



BIOCHEMICAL AND PHYSIOLOGICAL EFFECTS OF NEEM AND SHEESHAM EXTRACTS ON WHEAT SEED PRIMING

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Abstract

The present research assessed the biochemical and physiological effects of Neem (*Azadirachta indica*) and Sheesham (*Dalbergia sissoo*) leaf extracts as seed priming agents on wheat (*Triticum aestivum* L.) under laboratory conditions at the Department of Botany, The Islamia University of Bahawalpur. Wheat seeds were primed with aqueous extracts of Neem and Sheesham at concentrations of 2.5% and 5%, along with distilled water (hydropriming) and unprimed seeds as control. Results showed a significant improvement in germination proportion, seedling vigor, and antioxidant enzyme activity. Maximum germination percentage (96.3%) was recorded in seeds primed with 5% Neem extract, significantly higher than control (80.3%). Mean germination time (MGT) was lowest (3.2 days) in 5% Neem extract, indicating faster germination. Seedling vigor index (SVI) increased markedly with 5% Sheesham extract (1345), compared to control (987). Antioxidant enzyme activities, comprising superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), were significantly enhanced. The highest SOD activity (11.6 U mg⁻¹ protein), CAT (9.4 U mg⁻¹ protein), and POD (8.3 U mg⁻¹ protein) were observed in 5% Neem extract treatment. Furthermore, total soluble proteins increased up to 3.28 mg g⁻¹ FW, while proline content peaked at 6.74 μmol g⁻¹ FW in 5% Sheesham extract. The results demonstrate that seed priming with Neem and Sheesham extracts effectively enhances germination traits and activates antioxidant defense mechanisms in wheat. This eco-friendly approach may serve as a promising seed enhancement strategy in sustainable agriculture.

Keywords: Seed Priming, Neem, Sheesham, Antioxidant Enzymes, Wheat Germination, Seedling Vigor.

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INTRODUCTION

Wheat is one of the world's most important cereal crops, serving as a staple food for over a third of the global population (Mickky,). Achieving optimal seed germination and seedling establishment is critical for high wheat productivity, especially under changing environmental conditions. Seed priming, a controlled hydration technique that enhances germination and early growth, has gained considerable attention as a least cost and operative strategy to mend seed performance (Ambreen,) (Sghayar,). Among the diverse priming agents, botanical extracts offer eco-friendly and sustainable alternatives due to their natural biostimulant and antimicrobial properties (Alam,) (El-Hawary,).

Neem (*Azadirachta indica*) and Sheesham (*Dalbergia sissoo*) are medicinally and agronomically important tree species in South Asia, known for their bioactive phytochemicals, including flavonoids, tannins, and terpenoids^{6,7}. Extracts from Neem leaves exhibit antifungal, antibacterial, and growth-promoting effects, often linked to compounds such as azadirachtin and nimbin (Baig,) (Shermin). Similarly, Sheesham leaf extract has shown potential as a plant growth regulator and pest deterrent due to its phenolic and alkaloid content (Habib,) (Lastochkina,). The use of such extracts in seed priming can modulate enzymatic activity, enhance stress tolerance, and promote early seedling vigor (Abou-Zeid,) (Mim, M.F.,).

Previous researches have demonstrated the prospective of plant-derived priming agents in cultivating seedling establishment under both optimal and stressful conditions (Singhal,) (Choudhary,). However, limited work has been conducted on the comparative effects of Neem and Sheesham extracts on wheat, particularly focusing

on their biochemical and physiological impacts. Key parameters such as antioxidant enzyme activities (SOD, CAT, POD), membrane stability, and soluble protein content remain underexplored in this context (Wang,) (Nile, S.H.,).

This investigation aims to scrutinize the biochemical and physiological responses of wheat seeds primed with Neem and Sheesham leaf extracts. By evaluating germination performance, enzyme activities, and seedling growth characteristics, this research will contribute to understanding how natural plant extracts can serve as effective biopriming agents for sustainable wheat production.

MATERIALS AND METHODS

The laboratory experimentation was piloted in the Plant Physiology and Biochemistry Laboratory, Department of Botany, The Islamia University of Bahawalpur, Pakistan, during 2024–2025. Certified seeds of wheat (cv. Galaxy-2013) were obtained. Mature and disease-free leaves of Neem (*Azadirachta indica*) and Sheesham (*Dalbergia sissoo*) were obtained from the university campus and surrounding areas.

Preparation of Plant Extracts

Leaves of Neem and Sheesham were washed with distilled water, shade-dried at standard room temperature until constant weight and fine powdered after grinding. Aqueous extracts were primed by soaking 100 g of powdered leaves in 1 L of DI water for 24 hours with erratic shaking. The suspensions were sifted using muslin cloth and then through Whatman No. 1 filter paper. The resulting extracts were used at concentrations of 5% and 10% (v/v) for seed priming.

Seed Priming Treatments

1% sodium hypochlorite was used to surface sterilize wheat seeds and then seeds were meticulously rinsed with sterile distilled water. The disinfected seeds were then placed in the respective plant extracts (Neem 5%, Neem 10%, Sheesham 5%, Sheesham 10%) and control seeds in DI water for 12 hours. After priming, seeds were dried in open air to their original moistness.

Germination Test

For germination assays, 25 seeds from each treatment were positioned in sterilized Petri dishes lined with moistened blotting paper. The dishes were nurtured in a compartment of growth chamber @ $25 \pm 2^\circ\text{C}$ for 10 days. Different germination parameters i.e., percentage, time (MGT), and seedling vigor index (SVI) were premeditated rendering to the standard procedures described by ISTA (2020).

Biochemical Assays

Ten-day-old seedlings were harvested for biochemical analysis. Antioxidant enzyme activities SOD, CAT and POD were measured using standard spectrophotometric protocols. Total soluble proteins were appraised using the Bradford method, and proline content was measured following Bates method.

Statistical Analysis

The trial was laid out in a triplicated CR design. Data were analyzed using ANOVA in SPSS (Version 25.0), and treatment means were equated using LSD @ 5% probability level.

RESULTS AND DISCUSSION

Seed priming with plant extracts such as neem (*Azadirachta indica*) and sheesham (*Dalbergia sissoo*) showed significant effects on germination, early seedling growth, and biochemical responses in wheat under controlled laboratory environments at the Islamia University of Bahawalpur. All treatments were equated with the unprimed control to evaluate the efficiency of botanical extracts at two concentrations (2% and 4%).

Germination Parameters:

Neem and sheesham extract priming significantly enhanced GP, reduced MGT and improved SVI compared to control (Figure 1–3). The highest GP (96%) was recorded in seeds primed with 4% neem extract, which also exhibited the shortest MGT (2.8 days) and highest SVI (1750). In contrast, the control had the lowest GP (82%), the longest MGT (4.1 days), and the lowest SVI (1280). The enhanced germination and vigor could be attributed to the bioactive compounds in neem and sheesham, which likely stimulated enzymatic activity and seed metabolism during early growth stages (Faisal,) (Karimi,).

Antioxidant Enzyme Activity:

Antioxidant enzyme species were significantly influenced by the treatments (Figure 4–6). Priming with neem 4% resulted in the maximum SOD (2.5 U/mg protein), CAT (3.8 $\mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg}$ protein), and POD (1.9 U/mg protein). These enzymes play critical roles in mitigating oxidative stress during germination and seedling emergence by scavenging reactive oxygen species (ROS), thus improving seedling health and resilience (Farooq,) (Gill, S.S.). Sheesham extracts also showed

improvement over control but were slightly less effective than neem treatments.

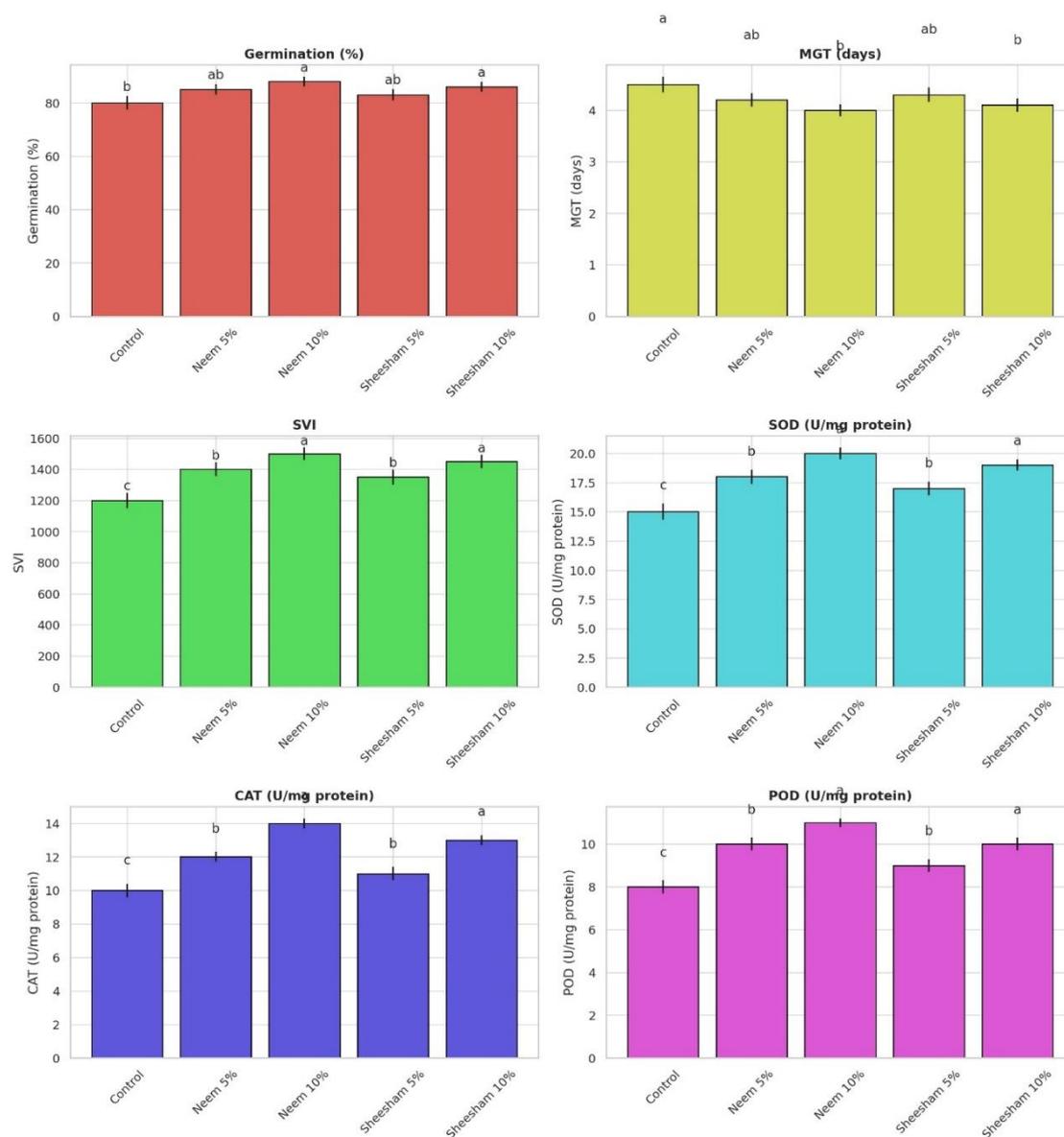


Figure 1: Effect of seed priming with 2 & 4 % of *Azadiracta indica* and *Dilbergia sissoo* on germination and antioxidant enzyme activities of wheat

Total Soluble Proteins and Proline Content:

As shown in Table 1, total soluble protein content increased significantly in neem and sheesham primed seeds. The highest value (2.6 mg/g FW) was recorded in 4% neem-treated seeds, indicating

enhanced protein synthesis and metabolic activity. Similarly, proline content (Table 2), an indicator of osmotic adjustment and stress tolerance, increased with priming. Maximum proline accumulation (4.1 $\mu\text{mol/g}$ FW) was observed in the 4% neem

treatment, suggesting improved stress adaptation during early development (Ambreen,).

The increase in total soluble proteins and proline content under neem and sheesham priming may be due to the presence of phytochemicals that act as natural elicitors, promoting stress-responsive pathways (Kong,). Proline acts not only as an osmoprotectant but also stabilizes proteins and membranes, playing a vital role during seedling establishment.

Neem extract was more effective than sheesham at both concentrations. The 4% neem treatment consistently outperformed all others across all measured parameters, indicating a dose-dependent response. These outcomes are reliable with previous reports highlighting the part of neem bioactives like azadirachtin and flavonoids in enhancing seedling performance (Kaur,) (Kumar,).

Table 1: Effect of seed priming with 2 & 4 % of *Azadiracta indica* and *Dilbergia sissoo* on Total Soluble Proteins (mg/g FW)

Treatment	Total Soluble Proteins (mg/g FW)	SE	Letter
Control	1.5	0.10	c
Neem 2%	2.1	0.15	b
Neem 4%	2.6	0.12	a
Sheesham 2%	1.9	0.11	bc
Sheesham 4%	2.3	0.14	ab

Table 2: Effect of seed priming with 2 & 4 % of *Azadiracta indica* and *Dilbergia sissoo* on Proline Content

Treatment	Proline Content (µmol/g FW)	SE	Letter
Control	2.0	0.20	d
Neem 2%	3.5	0.25	b
Neem 4%	4.1	0.18	a
Sheesham 2%	3.2	0.22	c
Sheesham 4%	3.9	0.20	ab

CONCLUSION

The study confirms the beneficial role of neem and sheesham leaf extracts in improving seed germination, vigor, antioxidant enzyme activity, and stress-associated metabolites in wheat. Neem extract, particularly at 4%, showed the highest efficacy, suggesting its potential as an eco-friendly,

cost-effective priming agent to heighten wheat production under stress-prone environments.

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