

## **Standardization of seed testing protocol and seed germination improvement treatment in Henna (*Lawsonia inermis* Linn.)**

### **Abstract**

A research endeavor aimed at standardizing the seed germination process for Henna involved a comprehensive exploration of various germination media, temperature regimes, and dormancy - breaking treatments. Three distinct media - top of paper, between paper and sand-were evaluated alongside constant temperatures of 20°C, 25°C, and 30°C, as well as an alternating temperature of 20/30°C. An array of pre-treatments, including water soaking, KNO<sub>3</sub>, thiourea, leaching, GA<sub>3</sub>, and chilling treatment, were administered with the objective of enhancing seed germination. The findings highlighted the top of paper method and an alternate temperature of 20/30°C as the most conducive for seed germination, yielding remarkable outcomes viz., 80% germination rate, root length of 1.4cm, shoot length of 1.6cm, dry matter production of 3.0 mg 10 seedlings<sup>-1</sup>, and a vigor index of 240. Furthermore, specific timelines for key germination stages were delineated: 5.5days for initiation of germination, 7.8days for 50% seed germination, 16.0days for the onset of seedling withering, and 17.8days for the initiation of seedling mortality. As a result, it recommended that the first count on 8th day and final count on 16th day for evaluation. Further investigation found that soaking seed in a 1% Thiourea solution for 24 hours was highly effective, resulting in an impressive 94% germination rate, with minimal abnormal seedlings (3%), fresh ungerminated seeds (2%), and dead seeds (1%). Additionally, it significantly improved speed of germination (4.6), root length (1.5cm), shoot length (1.4cm), dry matter production (3.43 mg 10 seedlings<sup>-1</sup>), vigor index I (277), and Vigor index II (324). In contrast, untreated seeds only attained a 64% germination rate.

**Key words:** Henna – *Lawsonia inermis*, Media, temperature, dormancy breaking treatments, germination

### **1. Introduction**

Henna (*Lawsonia inermis* Linn, syn. *L. alba* Lam.), commonly referred to as Mehandi, belongs to the family Lythraceae. It is a densely branched, smooth shrub native to subtropical regions of Asia and Africa. Cultivation of henna is widespread in the western parts of India, Pakistan, Morocco, Yemen, Iran, Sudan, and Libya. Notably, Rajasthan is the primary region for henna cultivation in India, followed by Gujarat, Uttar Pradesh, and Punjab. Henna holds significant economic importance as a medicinal plant renowned for its cosmetic and traditional medicinal uses. The

powdered leaves of henna are utilized for dyeing hair, staining nails, and acting as a preservative for leather and cloth (Chaudhary et al., 2010; Siva, 2007). The plant was reported to have analgesic, antibacterial, antifungal, anti-parasitic and many similar properties (Babu and Subhasree, 2009). Seeds have deodorant properties and are useful in menorrhagia, leucorrhoea. Additionally, females have traditionally utilized henna seeds to mitigate body odor during gynecological problems (Nawagish, 2005; Zafar et al., 2006).

Henna serves as an ornamental garden shrub and is intentionally cultivated as a hedge plant and live fencing to safeguard crop fields from grazing animals (Chaudhary et al., 2005). Furthermore, henna holds significant importance in India, ranking as the third most essential medicinal and aromatic plant, following Isabgol (*Plantago ovata* L.) and Senna (*Cassia angustifolia* Vahl.) (Parihar et al., 2009).

The increasing demand for henna in cosmetics and medicinal applications has led to a rise in large-scale cultivation, necessitating a substantial supply of seeds for commercial cultivation and cuttings for hedge plantation. However, despite its importance as a medicinal crop, henna faces challenges in seed availability due to factors such as poor seed production and limited knowledge regarding seed germination improvement methods and dormancy-breaking techniques. Addressing these challenges is crucial for ensuring successful crop growth and maintaining seed quality.

To tackle these issues, studies have been undertaken with the following objectives: (i) developing standardized methods for seed germination testing procedures in henna seeds. (ii) developing dormancy-breaking treatments to enhance seed germination in henna seeds. These efforts aim to improve the availability and quality of henna seeds, thereby supporting the sustainable cultivation of this valuable crop to meet the growing demand in various industries.

## **2. Materials and methods**

### **2.1. Standardizing method for seed germination testing procedure for Henna seeds**

Seeds of Henna (*Lawsonia inermis*) were sourced from the Department of Medicinal and Aromatic Crops, Tamil Nadu Agricultural University, Coimbatore. The laboratory experiments conducted at the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore. Prior to experimentation, the seeds were meticulously cleaned and dried to achieve an optimal moisture content, following which they were graded to ensure uniform size. Subsequently, these prepared seeds were utilized for the study. The details of the treatments and methods employed for observation are elaborated below.

### **2.1.1. Treatment details**

A germination study was conducted on Henna seeds using various germination media, including Roll towel media ( $M_1$  - placed between paper), Top of paper media ( $M_2$ ), and sterilized sand media ( $M_3$ ). Additionally, the study involved subjecting the seeds to three constant temperature conditions: 20°C ( $T_1$ ), 25°C ( $T_2$ ), and 30°C ( $T_3$ ), as well as one alternating temperature setting ( $T_4$  - 20/30°C). Under the alternating temperature regime ( $T_4$ ), the seeds experienced 16 hours of light exposure at the higher temperature and 8 hours of darkness at the lower temperature. Similarly, for the constant temperature regimes ( $T_1$ ,  $T_2$ ,  $T_3$ ), the seeds were exposed to a 16-hour light period followed by an 8-hour dark period. The germination tests were carried out in controlled germination chambers, meticulously set to maintain the specified temperature regimes consistently throughout the entire duration of the study.

### **2.1.2. Methods**

#### **2.1.2.1. Seed germination (%)**

A germination test was conducted in accordance with the rules outlined by the International Seed Testing Association (ISTA), with minor modifications. Four replications, each comprising 100 seeds, were placed on moist germination media and then incubated under various temperature regimes as specified in the treatment details. The seeds were placed in a germination room under fluorescent lighting with a relative humidity of  $90 \pm 2\%$ . After the designated test period, the number of normal seedlings that emerged was counted, and the germination percentage was calculated. The germination percentage was expressed as a percentage using the following formula:

Germination (%) =

#### **2.1.2.2. Root length (cm)**

During the germination count, ten normal seedlings were randomly selected from each replication for the measurement of root length. Root length was measured from the point of attachment of the seed to the tip of the primary root. Subsequently, the mean values of root length were calculated and expressed in centimeters (cm). This procedure provided insight into the average root development of the germinated seedlings under the specified experimental conditions.

#### **2.1.2.3. Shoot length (cm)**

The seedlings chosen for root length measurement were also utilized for measuring shoot length. Shoot length was measured from the point of attachment of the seed to the tip of the leaf. Similarly to root length, the mean values of shoot length were calculated and expressed in centimeters

(cm). This comprehensive approach allowed for the assessment of both root and shoot development in the germinated seedlings under the specified experimental conditions.

#### **2.1.2.4. Seedling dry weight (mg/10 seedlings)**

The ten seedlings selected for length measurements underwent a drying process in a hot air oven set at  $80\pm 2^{\circ}\text{C}$  for a duration of 24 hours. Following this, they were allowed to cool to laboratory ambient temperature within a closed desiccator containing silica gel. After cooling, the seedlings were weighed and the weight was expressed in milligrams (mg) per 10 seedlings. This procedure provided information regarding the dry weight of the seedlings and was crucial for understanding their physiological characteristics under the experimental conditions.

#### **2.1.2.5. Vigour indices**

Vigour index was calculated using formula provided by Abdul - Baki and Andreson (1973) and the resulting mean values were expressed as whole numbers.

Vigour Index - I = Germination percentage X Seedling length (cm)

Vigour Index -II = Germination percentage X Seedling dry weight (mg/10 seedlings)

#### **2.1.2.6. Number of days for initiation of germination (days)**

The average number of days required for radicle emergence was recorded for each replication, and subsequently, the mean values were calculated. These mean values represent the average number of days required for the initiation of germination.

#### **2.1.2.7. Days required for 50% of germination (days)**

The number of days required for the completion of 50 percent germination of seedlings, encompassing all essential structures, was noted. This duration was designated as the first count day and expressed in days.

#### **2.1.2.8. Start of withering of seedling (days)**

After the complete establishment of seedlings, the days when seedlings began to wither were recorded for each replication within each treatment. Subsequently, the mean value of these recorded days was calculated and expressed in days. This duration, at which the withering of seedlings commenced, is denoted as the final count day.

#### **2.1.2.9. Start of mortality of seedling (days)**

Once the final count is reached, seedlings start to show signs of drying and a decline in vitality, attributed to the depletion of available food reserves necessary for their growth. As a result, they become incapable of thriving in the germination medium, ultimately leading to seedling mortality. The onset of seedling mortality was recorded for each treatment across replications, and subsequently, the mean value was calculated and expressed in days.

## 2.2. Dormancy breaking treatments to improve seed germination of Henna seeds

### Dormancy breaking treatments:

T <sub>0</sub> - Control	T <sub>16</sub> - Soaking in 300 ppm GA <sub>3</sub> for 24 hours
T <sub>1</sub> - Water soaking for 12 hours	T <sub>17</sub> - Soaking in 400 ppm GA <sub>3</sub> for 12 hours
T <sub>2</sub> - Water soaking for 24 hours	T <sub>18</sub> - Soaking in 400 ppm GA <sub>3</sub> for 24 hours
T <sub>3</sub> - Soaking in 0.5 % KNO <sub>3</sub> solution for 12 hours	T <sub>19</sub> - Soaking in 500 ppm GA <sub>3</sub> for 12 hours
T <sub>4</sub> - Soaking in 0.5 % KNO <sub>3</sub> solution for 24 hours	T <sub>20</sub> - Soaking in 500 ppm GA <sub>3</sub> for 24 hours
T <sub>5</sub> - Soaking in 1 % KNO <sub>3</sub> solution for 12 hours	T <sub>21</sub> - Leaching in water for 12 hours
T <sub>6</sub> - Soaking in 1 % KNO <sub>3</sub> solution for 24 hours	T <sub>22</sub> - Leaching in water for 24 hours
T <sub>7</sub> - Soaking in 0.5 % Thiourea solution for 12 hours	T <sub>23</sub> - Chilling treatment at 5 °C for 2 days
T <sub>8</sub> - Soaking in 0.5 % Thiourea solution for 24 hours	T <sub>24</sub> - Chilling treatment at 5 °C for 4 days
T <sub>9</sub> - Soaking in 1 % Thiourea solution for 12 hours	T <sub>25</sub> - Chilling treatment at 5 °C for 6 days
T <sub>10</sub> - Soaking in 1 % Thiourea solution for 24 hours	T <sub>26</sub> - Chilling treatment at 5 °C for 8 days
T <sub>11</sub> - Soaking in 100 ppm GA <sub>3</sub> for 12 hours	T <sub>27</sub> - Chilling treatment at 5 °C for 10 days
T <sub>12</sub> - Soaking in 100 ppm GA <sub>3</sub> for 24 hours	T <sub>28</sub> - Chilling treatment at 10 °C for 2 days
T <sub>13</sub> - Soaking in 200 ppm GA <sub>3</sub> for 12 hours	T <sub>29</sub> - Chilling treatment at 10 °C for 4 days
T <sub>14</sub> - Soaking in 200 ppm GA <sub>3</sub> for 24 hours	T <sub>30</sub> - Chilling treatment at 10 °C for 6 days
T <sub>15</sub> - Soaking in 300 ppm GA <sub>3</sub> for 12 hours	T <sub>31</sub> - Chilling treatment at 10 °C for 8 days
	T <sub>32</sub> - Chilling treatment at 10 °C for 10 days

The seeds underwent different pre-treatment methods before germination. Initially, they were soaked in water for durations of 12 and 24 hours, followed by shade drying at room temperature. Subsequently, GA<sub>3</sub> treatments were administered by soaking seeds in varying

concentrations ranging from 100 to 500 ppm for durations of 12 and 24 hours, followed by shade drying. Furthermore, chilling treatments subjected seeds to temperatures of 5°C and 10°C for periods ranging from 2 to 10 days. After the application of these seed treatments, a standard germination test was conducted using the petri plate method (top of the paper method) following the guidelines outlined by ISTA (2022). Observations were recorded for various parameters including germination percentage, abnormal seedlings, fresh ungerminated seeds (FUG), dead seeds, root length (cm), shoot length (cm), seedling dry weight (mg/10 seedlings), and vigor indices. These observations provided insights into the effects of different pre-treatment methods on the germination and growth characteristics of the henna seeds.

### **3. Result and Discussion**

#### **3.1. Standardization of suitable media, temperature, first and final count days for seed germination testing in Henna**

The present study found that the germination percentage of Henna varied significantly depending on the method used, with the highest germination rate observed in the top of paper method (75%), and the lowest in sand and between paper methods (46% and 44% respectively). However, various seedling quality parameters such as speed of germination (2.8), root length (1.2 cm), shoot length (1.3 cm), seedling dry weight (3.5 mg per 10 seedlings), and vigour index (189) were found to be maximal in the top of paper method (Table 1, 2 & 3 and Fig 1 & 2). The superior germination and seedling quality observed in the top of paper method for Henna may be attributed to the small size of Henna seeds, facilitating better growth and emergence in this method compared to sand. Additionally, the enhanced penetration of light and oxygen supply to the seeds in the top of paper method likely contributed to these favorable outcomes. Conversely, the use of sand as a medium posed challenges for seedling emergence and caused damage during seedling evaluation. These findings align with previous studies, such as that of Fathima et al. (2003), who recommended the top of paper method for better and quicker germination in *Andrographis paniculate*, and Bharath (2008), who similarly advocated for the top of paper method for *Ocimum sanctum* and *Andrographis paniculate*.

**Table 1. Influence of media and temperature on speed of germination and seed germination in Henna (*Lawsonia inermis*)**

Speed of germination						Germination (%)				
Media (M) / Temperature (T)	20 °C Constant (T <sub>1</sub> )	25 °C Constant (T <sub>2</sub> )	30°C Constant (T <sub>3</sub> )	20/30 °C Alternate (T <sub>4</sub> )	Mean	20 °C Constant (T <sub>1</sub> )	25 °C Constant (T <sub>2</sub> )	30°C Constant (T <sub>3</sub> )	20/30 °C Alternate (T <sub>4</sub> )	Mean
Between paper (M <sub>1</sub> )	1.6	1.5	1.2	1.7	<b>1.5</b>	49(44.41)	41(39.80)	45(42.10)	46(42.69)	<b>44(42.25)</b>
Top of paper (M <sub>2</sub> )	2.8	2.7	2.6	3.0	<b>2.8</b>	71(57.43)	78(62.29)	69(56.15)	80(63.46)	<b>75(59.84)</b>
Sand (M <sub>3</sub> )	1.4	1.4	1.4	1.6	<b>1.4</b>	49(44.41)	45(42.11)	44(41.53)	46(42.69)	<b>46(42.68)</b>
<b>Mean</b>	<b>1.9</b>	<b>1.8</b>	<b>1.7</b>	<b>2.1</b>		<b>56 (48.75)</b>	<b>55(48.07)</b>	<b>53(46.59)</b>	<b>57(49.91)</b>	
		<b>M</b>	<b>T</b>	<b>M × T</b>		<b>M</b>	<b>T</b>	<b>M × T</b>		
	<b>SEd</b>	<b>0.065</b>	<b>0.075</b>	<b>0.130</b>		<b>0.886</b>	<b>1.023</b>	<b>1.771</b>		
	<b>CD (P=0.05)</b>	<b>0.133</b>	<b>0.153</b>	<b>0.265</b>		<b>1.804</b>	<b>2.083</b>	<b>3.607</b>		

(Figures in parentheses indicate arc sine transformed values)

**Table 2. Influence of media and temperature on root and shoot length in Henna (*Lawsonia inermis*)**

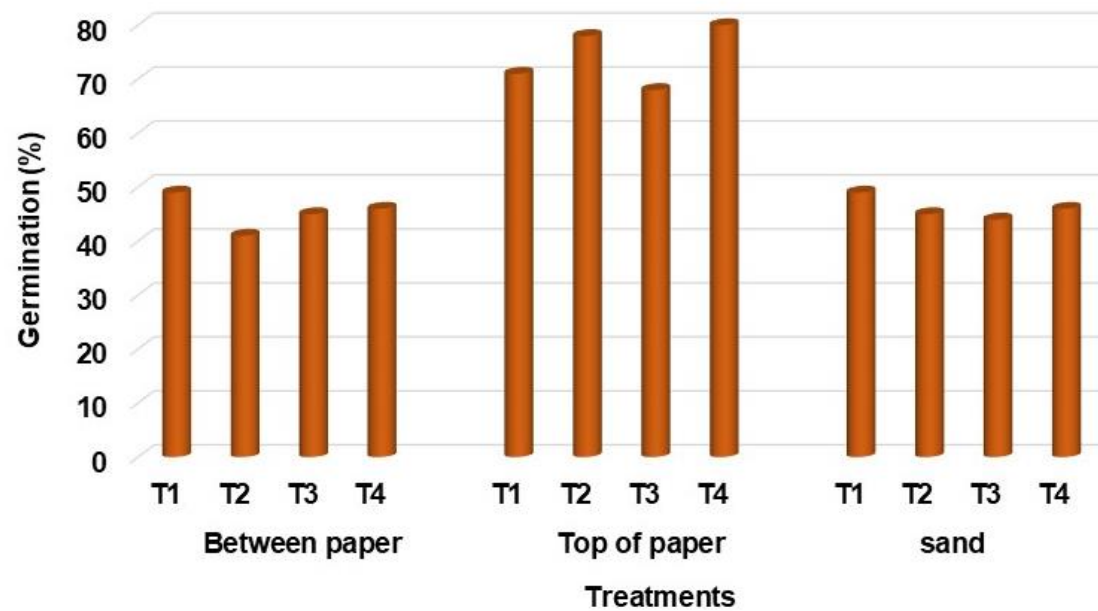
Root length (cm)						Shoot length (cm)				
Media (M) / Temperature (T)	20 °C Constant (T <sub>1</sub> )	25 °C Constant (T <sub>2</sub> )	30°C Constant (T <sub>3</sub> )	20/30 °C Alternate (T <sub>4</sub> )	Mean	20 °C Constant (T <sub>1</sub> )	25 °C Constant (T <sub>2</sub> )	30°C Constant (T <sub>3</sub> )	20/30 °C Alternate (T <sub>4</sub> )	Mean
Between paper (M <sub>1</sub> )	1.2	1.1	1.1	1.2	<b>1.1</b>	1.2	1.2	1.3	1.2	<b>1.3</b>
Top of paper (M <sub>2</sub> )	1.1	1.2	1.0	1.4	<b>1.2</b>	1.4	1.1	1.3	1.6	<b>1.3</b>
Sand (M <sub>3</sub> )	1.2	1.1	1.0	1.2	<b>1.1</b>	1.3	1.1	1.1	1.2	<b>1.2</b>
<b>Mean</b>	<b>1.2</b>	<b>1.1</b>	<b>1.0</b>	<b>1.3</b>		<b>1.3</b>	<b>1.2</b>	<b>1.2</b>	<b>1.3</b>	
		<b>M</b>	<b>T</b>	<b>M × T</b>		<b>M</b>	<b>T</b>	<b>M × T</b>		
	<b>SEd</b>	<b>0.071</b>	<b>0.083</b>	<b>0.143</b>		<b>0.038</b>	<b>0.044</b>	<b>0.076</b>		
	<b>CD(P=0.05)</b>	<b>0.146</b>	<b>0.168</b>	<b>0.291</b>		<b>0.078</b>	<b>0.090</b>	<b>0.155</b>		

**Table 3. Influence of media and temperature on dry matter production and vigour index in Henna (*Lawsonia inermis*)**

Dry matter production (mg 10 seedling <sup>-1</sup> )						Vigour index				
Media (M) / Temperature (T)	20 °C Constant (T <sub>1</sub> )	25 °C Constant (T <sub>2</sub> )	30°C Constant (T <sub>3</sub> )	20/30 °C Alternate (T <sub>4</sub> )	Mean	20 °C Constant (T <sub>1</sub> )	25 °C Constant (T <sub>2</sub> )	30°C Constant (T <sub>3</sub> )	20/30 °C Alternate (T <sub>4</sub> )	Mean
Between paper (M <sub>1</sub> )	3.2	3.3	4.1	3.3	<b>3.5</b>	118	94	108	110	<b>108</b>
Top of paper (M <sub>2</sub> )	3.3	3.3	3.2	3.0	<b>3.2</b>	178	179	159	240	<b>189</b>
Sand (M <sub>3</sub> )	2.3	3.2	3.1	3.0	<b>2.9</b>	123	99	92	110	<b>106</b>
Mean	<b>2.9</b>	<b>3.2</b>	<b>3.5</b>	<b>3.1</b>		<b>139</b>	<b>124</b>	<b>120</b>	<b>154</b>	
		M	T	M × T		M	T	M × T		
	SEd	0.344	0.397	0.687		4.935	5.699	9.870		
	CD (P = 0.05)	0.700	0.808	1.400		10.050	11.604	20.100		

**Table 4. Influence of media and temperature on number of days required for germination initiation and germination of 50 per cent of Henna (*Lawsonia inermis*)**

Germination initiation (days)						Germination of 50 % of seeds (days)				
Media (M) / Temperature (T)	20 °C Constant (T <sub>1</sub> )	25 °C Constant (T <sub>2</sub> )	30°C Constant (T <sub>3</sub> )	20/30 °C Alternate (T <sub>4</sub> )	Mean	20 °C Constant (T <sub>1</sub> )	25 °C Constant (T <sub>2</sub> )	30°C Constant (T <sub>3</sub> )	20/30 °C Alternate (T <sub>4</sub> )	Mean
Between paper (M <sub>1</sub> )	6.5	7.0	7.5	6.8	<b>6.9</b>	8.8	10.0	11.3	8.5	<b>9.6</b>
Top of paper (M <sub>2</sub> )	5.8	7.3	6.0	5.5	<b>6.1</b>	8.5	9.5	9.3	7.8	<b>8.8</b>
Sand (M <sub>3</sub> )	8.3	7.3	8.3	6.8	<b>7.6</b>	9.3	9.8	11.0	9.0	<b>9.8</b>
Mean	<b>6.8</b>	<b>7.2</b>	<b>7.3</b>	<b>6.4</b>		<b>8.8</b>	<b>9.8</b>	<b>10.5</b>	<b>8.4</b>	
		M	T	M × T		M	T	M × T		
	SEd	0.265	0.306	0.530		0.264	0.305	0.528		
	CD (P = 0.05)	0.540	0.624	1.080		0.537	0.621	1.075		



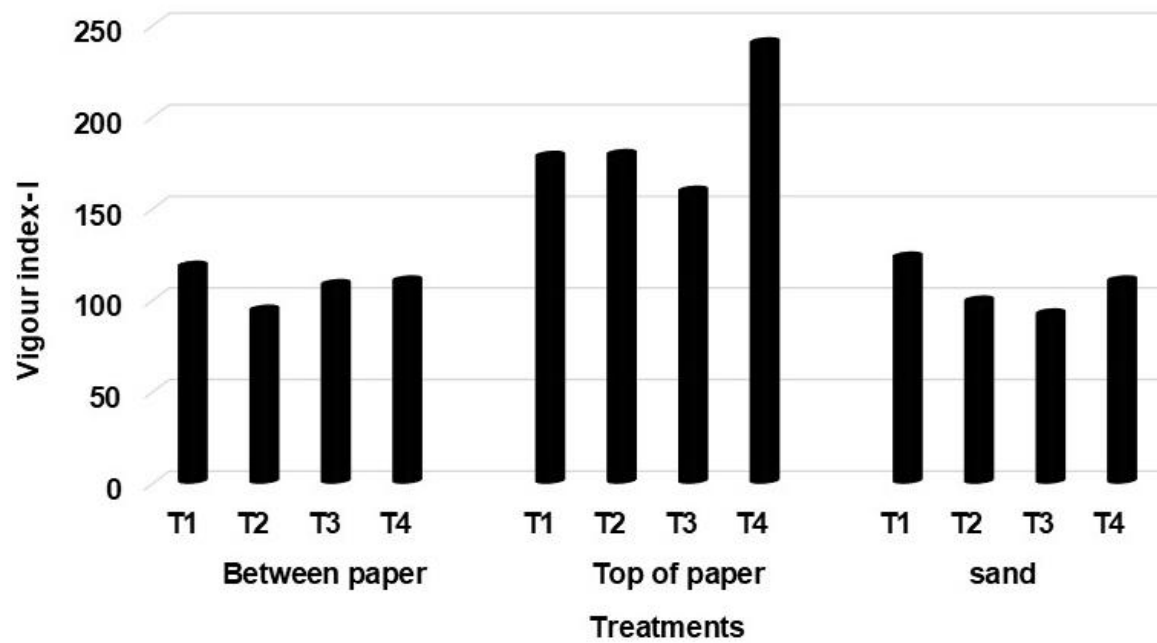
**Figure 1. Influence of media and temperature on seed germination in Henna (*Lawsonia inermis*)**

**T1** - 20°C Constant temperature

**T2** - 25°C Constant temperature

**T3** - 30°C Constant temperature

**T4** - 20/30°C Alternate temperature



**Figure 2. Influence of media and temperature on vigour index in Henna (*Lawsonia inermis*)**

**T1** - 20°C Constant temperature

**T3** - 30°C Constant temperature

**T2** - 25°C Constant temperature

**T4** - 20/30°C Alternate temperature

In the top of the paper method, the duration for various germination stages exhibited the shortest times: initiation of germination at 6.1 days, first count day when 50 percent of seeds germinated at 8.8 days, onset of seedling withering at 17.5 days, and commencement of seedling mortality at 19.2 days (Table 4 & 5). Conversely, these durations were maximal when utilizing sand media.

Among the various temperature regimes investigated, superior outcomes were observed in terms of speed of germination (2.1), seed germination rate (57%), root length (1.3 cm), shoot length (1.3 cm), dry matter production (3.1 mg per 10 seedlings), and vigour index values (154) under the 20/30 °C alternate temperature treatment. Additionally, the 20°C constant temperature treatment yielded results comparable to the alternate temperature regime. Conversely, the least favorable outcomes were recorded under the 30°C constant temperature treatment.

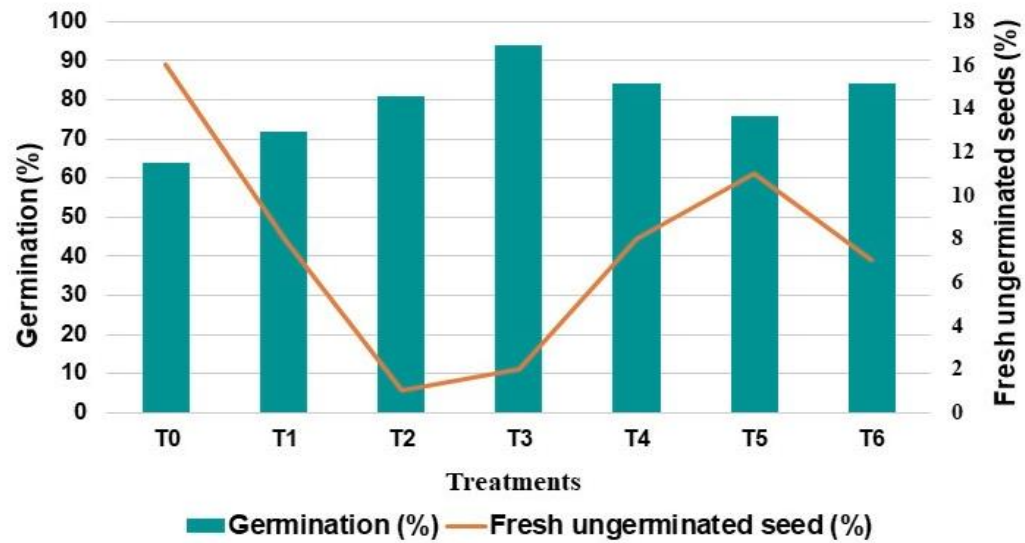
The duration for various germination stages, including initiation of seed germination (6.4 days) and the time taken for 50 percent germination (first count day) at 8.4 days, as well as the onset of seedling withering at 16.7 days and seedling mortality at 18.3 days, were significantly shorter under the 20/30°C alternate temperature regime followed by the 20°C constant temperature treatment, whereas these durations were notably delayed under the 30°C constant temperature treatment (Table 4 & 5; Figure 3 & 4). Ellis et al. (1985) conducted an extensive study on germination requirements in *Hibiscus sabdariffa* and *Catharanthus roseus*, reporting that alternate temperatures of 25/30 °C and 20/30 °C exhibited superior seed germination rates and other seedling quality parameters.

The study results demonstrated that the top of paper method was effective for both seed germination testing and evaluating seedling vigor parameters in Henna. Additionally, it was found that an alternate temperature of either 20/30°C or a constant temperature of 20°C was suitable for conducting germination tests on Henna seeds. However, considering the interaction between media and temperature, conducting germination tests using the top of paper method at 20/30°C alternate temperature emerged as the most effective approach for seed germination testing in Henna.

Furthermore, the study inferred that it would be appropriate to consider the 8<sup>th</sup> day as the first count day since 50 percent of seeds had germinated by this point. Similarly, the 16<sup>th</sup> day was suggested as the final count day since seedlings began to wither beyond this duration in Henna.

**Table 5. Influence of media and temperature on number of days on which withering and mortality of seedlings starts in Henna (*Lawsonia inermis*)**

Media (M) / Temperature (T)	Start of withering of seedling (days)					Start of mortality of seedling starts (days)				
	20 °C Constant (T <sub>1</sub> )	25 °C Constant (T <sub>2</sub> )	30°C Constant (T <sub>3</sub> )	20/30 °C Alternate (T <sub>4</sub> )	Mean	20 °C Constant (T <sub>1</sub> )	25 °C Constant (T <sub>2</sub> )	30°C Constant (T <sub>3</sub> )	20/30 °C Alternate (T <sub>4</sub> )	Mean
<b>Between paper (M<sub>1</sub>)</b>	19.0	18.5	19.8	17.3	<b>18.6</b>	20.8	19.8	21.0	18.5	<b>20.0</b>
<b>Top of paper (M<sub>2</sub>)</b>	17.8	18.5	17.8	16.0	<b>17.5</b>	19.3	20.3	19.5	17.8	<b>19.2</b>
<b>Sand (M<sub>3</sub>)</b>	18.0	18.0	18.3	16.8	<b>17.8</b>	19.3	20.3	19.5	18.5	<b>19.4</b>
<b>Mean</b>	<b>18.3</b>	<b>18.3</b>	<b>18.6</b>	<b>16.7</b>		<b>19.8</b>	<b>20.1</b>	<b>20.2</b>	<b>18.3</b>	
		<b>M</b>	<b>T</b>	<b>M × T</b>		<b>M</b>	<b>T</b>	<b>M × T</b>		
	<b>SEd</b>	<b>0.298</b>	<b>0.344</b>	<b>0.596</b>		<b>0.266</b>	<b>0.307</b>	<b>0.532</b>		
	<b>CD (P = 0.05)</b>	<b>0.606</b>	<b>0.700</b>	<b>1.212</b>		<b>0.241</b>	<b>0.625</b>	<b>1.083</b>		



**Figure 3. Effect of dormancy breaking treatments on fresh ungerminated seeds and germination percentage in Henna (*Lawsonia inermis*)**

**T0** - Control

**T1** - Water soaking for 24 hours

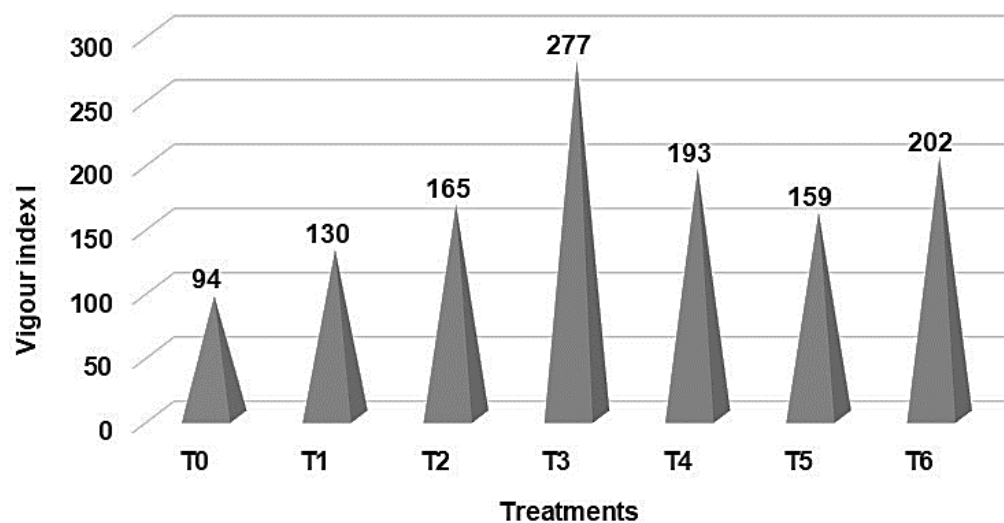
**T2** - 0.5% KNO<sub>3</sub> for 24 hours

**T3** - 1% Thiourea for 24 hours

**T4** - 300 ppm GA3 for 12 hours

**T5** - Leaching in water for 24 hours

**T6** - Chilling treatment @ 8days



**Figure 4. Effect of dormancy breaking treatments on vigour index in Henna (*Lawsonia inermis*)**

**T0** - Control

**T1** - Water soaking for 24 hours

**T2** - 0.5% KNO<sub>3</sub> for 24 hours

**T3** - 1% Thiourea for 24 hours

**T4** - 300 ppm GA3 for 12 hours

**T5** - Leaching in water for 24 hours

**T6** - Chilling treatment @ 8days

### 3.2. Standardization of suitable seed treatment to improve seed germination

The results demonstrated that soaking Henna seeds in a 1% Thiourea solution for 24 hours (T10) led to a notable improvement in seed germination by 94%, accompanied by enhanced speed of germination (4.6), root length (1.5 cm), shoot length (1.4 cm), dry matter production (3.43 mg per 10 seedlings), vigour index I (277), and vigour index II (324). Additionally, this treatment reduced the occurrence of abnormal seedlings to 3%, fresh ungerminated seeds to 2%, and dead seeds to 1%. In contrast, control seeds exhibited only 64% germination, along with a higher incidence of abnormal seedlings (12%), fresh ungerminated seeds (16%), and dead seeds (8%). The control group also showed reduced speed of germination (2.3), root length (0.6 cm), shoot length (0.9 cm), dry matter production (2.30 mg per 10 seedlings), vigour index I (94), and vigour index II (147).

Light was identified as a crucial factor in releasing dormancy from seeds, particularly for small seeds requiring light for germination. Thiourea, widely utilized to promote germination in light-sensitive seeds, was employed in this study as a germination-stimulating substance, effectively releasing dormancy and enhancing germination in Henna seeds. These findings were consistent with previous reports by Hartmann et al. (1997) in *Prunus* seeds, Arularasu and Sambandamurthi (1999) in *Ocimum sanctum*, and Revathi (2001) in *Phyllanthus amarus*.

Furthermore, although chilling treatment at 5°C for 10 days also improved seed germination to 85% compared to untreated control seeds (64%), it resulted in the development of more abnormal seedlings (5%) and fresh ungerminated seeds (8%). Chilling treatment is believed to activate the gibberellin synthesizing mechanism in seeds, as reported by Gashi et al. (2012). Pre-chilling was reported to affect metabolic and physiological activities, including hormone changes, disappearance of abscisic acid, and activation of GA<sub>3</sub>, consequently initiating seed germination, according to Greipsson (2001). In summary, the study found that soaking Henna seeds in a 1% Thiourea solution for 24 hours greatly enhanced seed germination rates and seedling vigor. This treatment resulted in significant improvements in various parameters such as speed of germination, root and shoot length, dry matter production, and vigor indices. Additionally, it reduced the occurrence of abnormal and dead seeds compared to untreated controls. The findings align with previous research indicating Thiourea's effectiveness in promoting germination, particularly in light-sensitive seeds like Henna. While chilling treatment also improved germination rates, it led to the development of more abnormal seedlings and fresh ungerminated seeds.

Overall, the study underscores the importance of Thiourea treatment for maximizing Henna seed germination and seedling quality.

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