



Herbicide-induced Activation of Glutathione S-transferase in Garlic (*Allium sativum* L.) Exposed to Oxadiazon

Ahmad Al Hadidi¹, Muath Bani Melhim²

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ABSTRACT

Background: Herbicides are widely used in agriculture and may induce detoxification mechanisms in plants through activation of defense-related enzymes. Glutathione S-transferases (GSTs) play a crucial role in xenobiotic detoxification by conjugating toxic compounds with glutathione, thereby enhancing plant tolerance to chemical stress. The present study aimed to investigate the induction of GST activity in garlic (*Allium sativum*) following exposure to the herbicide oxadiazon and to compare the response among different plant species and tissues.

Methods: Garlic plants were treated with varying concentrations of oxadiazon [2-tert-butyl-4-(2,4-dichloro-5-isopropoxyphenyl)-1,3,4-oxadiazol-5-one]. GST activity was measured in multiple plant tissues, including seedlings, roots, shoots, leaves and stems. Time-dependent enzyme activity was evaluated in eight-day-old plants after herbicide exposure. Comparative analyses were also conducted in shoots of broad bean (*Vicia faba*), corn (*Zea mays*), lentil (*Lens esculenta*), bitter vetch (*Vicia ervilia*), wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*).

Result: Oxadiazon treatment caused a progressive increase in GST activity in all examined garlic tissues. The lowest effective concentration inducing detectable enzyme activation was 0.025 ppm. Maximum GST activity exceeded 275% of control values at concentrations between 10 and 20 ppm. In eight-day-old garlic plants, GST activity in shoots increased significantly within 6 hours of exposure, reaching peak levels between 24 and 26 hours. Moderate increases in GST activity were observed in shoots of broad bean and corn, whereas lentil, bitter vetch, wheat and barley showed no significant enzymatic response following treatment. Oxadiazon effectively induces GST activity in garlic, demonstrating a species and tissue-specific detoxification response. The marked enhancement of GST activity suggests an adaptive biochemical mechanism contributing to herbicide tolerance in garlic and highlights GST as a potential biomarker for herbicide exposure in plants.

Key words: Alliaceae, *Allium sativum*, Garlic, Glutathione S-transferase, Induction, Oxadiazon.

INTRODUCTION

The conjugation of glutathione with herbicides and insecticides is a well-recognized detoxification mechanism in plants (Edwards and Walbot, 2000; Dixon and Edwards, 2002; Cummins *et al.*, 2011; Marrs, 1996). This process is mediated 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 (Riechers and Molin, 2005; Kumar and Trivedi, 2019). GST activity has been shown to increase in plants following exposure to several herbicides and herbicide antidotes (DeRidder and Goldsbrough, 2002; Gronwald *et al.*, 1987; Jain *et al.*, 2001). For example, enzyme levels in sorghum rise after treatment with the safener CGA-92194 (Gronwald *et al.*, 1987) and dichloroacetamide antidotes or thiocarbamate herbicides applied to corn also enhance GST activity (DeRidder and Goldsbrough, 2002). In many plant species, metabolites of chloroacetanilides, atrazine, simazine, cyprazine, propachlor, fluorodifen, metachlor, pentachloronitrobenzene and EPTC form conjugates with glutathione or homoglutathione, often catalyzed by GSTs (Cummins *et al.*, 2011; Riechers and Molin, 2005; Marrs, 1996).

Oxadiazon [2-tert-butyl-4-(2,4-dichloro-5-isopropoxyphenyl)-1,3,4-oxadiazol-5-one] is a selective pre-emergence herbicide used to control annual grasses and

¹Department of Basic Medical Sciences, Nursing College, Irbid National University, Irbid, Jordan.

²Department of Medical Laboratory Sciences, Applied Sciences College, Irbid National University, Irbid, Jordan.

Corresponding Author: Ahmad A. Alhadidi, Department of Basic Medical Sciences, Nursing College, Irbid National University, Irbid, Jordan. Email: a.hadidi@inu.edu.jo

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broadleaf weeds (Hunaiti and Ali, 1991; Hunaiti, 1990). In rice, oxadiazon is metabolized to produce dealkylated compounds, oxidized alcohols and carboxylic acids as major metabolites (Hu *et al.*, 2014), yet studies on its enzymatic biotransformation remain limited.

GSTs enzymes also play an essential role in protecting plant tissues against oxidative damage generated during environmental and chemical stress (Gullner *et al.*, 2018; Kumar and Trivedi, 2019). Herbicide exposure frequently induces reactive oxygen species (ROS), leading to activation of antioxidant defense pathways including GSTs,

catalase and peroxidases. Previous studies demonstrated that antioxidant and detoxification systems are closely associated with stress tolerance and adaptive metabolic responses in plants (Pradhan *et al.*, 2021; Autukait *et al.*, 2021; Soni *et al.*, 2024). Mechanical and environmental stresses were also shown to induce antioxidant-related genes and enzymatic defense pathways in plants (Abu-Romman and Ammari, 2022). Furthermore, oxidative stress biomarkers and detoxification-related biochemical responses have been widely used to evaluate physiological damage and adaptive responses under stress conditions (Carvalho-Moore *et al.*, 2024; Soni *et al.*, 2024).

Despite the recognized importance of GST-mediated detoxification, limited information is available regarding the induction of GST activity in garlic following oxadiazon exposure. Therefore, the present study aimed to investigate the induction and distribution of GST activity in garlic and several crop species after treatment with oxadiazon, with particular emphasis on concentration-dependent responses, developmental stage, organ-specific induction patterns and the time course of enzyme activation.

MATERIALS AND METHODS

Plant materials and growth conditions

The experiment was conducted at Jordan University of Science and Technology. The study was carried out during April to June 2024. Plant species used in this study included garlic (*Allium sativum* L.), broad bean (*Vicia faba*), lentil (*Lens esculenta*), bitter vetch (*Vicia ervilia*), corn (*Zea mays*), wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*). Garlic cloves 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°C±2°C during the light cycle and 17°C±2°C during the dark cycle and relative humidity levels of 65% during the light period and 80% during the dark period.

Herbicide treatment

Oxadiazon (Société des Usines Chimiques Rhône-Poulenc, France) was dissolved in distilled water containing 0.1% (v/v) Tween-20 as a surfactant. The herbicide was applied to the plants via a single spray treatment at a concentration of 10 µg/mL (unless otherwise specified in concentration-response experiments) at a volume of 0.1 mL per cm² of tray surface area. Control plants were sprayed with distilled water containing 0.1% Tween-20 only. Plants were harvested at specified time points (0, 3, 6, 12, 18, 24, 26, 36 and 48 hours post-treatment) for time-course experiments.

Tissue preparation and enzyme extraction

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 0.1 M potassium phosphate buffer (pH 7.0) containing 5% (w/v) polyvinylpyrrolidone at a ratio of 1:1 (w/v), except for wheat and barley tissues where a ratio of 1:1.5 (w/v) was used. The homogenates were filtered through glass wool and centrifuged at 12,000 × g for 20 min at 4°C. The resulting supernatants were collected and used as crude enzyme extracts for glutathione S-transferase (GST) activity measurements. Further enzyme purification was achieved via cibacron agarose affinity chromatography, as described previously (Hunaiti, 1990).

GST activity assay

GST activity was measured spectrophotometrically at 340 nm using a PYE Unicam SP3-400 spectrophotometer by monitoring the conjugation of reduced glutathione (GSH) with 1-chloro-2,4-dinitrobenzene (CDNB). The standard reaction mixture consisted of 2.92 mL of 0.1 M potassium phosphate buffer (pH 7.0) containing 1 mM reduced glutathione, 30 µL of 0.1 M CDNB dissolved in ethanol and 50 µL of enzyme extract in a final reaction volume of 3 mL. The reaction was initiated by the addition of CDNB and the increase in absorbance at 340 nm was monitored continuously for 3 min at 25°C. One unit of GST activity was defined as the amount of enzyme catalyzing the formation of 1 nmol of glutathione-CDNB conjugate per minute under the assay conditions. GST activity was expressed as nmol min⁻¹, while specific activity was expressed as nmol min⁻¹ mg⁻¹ protein. The assay conditions were adapted from those described previously (Hunaiti and Ali, 1991).

Protein determination

Protein concentrations were determined according to the method of Bradford (1976), using bovine serum albumin as the standard.

Statistical analysis

All experiments were conducted using three independent biological replicates, with duplicate technical measurements for each sample. Data are presented as mean± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post-hoc test for multiple comparisons. A probability value of p<0.05 was considered statistically significant. All statistical analyses were conducted using SPSS version 25.0 (IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

Oxadiazon treatment resulted in a progressive increase in glutathione S-transferase (GST) activity in garlic shoots after 24 hours of exposure (Table 1). The lowest concentration producing a significant elevation in enzyme activity compared to control was 0.02 µg/mL (p<0.05), indicating high sensitivity of garlic tissues to the herbicide.

GST activity increased steadily with increasing oxadiazon concentration and reached maximum induction between 10 and 20 µg/mL, where enzyme activity exceeded 300% of control values ($p < 0.001$). At higher concentrations (100-200 µg/mL), GST activity declined slightly but remained markedly higher than untreated controls ($p < 0.01$).

These findings are consistent with the known role of GSTs as inducible detoxification enzymes. GST enzymes catalyze the conjugation of glutathione with xenobiotic compounds, facilitating their detoxification and compartmentalization within plant cells (Edwards and Walbot, 2000; Cummins *et al.*, 2011; Marrs, 1996). Similar increases in GST activity have been reported in plants exposed to herbicides and safeners, where GSTs function as important stress-responsive enzymes involved in cellular protection (DeRidder and Goldsbrough, 2002; Gronwald *et al.*, 1987; Jain and Bhalla-Sarin, 2001). The slight decline in GST activity at the highest oxadiazon concentrations (100-200 µg/mL) may reflect metabolic disruption or partial inhibition of enzyme synthesis caused by excessive herbicide stress. Comparable biphasic responses have previously been described in herbicide-treated plants, where moderate stress stimulates antioxidant defense systems while severe stress suppresses normal metabolism (Hunaiti and Ali, 1991; Carvalho-Moore *et al.*, 2024).

Table 1: Glutathione S-transferase activity in 6-day-old garlic shoots exposed for 24 hours to different concentrations of oxadiazon.

Oxadiazon (µg/mL)	Glutathione S-transferase activity (nmol min ⁻¹ mg ⁻¹ 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

Values are mean±SD of three experiments, each with duplicate determinations.

Effect of plant developmental stage on GST induction

Plant developmental stage significantly influenced GST activity following exposure to 10 µg/mL oxadiazon (Table 2). The highest enzyme activity was observed in garlic shoots aged 4-10 days, with peak induction at day 6, reaching nearly threefold higher activity compared with controls ($p < 0.001$). Younger seedlings showed stronger responses than older plants, while GST induction gradually declined after 12 days of growth ($p < 0.05$ for days 14-18 compared to day 6).

These findings suggest that young, actively growing tissues possess greater detoxification potential and metabolic flexibility than mature tissues. Rapidly dividing cells generally require stronger antioxidant and detoxification systems to protect against oxidative and xenobiotic damage. Developmental regulation of GST activity has been reported in several plant systems and is considered an important determinant of herbicide tolerance and stress adaptation (Marrs, 1996; Kumar and Trivedi, 2019). The reduced induction observed in older seedlings may reflect physiological stabilization and lower metabolic responsiveness to herbicide stress.

Time course of GST induction in eight-day-old garlic shoots

In eight-day-old garlic plants, GST activity in shoots increased significantly within 6 hours of exposure to 10 µg/mL oxadiazon ($p < 0.05$), reaching peak levels between 24 and 26 hours ($p < 0.001$), followed by a gradual decline at 36 and 48 hours (Table 3).

These data demonstrate that GST induction is a relatively rapid response to herbicide exposure, consistent possibly reflecting activation of pre-existing GST isoforms followed by de novo enzyme synthesis. The sustained elevation of GST activity for up to 48 hours indicates prolonged detoxification capacity following a single herbicide application.

Organ-specific distribution of GST activity following oxadiazon treatment

Analysis of different garlic organs revealed marked variation in GST induction following exposure to 10 µg/mL oxadiazon for 12 and 24 hours (Table 4). Roots showed the highest absolute increase in GST activity after 24 hours of treatment (380% of control). Leaves, shoots and stems also exhibited significant increases, although to a lesser extent.

Table 2: GST activity at different developmental stages following 10 µg/mL oxadiazon treatment.

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

When enzyme activity was expressed per gram of fresh tissue, leaves displayed the highest specific activity, while roots showed comparatively lower values per mg protein but higher values per gram tissue.

The strong GST induction in roots likely reflects their direct exposure to the herbicide, making them the primary site of xenobiotic perception and detoxification. Elevated GST activity in leaves and shoots suggests that detoxification responses occur systemically throughout the plant and may contribute to protection against reactive oxygen species generated during herbicide metabolism. Similar organ-specific patterns of GST induction have been reported in plants exposed to herbicides and environmental pollutants (Hu *et al.*, 2014; Cummins *et al.*, 2013).

Comparative GST induction among plant species

Comparative experiments demonstrated substantial variation in GST induction among plant species exposed to 10 µg/mL oxadiazon for 24 hours (Table 5). Garlic exhibited the strongest response, with GST activity increasing approximately

threefold relative to control levels (300%, $p < 0.001$). Bean (*Vicia faba*) and corn (*Zea mays*) showed moderate induction (225% and 200% of control, respectively; $p < 0.01$), whereas lentil (*Lens esculenta*), bitter vetch (*Vicia ervilia*), wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) displayed little or no significant change in enzyme activity ($p > 0.05$).

The pronounced GST induction observed in garlic suggests a highly efficient detoxification system capable of metabolizing oxadiazon more effectively than other tested species. In contrast, limited GST activation in wheat and barley may indicate lower detoxification efficiency or the involvement of alternative metabolic pathways. Species-specific variability in GST induction has frequently been associated with herbicide selectivity and resistance mechanisms in plants (Cummins *et al.*, 2013; Riechers and Molin, 2005; Carvalho-Moore *et al.*, 2024). These findings emphasize the importance of GST regulation in determining plant sensitivity or resistance to herbicides (DeRidder and Goldsbrough, 2002; Gronwald and Egli, 1987; Jain and Bhalla-Sarin, 2001; Hu *et al.*, 2014).

Table 3: Organ-specific GST response to 10 µg/mL oxadiazon.

Plant organ	0 hr (nmol/min/ /mg protein)	0 hr (nmol/min/ tissue)	12 hr (nmol/min mg protein)	12 hr (nmol/min/ g tissue)	% of control	24 hr (nmol/min/ mg protein)	24 hr (nmol/min/g g tissue)	% of control
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

Table 4: Effect of 10 µg/mL oxadiazon on glutathione S-transferase activity in different plant species after 24 h Exposure.

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

Table 5: Comparative changes in glutathione S-transferase activity in plant shoots following 24 h exposure to 10 µg/mL oxadiazon.

Plant species	Control GST activity (nmol min ⁻¹ mg ⁻¹ protein)	Oxadiazon treated GST activity (nmol min ⁻¹ mg ⁻¹ protein)	% of control
Garlic (<i>Allium sativum</i>)	22.0±1.5	66.0±5.2**	300
Bean (<i>Vicia faba</i>)	12.0±1.1	27.0±2.4**	225
Lentil (<i>Lens esculenta</i>)	8.7±0.8	9.0±0.9	103
Bitter vetch (<i>Vicia ervilia</i>)	2.8±0.3	3.0±0.4	107
Corn (<i>Zea mays</i>)	50.0±4.2	101.0±8.5**	202
Wheat (<i>Triticum aestivum</i>)	73.0±6.1	66.0±5.8	90
Barley (<i>Hordeum vulgare</i>)	61.0±5.4	60.0±5.2	98

Values are mean±SD of three independent experiments. ** $p < 0.001$ compared to respective control; ns = Not significant ($p > 0.05$) (one-way ANOVA with Tukey's HSD post-hoc test).

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 µg/mL. The time-course analysis revealed significant GST induction within 6 hours and peak activity at 24-26 hours post-treatment. The results indicate that garlic roots have a higher capacity for initial detoxification (380% of control), while leaves contribute significantly to further enzymatic detoxification (283% of control). 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.

DECLARATIONS

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Ethical approval

Not applicable (plant study only).

Data availability

The data supporting this study are available from the corresponding author upon reasonable request.

Conflict of interest

The authors declare no conflict of interest.

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