ID: 559

Effects of GA_3 and PLGA-Controlled Release of GA_3 on the Germination of Caraway (*Carum carvi* L.)

Münüre TANUR ERKOYUNCU[1]

[1]Department of Field Crop, Faculty of Agriculture, Selcuk University, Konya, Türkiye, mtanur@selcuk.edu.tr

Abstract

This study was conducted to evaluate the effects of gibberellic acid (GA_3) applied at different concentrations and its combination with PLGA (poly(lactic-co-glycolic acid)) controlled-release systems on the germination of *Carum carvi* L. seeds. The experiment was designed as a factorial trial in a completely randomized plot design using five treatments: control (distilled water), GA_3 at 150 and 300 mg/L, and GA_3 + PLGA combinations at 150 and 300 mg/L concentrations. Germination percentage (%), mean germination time (days), and germination index (speed) were analyzed. The results showed that GA_3 applied alone resulted in poor or no germination. In contrast, the combination of GA_3 with PLGA significantly improved both the germination rate and speed, providing more stable germination compared to the control group ($P \leq 0.05$). Notably, the PLGA + 300 mg/L GA_3 treatment exhibited the highest performance, achieving a germination rate of 42.86% and a germination index of 5.01. No significant differences were observed between the control and PLGA + GA_3 treatments in terms of mean germination time. Moreover, while growth regression was observed in the control group after the 14th day, the PLGA + GA_3 treatments resulted in healthier and more sustained seedling development. These findings suggest that PLGA is an effective carrier for the controlled release of growth regulators such as GA_3 and can positively influence germination and early seedling development in medicinal and aromatic plants like *Carum carvi* L.

Keywords: Carum carvi *L., GA_3, PLGA, Germination*

Introduction

Carum carvi L. (commonly known as caraway) is an annual or biennial aromatic and medicinal plant belonging to the Apiaceae (Umbelliferae) family. Its natural distribution spans Western Asia, Europe, and North Africa, and it is also an integral part of the Turkish flora (Goyal et al., 2018). The seeds of *Carum carvi* L. are particularly notable for their high essential oil content, with carvone (55–60%) and limonene (30–40%) being the primary constituents (Öztürk et al., 2018). Pharmacologically, this species exhibits a wide spectrum of bioactivities, including antioxidant, antimicrobial, anticancer, hepatoprotective, anticholinergic, analgesic, and antidiabetic properties (Agnihotri et al., 2024). Consequently, *C. carvi* L. continues to be widely used in traditional medicine and has found increasing applications in modern pharmaceutical formulations.

As is common in many aromatic and medicinal plants, the germination rate of *C. carvi* L. seeds can be adversely affected by factors such as genetic variation, seed dormancy, and environmental conditions (Galambosi & Peura, 1996). In particular, low and delayed germination rates constitute major limiting factors for the commercial cultivation of this species. Seed germination represents a critical stage in the plant life cycle and directly influences agricultural productivity. Accordingly, various chemical and biological treatments have been developed to improve germination success. Among these treatments, gibberellic acid (GA_3) plays a pivotal role in breaking physiological dormancy and stimulating metabolic activation in the embryo (Miransari & Smith, 2014).

Despite the high essential oil content and medicinal value of *C. carvi* L., research focused on improving its germination through biostimulants and growth regulators remains limited. A study by Mirmazloum et al. (2020) demonstrated that osmopriming treatments using various chemical solutions (PEG, KNO_3, KCl) positively affected germination and early seedling development in *C. carvi* L. seeds. In contrast, the germination-promoting effects of GA_3 have been well-documented in numerous plant species. For example, GA_3 application significantly increased the germination rate of *Capsicum chinense* seeds (Mendoza-Gómez et al., 2021) and both improved the germination rate and reduced the germination period in *Sorghum bicolor* (Kamal et al., 2025).

Recent advancements in agricultural biotechnology have highlighted the potential use of biodegradable polymers, particularly poly(lactic-co-glycolic acid) (PLGA), in controlled-release delivery systems. PLGA is a biologically degradable polymer widely used in sustained-release nanosystems (Yang et al., 2024). Studies have shown that PLGA nanoparticles can be absorbed by plant roots and translocated within plant tissues, allowing active compounds such as GA_3 to be protected from environmental stressors and delivered to the seed surface in a more prolonged and effective manner (Vishwakarma et al., 2023). Experiments with *Arabidopsis thaliana* have demonstrated the effectiveness of PLGA in substance transport and its potential to enhance root development post-germination (De Angelis et al., 2023).

The present study aims to investigate the effects of different doses of GA_3 and GA_3 + PLGA combinations on germination rate, germination speed, and mean germination time in *C. carvi* L. seeds. Furthermore, this research will evaluate whether the controlled-release system provided by PLGA enables the physiological effects of GA_3 to be sustained in a more prolonged and balanced manner.

Materials and Methods

This study was conducted at the Plant Biotechnology Laboratory of the Department of Field Crops, Faculty of Agriculture, Selçuk University. The plant material used in this research was *Carum carvi* L. (caraway), an annual aromatic and medicinal species. The seeds were obtained from the Zeytinburnu Medicinal Plants Garden. For the experimental treatments, Resomer RG 503H-type poly(lactic-co-glycolic acid) (PLGA) (Sigma-Aldrich, 719870) and gibberellic acid (GA_3) (Sigma-Aldrich, 77065) were employed.

To evaluate seed germination performance, five different treatments were established: (1) a control treatment with distilled water, (2) GA_3 applied at two concentrations (150 mg/L and 300 mg/L), and (3) GA_3 + PLGA combinations prepared at the same two concentrations (150 mg/L and 300 mg/L). The experiment was arranged in a factorial design within a completely randomized block design (CRBD) with three replications per treatment. In each petri dish, 35 seeds were placed. Prior to germination, seeds were surface-sterilized using a 5% (v/v) sodium hypochlorite (NaClO) solution for 10 minutes, followed by three rinses with distilled water (Tanur & Yorgancılar, 2020). Subsequently, seeds were dried for 24 hours on filter paper at room temperature to restore their original moisture content. The dried seeds were uniformly arranged in sterile petri dishes lined with double layers of Whatman filter paper. Throughout the germination period, treatment solutions (GA_3 and GA_3 + PLGA) were applied at a volume of 10 mL every other day. Petri dishes were incubated under dark conditions at 25 ± 2 °C in a climate chamber for 14 days or until no further germination was observed. Daily observations were performed, and seeds were considered germinated when radicle emergence reached 5 mm (Demir & Mavi, 2008). At the end of the experiment, germination performance was assessed based on germination percentage, germination index, and mean germination time, calculated using the following formulas:

- **Germination Percentage (%)** = $(G / T) \times 100$

  G: Number of germinated seeds, T: Total number of seeds
- **Germination Index (GI)** = $N_1/T_1 + N_2/T_2 + ... + N_n/T_n$

N: Number of seeds germinated on a given day, T: Number of days (Kader & Jutzi, 2004)

- **Mean Germination Time (MGT)** = $\sum(f \times x) / \sum f$

f: Number of seeds germinated on a given day, x: Number of days (Ellis & Roberts, 1981)

The obtained data were subjected to analysis of variance (ANOVA) using the JUMP statistical software, and differences among treatment means were determined by Tukey's multiple comparison test at the $P<0.05$ significance level.

Results and Discussion

Effects of GA_3 and PLGA-Controlled Release Treatments on Germination Dynamics of *Carum carvi* L.

An evaluation of the germination dynamics of *Carum carvi* L. seeds revealed that germination commenced on the 7th day and no additional germination was observed beyond the 14th day. Notably, in the control treatment (distilled water), some seeds exhibited inhibited or regressed radicle and shoot development after the 14th day. In contrast, the PLGA + GA_3 combinations promoted post-germination development, resulting in healthier and more sustainable seedling growth. According to the findings, on the 7th day, the highest germination rate (26.67%) was recorded in the control group, while the PLGA + GA_3 treatments at 150 mg/L and 300 mg/L concentrations exhibited similar germination rates of 22.86% and 21.90%, respectively. In the groups treated solely with GA_3, germination rates remained below 1%, and in some cases, no germination was observed at all (Table 1).

Table 1. Effects of GA_3 and PLGA-Controlled Release GA_3 Treatments on Germination Percentage (%) of *Carum carvi* L. Seeds Over Time

Days	Control	150 mg/L GA_3	300 mg/L GA_3	PLGA +150 mg/L GA_3	PLGA +300 mg/L GA_3	Mean
7	26.67bd	0.95e	0.00e	22.86cd	21.90d	**14.48B**
9	34.29ac	1.90e	0.00e	37.14ab	40.95a	**22.86A**
12	38.10ab	3.81e	0.00e	40.95a	42.86a	**25.14A**
14	38.10ab	4.76e	0.00e	40.95a	42.86a	**25.33A**
Mean	**34.29A**	**2.86B**	**0.00B**	**35.48A**	**37.14A**	
Day:**, Treatment:**, Dayxtreatment:* (**$P \leq 0.01$, *$P \leq 0.05$)						

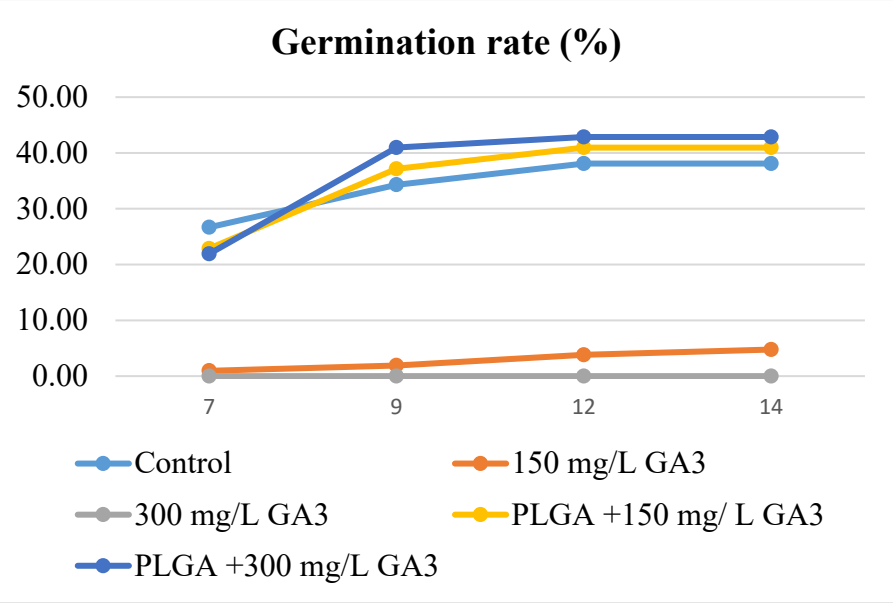

Figure 1. Time-Dependent Changes in Germination Rate (%) of *Carum carvi* L. Under GA_3 and PLGA-Controlled Release Treatments

During the subsequent days of the experiment, particularly on the 9th, 12th, and 14th days, the germination rate of the PLGA + GA_3 combinations increased sharply, exceeding 40%. On the 14th day, the highest germination rate (42.86%) was achieved with the PLGA + 300 mg/L GA_3 treatment, while the control group reached a maximum of 38.10%. In contrast, the GA_3-only treatments consistently showed germination rates below 5%, indicating the ineffectiveness of GA_3 alone in stimulating germination (Figure 1).

In general, the control and PLGA + GA_3 treatments were statistically grouped together, exhibiting similar germination rates, while the GA_3-only groups displayed significantly lower germination performance ($P \leq 0.01$). Additionally, it was observed that germinated seeds in the control group exhibited growth cessation or even regression after the 14th day. However, the PLGA + GA_3 treatments supported post-germination seedling development and ensured continued growth.

Analysis of variance (ANOVA) revealed that the main effects of day, treatment, and their interaction (day × treatment) were all statistically significant ($P \leq 0.01$, $P \leq 0.05$). These results indicate that both treatment type and temporal variation were critical determinants of the germination process.

Effects of GA_3 and PLGA-Controlled Release Treatments on Germination Index (GI) and Mean Germination Time (MGT) of *Carum carvi* L.

The effects of different treatments on the germination index (GI) and mean germination time (MGT) of *Carum carvi* L. seeds were investigated, and the results were found to be statistically significant ($P \leq 0.05$), with distinct differences detected among the treatments (Table 2).

In terms of germination speed, the highest GI value (5.01) was recorded in the PLGA + 300 mg/L GA_3 treatment, followed by the PLGA + 150 mg/L GA_3 (4.81) and the control (4.73) treatments. In contrast, the groups treated solely with GA_3 exhibited extremely low germination speeds, with a GI of 0.35 in the 150 mg/L GA_3 group and 0.00 in the 300 mg/L GA_3 group due to the absence of germination. These findings indicate that GA_3 applied alone negatively affected germination speed in *C. carvi* L., while its combination with PLGA mitigated this inhibitory effect and significantly improved germination performance.

Table 2. Effects of GA_3 and PLGA-Controlled Release GA_3 Treatments on Germination Index (GI) and Mean Germination Time (MGT) of *Carum carvi* L.

	GI	MGT (day)
Control	4.73a	10.83a
150 mg/L GA_3	0.35b	8.13ab
300 mg/L GA_3	0.00b	0.00b
PLGA +150 mg/ L GA_3	4.81a	10.99a
PLGA +300 mg/L GA_3	5.01a	11.01a
	*$P \leq 0.05$	*$P \leq 0.05$

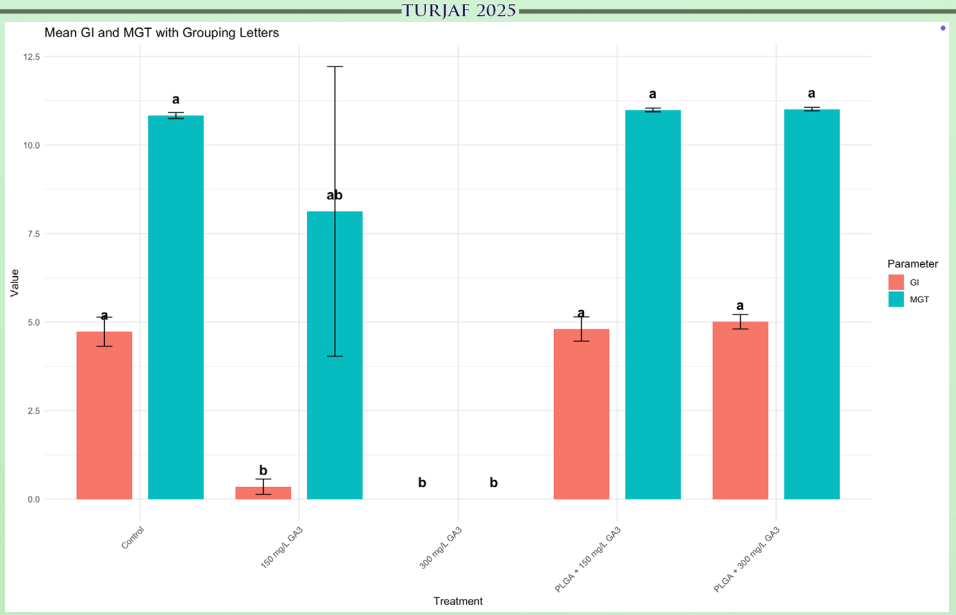

Figure 2. Comparison of GI and MGT Among Different GA_3 and PLGA-Controlled Release Treatments in *Carum carvi* L.

Regarding mean germination time (MGT), similar and statistically comparable values were observed among the control (10.83 days), PLGA + 150 mg/L GA_3 (10.99 days), and PLGA + 300 mg/L GA_3 (11.01 days) treatments. However, the 150 mg/L GA_3 treatment showed a shorter MGT of 8.13 days, although its biological relevance was limited due to the low germination speed and poor overall germination performance. In the 300 mg/L GA_3 group, no MGT value could be calculated due to the absence of germination (Figure 2).

Overall, the results revealed that GA_3, when combined with PLGA, enhanced the germination speed while maintaining a mean germination time comparable to the control group. In contrast, GA_3 applied alone resulted in reduced germination speed and poor germination performance. Statistical analysis confirmed significant differences among treatments for both germination index and mean germination time ($P \leq 0.05$).

The findings of this study clearly demonstrate that the direct application of GA_3 was insufficient to effectively promote germination and seedling development in Carum carvi L. However, when GA_3 was applied via a controlled-release system using PLGA, both germination rate and seedling development were significantly improved. This result is particularly noteworthy considering the presence of morphophysiological dormancy, which is common among species within the Apiaceae family. Similar patterns have been reported in the literature. For example, studies on *Ferula assa-foetida* (Rajabian et al., 2007), *Kelussia odoratissima* (Etemadi et al., 2010) and *Ducrosia anethifolia* (Ashtari et al., 2013) have shown that GA_3 alone had a limited effect on promoting germination, but its effectiveness improved when combined with additional environmental cues such as cold stratification.

In the present study, the combination of GA_3 with PLGA notably enhanced the germination rate (~42%) and germination speed (GI = 5.01) while supporting sustained seedling development. This improvement can be attributed to the controlled-release capabilities of PLGA. Biodegradable polymers like PLGA have gained attention in recent years for their potential use in seed coating and as carriers for plant growth regulators (Shakiba et al., 2020). PLGA adheres to the seed surface, creating a localized environment that retains the hormone near the root and shoot zones, thereby minimizing rapid degradation caused by environmental factors and enabling the gradual and balanced release of the hormone (Bonser et al., 2023).

Consistent with these findings, other studies have reported similar benefits of polymer-based delivery systems. For instance, GA_3 encapsulated within alginate/chitosan polymer matrices improved germination and yield in *Solanum lycopersicum* (Pereira et al., 2019). Likewise, the slow release of metolachlor using mPEG-PLGA nanocarriers was successfully achieved in plants (Gao et al., 2018).These findings highlight that polymer-based carriers are not only effective for pesticide delivery but also for the controlled release of plant growth regulators.

In this study, the slow-release effect provided by PLGA appears to have reduced the potential phytotoxicity of GA_3 in *C. carvi* L., a species characterized by strong seed dormancy, and allowed for the sustained delivery of physiologically tolerable doses of the hormone. This resulted in a significant improvement in both germination performance and early seedling development.

Turkish Science and Technology Publishing (TURSTEP)
www.turstep.com.tr

Conclusion

Controlled release systems present an effective strategy to overcome common challenges such as low germination rates and poor seedling development, especially in species belonging to the Apiaceae family. Our findings demonstrated that the use of GA_3 through controlled release formulations, rather than direct application, significantly optimizes both germination and seedling growth. Specifically, in *Carum carvi* L. seeds, GA_3 alone had limited and low impact on germination, whereas its combination with PLGA notably enhanced both germination and seedling vigor. These results are largely consistent with previous reports on various Apiaceae species, highlighting the potential of controlled release systems to improve propagation efficiency in this important plant family.

References

Agnihotri, V., Shashni, S., & Tripathi, M. 2024. Morphological, Phytochemical and Pharmacological Properties of Carum carvi (Caraway) and Bunium persicum (Black Caraway) Seeds: A Review. Journal of Food Engineering and Technology, 13(1), 25-31.

Ashtari, R., Heidari, M., Omidi, M., & Zare, A. 2013. Seed germination and dormancy breaking techniques for Ducrosia. Anethifolia (DC.). Trakia Journal of Sciences, 1, 82-87.

Bonser, C. A., Borgatta, J., White, J. C., Astete, C. E., Sabliov, C. M., & Davis, J. A. 2023. Impact of zein and lignin-PLGA biopolymer nanoparticles used as pesticide nanocarriers on soybean growth and yield under field conditions. Agrosystems, Geosciences & Environment, 6(2), e20350.

De Angelis, G., Badiali, C., Chronopoulou, L., Palocci, C., & Pasqua, G. 2023. Confocal microscopy investigations of biopolymeric PLGA nanoparticle uptake in Arabidopsis thaliana L. cultured cells and plantlet roots. Plants, 12(13), 2397.

Demir, I., & Mavi, K. 2008). Effect of salt and osmotic stresses on the germination of pepper seeds of different maturation stages. Brazilian Archives of Biology and Technology, 51, 897-902.

Ellis, R., & Roberts, E. 1981. The quantification of ageing and survival in orthodox seeds. Seed Science and Technology (Netherlands), 9(2).

Etemadi, N., HaghIghI, M., NIkbakht, A., & Zamani, Z. 2010. Methods to promote germination of Kelussia odoratissima Mozaff., an Iranian endemic medicinal plants. Herba polonica, 56(2).

Galambosi, B., & Peura, P. 1996. Agrobotanical features and oil content of wild and cultivated forms of caraway (Carum carvi L.). Journal of Essential Oil Research, 8(4), 389-397.

Gao, H., Qin, Y., Guo, R., Wu, Y., Qiu, D., & Fu, Y. 2018. Enhanced plant growth promoting role of mPEG-PLGA-based nanoparticles as an activator protein PeaT1 carrier in wheat (Triticum aestivum L.). Journal of Chemical Technology & Biotechnology, 93(11), 3143-3151.

Goyal, M., Gupta, V. K., Singh, N., & Mrinal, N. 2018. Carum carvi-an updated review. Indian J. Pharm. Biol. Res, 6, 14-24.

Kader, M., & Jutzi, S. 2004. Effects of thermal and salt treatments during imbibition on germination and seedling growth of sorghum at 42/19 C. Journal of Agronomy and Crop Science, 190(1), 35-38.

Kamal, M., Pramono, E., Hadi, M., Setiawan, K., Ardian, A., Muhtadi, S., & Andis, T. (2025). Effectiveness of the application of gibberellic acid (GA3) to stimulate the germination of artificially aged seeds of several sorghum (Sorghum bicolor (L.) Moench) genotypes. BIO Web of Conferences,

Mendoza-Gómez, A., Velázquez-Alcaraz, T., & López-Orona, C. 2021. Analysis of germination in seeds of different geno types of habanero chili pepper (capsicum chinense jacq.) treated with gibberellic acid. Horticult Int J, 5(3), 139-143.

Miransari, M., & Smith, D. 2014. Plant hormones and seed germination. Environmental and experimental botany, 99, 110-121.

Mirmazloum, I., Kiss, A., Erdélyi, É., Ladányi, M., Németh, É. Z., & Radácsi, P. (2020). The effect of osmopriming on seed germination and early seedling characteristics of Carum carvi L. Agriculture, 10(4), 94.

Öztürk, G., Demirci, F., & Demirci, B. 2018. Biological activity evaluation of Carvi aetheroleum and its major components. Facta Universitatis, Series: Physics, Chemistry and Technology, 16(1), 140.

Pereira, A. d. E. S., Oliveira, H. C., & Fraceto, L. F. 2019. Polymeric nanoparticles as an alternative for application of gibberellic acid in sustainable agriculture: a field study. Scientific reports, 9(1), 7135.

Rajabian, T., Saboora, A., Hassani, B., & Fallah Hosseini, H. 2007. Effects of GA3 and chilling on seed germination of Ferula assa-foetida, as a medicinal plant. Iranian Journal of Medicinal and Aromatic Plants Research, 23(3), 391-404.

Shakiba, S., Astete, C. E., Paudel, S., Sabliov, C. M., Rodrigues, D. F., & Louie, S. M. (2020). Emerging investigator series: polymeric nanocarriers for agricultural applications: synthesis, characterization, and environmental and biological interactions. Environmental Science: Nano, 7(1), 37-67.

Tanur, M., & Yorgancılar, M. 2020. Tuz stresine maruz bırakılan kanola (Brassica napus L.)'da priming uygulamalarının (salisilik asit ve askorbik asit) çimlenme üzerine etkisi. Journal of the Institute of Science and Technology, 10(4), 3109-3121.

Vishwakarma, V., Ogunkunle, C. O., Rufai, A. B., Okunlola, G. O., Olatunji, O. A., & Jimoh, M. A. (2023). Nanoengineered particles for sustainable crop production: potentials and challenges. 3 Biotech, 13(5), 163.

Yang, J., Zeng, H., Luo, Y., Chen, Y., Wang, M., Wu, C., & Hu, P. 2024. Recent Applications of PLGA in drug delivery systems. Polymers, 16(18), 2606.