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Research Article

Effect of Pre-Sowing Treatments on Seed Germination and Seedling Vigour of *Indigofera tinctoria* L. and Value Chain Analysis

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Abstract

Commercial cultivation of *Indigofera tinctoria* L. is hindered by seed coat physical dormancy. To resolve this propagation bottleneck, a study at AC&RI, Vazhavachanur, evaluated 12 pre-sowing treatments in a completely randomized design, identifying mechanical scarification with sandpaper as the most effective method for breaking physical dormancy. Mechanical scarification for 10 minutes yielded the highest germination percentage (24.0%) and seedling vigour index (276.0), whereas a 5-minute treatment produced the maximum total seedling length (13.0 cm). Complementing agronomic findings, a value chain analysis across five key stakeholder groups revealed that processing 800 kg of dry leaf biomass yields approximately 25 kg of natural indigo cake via biological fermentation and extraction. This value-addition chain generates a net profit of ₹28,000 per acre for farmers (Benefit-Cost Ratio of 2.04) and ₹20,000 per processing batch for processors, establishing a sustainable, economically viable roadmap for commercial natural indigo dye production.

Keywords: True Indigo; Physical Seed Coat Dormancy; Mechanical Scarification; Indigotin Extraction; Agronomic Economics; Dye Processing Profitability

Introduction

The rapidly escalating environmental and toxicological concerns surrounding synthetic colorants have catalyzed a major global transition toward sustainable, non-toxic, and eco-friendly natural dyes [1,2]. Synthetic dyes generate chemical-laden effluents that contribute significantly to water pollution and soil degradation, prompting stringent environmental regulations worldwide [2]. Consequently, *Indigofera tinctoria* L. (commonly known as true indigo or Indian indigo) a leguminous shrub renowned as the premier botanical source of indigo dye has regained immense economic and industrial importance [3,4]. Beyond its deep blue pigment (*indigotin*), *I. tinctoria* offers valuable agronomic benefits, such as atmospheric nitrogen fixation, which enhances soil health and supports sustainable cropping systems [5].

Despite its growing market demand in the textile, hair care, and cosmetic industries, the commercial-scale cultivation of *I. tinctoria* is severely constrained by poor and non-uniform seed germination [3]. Like many members of the Fabaceae family, the seeds possess a dense, hard, and impermeable seed coat that imposes physical dormancy [6,7]. This protective outer layer consists of tightly packed, lignified palisade cells impregnated with water-repellant substances that inhibit water absorption (imbibition) and gas exchange, leading to delayed emergence, uneven crop stands, and increased vulnerability to environmental stressors [8,9]. Breaking seed coat dormancy through optimized pre-sowing scarification treatments such as mechanical abrasion, thermal exposure, or chemical treatments is therefore essential to ensure rapid, uniform germination and strong early seedling vigour [10,11].

In addition to biological and propagation hurdles, the natural indigo sector faces commercial inefficiencies, including supply chain fragmentation, market volatility, and limited processing standardization [12,13]. To establish *I. tinctoria* as an economically viable alternative to synthetic dyes, agronomic standardizations must be integrated with value chain mapping and economic assessments [14,15]. Evaluating cost structures, profit margins across stakeholders, and processing conversion efficiency provides a clear roadmap for scaling production and enhancing financial returns for growers and processors alike [16,17].

Objectives

- Standardize effective pre-sowing scarification techniques for breaking seed dormancy in *Indigofera tinctoria*.
- Assess early seedling growth characteristics and vigour indices under different pre-sowing methods.
- Map the existing value chain of *I. tinctoria* from primary production to final consumer utilization.
- Evaluate market opportunities and economic feasibility across value chain stakeholders.

Methodology

Experimental site and seed procurement

The present investigation was conducted in the Department of Forestry at the Agricultural College and Research Institute (AC and RI), Vazhavachanur, Tamil Nadu. Mature pods of Indigofera tinctoria L. were harvested from healthy mother plants and processed to extract clean, viable seeds. Seed lots were cleaned manually to eliminate foreign matter, damaged seeds, and light seeds, and stored at ambient laboratory conditions prior to setting up the germination trials [10].

Laboratory setup and pre-sowing treatments

The experiment was laid out in a Completely Randomized Design (CRD) with 12 distinct pre-sowing treatments (T_1through T_{12}) in two replications, using 25 seeds per replication (25 seeds x 2 = 50 seeds per treatment). Initial attempts using seedling protrays yielded low germination (1–2 seeds); thus, the trial was shifted to the standardized Roll Paper Method inside a controlled germination room maintained at $25 \pm 2^{\circ}C$ and $75 \pm 5\%$ relative humidity [18,19].

The evaluated dormancy-breaking treatments included:

- T_1: Quick dip in 50% concentrated sulphuric acid () [10].
- T_2: Quick dip in 75% concentrated sulphuric acid () [11].
- T_3: Quick dip in 100% concentrated sulphuric acid () [6].
- T_4: Hot water treatment at for 5 minutes [7].
- T_5: Hot water treatment at for 5 minutes [20].
- T_6: Hot water treatment at for 5 minutes [8].
- T_7: Cold water soaking for 24 hours [9].
- T_8: Cold water soaking for 48 hours [9].

- T_9: Cold water soaking for 72 hours [9].
- T_{10}: Control (Untreated seeds) (ISTA, 1999).
- T_{11}: Mechanical scarification using sandpaper for 5 minutes [11].
- T_{12}: Mechanical scarification using sandpaper for 10 minutes [11].

Assessment of germination and seedling growth characteristics

Germination counts were recorded daily until no further germination occurred. A seed was scored as germinated upon the emergence of a normal radical reaching at least in length [18].

The following growth parameters were measured at the conclusion of the test:

Germination Percentage (%): Calculated following the standard ISTA protocol [18,19]:

$$\text{Germination Percentage (\%)} = \left(\frac{\text{Number of germinated seeds}}{\text{Total number of seeds sown}}\right) \times 100$$

Shoot and Root Lengths (cm): Five representative germinated seedlings were selected at random from each replication. Shoot length (collar region to apical bud) and primary root length (collar region to root tip) were measured using a ruler [10].

Total Seedling Length (cm): Calculated as the sum of shoot length and root length.

Seedling Vigour Index (SVI): Calculated according to the standard mathematical formula proposed by Abdul-Baki and Anderson [21]:

$$\text{Seedling Vigour Index} = \text{Germination Percentage (\%)} \times \text{Total Seedling Length (cm)}$$

Value chain mapping and economic feasibility analysis

To map the value chain and analyze financial returns, primary field surveys and stakeholder interactions were conducted using pre-tested semi-structured questionnaires [12].

- **Value Chain Framework**: Mapped across primary producers (farmers), industrial processors, wholesalers, retailers, and final consumers using the functional analysis framework [12,16].
- **Conversion Efficiency:** Calculated based on raw biomass processing yields (dry biomass to refined dye cake conversion ratio) recorded during traditional fermentation-based dye extraction [22,23].
- Economic Assessment: Evaluated using Cost-Benefit frameworks [15,17]. Indicators computed included:
  - Gross Returns: $\text{Total yield} \times \text{Unit market price}$
  - Net Returns: $\text{Gross returns} - \text{Total cost of production/processing}$
  - Benefit-Cost Ratio (BCR):
- $$\text{Benefit-Cost Ratio (BCR)} = \frac{\text{Gross Returns}}{\text{Total Production Cost}}$$
  - Profit Margin (%):
- $$\text{Profit Margin (\%)} = \left(\frac{\text{Selling Price} - \text{Total Cost}}{\text{Total Cost}}\right) \times 100$$

Results and Discussion

Effect of pre-sowing treatments on germination

Considerable variation in seed germination was observed among the pre-sowing treatments (Table 1). Mechanical scarification using sandpaper for 10 minutes (T_{12}) achieved the highest germination rate of 24.0% (6 seeds germinated), followed by quick dipping in 75% concentrated H_2SO_4 (T_2) at 16.0% (4 seeds germinated). Untreated control (T_{10}) yielded only 4.0% germination, confirming strong physical coat dormancy. Treatment T_1 (50% H_2SO_4) and T_5(hot water at 80^0c) failed to produce any germinated seeds (0.0%).

The low germination observed in the untreated control (T_{10}, 4.0%) confirms that Indigofera tinctoria seeds exhibit strong physical coat-imposed dormancy, a widespread trait among members of the Fabaceae family [6,7]. This physical dormancy is caused by a outer layer of palisade cells impregnated with suberin and cutin, which creates a hydrophobic barrier preventing water imbibition and gas exchange [8].

Mechanical scarification using sandpaper for 10 minutes (T_{12}) yielded the highest germination percentage (24.0%) and seed vigour index (276.0). Abrasion physically disrupts the impermeable outer seed coat, creating micro-fissures and exposing the internal

macrosclereid layer without damaging the embryo, thereby enabling rapid water uptake and physiological activation [10,11].

Chemical scarification with 75% concentrated sulphuric acid (T_2) achieved a moderate germination rate of 16.0%, as acid oxidation effectively dissolves protective cuticular waxes and softens the seed coat (Rolston, 1978). However, increasing the acid concentration to 100% (T_3) reduced germination to 12.0%, while 50% acid (T_1) failed to induce germination (0.0%). Excessive acid exposure can damage embryo tissues via chemical burning, whereas insufficient concentrations fail to weaken the thick seed coat [10,24]. Complete germination failure in the 80^{0}C hot water treatment (T_5) reflects thermal denaturation of vital seed proteins and embryo death [20]. Similarly, cold water immersion (T_7–T_9) showed poor performance (4.0–8.0%) because soaking alone cannot bypass the mechanical resistance of an unscarified seed coat [8,9].

S. No	Treatment Code	Treatment Details	No. of Seeds Germinated	Germination Percentage (%)
1	T_1	50% Conc. Sulphuric Acid (Quick Dip)	0	0.0
2	T_2	75% Conc. Sulphuric Acid (Quick Dip)	4	16.0
3	T_3	100% Conc. Sulphuric Acid (Quick Dip)	3	12.0
4	T_4	Hot Water (for 5 min)	2	8.0
5	T_5	Hot Water (for 5 min)	0	0.0
6	T_6	Hot Water (for 5 min)	2	8.0
7	T_7	Cold Water (24 hours)	2	8.0
8	T_8	Cold Water (48 hours)	2	8.0
9	T_9	Cold Water (72 hours)	1	4.0
10	T_{10}	Control (Untreated)	1	4.0
11	T_{11}	Sandpaper Scarification (5 min)	2	8.0
12	T_{12}	Sandpaper Scarification (10 min)	6	24.0

Table 1: Effect of pre-sowing treatments on germination percentage of *Indigofera tinctoria.*

Growth performance and seedling vigour

Pre-sowing scarification influenced early seedling growth parameters significantly (Table 2, 3 and Figure 2, 3). Mechanical scarification for 5 minutes (T_{11}) recorded the maximum shoot length (7.0 cm), root length (6.0 cm), and total seedling length (13.0 cm). This was followed by T_{12} (sandpaper for 10 min) with a total seedling length of 11.5 cm.

Calculating the vigour index (Table 3) showed that T_{12} attained the highest index value of 276.0, driven by its higher germination success. T_{11} followed with a vigour index of 130.0, while acid scarification (T_2) reached 96.0.

Although T_{12} recorded the highest overall vigour index due to higher germination, mechanical scarification for 5 minutes (T_{11}) resulted in the maximum shoot length (7.0 cm), root length (6.0 cm), and total seedling length (13.0 cm). Moderate mechanical scarification breaks dormancy sufficiently early to accelerate metabolic processes during imbibition while minimizing mechanical stress on embryonic tissues, promoting superior shoot and primary root development [11,21].

The minimal germination observed in untreated *Indigofera tinctoria* seeds (T_{10}: 4.0%) confirms that this species possesses

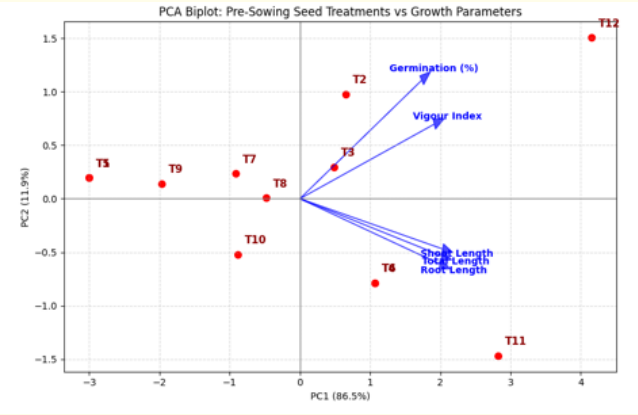

Figure 1: PCA biplot for pre sowing seed treatment and growth parameters.

severe physical seed coat dormancy. This dormancy mechanism is widespread across the Fabaceae family and is driven by an outer palisade layer impregnated with suberin, cutin, and waxes, which together create an impermeable barrier to water and oxygen.

Mechanical scarification with sandpaper for 10 minutes (T_{12}) was the most effective treatment for breaking dormancy, achieving the highest germination rate (24.0%) and seedling vigour index (276.0). Physical abrasion creates micro-cracks in the macro-sclereid layer without causing mechanical damage to the embryo, facilitating rapid imbibition and metabolic activation upon moisture exposure. Interestingly, while T_{12} maximized overall germination percentage, a shorter 5-minute mechanical scarification (T_{11}) resulted in superior seedling length metrics (shoot length: 7.0 cm; root length: 6.0 cm; total length: 13.0 cm). Moderate mechanical abrasion appears to break seed coat resistance sufficiently to initiate rapid metabolic activation while preventing micro-fractures in the embryonic axis, thereby promoting vigorous initial root and shoot elongation.

Chemical scarification using concentrated sulphuric acid showed mixed efficacy. Acid treatment at 75% H_2SO_4 (T_2) yielded 16.0% germination, as acid oxidation dissolves surface cuticular waxes. However, raising the concentration to 100% (T_3) decreased germination to 12.0%, and a 50% concentration (T_1) failed completely (0.0% germination). Concentrated acid treatments pose risks of chemical burning to embryonic tissues, whereas low concentrations fail to penetrate the thick coat. Hot water treatments at 80°C (T_5) resulted in complete embryo mortality (0.0% germination) due to thermal protein denaturation, while cold water soaking (T_7–T_9: 4.0–8.0% germination) failed to overcome mechanical seed coat resistance. Principal Component Analysis (PCA) biplot ordination confirmed that mechanical scarification treatments (T_{12} and T_{11}) align strongly with germination, vigour index, and shoot/root development vectors, proving their superiority over chemical or thermal methods.

S. No	Treatment Code	Shoot Length (cm)	Root Length (cm)	Total Seedling Length (cm)
1	T_1	0.0	0.0	0.0
2	T_2	3.5	2.5	6.0
3	T_3	3.5	3.0	6.5
4	T_4	5.0	4.0	9.0
5	T_5	0.0	0.0	0.0
6	T_6	5.0	4.0	9.0
7	T_7	2.2	1.8	4.0
8	T_8	2.8	2.3	5.1
9	T_9	1.1	1.0	2.1
10	T_{10}	2.7	2.3	5.0
11	T_{11}	7.0	6.0	13.0
12	T_{12}	6.5	5.0	11.5

Table 2: Effect of pre-sowing treatments on seedling length of *Indigofera tinctoria.*

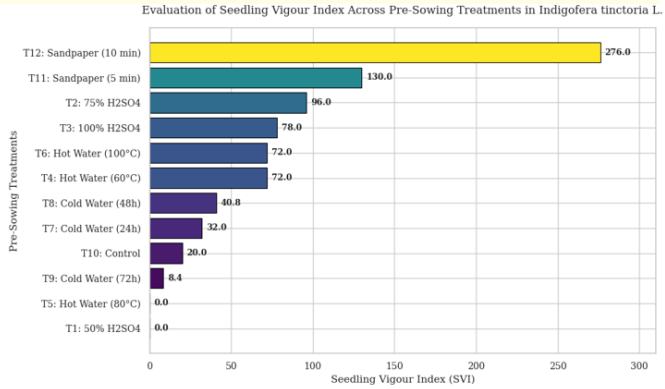

Figure 2: Evaluation of Seedling Vigour Index (SVI) across pre-sowing treatments in *Indigofera tinctoria* L.

S. No	Treatment Code	Germination (%)	Seedling Length (cm)	Vigour Index
1	T_1	0.0	0.0	0.0
2	T_2	16.0	6.0	96.0
3	T_3	12.0	6.5	78.0
4	T_4	8.0	9.0	72.0
5	T_5	0.0	0.0	0.0
6	T_6	8.0	9.0	72.0
7	T_7	8.0	4.0	32.0
8	T_8	8.0	5.1	40.8
9	T_9	4.0	2.1	8.4
10	T_{10}	4.0	5.0	20.0
11	T_{11}	8.0	13.0	130.0
12	T_{12}	24.0	11.5	276.0

Table 3: Effect of pre-sowing treatments on Seedling Vigour Index.

Value chain analysis

The value chain of *Indigofera tinctoria* relies on an integrated network of stakeholders to transition raw biomass into high-value commercial dye. At the foundational level, farmers cultivate and harvest approximately 7 tonnes of green biomass per acre, which is converted through shade-drying into 800 kg of dry leaves and sold to processors at ₹68/kg. Processors and manufacturers then transform this 800 kg of dry leaf biomass into roughly 25 kg of natural indigo cake using biological fermentation, oxidation, and filtration processes, selling the extracted dye cake at ₹3,000/kg. Finally, wholesalers and retailers manage the downstream operations, including bulk storage, transportation, pulverization, retail packaging, and brand distribution, ultimately selling the refined indigo powder to end consumers at retail prices reaching ₹4,000/kg.

Stakeholder	Primary Activities/Functions	Input Material	Output Product	Selling Price
Farmer	Cultivation, harvesting, shade-drying	Seeds, inputs, labour	Dry leaves (800 kg)	₹68/kg
Processor	Fermentation, oxidation, settling, drying	Dry leaves (800 kg)	Indigo cake (25 kg)	₹3,000/kg
Wholesaler	Bulk procurement, storage, transport	Indigo cake	Bulk indigo cake	₹3,500/kg
Retailer	Packaging, pulverization, branding	Bulk indigo cake	Indigo powder/retail cake	₹4,000/kg
Consumer	End application in textiles, cosmetics	Retail products	Dyeing and hair coloring	—

Table 4: Value Chain Mapping and Function Analysis of *Indigofera tinctoria.*

Bridging biological propagation with economic assessment demonstrates that overcoming seed dormancy is the initial enabler of a highly profitable value chain. Value chain mapping across primary producers, processors, wholesalers, retailers, and end consumers revealed substantial value addition during processing. Transforming 800 kg of shade-dried leaf biomass into 25 kg of natural indigo cake via traditional biological fermentation yields a high-value product that commands ₹3,000/kg.

Cultivation of *I. tinctoria* provides smallholder farmers with a net return of ₹28,000 per acre and a Benefit-Cost Ratio (BCR) of 2.04. At the processing level, converting a batch of 800 kg of dry leaves generates a net profit of ₹20,000, yielding a 26.67% profit margin. Incorporating *I. tinctoria* into smallholder farming systems offers dual agronomic and socio-economic benefits. As a leguminous crop, true indigo fixes atmospheric nitrogen, improving soil fertility, reducing dependence on synthetic fertilizers, and serving as an ideal rotation crop in rainfed and marginal agricultural landscapes. Establishing decentralized processing units near farming clusters can capture added value locally, insulate smallholders from raw biomass price fluctuations, and meet the growing global industrial demand for eco-friendly, non-toxic natural colorants.

Economic feasibility and market opportunities

Economic evaluation indicates strong profitability for both cultivation and processing sectors (Table 5). Farmers achieve a net return of ₹28,000 per acre with a B:C ratio of 2.04. Processors obtain a net profit of ₹50,000 per processing batch of 800 kg dry leaves.

Value chain mapping highlighted key structural stages from biomass production to final market distribution [12]. The primary value-addition step occurs during processing, where 800 kg of dry leaves yield approximately 25 kg of natural indigo cake through biological fermentation, oxidation, and settling [22,23].

Value-added processing significantly enhances profitability across supply chain nodes [15,16]. Raw biomass production provides farmers with a net return of ₹28,000 per acre and a Benefit-Cost Ratio (BCR) of 2.04 [17]. Transforming dry leaves into natural indigo cake generates a net profit of ₹20,000 per batch for processors, expanding total financial returns and demonstrating the commercial viability of natural indigo production [13]. Combining non-destructive mechanical seed scarification with integrated value chain links offers a reliable framework for expanding commercial cultivation of *I. tinctoria* to meet the growing global demand for eco-friendly colorants [3,4].

Level/Component	Cost Component	Value (₹)	Returns Component	Value (₹)	Net Return/ Profit	B:C Ratio/Profit Margin
Cultivation Level (Per Acre)	Total Cost of Cultivation	₹27,000	Gross Return (800 kg @ ₹68/kg)	₹55,000	₹28,000	2.04
Processing Level (Per Batch)	Leaf Purchase (800 kg) Processing and Labor Cost	₹55,000 ₹20,000	Gross Return (25 kg cake @ ₹3000/kg)	₹75,000	₹20,000	Profit Margin: 26.67%
Retail Level (Per kg)	Procurement and Retail Packaging	₹3,500	Gross Sales Price	₹4,000	₹500/kg	Profit Margin: 14.28%

Table 5: Economic analysis and value addition in *Indigofera tinctoria* production and processing.

Conclusion

The present investigation demonstrates that physical dormancy imposed by a hard, impermeable seed coat is the primary bottleneck restricting the rapid and uniform propagation of *Indigofera tinctoria* L. Among the evaluated pre-sowing treatments, mechanical scarification using sandpaper for 10 minutes (T_{12}) proved to be the most effective method for breaking physical seed coat dormancy, yielding the highest germination percentage (24.0%) and seedling vigour index (276.0). Meanwhile, mechanical scarification for 5 minutes (T_{11}) produced the maximum total

seedling length (13.0 cm), establishing sandpaper scarification for 5–10 minutes as a simple, eco-friendly, and highly efficient pre-sowing technique for commercial propagation. Complementing these biological findings, value chain mapping traced the flow of raw materials across five primary stakeholders—farmers, processors, wholesalers, retailers, and end consumers—revealing a conversion yield of approximately 25 kg of natural indigo cake from 800 kg of dry leaves. Economic feasibility analysis confirmed that value-added processing significantly elevates commercial profitability, offering farmers a net return of ₹28,000 per acre (Benefit-Cost Ratio of 2.04) and processors a net profit of ₹50,000 per unit. Overall, combining 5–10 minutes of mechanical sandpaper scarification with integrated value chain processing provides a sustainable, highly remunerative framework for large-scale *I. tinctoria* production to meet the expanding global market demand for eco-friendly natural colorants.

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