll b

Source	DF	SS	MS	F	P		
replication	2		5.333	2.6667			
factor	3		30.000	10.0000	1.87	0.1700	
Error	18		96.000	5.3333			
Total	23		131.333				
Grand Mean 7.8333		**CV 29.48**					

Table 10: Analysis of Variance Table for total Chlorophyll

Source	DF	SS	MS	F	P		
replication	2		1425	712.5			
factor	3		49612	16537.5	0.93	0.4477	
Error	18		321025	17834.7			
Total	23		372063				
Grand Mean 911.25		**CV 14.66**					

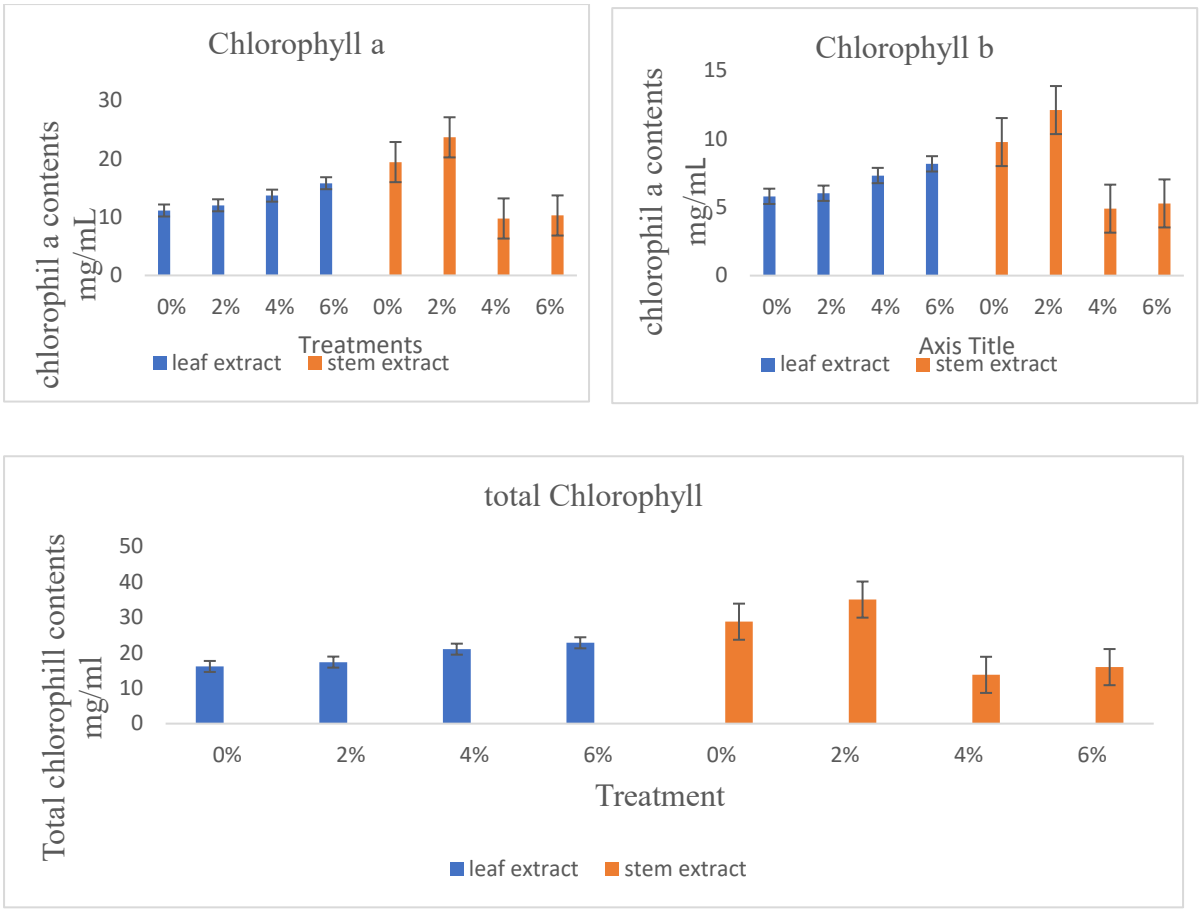

Figure 6: Effect of *P. australis* aqueous extracts (leaf and stem) on Chlorophyll a, b and total chlorophyll of *B. nigra*.

Carotenoids: Carotenoid content was measured across all concentrations of leaf and stem extracts. The leaf extract had a relatively stable carotenoid content, maintaining levels between 800 and 1000 mg/g across all concentrations. In contrast, the stem extract showed a marked decrease in carotenoid content as the concentration of the extract increased, starting at approximately 1000 mg/g at 0%, but dropping to around 600-700 mg/g at higher concentrations **(Figure 7).** The stem extract may exert a stronger inhibitory effect on carotenoid biosynthesis as the concentration increases, indicating a potential negative allelopathic effect.

Table 11: Analysis of Variance Table for carotenoids

Source	DF	SS	MS	F	P
replication	2	5.333	2.6667		
factor	3	283.500	94.5000	3.28	0.0450
Error	18	519.000	28.8333		
Total	23	807.833			

Grand Mean 18.583 CV 28.90

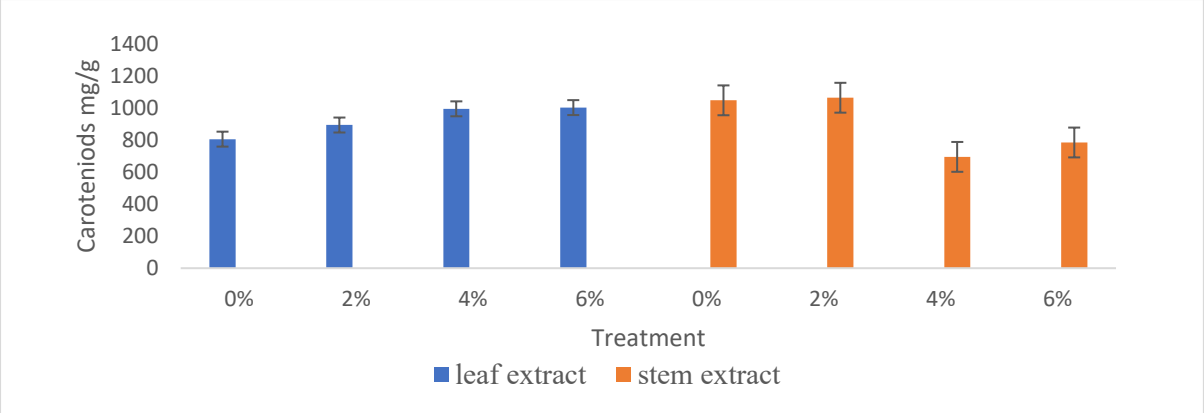

Figure 7: Effect of *P. australis* aqueous extracts (leaf and stem) on Carotenoids of *B. nigra*

Indole Acetic Acid (IAA): The concentration of IAA in *B. nigra* was higher in the leaf extract across all concentrations, with stable levels between 250-300 mg/g (Figure 8). The stem extract, however, exhibited higher IAA concentrations at lower concentrations (0% and 2%), reaching 300-350 mg/g, but decreased significantly to 150-200 mg/g at 4% and 6% concentrations (Figure 8). This indicates that the stem extract may affect the auxin (IAA) levels, potentially interfering with plant growth and development at higher concentrations.

Table 12: Analysis of Variance Table for IAA

Source	DF	SS	MS	F	P
replication	2		18557.2	9278.60	
factor	3		1368.2	456.06	0.14 0.9330
Error	18		57525.6	3195.87	
Total	23		77451.0		
Grand Mean 284.53	**CV 19.87**				

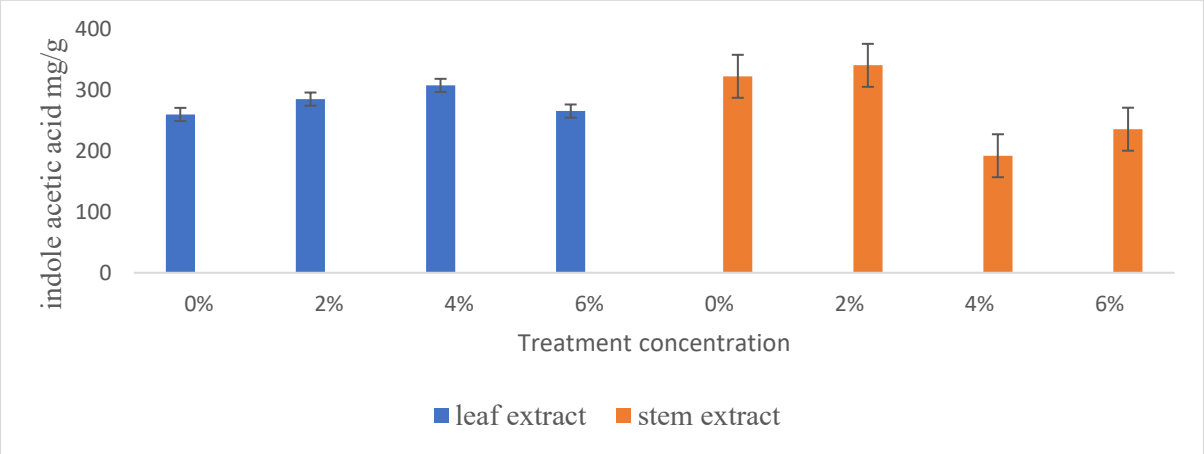

Figure 8: Effect of *P. australis* aqueous extracts of (leaf and stem) on IAA of *B. nigra*

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Flavonoids and Phenols: Flavonoid content in *B. nigra* was measured in mg/g and showed different responses to leaf and stem extracts. The leaf extract showed a consistent increase in flavonoid levels from 45 mg/g at 0% to 80 mg/g at 6%. The stem extract, on the other hand, exhibited a rapid initial increase at 0% and 2% (80-100 mg/g), followed by a marked decline at 4% and 6% (Figure 9). On the other hand, Phenolic content was significantly higher in the stem extract compared to the leaf extract, particularly at lower concentrations (0% and 2%). At these concentrations, the stem extract exhibited phenol levels exceeding 200 mg/mL. However, at higher concentrations (4% and 6%), phenol levels in both extracts decreased, although they remained above 150 mg/mL. These elevated phenolic levels in the stem extract may act as allelopathic agents, potentially interfering with *B. nigra's* growth and development by affecting germination and metabolic processes (Figure 10).

Table 12: Analysis of Variance Table for flavonoid content

Source	DF	SS	MS	F	P	
replication	2		400.00	200.000		
factor	3		975.00	325.000	1.13	0.3632
Error	18		5175.00	287.500		
Total	23		6550.00			
Grand Mean 70.000	**CV 24.22**					

Table 13: Analysis of Variance Table for phenol content

Source	DF	SS	MS	F	P	
replication	2		400.00	200.000		
factor	3		1312.50	437.500	1.22	0.3309
Error	18		6450.00	358.333		
Total	23		8162.50			
Grand Mean 153.75	**CV 12.31**					

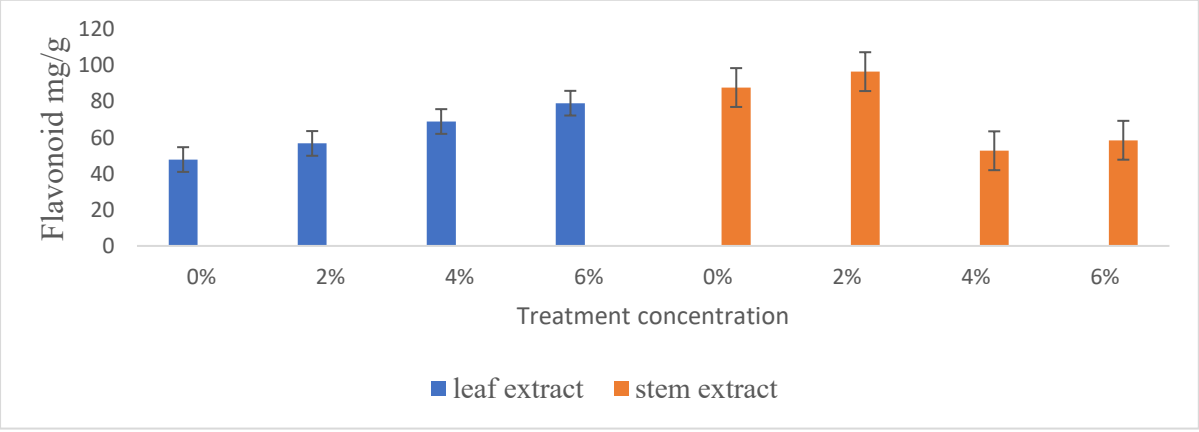

Figure 9: Effect of *P. australis* aqueous extracts (leaf and stem) on Flavonoid of *B. nigra.*

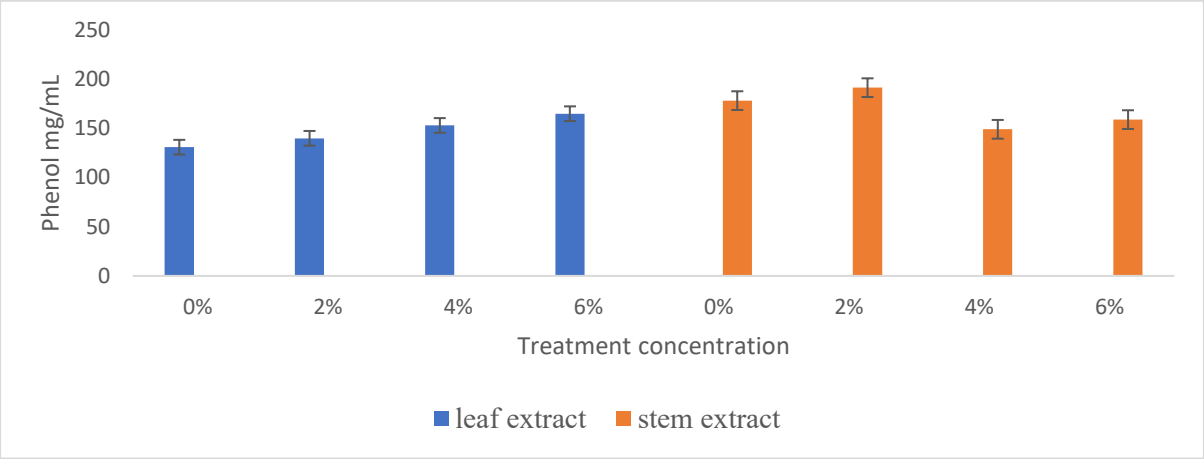

Figure 10: Effect of *P. australis* aqueous extracts (leaf and stem) on phenol of *B. nigra*

Sugars: Sugar concentrations in *B. nigra*treated with the leaf and stem extracts showed distinct patterns. The leaf extract resulted in a steady increase in sugar content from 12 mg/mL at 0% to 14 mg/mL at 6%. Conversely, the stem extract exhibited higher sugar levels at lower concentrations (16 mg/mL at 0%), which peaked at 18 mg/mL at 2% but then declined to around 10 mg/mL at 4% and 6% (Figure 11). This fluctuation in sugar concentrations suggests that both extracts may affect the plant's carbohydrate metabolism and stress responses, potentially impacting its growth.

Table 14: Analysis of Variance Table for Sugar Content

Source	DF	SS	MS	F	P
replication	2		10.5625	5.28125	
factor	3		23.5313	7.84375	2.17	0.1269
Error	18		65.0625	3.61458	
Total	23		99.1563		
Grand Mean 13.563	**CV 14.02**				

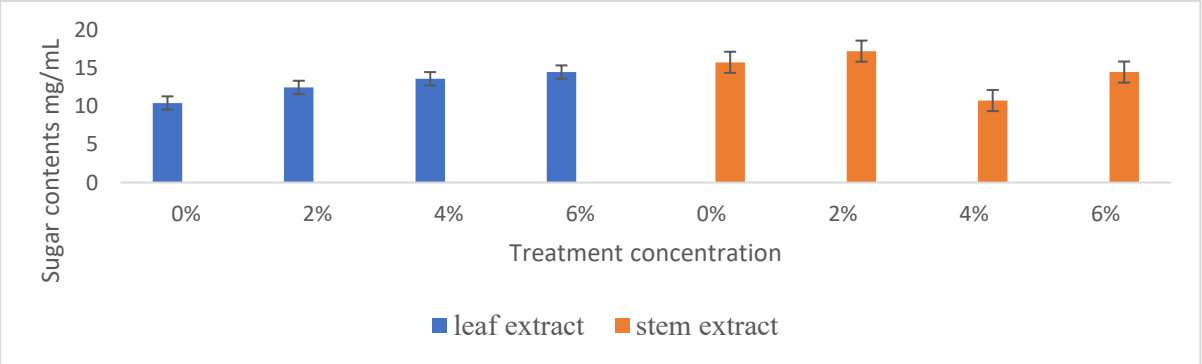

Figure 11: Aqueous leaf and stem extracts of *P. australis* affect *B. nigra* sugar.

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DISCUSSION

Allelopathy refers to the chemical interactions between plants, where the production of allelochemicals by one plant species influences the growth, survival, and reproduction of other species (Latif *et al*., 2017) . These allelochemicals, secondary metabolites that can either stimulate or inhibit the growth of neighboring species, have long been recognized for their ecological and agronomic significance (Bachheti *et al*., 2020). The present study investigates the allelopathic effects of aqueous extracts from *P. australis* on *B. nigra*, with a focus on germination, growth, biomass, and leaf development. The findings underscore the influence of allelopathic compounds in modulating plant interactions and the potential implications for invasive species management.

The impact of *P. australis* extracts on *B. nigra*was most evident in the germination rate. Similar to previous studies, the stem extract of *P. australis* exhibited stronger inhibitory effects compared to leaf extracts, with germination percentages being more significantly suppressed at higher concentrations. This finding aligns with the work of Uddin *et al*., (2014) who observed that allelopathic chemicals from invasive species typically hinder seed germination. However, it is also consistent with the hormesis theory, which suggests that low concentrations of allelochemicals can sometimes promote growth, as seen in the slight improvement in germination at moderate concentrations of leaf extract (Pysek *et al*., 2019; Xue *et al*., 2020) . The observed stimulation of germination at lower concentrations of the leaf extract further supports the notion that certain allelopathic compounds may enhance physiological processes in the early stages of plant development (Janusauskaite, 2023) .

In terms of growth measurements, both root and shoot lengths showed a dose-dependent response to the allelopathic treatments, with leaf extracts exhibiting stimulatory effects at low concentrations (2-4%), while higher doses (6%) led to inhibited growth. These findings mirror those reported by (53), who noted that allelochemicals from invasive species generally impair root development, particularly at higher concentrations. Moreover, the concentration-dependent pattern observed in fresh and dry biomass is consistent with findings from Sodaeizadeh and Hosseini (2012), who demonstrated that aqueous extracts from invasive species like *P. australis* can reduce biomass formation in sensitive plants, especially when high concentrations of allelochemicals are applied. However, the stimulation of biomass at moderate leaf extract concentrations (6%) suggests that allelopathy's effects can be species-specific, with potential positive outcomes at lower concentrations of certain compounds (Šoln *et al*., 2022) .

The number of leaves produced also exhibited a clear response to the type and concentration of *Phragmites australis* extract. While leaf extracts promoted leaf production at higher concentrations (6%), stem extracts delayed leaf emergence, particularly at lower doses (2%). These results align with those of Smith and Knapp (2001), who suggested that allelopathic stress can delay leaf emergence and reduce competitive abilities in target species. Furthermore, the increase in leaf production due to leaf extract exposure may indicate that certain allelochemicals enhance the plant's capacity for foliar growth, possibly through hormonal mediation, which could be an adaptive stress response (Kieta and Owens, 2019).

The biochemical analysis of chlorophyll and carotenoid levels revealed significant alterations in response to the *P. australis* extracts. Higher

concentrations of stem extract caused a marked reduction in chlorophyll a and carotenoids, suggesting that these extracts may impair photosynthetic efficiency and oxidative stress pathways. This result is consistent with previous studies (Deja-Muylle *et al*., 2020; Qu *et al*., 2021) , which found that allelochemicals, particularly phenolic compounds, could suppress chlorophyll synthesis and induce oxidative damage to the photosynthetic apparatus. The reduction in chlorophyll b levels, particularly in response to stem extracts, supports the idea that chlorophyll b is more sensitive to oxidative stress induced by allelochemicals (Bonilla-Bird et al., 2020) (38). These findings further corroborate the hypothesis that oxidative stress and cellular damage are significant mechanisms underlying allelopathy-induced plant growth inhibition.

In addition to chlorophyll degradation, the reduction in IAA (indole-3-acetic acid) levels, especially under stem extract treatments, indicates that allelopathic compounds from *Phragmites australis* can interfere with hormonal regulation. IAA is a key plant hormone involved in regulating root and shoot development, and a reduction in IAA concentrations is associated with stunted growth (Parra-Lobato et al., 2009) (39). The present study demonstrated that allelopathic effects from invasive species such as *Phragmites australis* lead to hormonal imbalances, hindering normal growth processes (Joo *et al*., 2017).

Regarding secondary metabolite production, the study observed an increase in flavonoid accumulation under leaf extract exposure, particularly at lower concentrations. This supports the findings of Khalil et al. (2020), who suggested that mild allelopathic stress can stimulate flavonoid synthesis as part of the plant's defense response to oxidative damage (Bhattarai, 2005) . On the other hand, stem extracts at higher concentrations impeded flavonoid production, which could reflect the toxic effects of high allelochemical levels on the plant's metabolic processes (Anwar *et al*., 2021). These findings are consistent with the work of Akbar et al. (Akbar *et al*., 2024), who reported that elevated allelochemical concentrations inhibit the synthesis of secondary metabolites, further emphasizing the concentration-dependent nature of allelopathic stress (Mangel *et al*., 2011)(43).

The sugar metabolism results also suggest that allelopathic stress from *P. australis* extracts disrupts carbon assimilation and glucose metabolism in *B. nigra*. High concentrations of stem extract were particularly effective at reducing sugar levels, aligning with findings from Bhatt *et al.* (2024), who described the disruption of photosynthetic and carbon assimilation processes under allelopathic stress. These results further support the hypothesis that allelopathy can limit the availability of energy substrates necessary for plant growth, thus impairing overall plant development (Latif *et al*., 2022) .

CONCLUSIONS

The current work shows that allelopathic interactions from invasive species like *P. australis* may greatly hinder the germination, root growth, and seedling development of target plants, including *nigra*. Specifically, aqueous extracts from *P. australis* organs-particularly leaves and rhizomes-exert severe phytotoxic effects, lowering germination rates and root elongation in related species Exposure to allelochemicals from invasive plants may inhibit germination and seedling root length for *B. nigra*, so verifying that allelopathy is a major mechanism by which *P. australis* affects the structure of plant communities and competitive dynamics. These results underline the need of include allelopathic effects in management plans for

invasive species and imply that further study should be on determining the particular allelochemicals engaged and their mechanisms of action.

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AUTHOR'S CONTRIBUTION

All authors have equal contributions in this manuscript.

DATA AVAILABILITY STATEMENT

All the data is primary.

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CONFLICT OF INTEREST

The authors declared that the present study was performed in the absence of any conflict of interest.

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