**Encapsulation of *Tinospora cordifolia* for *In Vitro* short-term storage and germplasm conservation**

**ABSTRACT**

*Tinospora cordifolia* (commonly known as giloy) is highly valued in traditional Ayurvedic medicine for their therapeutic properties. Giloy is known to enhance immunity, reduce inflammation, and support liver health. However, giloy is threatened by overharvesting and environmental factors, making it crucial to explore sustainable cultivation and conservation strategies. This study examined the application of encapsulation techniques for the controlled micropropagation and preservation of *Tinospora cordifolia* germplasm. The effects of different calcium chloride concentrations (50 mM, 75 mM, and 100 mM), sodium alginate concentrations (1%, 2%, 3%, and 4%), and storage temperatures (25°C and 4°C) on viability of nodes and growth were evaluated over 0, 7, 14, and 21 days.

Preliminary results indicated that a combination of 75 mM calcium chloride, 3% sodium alginate, and storage at 4°C resulted in the best encapsulation and growth outcomes with a regeneration frequency of 90 %. These findings highlight the potential of encapsulation for the genetic preservation, stress resistance, and sustainable cultivation of medicinal plants, addressing broader concerns such as biodiversity loss and climate change.

**Keywords: *Tinospora cordifolia*, encapsulation, germplasm conservation, sodium alginate, calcium chloride, micropropagation.**

**● INTRODUCTION** –

*Tinospora cordifolia* (commonly known as Giloy) hold significant importance in Ayurvedic medicine, being widely recognized for their diverse therapeutic benefits [1]. *Tinospora cordifolia* is particularly valued for its role in boosting the immune system, alleviating inflammation, and acting as a potent antioxidant [2]. These properties make it an effective remedy for managing conditions, such as diabetes, immune disorders, and inflammatory diseases. Its bioactive components, including alkaloids, glycosides, flavonoids, and terpenoids, contribute to its medicinal versatility and are continually being investigated for novel therapeutic applications [3,4].

Despite its therapeutic value, this plant faces threats owing to deforestation, habitat destruction, and overharvesting, leading to the depletion of its natural population. The

increasing demand for this species in the pharmaceutical and wellness industries further exacerbates pressure on its cultivation [5]. To address these challenges, innovative conservation strategies are essential for safeguarding genetic diversity and ensuring sustainable utilization [6]. Biotechnological advancements such as encapsulation offer promising methods for conserving endangered medicinal plants [7,8]. Encapsulation involves embedding plant tissues in a biocompatible gel-like material, which not only protects the samples but also facilitates their handling, storage, and regeneration under laboratory conditions [8,9].

This study focuses on refining encapsulation protocols for the conservation of *Tinospora cordifolia* by optimizing these methods, this study aimed to enhance the propagation, genetic preservation, and its sustainable utilization of this plant in traditional medicine, modern pharmacology, and agricultural practices. Additionally, this study underscores the critical need to integrate cutting-edge technologies with conservation efforts to mitigate the impact of environmental degradation and ensure the long-term availability of these valuable medicinal resources.

● **Material and Method**

This research aimed to investigate the effects of encapsulation on the preservation and germination of *Tinospora cordifolia*. To achieve this, a series of steps were carried out to prepare and assess the nodes of the plant under controlled conditions.

**Plant Material and Sterilization**

Fresh nodes of *Tinospora cordifolia* were obtained for this study. The nodes were initially sterilized to eliminate any surface contaminants. A 0.1% mercuric chloride solution was used for sterilization, with the nodes being immersed in this solution for 10 minutes. After sterilization, the nodes were thoroughly rinsed in sterile distilled water multiple times to remove any residual sterilizing agent, ensuring that they were clean and suitable for further processing [9,10].

**Encapsulation Process**

For the encapsulation of nodes, sodium alginate solutions at varying concentrations of 1%, 2%, 3%, and 4% were prepared. To ensure the complete dissolution of sodium alginate, each solution was heated to 50°C. After preparing the alginate solution, the sterilized nodes were carefully mixed with the solution. The resulting node-alginate mixture was then dropped into sterile calcium chloride solutions at concentrations of 50 mM, 75 mM, and 100 mM. This process allowed the formation of beads around the nodes through cross-linking with calcium

ions. The beads were then left to set for 20 minutes in the calcium chloride solution. After setting, the beads were rinsed in sterile distilled water and air-dried to prepare them for further storage and testing [11,12].

**Storage and Viability Assessment**

Post-treatment, the encapsulated nodes were stored under two different temperature conditions: 25°C (ambient room temperature) and 4°C (refrigerated conditions) to simulate different storage environments. The viability of the nodes was monitored at four intervals: 0, 7, 14, and 21 days.[13] Parameters such as node germination rate, root and shoot growth, and overall seedling health were observed and recorded to evaluate the effectiveness of the encapsulation.

**Data Analysis**

The data collected from the node viability and growth measurements were subjected to statistical analysis using one-way analysis of variance (ANOVA). This analysis was conducted to assess the effects of various treatments (sodium alginate concentration, calcium chloride concentration, and storage temperature) on the germination rates and growth of the nodes. A significance level of p < 0.05 was used to determine statistically significant differences between treatments.

**● RESULTS**

The encapsulation of *Tinospora cordifolia* was effectively accomplished using a 3% sodium alginate solution, leading to the formation of stable, well-structured beads. These beads remained intact and viable during the entire storage period, indicating that the encapsulation technique is suitable for the long-term preservation and propagation of *Tinospora cordifolia*.

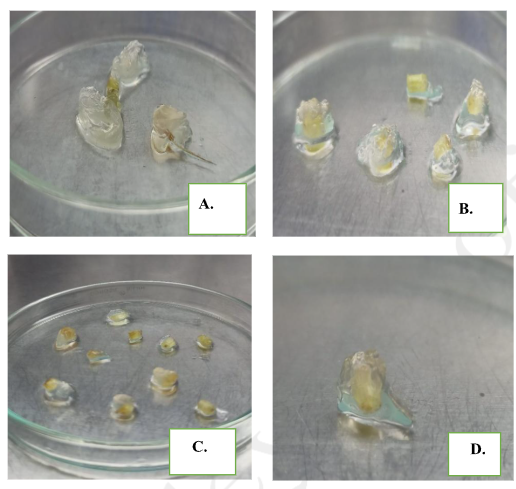
When the encapsulated material was cultured on MS medium, *Tinospora cordifolia* showed rapid growth and effective regeneration. The plant exhibited robust development, demonstrating the success of the encapsulation method in promoting healthy growth. This suggests that the encapsulated beads are not only viable but also capable of supporting rapid regeneration under in vitro conditions.

The consistency of the beads' stability throughout storage, combined with the positive regeneration response, confirms the efficiency of encapsulation as a propagation technique for *Tinospora cordifolia*. These results suggest that encapsulation could serve as a reliable method for both short- and long-term storage, as well as mass propagation of this species.

**Table 1. Effect of sodium alginate concentrations on morphology of encapsulated shoot tip**

|  |  |  |
| --- | --- | --- |
| **Different concentrations of sodium-**  **alginate (%) alongwith 100mM calcium chloride** | **Texture** | **Morphology of encapsulated nodes** |
| **1** | **++** | **Inefficient encapsulation** |
| **2** | **++** | **Cluster formation, soft for handling** |
| **3** | **++++** | **Uniform size encapsulation** |
| **4** | **+++** | **Firm and rigid encapsulation** |

**++, Poor quality; ++++, Best quality; +++, Good but very hard**

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**Fig. 1. Effect of sodium alginate concentrations on morphology of encapsulated shoot tip.**

1. **Encapsulated nodes using 1% concentration of sodium alginate.**
2. **Encapsulated nodes using 2% concentration of sodium alginate.**
3. **Encapsulated nodes using 3% concentration of sodium alginate.**
4. **Encapsulated nodes using 4% concentration of sodium alginate.**

**Table 2. Effect of different concentrations (1-4%) of sodium alginate on percent multiplication frequency of encapsulated nodes, stored at two different temperatures (4°C and 25°C) for the period of 15 days**

|  |  |  |
| --- | --- | --- |
| **Combinations of different concentrations of**  **sodium alginate (%) (w/v) with 100mM calcium chloride** | **Mean % frequency of**  **multiplication of artificial seeds stored at 4°C, 15 days** | **Mean % frequency of**  **multiplication of artificialseeds stored at 25°C, 15 days** |
| **1** | **45.33 bc** | **38.50 ab** |
| **2** | **58.60 b** | **48.33 a** |
| **3** | **90.00 a** | **58.65 a** |
| **4** | **36 c** | **15.60 b** |

**Data were analyzed by ANOVA and means were compared with DMRT (Duncan's Multiple Range test). Values followed by the same lower-case letter are non-significant at P<0.05**

**Table 3. Effect of various concentrations of calcium chloride on percentage multiplication frequency of encapsulated nodes stored at two different temperatures (4°C and 25°C) for a period of 15 days**

|  |  |  |
| --- | --- | --- |
| **Combinations of different concentrations of calcium chloride (mM) with 3% (w/v) sodium alginate** | **Mean % frequency of multiplication of artificial seeds stored at 4°C, 15 days** | **Mean % frequency of multiplication of artificial seeds stored at 25°C, 15 days** |
| **50** | **38 bc** | **30.33 bc** |
| **75** | **50.33 b** | **40.66 b** |
| **100** | **90.33 a** | **65.66 a** |
| **125** | **23.66 c** | **15 c** |

**Data were analyzed by ANOVA and means were compared with DMRT. Values followed by the same lower-case letter are non- significant at P<0.05Table 5. Influence of storage days and storage temperature on percentage multiplication frequency of encapsulated nodes.**

**Data were analysed by ANOVA and means were compared with DMRT. Values followed by same lower-case.**

|  |  |  |
| --- | --- | --- |
| **Storage duration (in Days)** | **Multiplication frequency of**  **encapsulated nodes stored at 4°C storage.** | **Multiplication frequency of**  **encapsulated nodes stored at 25°C storage.** |
| **15** | **91.33 a** | **86.66 a** |
| **30** | **62.66 b** | **60 .1b** |
| **45** | **51.33 bc** | **41.66 bc** |
| **60** | **40 cd** | **31.33 cd** |
| **75** | **22.66 de** | **20 de** |

**Table 4. Influence of storage days and storage temperature on percentage multiplication frequency of encapsulated nodes.**

Data were analysed by ANOVA and means were compared with DMRT. Values followed by same lower-case letter are non- significant at P<0.05

**● CONCLUSION**

* **Conclusion-**

The encapsulation technique has shown significant promise for short-term storage and the conservation of germplasm in medicinal plants. The successful preservation of plant material in encapsulated form supports its potential for maintaining plant viability and regeneration over extended periods. Further optimization of this method could improve its effectiveness across a wider variety of species, making it a valuable tool in both plant conservation and biotechnological applications.

In vitro culture remains an essential approach for producing high-quality planting materials, enhancing the production of secondary metabolites, and preserving the genetic diversity of medicinal plants, especially those that are rare or endangered. Despite its broad application, there is still a need for the development of standardized protocols for different medicinal species, requiring ongoing research to improve techniques.

Encapsulation provides an efficient means for storing plant genetic material, particularly in the short term, and offers a reliable method for conserving germplasm. By maintaining the integrity and viability of plant material, encapsulation contributes to the sustainability of medicinal plant resources for future cultivation and study.

● **REFERENCES**-

[1] K. Krishna, B. Jigar, and P. Jagruti, "Guduchi (*Tinospora cordifolia*): Biological and medicinal properties, a review," *The Internet Journal of Alternative Medicine*, vol. 6, no. 2, 2008, pp. 1-8.

[2] K. Arunachalam, X. Yang, and T. T. San, "Tinospora cordifolia (Willd.) Miers: Protection mechanisms and strategies against oxidative stress-related diseases," *Journal of Ethnopharmacology*, vol. 283, 30 January 2022, 114540.

[3] P. Sharma, B. P. Dwivedee, D. Bisht, A. K. Dash, and D. Kumar, "The chemical constituents and diverse pharmacological importance of *Tinospora cordifolia*," *Journal Name*, vol. 5, art. e02437, 2019.

[4] R. Verma and A. B. Khan, "Antioxidant, immunomodulatory and anticancer potential of *Tinospora cordifolia* - A review," *International Journal of Pharmacy and Biological Sciences*, vol. 8, no. 3, pp. 54-69, Jul.-Sept. 2018.

[5] Author(s), "Conservation of medicinal plants by tissue culture techniques," in *Medicinal Plants: Biodiversity, Biotechnology and Conservation, Sustainable Development and Biodiversity*, Springer, March 2023, pp. 1-24, doi:10.1007/978-981-19-9936-9\_27.

[6] A. Prasad, P. Patel, M. Kumari, G. Saxena, D. Chakrabarty, and S. S. Singh, "A literature update on strategies for harnessing and conserving the bioactive phytochemicals from *Tinospora cordifolia*: Current status, challenges, and future prospects," in *Plants for Immunity and Conservation Strategies*, pp. 1–20, First Online: 26 July 2023.

[7] Fathi, F., Ebrahimi, S. N., Rocha, F., & Estevinho, B. N. (2021). Encapsulation of extracts from Middle East medicinal plants and its advantages – A review article. *Department of Phytochemistry, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Iran, and LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Portugal*. Received January 22, 2021.

[8] M. B. Pithiya, S. K. Sharma, M. Sharma, M. Sharma, and N. Kotwal, "Advancements and challenges in plant tissue culture: A comprehensive overview,".

[9] S. Gantait, S. Kundu, N. Ali, and N. C. Sahu, "Synthetic seed production of medicinal plants: A review on influence of explants, encapsulation agent and matrix," *Acta Physiologiae Plantarum*, vol. 37, art. 98, 2015.

[10] S. Singh, M. K. Tripathi, S. Tiwari, N. Tripathi, G. Tejovathi, A. Ahuja, and S. Tiwari, "Production of synthetic seed from encapsulated nodal segments of Giloe (*Tinospora cordifolia* Willd.)," *Research Directions in Sustainable Technology*, vol. 2, article 2400B,

[11] V. Singh, A. Tyagi, P. K. Chauhan, P. Kumari, and S. Kaushal, "Identification and prevention of bacterial contamination on explant used in plant tissue culture labs," *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 3, no. 4, 2011, pp.

[12] Singh, P. R., & Singh, L. J. (2021). In vitro propagation for improvement of medicinal plants: A review. *Journal of Pharmacognosy and Phytochemistry*, 10(1).

[13] S. Saha, C. Sengupta, and P. Ghosh, "Encapsulation, short-term storage, conservation and molecular analysis to assess genetic stability in alginate-encapsulated microshoots of *Ocimum kilimandscharicum* Guerke," *Plant Cell, Tissue and Organ Culture (PCTOC)*, vol. 120, pp. 519-530, 2015, published 14 September 2014.

[14] Maruyama, E., Ishii, K., & Kinoshita, I. (1998). Alginate encapsulation technique and cryogenic procedures for long-term storage of the tropical forest tree *Guazuma crinita* Mart. in vitro cultures. *JARQ*, 32, 301-309.

[15] M. Micheli, I. A. Hafiz, and A. Standardi, "Encapsulation of in vitro-derived explants of olive (*Olea europaea* L. cv. Moraiolo): II. Effects of storage on capsule and derived shoots performance," *Scientia Horticulturae*, vol. 113, no. 3, pp. 286-292, 20 July 2007