



SYNTHETIC SEED PRODUCTION AND SHORT-TERM CONSERVATION OF HAWTHORN (*Crataegus monogyna*) USING NODAL SEGMENTS

Metin KOÇAK^{1*}

¹Van Yüzüncü Yıl University, Faculty of Agriculture, Department of Agricultural Biotechnology, 65030, Van, Türkiye

Abstract: Hawthorn (*Crataegus monogyna*) is an important fruit species with high nutritional and pharmacological value. Seed dormancy, low germination rates and variations in generative propagation make propagation of the species and conservation of its genetic resources difficult. In the present study, synthetic seeds were produced from nodal segments of *C. monogyna*, and the effect of different storage periods and Indole-3-acetic acid (IAA) on germination, micropropagation and rooting performance of the synthetic seeds was examined. Synthetic seeds were produced by 3% sodium alginate and hardened in a 100 mM CaCl₂ solution. The synthetic seeds obtained were cultured on hormone-free Woody Plant Media (HF-WPM) and on WPM supplemented with 2 mg/L IAA, either immediately or after storage for 1, 2 and 3 months in the dark at 4°C. Results indicated that storage period had no significant effect on germination rates, and the synthetic seeds retained their viability for up to 3 months. On the other hand, micropropagation and rooting were significantly influenced by storage duration. The highest micropropagation rates were observed after 2 and 3 months of storage, but rooting was observed only in synthetic seeds stored for 3 months. The addition of 2 mg/L IAA to the medium did not increase germination but induced micropropagation. The results indicated that the synthetic seed technology is an effective method for short-term conservation and in vitro propagation of hawthorn genetic resources.

Keywords: *Crataegus monogyna*, Synthetic seed, Storage duration, Germination, Micropropagation, Rooting

*Corresponding author: Van Yüzüncü Yıl University, Faculty of Agriculture, Department of Agricultural Biotechnology, 65030, Van, Türkiye

E mail: metinkocak@yyu.edu.tr (M. KOÇAK)

Metin KOÇAK  <https://orcid.org/0000-0002-8109-5245>

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1. Introduction

The genus *Crataegus* (hawthorn), a member of the Rosaceae family, includes numerous species naturally occurring across the temperate zones of the Northern Hemisphere. Rosaceae is a large plant family comprising around 85–90 genera and an estimated 2500–4800 species (Maghchiche, 2025). Hawthorn is a plant native to the mountainous regions of Türkiye. No cultural management is applied to these plants. Hawthorn trees are found in almost all regions of Türkiye due to their low water requirements and their natural growth on mountainous and rocky land. The Hawthorns exhibit a high level of genetic diversity and hybridization, and polymorphism, ranging from Turkey to Iran (Erkek et al., 2025). Nearly 20 species and subspecies of *Crataegus* grow throughout Türkiye. Like many natural products in Turkey, hawthorn fruit doesn't require fertilization or pesticide treatment (Yalçın Dokumacı et al., 2021).

Traditional and modern medicine have long utilized the hawthorn plant, including its flowers, leaves, and fruits. Uses documented include antioxidant activity, helping with digestion, relieving constipation and stomach pain, reducing inflammation, lowering blood pressure,

protecting against cardiovascular disease, and helping to manage type II diabetes (Zhang et al., 2002; Chang et al., 2006; Tassell et al., 2010; Aierken et al., 2017; Muradoğlu et al., 2021). The therapeutic potential of hawthorn is primarily associated with its abundance of bioactive constituents, such as flavonoids, tannins, and vitamin C, which possess strong antioxidant activity and support its medicinal properties (Bahorun et al., 2003; Martinelli et al., 2021; Yanakieva, 2023; Koçak et al., 2025). Plant-derived byproducts have recently attracted considerable interest as promising sources of antioxidants and various bioactive constituents, supporting the efficient and sustainable utilization of natural resources (Stabnikova et al., 2025; Kępińska-Pacelik and Biel, 2026).

Despite its considerable medicinal and horticultural value, conventional propagation of hawthorn is often constrained by factors such as seed dormancy, low germination rates, and variability among seedlings. Therefore, alternative biotechnological approaches, including synthetic seed technology, have attracted attention as potential tools to improve propagation efficiency and conserve valuable genetic resources. Synthetic seed technology is the encapsulation of



regenerative plant tissues such as somatic embryos, shoot buds, or other meristematic tissues in a protective matrix. These propagules can be stored and handled and subsequently induced to develop into complete plants, providing a seed-like system for propagation and germplasm conservation (Abbas et al., 2022). It is considered that synthetic seeds imitate natural seeds (Kocak et al., 2019). The endosperm of natural seeds can be simulated by adding nutrients, plant growth regulators, antimicrobial agents, biological control organisms and biofertilizers to the encapsulation matrix. Synthetic seed technology can reduce dependence on low-quality conventional seeds by providing a protected, nutrient-rich environment that can improve propagation success under unfavorable conditions (Singh et al., 2013; Ghosh et al., 2026). Synthetic seed technology has potential not only for plant propagation but also for efficient conservation and distribution of valuable germplasm, such as elite genotypes, rare hybrids, genetically engineered plants and sterile lines. The use of synthetic seed technology is particularly important for species with limitations in conventional seed propagation or specific symbiotic requirements. Encapsulation technology has attracted increasing attention in germplasm management and other experimental studies (Sharma et al., 2013). There are limited reports in the literature regarding synthetic seeds in *Crataegus*. Synthetic seed production in *Crataegus* has previously been reported by Majd et al. (2016) in *C. pseudoheterophylla* and *C. microphylla*, and Koçak et al. (2025) in *C. monogyna*.

Hawthorn (*C. monogyna*) is a species of high economic and pharmacological value, but propagation is difficult due to seed dormancy and low germination rates. This study aimed to examine the effects of different storage durations (0, 1, 2, and 3 months) and growth medium compositions on germination, micropropagation, and rooting of synthetic seeds derived from nodal segments. Moreover, the study was conducted to evaluate the feasibility of short-term storage of synthetic seeds and to highlight the potential of synthetic seed technology for the conservation of hawthorn genetic resources and for clonal propagation.

2. Materials and Methods

2.1. Materials

Nodal segments from sterile shoot cultures of *C. monogyna* were utilized for the production of synthetic seeds. The donor plant bears orange-colored fruit and is native to Van, Türkiye.

2.2. Methods

The nodal segments were cut to approximately 2 mm in size and added to an encapsulation gel matrix. The encapsulation matrix and propagules were gently and thoroughly mixed. A portion of the mixture, sufficient to create suitably sized beads, was transferred into a 100 mM CaCl₂ solution using a micropipette with a sterile tip. The CaCl₂ solution was agitated to facilitate the formation of well-shaped beads until the encapsulation hardened. Subsequently, the beads were extracted from the CaCl₂ solution by filtering through a sterile mesh and were rinsed several times in sterile water. After bead formation and the removal of CaCl₂ solution residues from the synthetic seeds, they were dried on sterile blotters. The synthetic seeds were then stored for 1, 2, or 3 months in sterile 50-ml centrifuge tubes at 4 °C in the dark, or cultured directly in growth medium without any storage period. The encapsulation matrix consisted of Woody Plant Media (WPM, Lloyd and McCown, 1980) basal salt mixture, WPM vitamins, 30 g/L sucrose, and 3% sodium alginate, or it was supplemented with 2 mg/L IAA (Table 1).

Two different growth media were used for germination, micropropagation and rooting propagules. The first medium was hormone-free WPM (HF-WPM), and the second was WPM supplemented with 2 mg/L IAA (WPM+IAA). Both of the growth media consisted of WPM vitamins and 30 g/L sucrose and were solidified with 3.7 g/L gelrite; the pH was adjusted to 5.7-5.8. The 50 mL of media was transferred to glass jars. Non-stored synthetic seeds were stored directly, and the other synthetic seeds, after the storage period, were transferred to growth media containing the same ingredients as those in the encapsulation matrix. Synthetic seeds were cultured at 24±1 °C and a 16 h light/8 h dark period.

Table 1. Medium composition in encapsulation matrix and growth culture, storage periods and storage temperature of the synthetic seeds

Medium composition (Encapsulation matrix and growth culture)	Storage period	Storage temperature
HF-WPM	Non-stored	at 4°C in dark
	1 month	
	2 months	
	3 months	
	Non-stored	
WPM+IAA	1 months	
	2 months	
	3 months	

2.3. Experimental Design and Statistical Analysis

The experiment was conducted in a completely randomized design, with 7 replicates and 5 synthetic seeds per replicate. This study investigated the effects of IAA and various storage periods (0, 1, 2, and 3 months) on the germination, micropropagation, and rooting of synthetic seeds. Average percentage values for germination, micropropagation, and rooting were recorded and subsequently arcsine-transformed before statistical analysis. The means were compared using analysis of variance (ANOVA), and significant differences among the factors were assessed with the least significant difference (LSD) test (Genç and Soysal, 2018). All analyses were conducted using the JMP® program (SAS Institute, Cary, NC) version 8.00.

3. Results and Discussion

Although the synthetic seed is often described as an encapsulated somatic embryo in numerous references, it is more broadly viewed as the encapsulation of any regenerable propagule (Kocak et al., 2019). Synthetic seed production through the encapsulation of non-embryogenic propagules in trees and shrubs has been previously documented in various species, including *Dalbergia sissoo* (Chand and Singh, 2004), *Erythrina variegata* (Javed et al., 2017), *Capparis decidua* (Siddique and Bukhari, 2018), *Salix tetrasperma* (Khan et al., 2018), *Allamanda cathartica* (Khanam et al., 2021), *Myrtus communis* (Dönmez, 2022), *Juglans regia* (Sota et al., 2023), *Pterocarpus marsupium* (Ahmad et al., 2023), and *Populus alba* (Hussien et al., 2024).

In the present study, synthetic seeds of *C. monogyna* produced by encapsulating nodal segments exhibited varying germination, micropropagation, and rooting percentages depending on medium composition and storage period. The synthetic seeds encapsulated and cultured with WPM+IAA exhibited a germination percentage of 32.85. The germination percentage in HF-WPM was 27.85. However, these percentages were not statistically significant. No micropropagation was observed in any of the explants cultured in HF-WPM, whereas 27.85% of the explants in WPM+IAA underwent micropropagation. According to these results, the addition of 2 mg/L IAA to the medium and encapsulation matrix did not increase the germination of synthetic seeds but did induce micropropagation. Dönmez (2022) reported that blueberry synthetic seeds exhibited the highest germination rate in a hormone-free medium and that micropropagation occurred at the highest level in a medium containing 0.5 mg/L BAP (Figure 1).

Although there was no rooting in WPM+IAA, the synthetic seeds cultured in HF-WPM showed a low rooting percentage of 4.28%, which was not statistically significant (Table 2). These results suggest that while IAA did not influence germination or rooting of the synthetic seeds, their micropropagation was dependent on IAA. Koçak et al. (2025) reported findings that contrast with the present study. They observed that synthetic seeds of *C. monogyna* encapsulated and cultured in hormone-free MS medium exhibited germination. However, no germination occurred in encapsulated synthetic seeds cultured in MS + 2 mg/L IAA. Furthermore, they did not provide any information regarding micropropagation or rooting in the synthetic seeds. The primary reason for differences in results between two separate studies of the same plant material lies in the base medium used. While the use of HF-WPM promoted rooting, WPM supplemented with 2 mg/L IAA induced micropropagation.

Several hydrogel-forming substances, such as agar, alginate, carrageenan, guar gum, and sodium pectate, have been tested for synthetic seed production. However, alginate has been recognized as the most effective encapsulation material because it combines favorable rheological properties with low toxicity, economic feasibility, biocompatibility, and rapid gelation. Furthermore, alginate capsules exhibit sufficient mechanical strength and structural integrity, which help safeguard the encapsulated propagules from external mechanical stresses (Ara et al., 2000; Saiprasad, 2001; Daud et al., 2008; Rihan et al., 2017). The concentration of sodium alginate plays a crucial role in the germination of synthetic seeds. Various reports have indicated that a 3% concentration of sodium alginate is optimal for the production of synthetic seeds (Bekheet et al., 2006; Adhikari et al., 2014; Gantait et al., 2017; Qahtan et al., 2023; Saadat et al., 2023). For many plant species, low alginate levels (1–2%) produce beads that are too soft to handle, whereas higher levels ($\geq 4\%$) lead to overly rigid beads that can inhibit shoot and root emergence (Ganapathi et al., 1992; Manjkhola et al., 2005; Sarmah et al., 2010; Tabassum et al., 2010; Cheruvathur et al., 2013; Sakhanokho et al., 2013). Similarly, in these reports and in the present study, the synthetic seeds encapsulated with 3% sodium alginate showed successful germination. Storage temperature is a key factor in the storage of synthetic seeds. In the present study, synthetic seeds of *C. monogyna* stored for up to 3 months in different media showed germination, micropropagation and rooting responses at varying rates.

Table 2. Germination, micropropagation, and rooting percentage of synthetic seeds for the medium composition

Medium	Germination	Micropropagation	Rooting
WPM+IAA	32.85 (31.44)	27.85 ^a (26.83)	4.28 (4.20)
HF-WPM	27.85 (27.90)	0 ^b (0)	0 (0)

LSD_{germination}=11.13, LSD_{micropropagation}=8.11, LSD_{rooting}=4.68, (P<0.05). Arcsine transforms of percentage values were presented in parentheses.

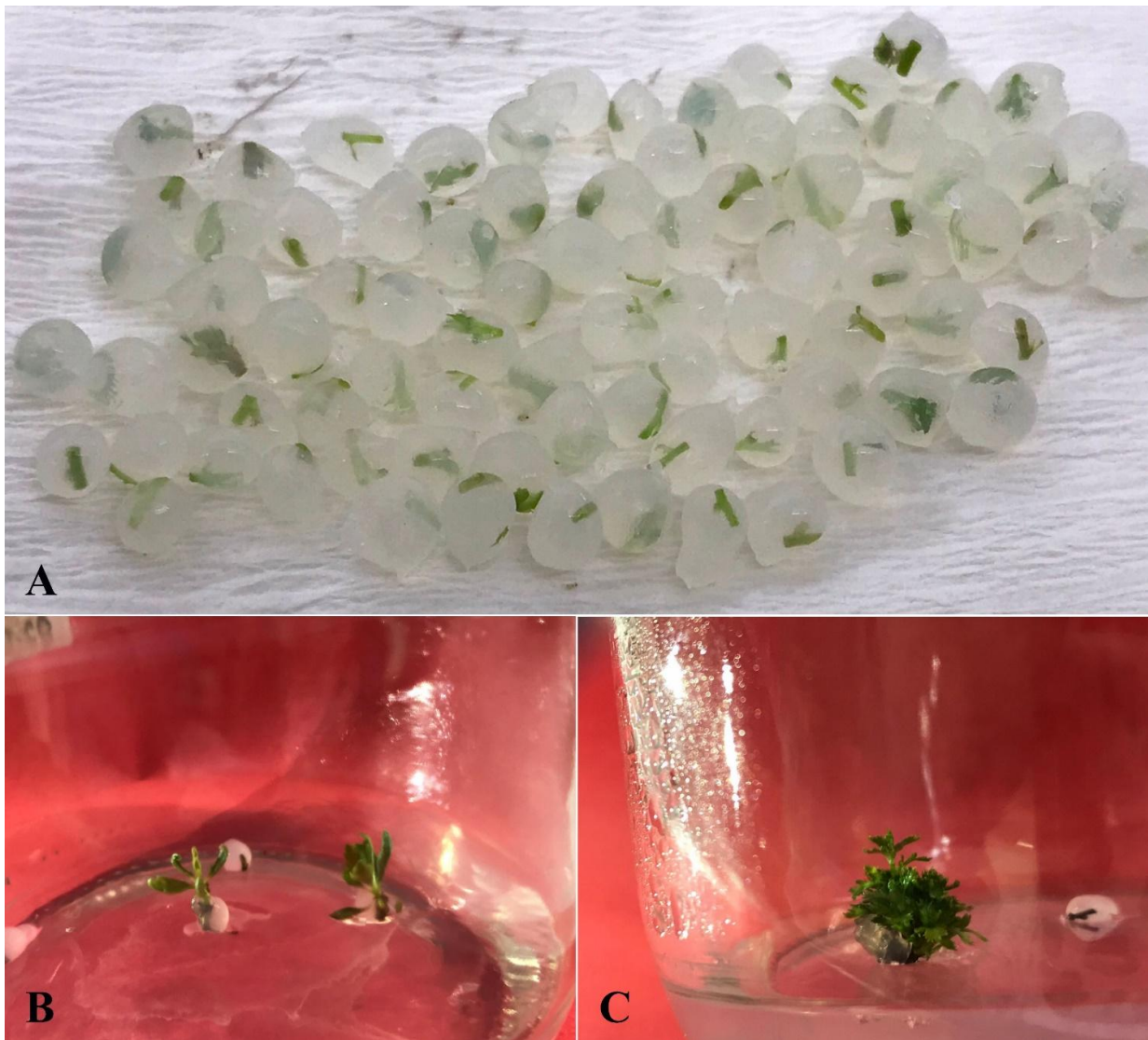


Figure 1. (a) Encapsulated nodal segments of *C. monogyna*, (b) Germinated synthetic seeds, (c) micropropagated synthetic seed.

Koçak et al. (2025) also indicated that the optimal storage temperature for *C. monogyna* was 4 °C. Furthermore, numerous studies in the literature report that 4°C is the most suitable temperature for storing synthetic seeds of various species. Sakhanokho et al. (2013) encapsulated begonia microshoots and stored them at 4 °C or 22 °C. They indicated that storage of begonia synthetic seeds at 4 °C is preferable to storage at 22 °C. Haque and Ghosh (2017) stored *Spathoglottis plicata* protocorm-like bodies in sodium alginate at 4, 15, and 24 °C. Despite having the best germination rate on the 30th day for synthetic seeds stored at 24 °C, synthetic seeds stored at 4 °C had the best germinability at 66.7% after 120 days.

The germination percentage of synthetic seeds varied with storage period, ranging from 22.85% to 40%, but this variation was not statistically significant. Generally, longer storage durations decrease the germination rates of synthetic seeds. However, the present study found no significant differences in germination rates across storage periods of up to 3 months. This suggests that

synthetic seeds of *C. monogyna* can be stored for more than 3 months without adversely affecting germination. Hatzilazarou et al. (2020) reported results similar to those of the present study. They stored synthetic seeds of *Viburnum dentatum* at 4 °C for up to 12 weeks, and 51.1% of the synthetic seeds successfully regenerated after this period. Hung and Trueman (2012) reported that shoot regrowth was 92–100% for *Corymbia torelliana* × *C. citriodora* and 71–98% for *Khaya senegalensis* synthetic seeds following a 12-month storage period, which is longer than the storage period in the present study.

The two- and three-month storage periods resulted in the highest micropropagation rates. In contrast, non-stored synthetic seeds exhibited the second-best micropropagation rate, whereas those stored for 1 month showed the lowest. The results indicate that storage for up to three months at 4 °C enhances micropropagation. However, Hatzilazarou et al. (2021) reported that encapsulated shoot explants of *Gardenia jasminoides* stored at 4 °C for 4, 8, or 12 weeks experienced a gradual

decline in viability as storage duration increased. Notably, only the synthetic seeds stored for 3 months demonstrated rooting, with an 8.57 percent success rate (Table 3). Although only to a small extent, the increase in storage time has induced rooting. These findings indicate

that the storage period did not affect the germination of the synthetic seeds. However, varying storage durations yielded significant differences in micropropagation and rooting outcomes for the synthetic seeds.

Table 3. Germination, micropropagation, and rooting percentage of the synthetic seeds dependent on the storage period

Storage period	Germination	Micropropagation	Rooting
Non-stored	22.85 (23.24)	14.28 ^{ab} (14.92)	0 ^b (0)
1 month	24.28 (25.22)	1.42 ^b (1.89)	0 ^b (0)
2 months	40.00 (36.76)	21.42 ^a (19.28)	0 ^b (0)
3 months	34.28 (33.46)	18.57 ^a (17.55)	8.57 ^a (8.40)

LSD_{germination}=15.5, LSD_{micropropagation}=14.81, LSD_{rooting}=6.33, (P<0.05). Arcsine transforms of percentage values were presented in parentheses.

The interaction between storage period and hormone on synthetic seed germination was found to be statistically significant. The combinations of nonstored WPM+IAA, WPM+IAA after 2 months of storage, HF-WPM after 1 month of storage, and HF-WPM after 3 months of storage exhibited the highest germination performance. On the contrary, non-stored synthetic seeds cultured in HF-WPM and the synthetic seeds cultured in WPM+IAA after one month's storage exhibited the lowest level of germination (Table 4). The results suggest that, with an approach anticipating both a long storage period and a high germination rate, the optimal outcomes were achieved with synthetic seeds cultured on HF-WPM after three months of storage. Faisal et al. (2012) reported achieving the highest conversion rate of synthetic seeds to plantlets when the seeds were stored for four weeks and then cultured on a woody plant medium enriched with 5.0 µM BA and 1.0 µM NAA.

The interaction between storage period and hormone was found to be statistically significant for the micropropagation of encapsulated propagules. None of the synthetic seeds cultured in HF-WPM, irrespective of the storage period, exhibited micropropagation. The synthetic seeds that were not stored, as well as those stored for 2 and 3 months, demonstrated the greatest potential for micropropagation (Table 5).

Table 4. Germination percentage of the synthetic seeds dependent on storage period - hormone interaction

Germination	WPM+IAA	HF-WPM
Non-stored	34.28 ^a (35.45)	11.42 ^b (11.04)
1 month	14.28 ^b (15.00)	34.28 ^a (35.45)
2 months	51.42 ^a (45.82)	28.57 ^{ab} (27.70)
3 months	31.42 ^{ab} (29.50)	37.14 ^a (37.42)

LSD = 20.11, (P<0.05). Arcsine transforms of percentage values were presented in parentheses.

The rooting of encapsulated propagules in relation to the interaction between storage period and medium composition was significant. However, it is important to note that only the synthetic seeds stored for 3 months

before being cultured in HF-WPM exhibited rooting (Table 6).

Table 5. Micropropagation percentage of the synthetic seeds dependent on storage period - hormone interaction

Micropropagation	WPM+IAA	HF-WPM
Non-stored	28.57 ^a (29.84)	0 ^b (0)
1 month	2.85 ^b (3.79)	0 ^b (0)
2 months	42.85 ^a (38.57)	0 ^b (0)
3 months	37.14 ^a (35.11)	0 ^b (0)

LSD = 13.11, (P<0.05). Arcsine transforms of percentage values were presented in parentheses.

Table 6. Rooting percentage of synthetic seeds dependent on storage period-medium composition interaction

Rooting	WPM+IAA	HF-WPM
Non-stored	0 ^b (0)	0 ^b (0)
1 month	0 ^b (0)	0 ^b (0)
2 months	0 ^b (0)	0 ^b (0)
3 months	0 ^b (0)	17.14 ^a (16.81)

LSD = 7.96, (P<0.05). Arcsine transforms of percentage values were presented in parentheses.

5. Conclusion

This study evaluated the effects of storage period and growth medium on germination, micropropagation, and rooting in synthetic seeds from nodal segments of *C. monogyna*. Results showed that germination rates were not significantly affected and that the synthetic seeds maintained their viability when stored in the dark at 4°C for up to three months. Also, germination was not improved by adding 2 mg/L IAA, but micropropagation was promoted. It was observed that the storage time influenced the micropropagation and rooting. The highest rates of micropropagation were observed after 2 and 3 months of storage, while rooting occurred only in synthetic seeds stored for 3 months. Moreover, the interaction between storage duration and medium composition was significant for germination, micropropagation, and rooting. In conclusion, the study revealed successful use of synthetic seeds of *C. monogyna*

for short-term storage and in vitro propagation. The results laid an important foundation for future studies on the conservation and propagation of hawthorn genetic resources.

Author Contributions

The percentages of the author' contributions are presented below. The author reviewed and approved the final version of the manuscript.

	M.K.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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