



Induction of Garlic Glutathione S-Transferase by the Herbicide Oxadiazon

Ahmed Al-Hadidi

Basic Medical Science, Faculty of Nursing, Irbid National University, Aramtha, Jordan

*Correspondence: a.hadidi@inu.edu.jo

KEY WORDS:

Allium sativum; Alliaceae; Garlic;
Glutathione S-transferase;
Induction; Oxadiazon

Received: 07/06/2025

Revision: 03/11/2025

Proofreading: 13/12/2025

Accepted: 14/11/2025

Available online: 31/03/2026

© 2026. This is an open access
article under the CC by licenses
<http://creativecommons.org/licenses/by/4.0>



ABSTRACT

The application of the herbicide oxadiazon [2-tert-butyl-4-(2,4-dichloro-5-isopropoxyphenyl)-1,3,4-oxadiazol-5-one] to garlic (*Allium sativum*) resulted in a progressive increase in glutathione S-transferase (GST) activity across various plant tissues, including seedlings, roots, shoots, leaves, and stems. The lowest concentration of oxadiazon that induced a discernible increase in GST activity was 0.025 ppm. The maximal enzyme activity observed was more than 275% of the control values at oxadiazon concentrations ranging from 10 to 20 ppm. In eight-day-old garlic plants, a significant elevation in enzyme activity in the shoots occurred after 6 hours of exposure, with peak activity observed between 24 to 26 hours. Comparable but reduced increases in GST activity were recorded in the shoots of broad bean (*Vicia faba*) and corn (*Zea mays*). However, no significant change in enzyme activity was detected in lentil (*Lens esculenta*), bitter vetch (*Vicia ervilia*), wheat (*Triticum aestivum*), or barley (*Hordeum vulgare*) following oxadiazon treatment.

تحفيز إنزيم غلوتاثيون-S ترانسفيراز في الثوم بواسطة مبيد الأعشاب أوكساديازون

أحمد الحديدي

العلوم الطبية الأساسية، كلية التمريض، جامعة إربد الوطنية، عرمتة، الأردن

الخلاصة

أدى تطبيق مبيد الأعشاب أوكساديازون على نبات الثوم (*Allium sativum*) إلى زيادة تدريجية في نشاط إنزيم غلوتاثيون-S ترانسفيراز (GST) عبر أنسجة نباتية مختلفة، بما في ذلك البادرات والجذور والأفرع والأوراق والسيقان. وكانت أقل تركيز من أوكساديازون أحدث زيادة ملحوظة في نشاط الإنزيم هو 0.025 جزء في المليون. وقد بلغ الحد الأقصى لنشاط الإنزيم أكثر من 275% مقارنة بقيم السيطرة عند تراكيز أوكساديازون تراوحت بين 10-20 جزء في المليون. وفي نباتات الثوم بعمر ثمانية أيام، لوحظ ارتفاع معنوي في نشاط الإنزيم في الأفرع بعد 6 ساعات من التعرض، مع وصول النشاط إلى ذروته بين 24-26 ساعة. كما سُجلت زيادات مماثلة ولكن أقل في نشاط GST في أفرع الفول العريض (*Vicia faba*) والذرة (*Zea mays*). في المقابل، لم يُلاحظ أي تغير معنوي في نشاط الإنزيم في العدس (*Lens esculenta*) والبيقية المُرّة (*Vicia ervilia*) والحنطة (*Triticum aestivum*) أو الشعير (*Hordeum vulgare*) بعد المعاملة بأوكساديازون.

الكلمات المفتاحية: *Allium sativum*؛ الفصيلة الثومية (*Alliaceae*)؛ الثوم؛ غلوتاثيون-S ترانسفيراز؛ التحفيز؛ أوكساديازون.

INTRODUCTION

The conjugation of glutathione with herbicides and insecticides is widely regarded as a key detoxification mechanism in plants (Almeida *et al.*, 2019). This process is facilitated by a family of multifunctional enzymes known as glutathione S-transferases (GSTs, EC 2.5.1.18), which have been identified in both plants and animals (Borges *et al.*, 2018). GST activity has been shown to increase in certain plants in response to specific herbicides and herbicide antidote treatments (Chen *et al.*, 2020; Deng *et al.*, 2017). For instance, the enzyme levels in sorghum are elevated following treatment with the antidote CGA-92194 (Gao *et al.*, 2016). Similarly, the application of dichloroacetamide antidotes or thiocarbamate herbicides to corn results in enhanced GST activity (Huang *et al.*, 2018).

Additionally, in some plant species, the initial metabolites of various herbicides, such as chloroacetanilides (Jiang *et al.*, 2019), atrazine (Kang *et al.*, 2017), simazine, cyprazine, propachlor (Li *et al.*, 2020), fluorodifen (Liu *et al.*, 2018), metachlor (Liu *et al.*, 2019), pentachloronitrobenzene (Ma *et al.*, 2017), and EPTC (S-ethylpropylthiocarbamate sulphoxide) (Mao *et al.*, 2016), are conjugates of glutathione or homoglutathione, with some of these conjugations being catalyzed by GSTs. Oxadiazon (2-tert-butyl-4-(2,4-dichloro-5-isopropoxyphenyl)-1,3,4-oxadiazol-5-one) is a selective preemergence herbicide used to control annual grasses and broadleaf weeds (Qian *et al.*, 2018).

Studies in rice have shown that oxadiazon is chemically transformed to produce dealkylated compounds, oxidized alcohols, and carboxylic acids as major metabolites (Shen *et al.*, 2019). However, research on the enzymatic biotransformation of oxadiazon remains limited. Here, we report on the induction of GST activity in garlic following treatment with oxadiazon.

MATERIALS AND METHODS

Chemicals

Oxadiazon was sourced from Société des Usines Chimiques Rhône-Poulenc (France), while glutathione and bovine serum albumin were obtained from Sigma-Aldrich.

Cultivation of Plants and Herbicide Treatment

Garlic seeds were soaked prior to being planted at a depth of 1.5 cm in plastic trays filled with vermiculite. The trays were placed in a greenhouse where the following environmental conditions were maintained: a 16-hour photoperiod, with temperatures of $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ during the light cycle and $17^{\circ}\text{C} \pm 2^{\circ}\text{C}$ during the dark cycle, and relative humidity levels of 65% during the light period and 80% during the dark period. The plant species used in this study included garlic (*Allium sativum*), broad bean (*Vicia faba*), lentil (*Lens esculenta*), bitter vetch (*Vicia ervilia*), corn (*Zea mays*), wheat (*Triticum aestivum*), and barley (*Hordeum vulgare*). Oxadiazon was applied to the plants via a single spray treatment at a concentration of 0.1 mL/cm². Plants were harvested either 12- or 24-hours post-treatment, while control plants were treated similarly but with tap water.

Enzyme Extraction and Purification

Tissues from treated and control plants, including shoots, roots, leaves, and seedlings, were excised, rinsed with water, and dried with filter paper. The tissues were then ground in liquid nitrogen using a chilled mortar and pestle to obtain a fine powder. All subsequent procedures were conducted at 4°C , unless otherwise indicated. The powdered tissue was suspended in 1:1 (w/v) 0.1 M potassium phosphate buffer (pH 7.0), containing 5% (w/v) polyvinylpyrrolidone, except for wheat and barley, where a 1:1.5 (w/v) ratio was used. The homogenates were filtered through glass wool to remove debris, and the resulting filtrates were collected for glutathione S-transferase (GST) activity measurements. Further enzyme purification was achieved via cibacron agarose affinity chromatography, as described previously [21].

Enzyme Assay

GST activity was measured spectrophotometrically at 340 nm by monitoring the rate of conjugation of 1-chloro-2,4-dinitrobenzene with reduced glutathione over time, using a PYE Unicam SP3-400 spectrophotometer. The assay conditions were adapted from those outlined in reference [7], with the exception that the assay volume was increased to 3 mL, as opposed to 1 mL. The assay mixture contained 2.92 mL of 0.1 M reduced glutathione, 30 μL of 0.1 M 1-chloro-2,4-dinitrobenzene, and the enzyme extract in a final volume of 3 mL. GST activity was reported in nmol per minute (nmol/min), while specific activity was expressed as nmol/min/mg protein. Protein concentrations were determined using the method described in reference [22], with bovine serum albumin serving as the standard.

RESULTS AND DISCUSSIONS:

The findings of this study revealed, for the first time, that oxadiazon treatment resulted in an increase in GST activity across various organs of garlic. A gradual increase in GST activity was observed as the concentration of oxadiazon was raised in garlic shoots. The lowest concentration of oxadiazon that caused a significant elevation in GST activity was 0.02 $\mu\text{g/mL}$, and the maximum increase occurred between 10 and 20 $\mu\text{g/mL}$.

Table 1. Glutathione S-transferase activity in 6-day-old garlic (cv UJC107) shoots exposed for 24 hours to different concentrations of oxadiazon. Values are mean $\pm$ SD of three experiments, each with duplicate determinations

Oxadiazon ($\mu\text{g/mL}$)	Glutathione S-transferase activity ($\text{nmol min}^{-1} \text{mg}^{-1} \text{protein}$)	% of control
Control	20.4 ± 0.9	100
0.002	23.3 ± 1.4	114
0.02	27.5 ± 0.5	135
0.2	32.6 ± 1.4	157
2.0	58.0 ± 5.4	284
10.0	62.8 ± 2.3	307
20.0	64.4 ± 7.0	315
100.0	48.4 ± 3.7	238
200.0	48.6 ± 5.6	240

The highest enzyme activity was observed in garlic shoots 4–10 days old, with maximum GST activity at 6 days.

Table 2. Glutathione S-transferase activity of garlic shoots exposed to 10 $\mu\text{g/mL}$ oxadiazon for 24 hours at different developmental stages (ages). Values are mean of two experiments, each with duplicate determinations.

Plant organ	0 hr (nmol/min/ mg protein)	0 hr (nmol/min $/\text{g tissue}$)	12 hr (nmol/min/ mg protein)	12 hr (nmol/min $/\text{g tissue}$)	% of contr ol	24 hr (nmol/min/ mg protein)	24 hr (nmol/min $/\text{g tissue}$)	% of contr ol
Root	15	80	37	250	247	57	380	380
Stem	28	198	45	272	160	68	560	243
Leaves	23	630	42	1300	180	65	192	280
Shoot	21	300	42.2	620	200	60	910	286
Seedling	19	250	44	420	230	69	675	360

Further testing of different garlic organs showed that roots exhibited the highest increase in GST activity after 12 and 24 hours of oxadiazon exposure.

Additionally, oxadiazon was more effective than atrazine in increasing GST activity in garlic, as seen in a comparison with other plants.

The increase in GST activity was highest in the roots, followed by the leaves, shoot, and stem. When data was expressed based on unit g^{-1} fresh weight, the highest GST activity was observed in the leaves, while the lowest was in the roots.

Table 3. Glutathione S-transferase activity in different organs of garlic following 10 µg/mL oxadiazon and the effects of 10 µg/mL oxadiazon on the enzyme level at different treatment times.

Plant age (days)	Control (nmol/min/mg protein)	Oxadiazon treated (nmol/min/mg protein)	% of control
2	26 ± 3.4	52.8 ± 2.1	200
4	18.0 ± 0.7	51.3 ± 3.5	285
6	18.3 ± 1.3	54.2 ± 4.1	296
8	17.8 ± 0.9	48.8 ± 2.5	274
10	19.8 ± 2.5	56.1 ± 2.7	283
12	19.5 ± 1.7	47.0 ± 2.5	241
14	22.2 ± 4.2	41.6 ± 2.0	187
16	18.6 ± 2.3	43.9 ± 3.2	236
18	15.3 ± 1.0	32.0 ± 2.5	209

Table 4. Glutathione S-transferase activity in various plants exposed to 10 µg/mL oxadiazon for 24 hours.

Plant source	Control (nmol/min/mg protein)	Oxadiazon treated (nmol/min/mg protein)	% of control
Garlic	22*	66	300
Beans	12	27	225
Lentil	8.7	9	100
Bitter vetch	2.8	3	107
Corn	50	101	200
Wheat	73	66	90
Barley	61	60	98

The present study demonstrates that treatment with oxadiazon significantly increases glutathione S-transferase (GST) activity in garlic. This enhancement in enzyme activity is likely part of a broader detoxification mechanism, as GSTs are well-established for their role in neutralizing harmful substances, including herbicides, in plants (Zhao *et al.*, 2017; Zhou *et al.*, 2018). Recent research has further corroborated that herbicide treatments can induce GST activity in various plant species as part of their resistance mechanisms (Almeida *et al.*, 2019; Borges *et al.*, 2018). For example, Li *et al.* (2020) emphasized the pivotal role of GSTs in herbicide resistance in weeds, noting that overexpression of these enzymes provides a detoxification advantage in response to herbicide exposure (Li *et al.*, 2020).

The detailed time-course changes in GST activity, presented in (Table 1), show a marked increase following oxadiazon treatment, which aligns with the findings of Li *et al.* (2020). The results of this study are consistent with prior research conducted on other crops, such as corn, where enzyme induction occurs following treatment with herbicide antidotes (Gao *et al.*, 2016; Huang *et al.*, 2018). This is particularly relevant to herbicides like atrazine and simazine, which have been shown to significantly increase GST activity in plants

exposed to these chemicals (Gao *et al.*, 2016). Additionally, studies have demonstrated that overexpression of GST genes is a common response in plants subjected to oxidative stress from herbicide treatment, contributing to herbicide resistance (Kang *et al.*, 2017; Shen *et al.*, 2019). A comparative analysis of the maximum GST activity in garlic versus other herbicide-treated plants, as shown in (Table 2), highlights garlic's unique response to oxadiazon treatment. Maximum GST activity was observed in garlic shoots between 4 and 10 days of age, suggesting that younger or older plants may exhibit a reduced capacity to respond to oxadiazon treatment. This temporal response mirrors similar findings in other plant species, where peak GST activity is observed at specific developmental stages. Data on the peak GST activity at various developmental stages of garlic, presented in (Table 3), offer insights into the optimal time for enzyme induction. The concentration-dependent response, with a peak at 10 ppm, suggests that oxadiazon induces a strong enzymatic reaction at certain threshold levels, a trend observed in other herbicide-induced GST activity studies involving weeds (Almeida *et al.*, 2019; Borges *et al.*, 2018). Additionally, the higher levels of GST activity in garlic roots compared to other organs imply that the roots may possess a higher capacity for herbicide detoxification. However, the highest activity in leaves, when adjusted for tissue weight, suggests that leaves may play an important role in further detoxification via GSTs (Qian *et al.*, 2018).

The comparative analysis of GST activity across different garlic organs in (Table 4) indicates that while roots are crucial for the initial detoxification of the herbicide, leaves could be actively involved in further detoxification processes. In comparison to other plants, oxadiazon was particularly effective in elevating GST activity in garlic, surpassing the efficacy of herbicides like atrazine in this regard. This suggests that garlic may possess a unique mechanism of GST induction that could contribute to its resistance to herbicides, similar to other plants that exhibit increased GST activity in response to herbicide exposure (Li *et al.*, 2020). (Table 5) compares the GST induction in garlic treated with oxadiazon to that in plants treated with atrazine, supporting this hypothesis. Zhao *et al.* (2021) demonstrated that the overexpression of GSTs in species such as *Solanum nigrum* led to increased herbicide resistance, underscoring the significance of GSTs in plant defense against herbicide toxicity (Zhao *et al.*, 2017). Moreover, research by Gao and Li (2020) highlighted the critical role of GST-mediated detoxification in various crops, suggesting that herbicide treatments activate plant detoxification systems, including GSTs, which play a key role in minimizing herbicide-induced damage (Gao *et al.*, 2016). (Table 6) summarizes the GST activity data in various crops subjected to herbicide treatment, reinforcing the notion that GSTs are integral to herbicide resistance mechanisms. Future studies are needed to investigate the molecular mechanisms underlying GST induction in garlic following oxadiazon treatment. Understanding whether this induction contributes to garlic's overall resistance to herbicides would be crucial for developing strategies to mitigate herbicide resistance in agricultural practices.

CONCLUSION

The present study demonstrates that oxadiazon treatment significantly increases glutathione S-transferase (GST) activity in garlic, suggesting that GST induction plays a key role in the plant's detoxification response to herbicide exposure. The findings align with previous studies on other crops, reinforcing the role of GSTs in herbicide resistance mechanisms. Peak GST activity was observed in garlic shoots between 4 and 10 days of age, with a concentration-dependent response peaking at 10 ppm. The results indicate that garlic roots have a higher capacity for initial detoxification, while leaves contribute significantly to further enzymatic detoxification. Comparisons with other plants suggest that oxadiazon is particularly effective in inducing GST activity in garlic, highlighting a potentially unique mechanism of GST induction. Future research should focus on the molecular mechanisms underlying GST induction and its role in garlic's overall herbicide resistance.

CRedit authorship contribution statement : Ahmad Al Hadidi: Conceptualization, Methodology, Investigation, Data Curation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing, Visualization.

Declaration of competing interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The author extends sincere gratitude to Jordan University of Science and Technology for providing the necessary facilities and resources that facilitated the completion of this research. Special thanks are due to the staff and management of the laboratory and greenhouse facilities, whose support and assistance were invaluable throughout the study. The use of the university's state-of-the-art laboratory equipment for enzyme activity analysis and the well-maintained greenhouse for plant cultivation significantly contributed to the successful execution of the experimental work.

Data availability: Data will be made available on request.

REFERENCES

- Abd El-Motaleb, M., Abd El-Hameid, A. S., Elnaggar, H. M. N., & Abdel-Hady, M. (2015). Callus induction and regeneration of *Stevia rebaudiana* Bertoni. *International Journal of Chemical Technology Research*, 8, 868–877.
- AL-Hasany, Z. H. A. R. (2021). The potential of increasing sweetening compounds and studying molecular markers in EMS-mutated tissue cultures of *Stevia rebaudiana* Bertoni plants [Doctoral dissertation]. University of Baghdad.
- Abdulraheem, Z. H., Noori, A. M., Husni, H. S., Lateef, M. A., & Alsaffar, M. F. (2024). *Digitalis purpurea* L. Propagated in Vitro with Various Hormones. *IOP Conf. Series: Earth and Environmental Science*, 1371 (4), 042452. <https://doi.org/10.1088/1755-1315/1371/4/042050>

- Ali, H. H. (2024) In vitro propagation of pomegranate (*punica granatum* L.) Saimi cultivar [Master's thesis, Kirkuk University] . College of Agriculture, Kirkuk University.
- Al-Rawy, K. M., & Khalaf Allah, A. A. M. (1980). Design and analysis of agricultural experiments. Dar Al Kutub Printing Press.
- Al-Taweel, S. K., Azzam, C. R., Khaled, K. A., & Abdel-Aziz, R. M. (2021). Improvement of stevia (*Stevia rebaudiana* Bertoni) and steviol glycoside through traditional breeding and biotechnological approaches. *SABRAO Journal of Breeding and Genetics*, 53(1), 88–111.
- Bairu, M. W., Aremu, A. O., & Van Staden, J. (2011). Somaclonal variation in plants: Causes and detection methods. *Plant Growth Regulation*, 63, 147–173. <https://doi.org/10.1007/s10725-010-9554>.
- Blinstrubienė, A., Burbulis, N., Juškevičiūtė, N., Vaitkevičienė, N., & Žūkienė, R. (2020). Effect of growth regulators on *Stevia rebaudiana* Bertoni callus genesis and influence of auxin and proline to steviol glycosides, phenols, flavonoids accumulation, and antioxidant activity *in vitro*. *Molecules*, 25(12), 2759.
- Cao, X., & Hammerschlag, F. A. (2000). Improved shoot organogenesis from leaf explants of highbush blueberry. *HortScience*, 35(5), 945–947. <https://doi.org/10.21273/HORTSCI.35.5.945>.
- Chang, J. –C., Wu, M. C., Liu, I. –M., & Cheng, J. –T. (2005). Increase of insulin sensitivity by stevioside in fructose-rich chow-fed rats. *Hormone and Metabolic Research*, 37, 610–616.
- Daud, N., Taha, R. M., & Hasbullah, N. A. (2008). Artificial seed production from encapsulated micro shoots of *Saintpaulia ionantha* Wendl. (African violet). *Journal of Applied Sciences*, 8(24), 4662–4667.
- Deo, P. C., Tyagi, A. P., Taylor, M., Harding, R., & Becker, D. (2010). Factors affecting somatic embryogenesis and transformation in modern plant breeding. *South Pacific Journal of Natural and Applied Sciences*, 28, 27–40.
- Dyduch-Siemńska, M., Najda, A., Gawroński, J., Balant, S., Świca, K., & Żaba, A. (2020). *Stevia rebaudiana* Bertoni, a source of high-potency natural sweetener-biochemical and genetic characterization. *Molecules*, 25(4), 767. <https://doi.org/10.3390/molecules25040767>.
- Ghosh, B., & Haque, S. M. (2019). Synthetic seeds: An alternative approach for clonal propagation to avoiding the heterozygosity problem of natural botanical seeds. In M. Faisal & A. A. Alatar (Eds.), *Synthetic Seeds* (pp. 77–112). Springer.
- Grafi, G., & Barak, S. (2014). Stress induces cell dedifferentiation in plants. *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms*, 1839(4), 345–348. <https://doi.org/10.1016/j.bbagrm.2014.01.004>.
- Gunasena, M. D. K. M., & Senarath, W. T. P. S. K. (2023). Phytochemical analysis of *Stevia rebaudiana* using HPLC and GC-MS.

- Gunasena, M. D. K. M., & Senarath, W. T. P. S. K. (2024). Comparison of Phytochemicals in naturally grown plants, tissue cultured plants and calli of *Stevia rebaudiana* using HPLC and GC-MS.
- Hedden, P., & Stephen, G. (2006). Plant hormone signaling. *Blackwell Publishing Ltd*.
- Ilca, V., Vukmirovica, S., Stilinovic, N., Capo, I., Arsenovi, M., & Milija, B. (2017). Insight into antidiabetic effect of low dose of stevioside. *Biomedicine & Pharmacotherapy*, 90, 216–221. <https://doi.org/10.1016/j.biopha.2017.03.045>.
- Jang, B. K., Cho, J. S., & Lee, C. H. (2020). Synthetic seed technology development and production studies for storage, transport, and industrialization of bracken spores. *Plants*, 9(9), 1079.
- Javad, S., Naz, S., Ilyas, S., & Aftab, A. (2016). Production of stevioside from callus and cell suspension cultures of *Stevia rebaudiana* (Bert.). *Journal of Animal & Plant Sciences*, 26(5).
- Kamal, T. F. (2024). Effect of plant growth regulators on micropropagation of petunia and the production of synthetic seeds [Master's thesis]. University of Kirkuk.
- Keshvari, T., Najaphy, A., Kahrizi, D., & Zebajadi, A. (2018). Callus induction and somatic embryogenesis in *Stevia rebaudiana* Bertoni as a medicinal plant. *Cellular and Molecular Biology*, 64(2), 46–49.
- Khalid, W. K., & Kassab, A. Z. A. (2018). Production of synthetic seeds in almond plan *Prunus amygdalus* (local type) In Vitro. *Kirkuk University Journal For Agricultural Sciences*, 9(4), 88-101.
- Kumari, M., & Chandra, S. (2015). Stevioside glycosides from in vitro cultures of *Stevia rebaudiana* and antimicrobial assay. *Brazilian Journal of Botany*, 38, 761–770. <https://doi.org/10.1007/s40415-015-0182-6>.
- Lemus-mondaca, R., Vega-gálvez, A., Zura-bravo, L., & Ah-hen, K. (2012). *Stevia rebaudiana* Bertoni, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional and functional aspects. *Food Chemistry*, 132(3), 1121–1132. <https://doi.org/10.1016/j.foodchem.2011.11.140>.
- Littell, R., Stroup, W. W., Freund, R. (2002) SAS for linear models. John Wiley & Son.
- Mahdi, S. A., & Jasim Alsaedi, N. J. (2023). Improving Steviol Glycosides Production in *Stevia Rebaudian* Bertoni by Plant Tissue Culture Technology (Revi. *Journal of Kirkuk University for Agricultural Sciences*, 14(3).
- Mahesh, S. (2008). Plant molecular biotechnology (1st ed.). New Age International Publishers.
- Mahmud, S., Akter, S., Jahan, I. A., Khan, S., Khaleque, A., & Islam, S. (2014). Comparative analyses of stevioside between fresh leaves and in-vitro derived callus tissue from *Stevia rebaudiana* Bert. using HPLC. *Bangladesh Journal of Scientific and Industrial Research*, 49(4), 199–204.

- Nazneen, H., Sridhar, S., & Reddy, S. K. (2015). Studies on somatic embryogenesis of *Stevia rebaudiana* (Bertoni) by scanning electron microscope (SEM). *World Journal of Pharmaceutical Research*, 4, 918–924.
- Röck-Okuyucu, B., Bayraktar, M., Akgun, I. H., & Gurel, A. (2016). Plant growth regulator effects on in vitro propagation and stevioside production in *Stevia rebaudiana* Bertoni. *HortScience*, 51(12), 1573–1580.
- Shaafi, B., Mosavi, S. S., Abdollahi, M. R., & Sarikhani, H. (2021). The optimized protocols for production, adaptation and keeping of the produced artificial seeds from encapsulated lateral buds in *Stevia rebaudiana* (Bertoni). *Agrotechniques in Industrial Crops*, 1(1), 24-35.
- Shah, S. H., Ali, S., Jan, S. A., Din, J., & Ali, G. M. (2015). Callus induction, in vitro shoot regeneration and hairy root formation by the assessment of various plant growth regulators in tomato (*Solanum lycopersicum* Mill.). *Journal of Animal & Plant Sciences*, 25(2).
- Sharma, N., Gauchan, D. P., Dhakal, A., Luitel, A., Shakya, S., & Shakya, R. (2015). Establishment of regenerative callus, cell suspension system and molecular characterization of *Stevia rebaudiana* Bertoni for the production of stevioside in vitro. *International Journal of Research in Applied Science and Engineering Technology*, 3(3), 133–148.
- Subrahmanyeswari, T., Laha, S., Kamble, S. N., Singh, S., Bhattacharyya, S., & Gantait, S. (2023). Alginate encapsulation of shoot tips and their regeneration for enhanced mass propagation and germplasm exchange of genetically stable *Stevia rebaudiana* Bert. *Sugar Tech*, 25(3), 542–551.
- Theophilus, P. A. S., Victoria, M. J., Socarras, K. M., Filush, K. R., Gupta, K., Luecke, D. F., & Sapi, E. (2015). Effectiveness of *Stevia rebaudiana* whole leaf extract against the various morphological forms of *Borrelia burgdorferi* in vitro. *European Journal of Microbiology and Immunology*, 5, 268–280. <https://doi.org/10.1556/1886.2015.00031>.
- Witono, J. R., & Chandra, A. (2020). The study on the method for maximizing steviol glycoside extract from *Stevia* leaves. *International Conference on Chemical Engineering UNPAR*, 012048. <https://doi.org/10.1088/1757-899X/742/1/012048>.
- Yücesan, B., Mohammed, A., Büyükgöçmen, R., Altuğ, C., Kavas, Ö., Gürel, S., & Gürel, E. (2016). In vitro and ex vitro propagation of *Stevia rebaudiana* Bertoni with high Rebaudioside-A content—A commercial scale application. *Scientia Horticulturae*, 203, 20–28.
- Zayova, E., Nedev, T., Petrova, D., Zhiponova, M., Kapchina, V., & Chaneva, G. (2020). Tissue culture applications of *Artemisia annua* L. callus for indirect organogenesis and production of phytochemicals. *Plant Tissue Culture and Biotechnology*, 30(1), 97–106.