J. Mountain Res. P-ISSN: 0974-3030, E-ISSN: 2582-5011 DOI: https://doi.org/10.51220/jmr.v19-i1.44
Vol. 19(1), (2024), 441-451

Studies On Standardization of Seed Germination Procedure *of Lawsonia inermis* Linn. (Henna).

Maryada Garg*

Department of Biotechnology, Pt. J.L.N. Govt. College, Faridabad

*Corresponding Author Email id: maryada.goyal@gmail.com

Received: 15.05.2024; Revised: 16.06.2024; Accepted: 16.06.2024

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. A steady temperature of 20°C, 25°C, and 30°C was maintained; at the same time an alternating temperature of 20/30°C was also maintained. An array of pre-treatments, including water soaking, KNO_3, Thio-urea, leaching, GA_3, and Vernalization, were administered with the objective of enhancing seed germination. The findings proved that the "top-of-paper" method and an alternate temperature of 20/30°C are the best conditions for seed germination. These methods showed remarkable outcomes, i.e. germination rate of 80%, root length of 1.4cm, shoot length of 1.6cm, dry matter production of 3.0 mg 10 seedlings^{-1}, 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. The first count of seeds is done on the 8th day and final germination count should be on the 16th day for evaluation purpose. Further investigation found that soaking seed in a 1% Thio-urea solution for 24 hrs. was highly effective, resulting in an 94% germination rate, with minimal abnormal seedlings (3%), fresh ungerminated seeds (2%), and dead seeds (1%). Other indices like speed of germination, length of root, length of shoot, dry matter, and vigor index were also improved. Untreated seeds had only 64% germination rate.

Key words: henna – *Lawsonia inermis* • media • temperature • dormancy breaking treatments • germination

Introduction

Henna plant (botanical name: *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; mostly, Rajasthan, Gujarat, Uttar Pradesh, Haryana, and Punjab. Henna is a medicinal plant renowned for its cosmetic and traditional medicinal uses. The powdered leaves of henna are utilized for dyeing hair and staining nails. It can act as a preservative for leather and cloth (Chaudhary et al., 2010; Siva, 2007). Further, the plant also has analgesic, antibacterial, antifungal, anti-parasitic properties (Babu and Subhasree, 2009). Females have used henna since times immemorial to get rid of body odor during gynecological problems (Nawagish, 2005; Zafar et al., 2006).

Henna serves as an ornamental garden shrub. It is grown over the fences because grazing animals do not consume these leaves; as a result, these leaves act as a safeguard to crop fields (Chaudhary et al., 2005). Furthermore, henna ranks on the third position for being the most essential medicinal and aromatic plant; while Isabgol (*Plantago ovata* L.) and Senna (*Cassia angustifolia* Vahl) rank first (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:

- Developing standardized methods for seed germination testing procedures in henna seeds.
- 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.

Materials and methods

Standardizing method for seed germination testing procedure for Henna seeds

Before starting the experiments, seeds of Henna (*Lawsonia inermis*) were first taken and 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.

Treatment details: A germination study was conducted on Henna seeds using various germination media, including Roll towel media (M_1 - placed between papers), 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 were given alternating light and dark periods; 16 hrs. of light exposure at the higher temperature followed by 8 hrs. of dark period at the lower temperature. Similarly, for the constant temperature regimes also (T_1, T_2, T_3), the seeds were exposed to a 16-hour light period and an 8-hour dark period. Controlled germination chambers were used to carry out the tests. The chambers were always maintained at specified temperature regimes.

Methods

Seed germination (%): A germination tests were conducted in conformity with the rules laid down by the International Seed Testing Association (ISTA); although slight modifications were done with respect to the laboratory conditions. 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±2%. After the test, the total number of normal seedlings that spurted up were counted. Germination percentage was calculated on this basis. The germination percentage was expressed as a percentage using the following formula:

Germination (%) = (normal seeds germinated/total seeds) *100

Root length (in cm): During the germination count, ten normal seedlings were randomly selected from each replication for the measurement of root length. The point of attachment of the seed to the ground till the tip of the primary root was considered as the root length. Using the root length of 10 seedlings, the mean value was calculated; it was expressed in centimeter (cm). This procedure provided insight into the average root development of the germinated seedlings under the specified experimental conditions.

Shoot length (in cm)

The above mentioned ten seedlings (chosen for root length measurement) were checked for the measurement of shoot length. Shoot length was measured from the ground attachment point of the seed to the leaf tip. The mean values of shoot length were also calculated in the same way as the root length; these were 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.

Seedling dry weight (mg/10 seedlings)

The ten seedlings selected for length measurements were dried in a hot air oven set at 85±2°C for a duration of 24-36 hrs.. Following this, they were allowed to cool to laboratory ambient temperature within a closed desiccator containing silica gel. After cooling, the weight of the seedlings was determined and 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 condition

Vigor indices: Vigor index was calculated using the given formula (reference: Abdul - Baki and Andreson (1973). Again mean values were calculated.

Vigor Index - I = {Germination percentage * Seedling length (cm)}

Vigor Index -II = {Germination percentage * Seedling dry weight (mg/10 seedlings)}

Number of days for initiation of germination (in days)

For each seedling, the number of days required for radicle emergence was recorded; again mean of all seedling's radicle emergence time was calculated. These mean values represent the average number of days required for the initiation of germination.

Days required for 50% of germination (in days)

Further, the time (in days) required for the completion of 50% germination of seedlings, was noted. This duration was designated as the first count day and expressed in days.

Start of withering of seedling (in 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.

Start of mortality of seedling (in 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 expressed in days.

The seeds underwent different pre-treatment methods before germination. Initially, they were soaked in water for durations of 12 and 24 hrs., followed by shade drying at room temperature. Subsequently, GA_3 treatments were administered by soaking seeds in varying concentrations ranging from 100 to 500 ppm for durations of 12 and 24 hrs., followed by shade drying. Furthermore, Vernalizations 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, total abnormal seedlings, total fresh ungerminated seeds (FUG), total number of dead seeds, mean root length (in cm), mean shoot length (in cm), mean seedling dry weight (expressed in 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.

Dormancy breaking treatments to improve seed germination of Henna seeds
Dormancy breaking treatments:

T_0 - Control
T_1 – Soak in water for 12 hrs.
T_2 – Soak in water for 24 hrs.
T_3 - Soak in 0.5 % KNO_3 solution for 12 hrs.
T_4 - Soak in 0.5 % KNO_3 solution for 24 hrs.
T_5 - Soak in 1 % KNO_3 solution for 12 hrs.
T_6 - Soak in 1 % KNO_3 solution for 24 hrs.
T_7 - Soak in 0.5 % Thio-urea solution for 12 hrs.
T_8 - Soak in 0.5 % Thio-urea solution for 24 hrs.
T_9 - Soak in 1 % Thio-urea solution for 12 hrs.
T_{10} - Soak in 1 % Thio-urea solution for 24 hrs.
T_{11} - Soak in 100 ppm GA_3 for 12 hrs.
T_{12} - Soak in 100 ppm GA_3 for 24 hrs.
T_{13} - Soak in 200 ppm GA_3 for 12 hrs.
T_{14} - Soak in 200 ppm GA_3 for 24 hrs.
T_{15} - Soak in 300 ppm GA_3 for 12 hrs.
T_{16} – Soak in 300 ppm GA_3 for 24 hrs.
T_{17} – Soak in 400 ppm GA_3 for 12 hrs.
T_{18} - Soak in 400 ppm GA_3 for 24 hrs.
T_{19} - Soak in 500 ppm GA_3 for 12 hrs.
T_{20} - Soak in 500 ppm GA_3 for 24 hrs.
T_{21} - Leaching in water for 12 hrs.
T_{22} - Leaching in water for 24 hrs.
T_{23} – Vernalization treatment at 5 °C for 2 days
T_{24} - Vernalization at 5 °C for 4 days
T_{25} - Vernalization at 5 °C for 6 days
T_{26} - Vernalization at 5 °C for 8 days
T_{27} - Vernalization at 5 °C for 10 days
T_{28} - Vernalization at 10 °C for 2 days
T_{29} - Vernalization at 10 °C for 4 days
T_{30} - Vernalization at 10 °C for 6 days
T_{31}- Vernalization at 10 °C for 8 days
T_{32} - Vernalization at 10 °C for 10 days

Results and Discussion

Standardization of benchmarks: suitable media, temperature, first and final count days for seed germination testing in Henna

In the given study, it was found that the germination percentage of Henna varied notably depending on the method used, with the highest germination rate observed in the "top-of-paper" method; reaching a remarkable rate of 75%. The lowest germination rate was found in "sand and between paper methods"; (46% and 44% respectively). Various seedling quality parameters were tested. These included speed of germination, mean root length (1.2 cm), mean shoot length (1.3 cm), mean seedling dry weight (3.5 mg/10 seedlings), and vigor index (189). All these were maximum 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, it can be elucidated that 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 as the best method for seed germination in *Andrographis paniculate*. Further, Bharath (2008), also stated that the top-of-paper method is the best method for seed germination in *Ocimum sanctum* and *Andrographis paniculate.*

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, better outcomes were observed in terms of speed of germination, seed germination rate (57%), mean root length (1.3 cm), mean shoot length (1.3 cm), dry matter production (3.1 mg per 10 seedlings), and vigor index values (154) under the 20/30 °C alternate temperature treatment.

Note that the results of 20°C constant temperature treatment were almost equivalent to alternate temperature treatment 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 (refer to table 4 & 5; Figure 3 & 4). Earlier, Ellis *et. al.* (1985) reported that alternate temperatures of 25/30 ºC and 20/30 ºC exhibited superior seed germination rates and other seedling quality parameters in *Hibiscus sabdariffa* and *Catharanthus roseus,*

Finally, the study results concluded that the top-of-paper method was effective for both seed germination testing and evaluating seedling vigor parameters in Henna. It was further 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 8th day as the first count day since 50 percent of seeds had germinated by this point. Similarly, the 16th day was suggested as the final count day since seedlings began to wither beyond this duration in Henna.

Standardization of seed treatment method for improvement of seed germination.

The results demonstrated that soaking Henna seeds in a 1% Thio-urea solution for 24 hrs (T10) led to a notable improvement in seed germination by 94%, accompanied by enhanced germination speed, mean root length (1.5 cm), mean shoot length (1.4 cm), mean dry matter production (3.43 mg per 10 seedlings), vigor index I (277), and vigor 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. In case of control, there was a higher incidence of fresh ungerminated seeds (16%), abnormal seedlings (12%), and dead seeds (8%). The control group also showed reduced germination speed, mean root length (0.6 cm), mean shoot length (0.9 cm), mean dry matter production (2.30 mg/10 seedlings), vigor index I (94), and vigor index II (147).

Light was identified as a crucial factor in releasing dormancy from seeds, particularly for small seeds requiring light for germination. Thio-urea, 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. Previous reports by Hartmann et al. (1997) in *Prunus* seeds, Revathi (2001) in *Phyllanthus amarus*, and Arularasu and Sambandamurthi (1999) in *Ocimum sanctum*, showed almost similar findings.

Furthermore, vernalization at 5°C for 10 days also improved seed germination to 85% compared to untreated control seeds (64%). In control experiment, the number of abnormal seedlings (5%) and fresh ungerminated seeds (8%) was enhanced. Vernalization activates the gibberellin synthesizing mechanism in seeds, as reported by Gashi *et. al.* (2012).

Table 1. Effect 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_1)**	**25 °C Constant (T_2)**	**30°C Constant (T_3)**	**20/30 °C Alternate (T_4)**	**Mean**	**20 °C Constant (T_1)**	**25 °C Constant (T_2)**	**30°C Constant (T_3)**	**20/30 °C Alternate (T_4)**	**Mean**
Between paper (M_1)	1.6	1.5	1.2	1.7	**1.5**	49(44.41)	41(39.80)	45(42.10)	46(42.69)	**44(42.25)**
Top-of-paper (M_2)	2.8	2.7	2.6	3.0	**2.8**	71(57.43)	78(62.29)	69(56.15)	80(63.46)	**75(59.84)**
Sand (M_3)	1.4	1.4	1.4	1.6	**1.4**	49(44.41)	45(42.11)	44(41.53)	46(42.69)	**46(42.68)**
Mean	**1.9**	**1.8**	**1.7**	**2.1**		**56 (48.75)**	**55(48.07)**	**53(46.59)**	**57(49.91)**	
		M	**T**	**M × T**			**M**	**T**	**M × T**	
	SEd	**0.065**	**0.075**	**0.130**			**0.886**	**1.023**	**1.771**	
	CD (P =0.05)	**0.133**	**0.153**	**0.265**			**1.804**	**2.083**	**3.607**	

(Figures in parentheses indicate arc sine transformed values)

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

	Root length (in cm)					Shoot length (in cm)				
Media (M) / Temperature (T)	**20 °C Constant (T_1)**	**25 °C Constant (T_2)**	**30°C Constant (T_3)**	**20/30 °C Alternate (T_4)**	**Mean**	**20 °C Constant (T_1)**	**25 °C Constant (T_2)**	**30°C Constant (T_3)**	**20/30 °C Alternate (T_4)**	**Mean**
Between paper (M_1)	1.2	1.1	1.1	1.2	**1.1**	1.2	1.2	1.3	1.2	**1.3**
Top-of-paper (M_2)	1.1	1.2	1.0	1.4	**1.2**	1.4	1.1	1.3	1.6	**1.3**
Sand (M_3)	1.2	1.1	1.0	1.2	**1.1**	1.3	1.1	1.1	1.2	**1.2**
Mean	**1.2**	**1.1**	**1.0**	**1.3**		**1.3**	**1.2**	**1.2**	**1.3**	
		M	**T**	**M × T**			**M**	**T**	**M × T**	
	SEd	**0.071**	**0.083**	**0.143**			**0.038**	**0.044**	**0.076**	
	CD(P=0.05)	**0.146**	**0.168**	**0.291**			**0.078**	**0.090**	**0.155**	

Table 3. Effect of media and temperature on the production of dry matter and vigor index in Henna (*Lawsonia inermis*)

Dry matter production (mg 10 seedling^{-1})						Vigour index				
Media (M) / Temperature (T)	**20 °C Constant (T_1)**	**25 °C Constant (T_2)**	**30°C Constant (T_3)**	**20/30 °C Alternate (T_4)**	**Mean**	**20 °C Constant (T_1)**	**25 °C Constant (T_2)**	**30°C Constant (T_3)**	**20/30 °C Alternate (T_4)**	**Mean**
Between paper (M_1)	3.2	3.3	4.1	3.3	**3.5**	118	94	108	110	**108**
Top-of-paper (M_2)	3.3	3.3	3.2	3.0	**3.2**	178	179	159	240	**189**
Sand (M_3)	2.3	3.2	3.1	3.0	**2.9**	123	99	92	110	**106**
Mean	**2.9**	**3.2**	**3.5**	**3.1**		**139**	**124**	**120**	**154**	
		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. Effect of media/temperature on total number of days needed for germination initiation and germination of 50% Henna seeds (*Lawsonia inermis*)

Germination initiation (days)						Germination of 50 % of seeds (days)				
Media (M) / Temperature (T)	**20 °C Constant (T_1)**	**25 °C Constant (T_2)**	**30°C Constant (T_3)**	**20/30 °C Alternate (T_4)**	**Mean**	**20 °C Constant (T_1)**	**25 °C Constant (T_2)**	**30°C Constant (T_3)**	**20/30 °C Alternate (T_4)**	**Mean**
Between paper (M_1)	6.5	7.0	7.5	6.8	**6.9**	8.8	10.0	11.3	8.5	**9.6**
Top-of-paper (M_2)	5.8	7.3	6.0	5.5	**6.1**	8.5	9.5	9.3	7.8	**8.8**
Sand (M_3)	8.3	7.3	8.3	6.8	**7.6**	9.3	9.8	11.0	9.0	**9.8**
Mean	**6.8**	**7.2**	**7.3**	**6.4**		**8.8**	**9.8**	**10.5**	**8.4**	
		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**	

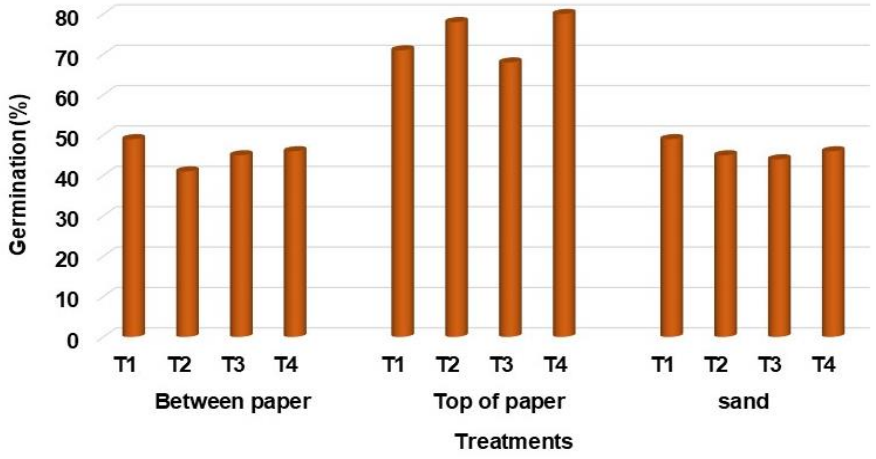

Figure 1. Influence of media and temperature on seed germination in Henna (*Lawsonia inermis*)

T1 - 20ºC Constant temperature
T2 - 25ºC Constant temperature
T3 - 30ºC Constant temperature
T4 - 20/30ºC Alternate temperature

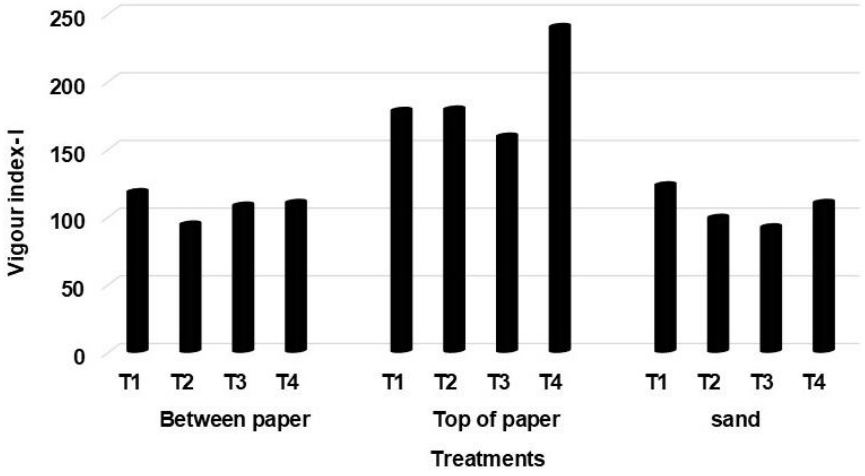

Figure 2. Influence of media and temperature on vigour index in Henna (*Lawsonia inermis*)

T1 - 20ºC Constant temperature
T2 - 25ºC Constant temperature
T3 - 30ºC Constant temperature
T4 - 20/30ºC Alternate temperature

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

	Start of withering of seedling (days)					Start of mortality of seedling starts (days)				
Media (M) / Temperature (T)	**20 °C Constant (T_1)**	**25 °C Constant (T_2)**	**30°C Constant (T_3)**	**20/30 °C Alternate (T_4)**	**Mean**	**20 °C Constant (T_1)**	**25 °C Constant (T_2)**	**30°C Constant (T_3)**	**20/30 °C Alternate (T_4)**	**Mean**
Between paper (M_1)	19.0	18.5	19.8	17.3	**18.6**	20.8	19.8	21.0	18.5	**20.0**
Top-of-paper (M_2)	17.8	18.5	17.8	16.0	**17.5**	19.3	20.3	19.5	17.8	**19.2**
Sand (M_3)	18.0	18.0	18.3	16.8	**17.8**	19.3	20.3	19.5	18.5	**19.4**
Mean	**18.3**	**18.3**	**18.6**	**16.7**		**19.8**	**20.1**	**20.2**	**18.3**	
	SEd	**M 0.298**	**T 0.344**	**M × T 0.596**			**M 0.266**	**T 0.307**	**M × T 0.532**	
	CD (P = 0.05)	**0.606**	**0.700**	**1.212**			**0.241**	**0.625**	**1.083**	

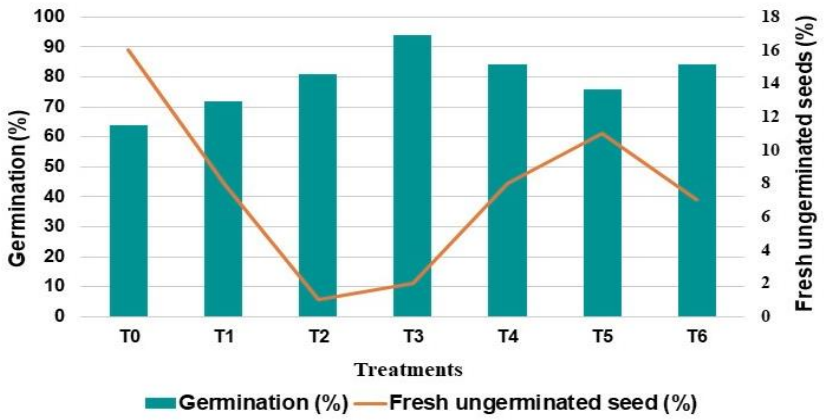

Figure 3. Effect of dormancy breaking treatments on fresh ungerminated seeds and germination percentage in Henna (*Lawsonia inermis*)

T0 - Control
T1 - Water soaking for 24 hrs.
T2 - 0.5% KNO_3 for 24 hrs.
T3 - 1% Thio-urea for 24 hrs.
T4 - 300 ppm GA3 for 12 hrs.
T5 - Leaching in water for 24 hrs.
T6 - Vernalization @ 8days

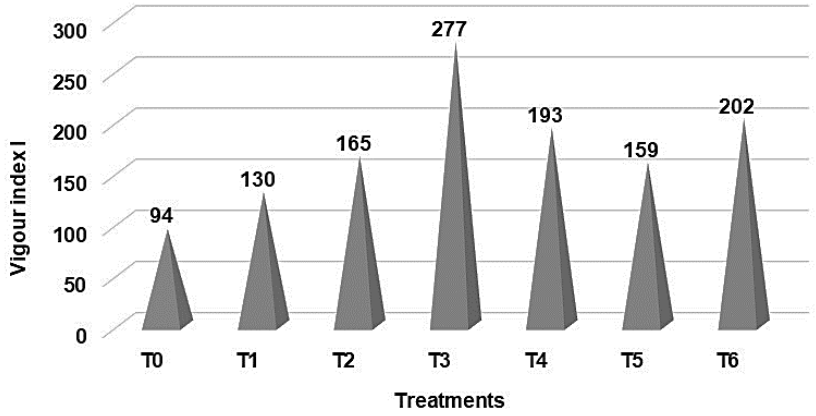

Figure 4. Effect of dormancy breaking treatments on vigour index in Henna (*Lawsonia inermis*)

T0 - Control
T1 - Water soaking for 24 hrs.
T2 - 0.5% KNO3 for 24 hrs.
T3 - 1% Thio-urea for 24 hrs.
T4 - 300 ppm GA_3 for 12 hrs.
T5 - Leaching in water for 24 hrs.
T6 - Vernalization @8 days

Pre-vernalization process affects metabolic and physiological activities, like hormone changes, removal of abscisic acid (ABA), and activation of GA_3. This results in initiation of seed germination (Greipsson, 2001).

In summary, the current study concluded that soaking Henna seeds in a 1% thio-urea solution for 24 hrs. greatly enhanced seed germination rates and seedling vigor. This treatment resulted in significant improvements in various parameters such as speed of germination, mean dry matter production, mean root and shoot length, and vigor indices. Further, it reduced the occurrence of abnormal and dead seeds compared to untreated controls. The findings align with previous research indicating thio-urea's effectiveness in promoting germination, particularly in light-sensitive seeds like Henna. While Vernalization also improved germination rates, it led to the development of more abnormal seedlings and fresh ungerminated seeds. Overall, the study underscores the importance of thio-urea treatment for maximizing Henna seed germination and seedling quality.

References

Abdul-Baki, AA, Anderson, JD. Vigour determination in soybean seed by multiple criteria. Crop Science. 1973; 13: 630-33

Arularasu P, Sambandamurthi S. Effect of germination nitrogen and spacing on yield of herbage and oil yield in Tulasi (Ocimum sanctum L.). 1999.

Babu PD, Subhasree RS. Antimicrobial activities of Lawsonia inermis-a review. Acad J Plant Sci. 2009; 2(4):231-2.

Bharath VL. Standardization of seed testing procedures and storage studies in selected medicinal crops (Doctoral dissertation, UAS, Dharwad). 2008.

Chaudhary G, Goyal S, Poonia P. *Lawsonia inermis* Linnaeus: a

phytopharmacological review. Int J Pharm Sci Drug Res. 2010; 2(2):91-8.

Ellis RH, Hong TD, Roberts EH. Handbook of seed technology for genebanks: Volume II: compendium of specific germination information and test recommendation. 1985

Fathima Gani, Balasubramanian AM, Swaminatha V, Balakrishna K, Gururajan B. Seed germination studies on Kalmegh (*Andrographis pariculata*). Nat. Sem. New Presp. Sps., Med. Arom. Pl., pp. 2003; 27-29

Gashi B, Abdullai K, Mata V, Kongjika E. Effect of gibberellic acid and potassium nitrate on seed germination of the resurrection plants *Ramonda serbica* and *Ramonda nathaliae*. African Journal of Biotechnology. 2012; 11(20):4537-42.

Greipsson S. Effects of stratification and GA_3 on seed germination of a sand stabilizing grass *Leymus arenarius* used in reclamation. Seed Science and Technology. 2001; 29(1):1-0.

Hartmann K, Kroob C, Mollwo A. Phytochrome-mediated photocontrol of the germination of the Scentless Mayweed, *Matricaria inodora* L., and its sensitization by nitrate and temperature. Journal of Photochemistry and Photobiology B: Biology. 1997; 40(3): 240-252.

International Seed Testing Association. "International Rules for Seed Testing. International Seed Testing Association"; International Seed Testing Association: Bassersdorf, Switzerland. 2017.

Nawagish M. Standardisation and tissue culture studies on *Lawsonia inermis* Linn. M. Pharm (Doctoral dissertation, Thesis, Jamia Hamdard, New Delhi). 2005.

Parihar SS, Dadlani M, Yadav NK, Debarati. Seed morphology, dormancy, and germination in Henna (*Lawsonia inermis* L.). Abstract International Conference on nurturing arid Zone for People and Environment, CAZRI, Jodhpur, India. 2009; 159–60.

Revathi R. Seed production, testing and storage studies in *Phyllanthus amarus* (Schum and Thom.). M. Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore. 2001.

Siva R. Status of natural dyes and dye-yielding plants in India. Current science. 2007; 10:916-25.

Zafar S, Ahmad S, Ahmad SJ. *Lawsonia inermis* Linn: Medicinal plants traditional knowledge. IK International Publishing House, New Delhi. 2006.