**Investigation of Seed Extraction Methods to Enhance Seed Quality and Viability in Nightshade (*Solanum trilobatum* L.)**

**Abstract**

*Solanum trilobatum* L. (nightshade) is a medicinally important plant, yet its cultivation is limited due to challenges in seed germination and poor seed quality. This study aimed to standardize effective seed extraction methods and assess their impact on seed quality and viability. Three categories of extraction treatments such as fermentation, acidand alkali were evaluated. Among these, the acid method using commercial HCl @ 20 ml kg⁻¹ for 2 minutes (T5) resulted in the highest seed recovery (7.76%), germination (42%), root length (2.21 cm), shoot length (3.11 cm), dry matter production (2.74 mg/10 seedlings)and vigour index (224). Tetrazolium viability testing revealed that embryo excision soaked in 0.5% TZ solution for 2 hours showed the highest viability at 88%. The findings suggest that acid extraction, particularly HCl @ 20 ml kg⁻¹ for 2 minutes, is the most effective method for improving seed quality in nightshade and can aid in its large-scale cultivation and conservation.

**Keywords:** *Solanum trilobatum*, Tetrazolium test, Seed vigour, Fermentation method, Alkali treatment

**1.0 Introduction**

Nightshade (*Solanum trilobatum* L.), a medicinal plant belonging to the family Solanaceae, is known by various names across regions: *Alarka* in Sanskrit, *Tuduvalai* in Tamil, *Alarkapatramu* in Telugu, *Tutuvalam* in Malayalam and *Purple-fruited pea eggplant* in English. Native to the Indo-Malaysian region, it is widely distributed across India, particularly in Maharashtra, Kerala, Karnataka and most districts of Tamil Nadu. Traditionally, many medicinal plants such as Aloe, Kalmegh and Tulsi are valued for their analgesic and antibacterial properties. Among them, *Solanum trilobatum* is - known for its immunomodulatory, anti-diabetic, anti-ulcer and hepatoprotective effects. Its leaf extracts are traditionally used to neutralize snake venom and enhance male fertility (Kumar *et al*., 2011). Additionally, a decoction of the entire plant is commonly used in the treatment of acute and chronic bronchitis.

The demand for medicinal plants is increasing day by day (Bhattacharjee *et al*., 2020) and there is a drastic increase in the usage of herbal medicines was found in the last few years (Vethanarayanan *et al*., 2011). To meet out the emerging demand, it is an urgent need to cultivate and conserve the useful medicinal plants. For effective cultivation and conservation, the medicinal plants have to be identified about their habit, habitat, growth characteristics and mode of propagation. In vitro method of propagation is mainly adopted for regeneration in medicinal plants, but the mode of seed propagation is highly multiplicative and economically viable.

An international study of cancer research discovered that the plant *Solanum trilobatum* can be used to treat lung cancer (Sharma *et al*., 2015). Phytochemical compounds such as solasoline are extracted from - its leaves, fruits, seeds and stems, which are widely used in the production of steroid drugs (Rashid *et al*., 2014). The other phytochemical compounds found in this plant are sobatum, β-solamarine, solanine, solasodine, glycoalkaloid, disogenin and tomatidine.

Seed extraction is the process of separating or removing seeds from the fruit. Before sowing, the seeds from the fleshy fruits could be removed. The proper method of extraction of seeds needs to be identified for efficient seed recovery without damaging the seeds. So, it is one of the important aspects of seed technology to study the scientific method of extraction to separate quality seeds. Despite its well-documented medicinal value, systematic cultivation of *Solanum trilobatum* L. is not widely practiced, limiting the full utilization of its traditional benefits. Given its ethnomedicinal significance, there is a pressing need to promote the cultivation and conservation of this species for future generations. The crop is primarily propagated through seeds, which offers an effective means for conservation. Although numerous studies have focused on the medicinal properties of *S. trilobatum*, there is limited information available regarding its seed quality characteristics. The existing evidence on seed quality parameters in nightshade remains scarce. Therefore, this study was undertaken to evaluate different seed treatment methods, extract seeds efficiently and assess their viability and overall seed quality.

**2.0 MATERIALS AND METHODS**

**2.1 Experimental sit and Treatment details**

The work has been carried out in the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore, in the year 2024-2025. The fully ripened berries of *Solanum trilobatum* L. were collected from ARS, Vaigaidam. The berries were graded based on their size and berries were surface sterilized with 2% Sodium oxychloride (NaOCl) for 5 min and then treated with 80% ethanol for 5 min, followed by rinsing thrice with distilled water. The seeds were extracted using the following methods such as Fermentation- fully ripened fruits were gently squeezed to liberate the seeds with pulp. The seeds and mucilage were allowed for fermentation for 12 and 24 hrs. In acid method the fully ripened fruits were gently squeezed to liberate the seeds with pulp. Twenty grams of pulp was taken and 2% hydrochloric acid @ 20 ml/kg and 25 ml/kg was added. After continuous stirring, the pulp and acid mixture were allowed for 1,2,3 min (Fig. 1). In alkali method, the fully ripened fruits were gently squeezed to liberate the seeds with pulp. Twenty gram of pulp was taken and sodium bi-carbonate (0.5 %) were added and allowed for 12 and 24 h. The following treatments were tested: T1: Squeezing of fruits to liberate the seeds, T2: T1+ Fermentation of seeds for 12 hrs, T3:T1+ Fermentation of seeds for 24 hrs, T4: Extraction by Acid method (HCl @ 20ml/kg for 1 mins), T5: Extraction by Acid method (HCl @ 20ml/kg for 2 mins), T6: Extraction by Acid method (HCl @ 20ml/kg for 3 mins), T7: Extraction by Acid method (HCl @ 25ml/kg for 1 mins), T8: Extraction by Acid method (HCl @ 25ml/kg for 2 mins), T9: Extraction by Acid method (HCl @ 25ml/kg for 3 mins), T10: Extraction by Alkali method (Sodium bicarbonate @ 0.5% for 12 hrs), T11: Extraction by Alkali method (Sodium bicarbonate @ 0.5% for 24 hrs). After the seeds were extracted using different treatment, the seeds were washed repeatedly for 4 to 5 times to remove the traces of impurities present in the seed coat seeds were dried under shade at room temperature.

**2.2 Seed recovery percentage and 100-seed weight**

The seeds extracted from the berries with the different treatments, such as fermentation and acid, alkaline and the seeds were shade dried for 48 hr as recommended (Murugeshwari *et al*., 2022). The seed recovery was calculated using following formula and expressed in percentage (%). Followed by the weight of 100 seeds was calculated and the mean value was expressed in gram. Seed recovery (%) = Dry weight of seed/ Weight of pulp ×100

**2.3 Germination percentage and seedling growth parameters**

The seeds were subjected to germination test using paper medium (top of paper method). Four replicates of 100 seeds each were placed in a germination room maintained with 25±20C temperature and 95±2% relative humidity. After 21 days, the normal seedlings were counted and it is expressed as percentage (ISTA., 2019).

 Germination (%) = Number of normal seedlings/ Total number of seeds sown ×100

Speed of emergence was calculated using the following formula and expressed in number (Maguire, 1962).

Speed of germination = X1Y1+X2-X1Y2+…+Xn- Xn-1Yn

Here X1, X2… Xn are the frequency of germinated seeds on the first, second and final day. While Y1, Y2 … Yn are the days from sowing to first, second and up to last day.

Extracted seeds were estimated for other seed quality parameters such as abnormal seedlings (%), Dead seeds (%), Hard seeds (%), Fresh ungerminated seeds (%), root length (cm), shoot length (cm) and dry matter production (g 10 seedlings-1), following the established protocol (ISTA, 2019). The seed vigor index was also determined using the standardized method (Abdul-Baki and Anderson, 1972).

Vigour index = Germination (%) × Mean seedling length (cm).

**2.4 Tetrazolium (Tz) test for estimating seed viability**

The seed viability was assessed by tetrazolium test in which four replicates of 25 seeds were preconditioned by soaking in distilled water for a period of 24 hrs. After preconditioning, the seeds were prepared for assessing viability such as by removing seed coat and cutting the seed, after that transfer the seeds to the petri dishes. The 2,3,5 triphenyl tetrazolium chloride solution under different concentration such as 0.1, 0.2, 0.5% was added and kept in hot air oven at 350 C under dark condition for different duration such as 1,2 hrs. The excess solution was then decanted to assess the staining pattern of the embryo. Based on the staining pattern the embryo has been grouped into viable and non-viable. The viability seeds of each treatment was recorded and the mean viability was calculated and expressed in percentage (Murugeshwari *et al*., 2022).

Viable seed (%) = Number of fully stained seeds/ Total number of seeds placed ×100

**3.0 Statistical analysis**

Statistical analyses were conducted using SPSS version 21.0 (Chicago, USA). One-way analysis of variance (ANOVA) was employed to determine significant differences among the treatments, followed by Duncan’s multiple comparison test for mean separation at P < 0.05. Bar graphs were generated and visualized using GraphPad Prism version 8.

**4.0 Results and discussion**

Seed extraction is a critical post-harvest operation that significantly influences seed quality, especially for crops with fleshy fruits like *Solanum trilobatum* L. Several seed extraction methods are encountered for extraction of seeds such as wet, dry, natural fermentation, chemical fermentation and mechanical means of seed extraction. Understanding the proper seed extraction method is essential for maintaining high seed quality. In this study, different seed extraction techniques were evaluated to identify the most effective method for maintaining seed quality standards in nightshade. The results highlight the importance of selecting an appropriate extraction procedure to ensure optimal seed performance.

The berries of nightshade were subjected with different extraction methods such as squeezing, fermentation, acid and alkali. Among, the methods of seed extraction, acid (Comm. HCl @ 20 ml/kg for 2 min) recorded higher amount of seed recovery (7.76 per cent) followed by (7.25 per cent in Comm. HCl @ 20 ml/kg for 3 min ) when compared with other treatments such as alkali (NaHCO3 @ 0.5% for 24 h) recorded 3.84 per cent and fermentation for 12h recorded 2.90 per cent, while control recorded 2.13 per cent seed recovery (Fig. 2). The higher percent of seed recovery by HCl clearly demonstrates that the seeds are completely separated by the action of acid on the colloidal pulp. The seeds extracted by acid improves the seed colour in to golden yellow, which gave lustrous appearance and were free from gelatinous pulp material, whereas the seeds removed by alkali had dusky coloured seed coat which may affects the marketability of seeds. Similar results were reported by Gunasekaran (2003) in Solanum nigrum, where the seeds extracted with acid (HCl @ 25 ml/kg of pulp) recorded high seed recovery (7.28 per cent) when compared with manual extraction (6.99 per cent) and also Raval *et al*., (2016) reported in tomato for higher seed recovery in acids compared to fermentation method. No significant difference was observed in 100-seed weight (g).

The seeds extracted from different treatments were subjected for germination to observe the seedling growth characteristics. Among the following treatments the seeds extracted by acid (Comm. HCl @ 20 ml/kg for 2 min) recorded highest germination per cent (42 per cent) followed by 25 per cent (Comm. HCl @ 20 ml/kg for 2 min, when it is compared with other treatments such as alkali (NaHCO3 @ 0.5% for 24 h) recorded 6 per cent of germination and fermentation for 12 h recorded 8 per cent while the control did not germinate (Table. 1). The results are in conformity with Das *et al*. (1997) in tomato. The increase in germination percentage over control is mainly due to high acidity which rapidly neutralize the germination inhibitors present around the seed and the result indicating nil germination in control clearly depicts that the presence of germination inhibitor which might inhibit the seed germination.

The present study also indicates that when the concentration of acid increases (Comm. HCl @ 25 ml/kg for 1,2 and 3 min) the germination percentage also reduced as 13, 15 and 12 per cent, respectively (Table. 1). These findings were notably indicating a decrease in germination percentage when the concentration of acid increases. The same findings were also reported by Raval *et al*., (2016) and Degwale *et al*., (2023) in tomato. In alkali (NaHCO₃ @ 0.5% for 12 and 24 hrs) method of extraction recorded low amount of germination percentage such as 2 and 6 per cent respectively. The decrease in germination percentage is primarily related to the duration of the alkali treatment or the alkalinity may be detrimental to nightshade seeds. The results indicated that seeds extracted using alkali were not significantly improved the germination and other seed quality parameters. Similar findings were reported by (Javaregowda *et al*., 1994) in brinjal (Solanum melongena).

Abnormalities in seedlings were increased when the concentration and duration of the alkali treatment were increased. The maximum number of abnormal seedlings (23%) were reported on alkali (NaHCO₃ @ 0.5% for 24 hrs.) when compared with other treatments such as fermentation for 24 h recorded 15 per cent of abnormal seedlings and acid (Comm. HCl @ 20 ml/kg for 2 mins) recorded 4 per cent of abnormal seedlings. Due to the deleterious effect of alkali, a greater number of dead seeds were reported when the seeds are extracted with alkali (NaHCO₃ @ 0.5% for 12 and 24 hrs) such as 61 and 65 per cent respectively (Table. 1). Root length and shoot length are prime indicators of seedling vigour that aid in performance and growth of the seed under certain environmental conditions. The seed extracted using acid (Comm. HCl @ 20 ml/kg for 2 mins) recorded maximum root and shoot length (2.21 cm, 3.11 cm) respectively (Table. 2). The dry matter production was escalated when the seeds extracted with acid (Comm. HCl @ 20 ml/kg for 2 mins) has recorded 2.74 mg seedlings-10 (Table. 2). It indicates that the acid method of extraction expresses it superiority over other methods of seed extraction. The other seedling quality parameters were decreased when the concentration of acid increases. The increase in concentration and duration of acid will have a negative impact on seed quality (Desai, 2004 and Degwale *et al*. 2023).

Seed vigour is an inherent ability of the seed to survive under a wide range of climatic conditions. The computed vigour index value of the present investigation recorded as 224, was found to be higher in the seeds extracted by acid (Comm. HCl @ 20 ml/kg for 2 mins). Similar results were reported by Singh (2002) in tomato where the tomato seeds extracted under acid treatment (HCl @ 20 ml/kg of pulp) reported high vigour index (1420) (Table. 2). Hence, the present study inferred that the fully matured berries of nightshade treated with acid (Comm. HCl @ 20 ml/kg for 2 mins) recorded high amount of seed recovery (7.76 per cent) followed by high germination percentage (42 per cent), root and shoot length (2.21 and 3.11 cm respectively) and vigour index (224) than other treatments. Therefore, it could be concluded that seeds of nightshade could be extracted with commercial hydrochloric acid @ 20 ml/kg for 2 mins for better seed quality parameters (Table. 1, 2).

**4.1 Assessment of topographical pattern of staining embryo in nightshade (Quick viability test)**

Tetrazolium test is a biochemical test which determines the presence of viable seed through the activity of dehydrogenase enzyme with a duration of 24-48hrs., which is irrespective of dormancy level of the seed (ISTA, 2019). The main advantage of tetrazolium test is simple, rapid and consistent approach which provides an accurate assessment on seed viability even when the seeds do not germinate due to dormancy. The Tz test distinguishes between viable and dead embryonic tissues based on relative respiration rate in the hydrated condition. The tetrazolium test makes use of the activity of dehydrogenase enzyme which acts as an index of respiration rate and seed viability. The oxidized, colourless 2,3,5 triphenyl tetrazolium salt solution is reduced by hydrogen ions due to the activity of dehydrogenase, which results in the formation of stable and non-diffusible compound such as formazan (reddish colour). The formation of reddish coloured formazan indicates the respiratory activity takes place in mitochondria, which enables the separation of live seeds (stained) from those that are unstained and abnormally coloured (Marcos Filho, 2005).

The concentration and duration of soaking in Tz solution was positively influenced in the conversion of colourless 2,3,5 triphenyl tetrazolium chloride into red coloured formazan. In this method, the seeds were prepared by longitudinal cutting and embryo excision. The prepared seeds were placed in 0.1,0.2 and 0.5 per cent of tetrazolium solution for 1 and 2h. Among the seed preparation methods, embryo excision is ideal for distinguishing viable and non-viable seeds. The seeds prepared by longitudinal cutting results in lower number of viable seed (44%) even placed in higher concentration (0.5%) for 2hrs. But the seeds prepared by separation of embryo from the seeds results higher number of viable seeds (88%) when placed in 0.5% TZ for 2h (Fig. 3), the test also records 72 per cent of viable seeds when placed in 0.5% TZ for 1h (Table. 3). Lower concentration (0.1%) with duration of exposure (2 h.) reported only 12 per cent of viable seeds and also results in improper pattern of staining in seeds which made it difficult to separate viable and non-viable seeds. Similar results were reported by Jayamani (2020) in black cumin where the seeds were preconditioned by soaking in water for 24 hrs and the embryo alone separated from the seed and placed in 1.0% Tz solution for 3 hrs, which clearly distinguish viable and non-viable seeds.

The concentration of solution and the time of soaking may alter the staining pattern and it has been noted that increasing the concentration and duration of soaking results in overstaining of seeds which makes it difficult to interpret the results. The possibility of utilizing a lower concentration (0.1% or 0.5%) of tetrazolium chloride solution allows for an insufficient pattern of staining on seed tissues without affecting viability visualization (Grzybowski *et al.,* 2012). The concentration of tetrazolium solution, temperature and duration of soaking may vary according to the species and it influences the results of the crop being tested. During the period of staining, the temperature should be maintained above 30o C results in proper staining of an embryo in shorter periods (Marcos Filho *et al.,* 1999) (Novembre *et al.,* 2006). The usage of lower concentration of tetrazolium salt solution for assessing seed viability was reported by Grzybowski *et al.,* (2012) in barley (0.1%). The expensive cost of tetrazolium salt and the clear visualisation of living tissues in seeds are the major factors that warrant the usage of tetrazolium salt at lower concentrations (Santos *et al.,* 2007, Poovizhi, 2020).

|  |  |  |
| --- | --- | --- |
|  |  |  |
| Fully ripened berries | Seeds | External view of seed |

**Figure 1. Berries and seeds of Nightshade (*Solanum trilobatum* L.)**

**Figure 2. Effect of different seed extraction methods on seed recovery (%) and 100 seed weight (g) in nightshade**

T1 – Control (Squeezing of fruits to liberate the seeds), T2 – T1+ Fermentation of seeds for 12 hrs, T3– T1+ Fermentation of seeds for 24 hrs, T4 – Comm. HCl @ 20 ml kg-1 for 1 min, T5 – Comm. HCl @ 20 ml kg-1 for 2 min, T6 – Comm. HCl @ 20 ml kg-1 for 3 min, T7 – Comm. HCl @ 25 ml kg-1 for 1 min, T8 – Comm. HCl @ 25 ml kg-1 for 2 min, T9 – Comm. HCl @ 25 ml kg-1 for 3 min, T10 – NaHCO₃ @0.5% for 12 hrs, T11 – NaHCO₃ @0.5% for 24 hrs.

**Table 1. Effect of different methods of seed extraction on germination of nightshade (*Solanum trilobatum* L.) seeds**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatment** | **Germination****(%)** | **Speed of****germination** | **Abnormal seedlings****(%)** | **Dead seeds****(%)** | **FUG****(%)** |
| T1 | 0  | 0.0 | 0  | 0  | 100 (89.71)d |
| T2 | 8 (16.43)e | 0.2de | 12 (20.26)b | 40 (39.08)b | 40 (41.55)bc |
| T3 | 4 (11.53)ef | 0.1e | 15 (22.78)c | 45 (41.10)b | 36 (34.44)b |
| T4 | 20 (26.56)c | 1.4c | 13 (21.13)b | 7 (16.69)a | 60 (50.78)c |
| T5 | 42 (40.39)a | 2.9a | 4 (11.54)a | 3 (11.45)a | 51 (47.87)bc |
| T6 | 25 (30.00)b | 2.2b | 10 (18.40)a | 9 (18.68)a | 56 (48.34)c |
| T7 | 13 (21.13)d | 0.3de | 18 (25.10)cd | 10 (19.64)a | 59 (50.19)c |
| T8 | 15 (22.78)d | 0.5d | 20 (26.56)d | 8 (17.65)a | 57 (49.03)c |
| T9 | 12 (20.26)d | 0.2de | 22 (28.72)d | 8 (16.40)a | 58 (49.50)c |
| T10 | 2 (8.13)f | 0.1e | 21 (27.27)d | 61 (50.53)c | 16 (23.58)ab |
| T11 | 6 (14.17)e | 0.2de | 23 (28.71)de | 65 (52.72)c | 6 (14.20)a |
| **Mean** | 13 (21.13) | 0.75 | 14 (21.21) | 26 | 49 (45.38) |
| **SEd** | 2.83 | 0.07 | 0.44 | 0.67 | 0.99 |
| **CD (P=0.05)** | 5.76 | 0.14 | 0.90 | 1.36 | 2.03 |

(Figures in parentheses indicate arc-sine value and data with different lowercase letters indicate significant differences at p < 0.05)

T1 – Control (Squeezing of fruits to liberate the seeds), T2 – T1+ Fermentation of seeds for 12 hrs, T3– T1+ Fermentation of seeds for 24 hrs, T4 – Comm. HCl @ 20 ml kg-1 for 1 min, T5 – Comm. HCl @ 20 ml kg-1 for 2 min, T6 – Comm. HCl @ 20 ml kg-1 for 3 min, T7 – Comm. HCl @ 25 ml kg-1 for 1 min, T8 – Comm. HCl @ 25 ml kg-1 for 2 min, T9 – Comm. HCl @ 25 ml kg-1 for 3 min, T10 – NaHCO₃ @0.5% for 12 hrs, T11 – NaHCO₃ @0.5% for 24 hrs.

**Table 2. Effect of different methods of seed extraction on seedling characteristics in nightshade (*Solanum trilobatum* L.)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatment** | **Root length****(cm)** | **Shoot length****(cm)** | **DMP****(mg/10 seedlings)** | **Vigour index** |
| T1 | 0.00 | 0.00 | 0.00 | 0 |
| T2 | 1.43 | 2.11 | 0.67 | 28 |
| T3 | 1.32 | 2.06 | 0.52 | 14 |
| T4 | 1.94 | 2.91 | 1.82 | 97 |
| T5 | 2.21 | 3.11 | 2.74 | 224 |
| T6 | 2.00 | 2.73 | 1.77 | 118 |
| T7 | 1.70 | 2.63 | 0.94 | 56 |
| T8 | 1.84 | 2.51 | 1.11 | 65 |
| T9 | 1.49 | 2.41 | 0.80 | 47 |
| T10 | 1.63 | 2.25 | 0.22 | 8 |
| T11 | 1.55 | 2.32 | 0.54 | 23 |
| **Mean** | 1.55 | 2.27 | 1.01 | 61.81 |
| **SEd** | 0.05 | 0.08 | 0.04 | 12.24 |
| **CD (P=0.05)** | 0.11 | 0.17 | 0.09 | 24.91 |

T1 – Control (Squeezing of fruits to liberate the seeds), T2 – T1+ Fermentation of seeds for 12 hrs, T3– T1+ Fermentation of seeds for 24 hrs, T4 – Comm. HCl @ 20 ml kg-1 for 1 min, T5 – Comm. HCl @ 20 ml kg-1 for 2 min, T6 – Comm. HCl @ 20 ml kg-1 for 3 min, T7 – Comm. HCl @ 25 ml kg-1 for 1 min, T8 – Comm. HCl @ 25 ml kg-1 for 2 min, T9 – Comm. HCl @ 25 ml kg-1 for 3 min, T10 – NaHCO₃ @0.5% for 12 hrs, T11 – NaHCO₃ @0.5% for 24 hrs.

**Table 3. Influence of Tetrazolium (TZ) solution on seed viability in nightshade (*Solanum trilobatum* L.)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Preparation of seed sample** | **Concentration of tetrazolium solution (%)** | **Duration of staining (h)** | **Viable seed (%)** | **Non-viable seed (%)** |
| Longitudinal cut method | 0.1 | 1 | 0 | 100 |
| 2 | 0 | 100 |
| 0.2 | 1 | 0 | 100 |
| 2 | 22 | 78 |
| 0.5 | 1 | 36 | 64 |
| 2 | 44 | 56 |
| Excision of embryo method | 0.1 | 1 | 0 | 100 |
| 2 | 12 | 88 |
| 0.2 | 1 | 38 | 62 |
| 2 | 56 | 44 |
| 0.5 | 1 | 72 | 28 |
| 2 | 88 | 12 |

 

**Longitudinal cutting of seed Fully stained embryo (Embryo excision)**

**Figure 3. Topographical pattern of the staining embryo**

**5. Conclusion**

The study demonstrates that seed extraction methods significantly influence the seed quality of *Solanum trilobatum* L. Among the methods evaluated, acid extraction using commercial HCl @ 20 ml kg⁻¹ for 2 minutes was found to be the most effective, recording the highest seed recovery, germination percentage, vigour indexand superior seedling traits including root length and shoot length. In contrast, fermentation for 12 hours showed moderate seed quality parameters such as seed recovery, germination and a vigour index compared to alkali treatment with NaHCO₃ @ 0.5% for 24 hours. Furthermore, tetrazolium viability testing confirmed that the embryo excision method with 0.5% TZ solution for 2 hours was the most effective for determining seed viability, with 88% viability. Therefore, acid treatment at the optimized concentration and duration can be recommended as a standard protocol for enhancing seed quality and promoting successful cultivation of nightshade, supporting its commercial propagation and conservation.

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**References**

Abdul-Baki, A. A., & Anderson, J. D. (1972). Physiological and biochemical deterioration of seeds. *Seed Biology*, 2, 283–316. <https://doi.org/10.1016/B978-0-12-424303-3.5010-5>

Bhattacharjee, T., Sen, S., Chakraborty, R., Maurya, P. K., & Chattopadhyay, A. (2020). Cultivation of medicinal plants: Special reference to important medicinal plants of India. In *Herbal Medicine in India* (pp. 101–115). <https://doi.org/10.1007/978-981-13-7248-3_8>

Das, R. K., Baruah, G. K. S., & Paul, S. R. (1997). Effect of extraction techniques on seed quality of tomato (*Lycopersicon esculentum* Mill.). *Annual Agricultural Research*, 18, 220–221.

Degwale, A., Tesfa, T., Meseret, B., & Fantaw, S. (2023). Seed extraction methods affect the physiological quality of tomato seed and developing seedlings. *International Journal of Vegetable Science*, 29(1), 16–24. <https://doi.org/10.1080/19315260.2022.2083042>

Desai, B. B. (2004). *Seeds handbook: Processing and storage*. CRC Press.

Grzybowski, C. R. S., Ohlson, O. C., da Silva, R. C., & Panobianco, M. (2012). Viability of barley seeds by the tetrazolium test. *Revista Brasileira de Sementes*, 34, 47–54. <https://doi.org/10.1590/S0101-31222012000100006>

Gunasekaran, S. (2003). *Seed development and maturation, extraction methods, germination test and storage in* *Solanum nigrum* Linn. (M.Sc. Ag. Thesis). Tamil Nadu Agricultural University, Coimbatore.

ISTA. (2019). *International rules for seed testing*. International Seed Testing Association, Bassersdorf, Switzerland, i-19-8.

Javaregowda, M. A. K., Jagdish, G. V. (1994). Optimization of seed extraction methods in brinjal. *Seed Technology News*, 24, 102.

Jayamani, S. (2020). *Seed technological studies in black cumin (Nigella sativa)* (M.Sc. Ag. Thesis). Tamil Nadu Agricultural University, Coimbatore.

Kumar, S. R. S., Sakthivel, M., Karthik, L., Mythili, S., & Sathiavelu, A. (2011). Phytochemical composition, antimicrobial and hemolytic activity of *Solanum trilobatum* Linn. *Asian Journal of Plant Science and Research*, 1(1), 48–56.

Maguire, J. D. (1962). Speed of germination – Aid in selection and evaluation for seedling emergence and vigor. *Crop Science*, 2, 176–177. [https://doi.org/10.2135/cropsci1962. 0011183X000200020033x](https://doi.org/10.2135/cropsci1962.%200011183X000200020033x)

Marcos Filho, J., Krzyzanowski, F. C., Vieira, R. D., & França Neto, J. B. (1999). *Vigor de sementes: conceitos e testes*. ABRATES, Londrina.

Marcos Filho, J. (2005). Dormência de sementes. In *Fisiologia de sementes de plantas cultivadas* (pp. 253–289). FEALQ.

Murugeshwari, T. (2022). Seed technological studies in yellow berried nightshade *(Solanum surattense Burm. f.)* (M.Sc. Ag. Thesis). Tamil Nadu Agricultural University, Coimbatore.

Murugeshwari, T., Sasthri, G., Jerlin, R., & Nalina, L. (2022). Germination improvement in *Solanum surattense* seeds through dormancy breaking treatments. *Biological Forum – An International Journal*, 14(3), 123–128.

Novembre, A. D. L. C., Chamma, H. M. C. P., & Gomes, R. B. R. (2006). Viabilidade das sementes de braquiaria pelo teste de tetrazolio. *Revista Brasileira de Sementes*, 28, 147–151. <https://doi.org/10.1590/S0101-31222006000200020>

Poovizhi, M., & Sujatha, K. (2020). Improvement in seed germination by priming treatments in black nightshade (*Solanum nigrum* L.). *Journal of Applied and Natural Science*, 12(2), 84. <https://doi.org/10.31018/jans.vi.2244>

Rashid, A. N. M., Hossain, M. S., Hassan, N., Dash, B. K., Sapon, M. A., & Sen, M. K. (2014). A review on medicinal plants with antidiabetic activity. *Journal of Pharmacognosy and Phytochemistry*, 3(4), 149–159.

Raval, A. N. K. I. T., Sasidharan, N., & Rao, K. A. L. Y. A. N. (2016). Effect of seed extraction procedures on seed quality parameters in tomato. *Advances in Life Sciences*, 5(20), 9020–9024.

Santos, M. A. O., Novembre, A. D. L. C., & Marcos-Filho, J. (2007). Tetrazolium test to assess viability and vigour of tomato seeds. *Seed Science and Technology*, 35(1), 213–223. <https://doi.org/10.15258/sst.2007.35.1.19>

Sharma, D. K., Gupta, V. K., Mondal, D. B., Mamta, S., Mandal, R. S. K., & Raguvaran, R. (2015). Evaluation of in vitro antioxidant capacity of aqueous and ethanolic extracts of eight different plant materials. *International Journal of Pharmaceutical Sciences and Research*, 6(9), 4086–4091.

Vethanarayanan, P., Unnikannan, P., Baskaran, L., & Sundaramoorthy, P. (2011). Survey on traditional medicinal plants used by the village peoples of Cuddalore district, Tamil Nadu, India. *Asian Journal of Biochemistry and Pharmaceutical Research*, 1(3), 351–361.