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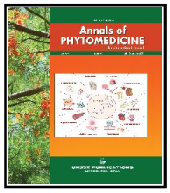

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Jasmonic acid priming to enhance glutathione-s-transferase mediated mancozeb detoxification in *Vicia faba* L. and *Vigna radiata* (L.) R. Wilczek

Nida Sultan*, Ayeesha Khatoon*, Sazia Siddiqui*, Azhar Kamal and Aisha Kamal****◆

**Department of Biosciences, Integral University, Lucknow-226026, Uttar Pradesh, India*
***Department of Bioengineering, Integral University, Lucknow-226026, Uttar Pradesh, India*

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Abstract

Glutathione-S-transferase (GST) are a group of enzymes essential for detoxification processes in living organisms, stress tolerance, and cell signalling in plants. This study investigates the effect of Jasmonic acid (JA) on GST activity and gene expression in *Vicia faba* L. (*V. faba*) and *Vigna radiata* (L.) R. Wilczek (*V. radiata*) exposed to Mancozeb (MNZ) toxicity. *V. faba* and *V. radiata* seeds were exposed to different levels of MNZ and primed with JA. The GST activity and gene expression were assessed using spectrophotometry and RT-qPCR, respectively. The results demonstrated that MNZ exposure significantly increased GST activity up to 90 ppm in *V. faba* and 70 ppm in *V. radiata*, with JA priming further enhancing this activity. Specifically, 1mM JA resulted in a notable upregulation of GST activity in both plants. Additionally, JA priming significantly increased the relative gene expression of the GST gene, with a 7.9-fold increase observed in *V. radiata* at 50 ppm MNZ and a 9.7-fold increase in *V. faba* at 90 ppm MNZ. These findings suggest that JA enhances the plant defence mechanisms by upregulating GST activity and gene expression, thereby mitigating MNZ-induced oxidative stress. This study highlights the potential of JA as a protective agent against pesticide toxicity in agricultural settings, offering insights into sustainable farming practices. The complex interplay between JA signalling pathways and pesticides requires further investigation to understand their combined effects, with implications for crop resilience and global food security.

1. Introduction

Glutathione-S-transferases (GST), a versatile family of isozymes, play crucial roles in plants by participating in primary and secondary metabolism, stress tolerance, and cell signalling. They are best known for their ability to conjugate the reduced tripeptide glutathione (GSH) to a wide range of polar and electrophilic substrates, facilitating cellular detoxification and enhancing the ability of cells to withstand oxidative stress (Parcharidou, 2024; Hadi and Al-Lami, 2024). This enzymatic activity enables plants to metabolize harmful exogenous chemicals (xenobiotic), including pesticides, which is a vital aspect of their defence mechanisms (Naidu and Kumar, 2021). GST constitute approximately 2% of the soluble proteins in plants and are especially recognized for their role in the detoxification of herbicides (Muslu *et al*., 2024). Their involvement in various cellular functions underlines their importance in plant biology (Hadi and Al-Lami, 2024). The study of GST activity is particularly relevant in crops such as *V. faba* and *V. radiata*, which are not only nutritionally significant but also crucial for understanding the plant's response to environmental stressors, including pesticide exposure.

Corresponding author: Dr. Aisha Kamal
Professor; Department of Bioengineering, Integral University, Lucknow-226026, Uttar Pradesh, India
E-mail: aishakamal04@gmail.com
Tel.: +91-9415147614

V. faba, is an annual herbaceous legume that plays a significant role in human and animal nutrition due to its high protein content, complex carbohydrates, dietary fibre, and a range of antioxidants and bioactive compounds (Khan *et al*., 2015; Singh *et al.,* 2014; USDA, 2021; Valentae *et al.,* 2018). Similarly, *V. radiata*, has been a staple in traditional diets for over 3500 years, valued for its high protein content and detoxification properties (Sengupta, 2018; Sehrawat *et al*., 2021). The bioactive compounds in *V. radiata* contribute to their recognized health benefits, including antioxidant, anti-inflammatory, and chemopreventive properties (Kurella *et al*., 2022).

MNZ, a commonly employed organometallic fungicide with broad-spectrum efficacy, has been linked to genotoxic and cytotoxic harm in humans, animals, and plants (Costa *et al*., 2022). The potential hazards posed by MNZ necessitate the development of approaches to bolster plant detoxification capabilities.

JA is a plant growth regulator that has gained attention for its role in enhancing plant defence and stress responses (Sabagh *et al*., 2022). Earlier research has shown that JA application can enhance antioxidant enzyme activity and boost the production of non-enzymatic antioxidants, leading to improved plant growth, photosynthesis, and crop yield (Sirhindi *et al*., 2022). This research seeks to determine the potential of JA priming as a strategy to enhance GST-mediated detoxification of mancozeb in *V. faba* and *V. radiata*. By elucidating the mechanisms through which JA mitigates the deleterious effects of pesticide exposure on the GST gene, this research aims to contribute to the development of sustainable agricultural approaches that protect crop health and maintain productivity.

2. Materials and Methods

2.1 Plant materials and dose treatment

V. faba and *V. radiata* seeds were obtained from an agricultural supplier in Lucknow, India. The seeds, selected for uniformity, health, and viability, were initially disinfected using a 1% (v/v) sodium hypochlorite solution, followed by a thorough rinse with distilled water. These sterilized seeds were then soaked overnight in distilled water containing 0.5 mM and 1 mM of JA. MNZ was administered at concentrations of 10, 30, 50, 70, 90, 110, and 130 ppm, with a control group included for comparison. These concentrations were selected based on prior research by Fatma *et al.* (2018). Fifteen seeds per treatment were placed on sterile Petri plates lined with double-layered filter paper. Initially, the plates were kept in a dark culture environment. After the hypocotyls emerged, the plates were exposed to 100 µmol m^2s^{-1} of light under a 14/10 h photoperiod (day/night) to allow the seedlings to develop into fully grown plantlets. For each MNZ concentration, triplicate sets of seeds were maintained on three separate Petri plates, with *V. faba* being grown for 21 days and *V. radiata* for 14 days.

2.2 Total protein determination

The total protein content in the plant samples was quantified using a modified Lowry method employing Folin-Ciocalteu reagent, with bovine serum albumin (BSA) serving as the protein standard.

2.3 Evaluation of GST activity

1 g sample of the plant tissue was collected and washed in PBS buffer (0.01M, pH 7.4) at 2-8°C. After washing, the sample was blotted dry using filter paper and then weighed. The sample was then homogenized in PBS buffer at a volume ratio of 9:1. The homogenate was subjected to centrifugation at 15,000 rpm for 15 min at 4°C. The supernatant, collected cautiously and maintained on ice, was utilized for GST activity assessment. GST activity was evaluated using a GST activity assay kit (Elabscience). Spectrophotometric analysis was performed using a UV-spectrometer, with absorbance measured at 340 nm at regular intervals of 20 sec over a total duration of 5 min (20 sec-320 sec). The GST activity was then calculated using the appropriate formula, and the results were plotted in graphical form.

2.4 Relative gene expression analysis

Total RNA was extracted from 200 mg of fresh leaf tissue using the HiPur-ATM Plant and Fungal RNA Miniprep Purification Kit (Himedia). This RNA was subsequently converted into cDNA using the Himedia Maxima First Strand cDNA Synthesis Kit. Gene expression analysis was performed through RT-qPCR on an AriaMX Real-Time PCR system, employing SYBR Green master mix and specific primers (Table 1). Relative gene expression levels were calculated using the 2-ÄÄCT method.

Table 1: Primers used in relative gene expression analysis

Crop name	Gene name	Gene I.D.	Primer sequence (5¹-3¹)
V. radiata	GST	VR_GST_RTF	CAAGCAAGACGAGGGTGCCA
		VR_GST_RTR	GCGAGCAGATAGTGGGCGTT
V. radiata	Tubulin	VR_TUB_F	CTTGACTGCATCTGCTATGTTCAG
		VR_TUB_R	CCAGCTAATGCTCGGCATACTG
V. faba	GST	VF_GST_RTF	GCCTTGCTCCCACGACTACA
		VF_GST_RTR	GGAGGAGGACGAACGGTGAC
V. faba	EF1	VF_EF1aF	GACAACATGATTGAGAGGTCCACCT
		VF_EF1aR	GGCTCCTTCTCAATCTCCTTACC

2.5 Statistical analysis

Data were collected from three independent experiments, each with three replications. One-way ANOVA followed by Duncan's multiple range test was employed to identify significant differences among groups, with a significance level of $p< 0.05$.

3. Results

3.1 Effect of JA on GST activity of mancozeb exposed *V. faba* and *V. radiata*

The results depicted that in the control group of *V. radiata*, GST activity is low (0.12 ± 0.009), providing a baseline for comparison. GST activity increases with MNZ concentration, reaching a peak (0.38 ± 0.28 U/mg-protein) at 70 ppm before declining at higher concentrations. This suggests that MNZ induces GST activity up to a certain concentration, beyond which the enzyme's activity decreases, possibly due to higher toxic effects. In the 0.5 mM JA-primed seeds, GST activity is generally higher control across all concentrations. The activity also peaks (0.6 ± 0.11 U/mg-protein) at 70 ppm, indicating that JA priming enhances the plant's ability to detoxify MNZ. The increased GST activity suggests that 0.5 mM JA priming improves the plant's defensive response, enabling it to cope better with the oxidative stress caused by MNZ. While in 1 mM JA-primed seeds, activity is the highest among all treatments, consistently surpassing both D.W and 25 across the MNZ concentration range. The highest activity (0.81 ± 0.113 U/mg-protein) was observed at 90 ppm remains evident, showing that 1 mM JA priming significantly boosts GST activity, providing even greater protection against MNZ-induced stress.

Similarly, in *V. faba*, GST activity increases with the increasing concentration of MNZ, peaking at 70 ppm for all treatments. Specifically, in the D.W group, GST activity reached 0.59 U/mg-protein at 70 ppm, followed by a slight decrease at higher concentrations. In comparison, 25 (0.5 mM JA) showed an enhanced GST activity of 0.77 U/mg-protein at 70 ppm, and 0.79 U/mg-protein at 90 ppm, indicating that JA priming at this concentration

significantly boosts GST activity. The 50 group (1 mM JA) exhibited the highest GST activity, reaching 0.82 U/mg-protein at 70 ppm and 0.83 U/mg-protein at 90 ppm, demonstrating that a higher concentration of JA priming further amplifies the plant detoxification response. Overall, the results suggest that JA priming, particularly at 1 mM, substantially enhances GST activity in *V. radiata* and *V. faba*, thereby improving the plant's ability to detoxify MNZ and mitigate its oxidative stress effects. This suggests that JA priming could be a valuable strategy for improving the stress resilience of plants exposed to pesticides.

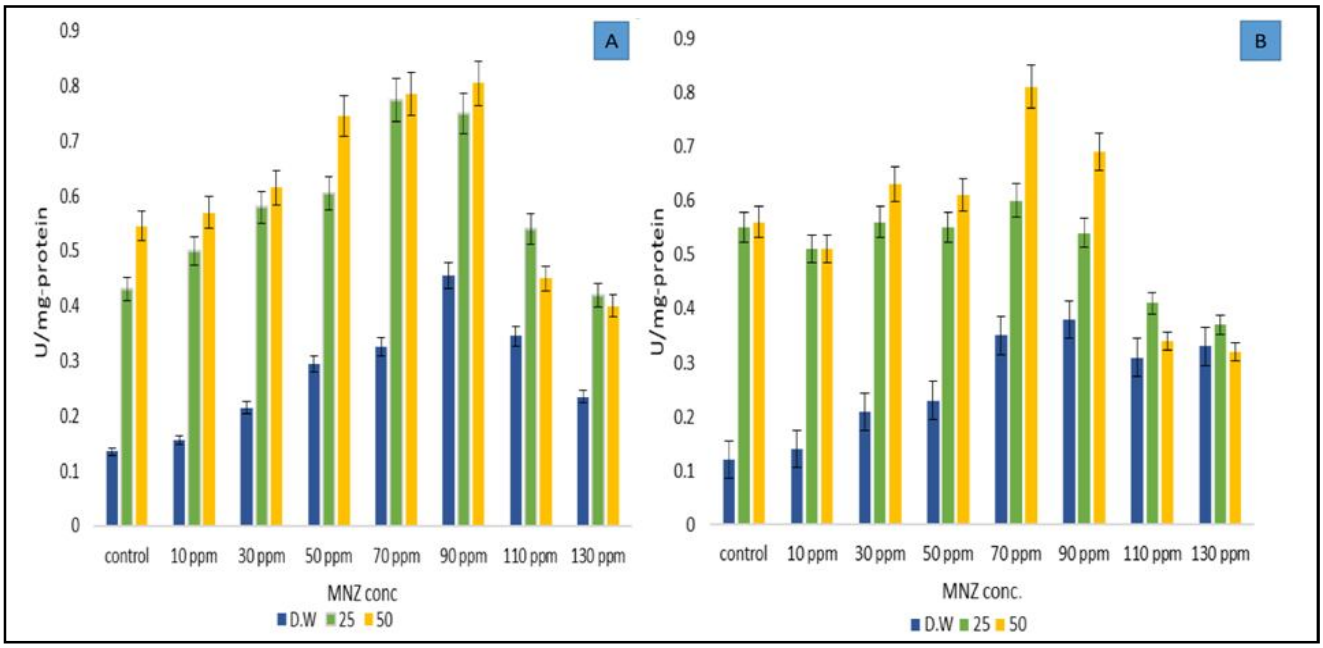

Figure 1: Impact on GST activity in test plants (A) *V. faba*, (B) *V. radiata* under Mancozeb toxicity. D.W. (DW soaked seeds + MNZ Treatments) 25 (0.5 mM JA primed seeds + MNZ Treatments), 50 (1 mM JA primed seeds + MNZ Treatment). Data represent SEM. of three replicates and significance ($p \leq 0.05$) was determined by ANOVA followed by Duncan's multiple range tests.

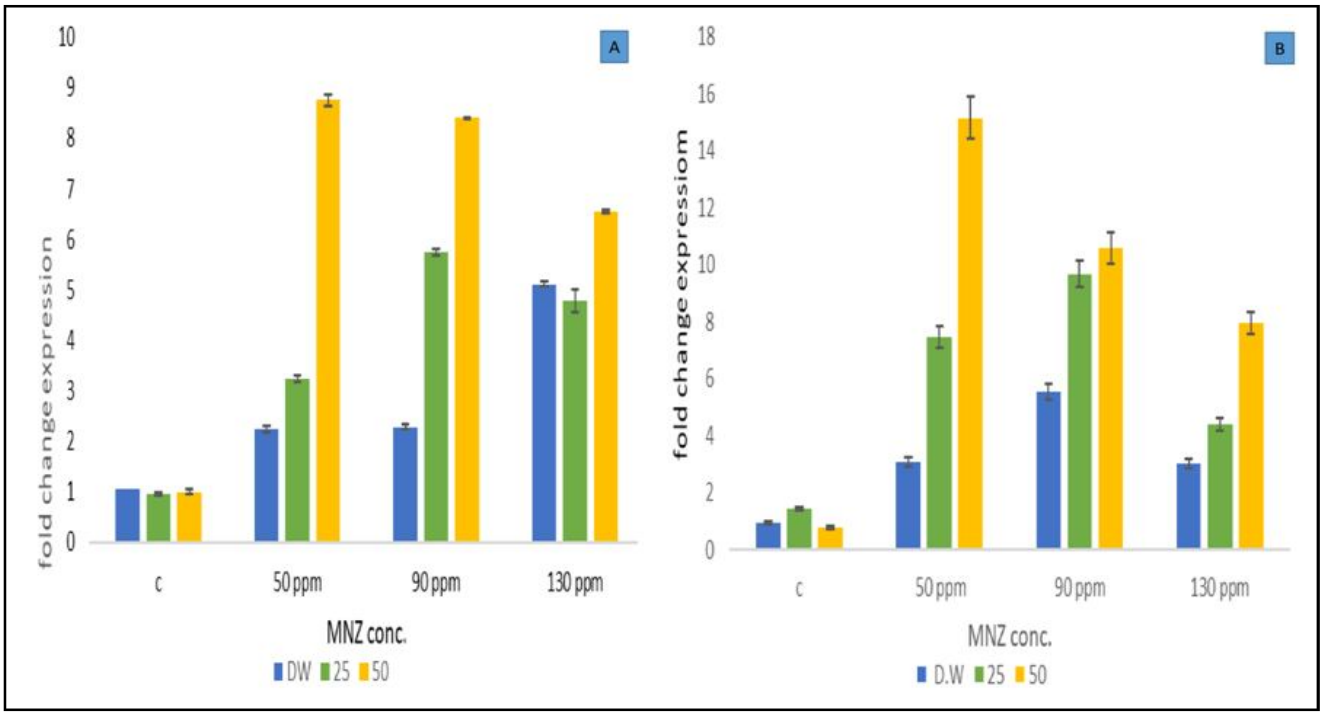

Figure 2: GST gene expression analysis in test plants (A) *V. faba,* (B) *V. radiata* under MNZ toxicity. D.W (DW soaked seeds + MNZ Treatments) 25 (0.5 mM JA primed seeds + MNZ Treatments), 50 (1 mM JA primed seeds + MNZ Treatments). Data represent SEM. of three replicates and significance ($p \leq 0.05$) was determined by ANOVA followed by Duncan's multiple range tests.

3.2 Impact of jasmonic acid on GST gene expression in *V. faba* and *V. radiata* under MNZ toxicity

The outcome of gene expression analysis revealed that JA treatment significantly enhances the expression of the GST gene. Both MNZ exposure and JA priming led to notable increases in GST gene expression. Specifically, MNZ exposure combined with 0.5 mM JA caused GST expression to increase by 3-fold at 50 ppm, 5.8-fold at 90 ppm and 4.4-fold at 130 ppm. In 1 mM JA primed seedlings, GST expression increased by 7.9-fold at 50 ppm, 9.7-fold at 90 ppm and 7.1-fold at 130 ppm compared to the control. Additionally, JA priming notably elevated GST expression levels, indicating that GST plays a crucial role in alleviating oxidative stress induced by MNZ. These results suggest that JA not only mitigates the harmful effects of MNZ toxicity but also boosts the plant's antioxidant defence mechanisms by significantly upregulating GST gene expression in *V. faba* and *V. radiata*.

4. Discussion

Fungicides play a critical role in modern agriculture by managing crop diseases and combating phytopathogenic fungi (Monika and Kidwai, 2017). MNZ, a widely used broad-spectrum fungicide, has been shown to influence the activity and expression of GST, which are essential for plant defence mechanisms. Our findings align with previous studies indicating that high concentrations of MNZ, particularly above 90 ppm, can adversely affect GST activity and gene expression in various crops. The observed inhibition of GST activity and gene expression is attributed to MNZ-induced seed reserve dissolution, reduced water absorption, and osmotic stress. This was because the toxicity caused seed reserve dissolution, which decreased the ability of the seeds to absorb water, and osmotic stress, which in turn affected the GST activity (Sehr *et al*., 2010, Wei Feng *et al*., 2016). The application of JA promotes cell expansion, elongation, and vascular tissue differentiation (Sehr *et al*., 2010). GST are pivotal in detoxifying xenobiotics and mitigating stress responses in plants. Previous research highlights that GST activity is modulated by environmental stressors, including heavy metals and pesticides. For instance, *V. radiata* seedlings exhibited enhanced GST activity when pretreated with exogenous glutathione (GSH) under high-temperature stress (Salman *et al*., 2022) and when administered exogenous spermine to alleviate cadmium (Cd) toxicity (Nahar *et al*., 2016). Similarly, maize seedlings showed increased GST activity following treatment with safeners and 1-aminobenzotriazole (ABT) derivatives (Saidaiah *et al*., 2021). In transgenic *Arabidopsis thaliana*, containing the maize GST F4 gene, increased resistance and GST activity were observed under heavy metal and herbicide stress (Bittsánszky and Gyulai, 2017). Notably, GST in spinach exhibit high catalytic efficiency for acrolein detoxification, a hazardous aldehyde generated by lipid peroxidation (Mano *et al*., 2016). Our study extends these findings by examining the effects of MNZ on GST activity and gene expression in *V. faba*. While previous research has extensively documented GST activity in other species, data on *V. faba* remains sparse. Our results indicate that MNZ application, coupled with JA treatment, significantly elevates GST gene expression, suggesting that JA plays a crucial role in enhancing plant stress responses under pesticide toxicity conditions. JA is known to regulate various stress response-related genes and enhance cellular machinery to degrade pesticide residues, including cyt450 and NADH (ADH-ubiquinone oxidoreductase) (Sharma *et al*., 2018). This regulatory role highlighted JA's potential in mitigating the adverse effects of pesticide-induced stress and improving plant resilience.

GST are dynamic components of the plant stress response, exhibiting increased activity and gene expression in response to various environmental and chemical stresses (Anderson and Davis, 2004; Barta and Dutka, 1996; Bittsánszky and Gyulai, 2017; Mano *et al.,* 2016). Our findings contribute to the growing understanding of GST function and highlight the importance of JA in modulating GST activity under pesticide stress. However, further research is needed to fully elucidate the GST activity in *V. faba* and to explore potential applications for enhancing crop protection and resilience through genetic and chemical interventions

5. Conclusion

This study demonstrates that MNZ adversely affects both gene expression patterns and GST activity in *V. faba* and *V. radiata*. Our findings highlighted the critical role of JA signalling pathways in mitigating pesticide-induced stress. Specifically, JA regulates GST activity and gene expression, thereby enhancing plant resilience against pesticide exposure. The observed negative impact of MNZ on GST activity highlights the complex interactions between plant physiology and pesticide application. Understanding these interactions is essential for developing sustainable agricultural practices. Our results suggest that JA signalling pathways can potentially alleviate pesticide stress, offering a pathway to improve crop resilience and performance. Future research should explore the synergistic effects of JA signalling and pesticides more comprehensively. Such studies could reveal novel strategies for optimizing crop yield and quality while minimizing environmental impact. By leveraging insights into these interactions, we can advance towards agricultural systems that not only boost productivity but also ensure environmental sustainability and global food security.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Anderson J.V., and Davis, D.G. (2004). Abiotic stress alters transcript profiles and activity of glutathione S-transferase, glutathione peroxidase, and glutathione reductase in *Euphorbia esula*. Physiologia Plantarum, **120**(3):421-433.

Bittsanszky, A. and Gyulai, G. (2017). Increased glutathione S-transferase activity in 35S(CaMV)- Zmgstf4 transgenic *Arabidopsis thaliana*. Ecocycles, **3**(2):13-16.

Boyapati, R.T.N. and Manoj, K.B. (2021). Field evaluation of fungicides for control of blast of rice (*Pyricularia grisea* Sacc.). Ann. Phytomed., **10**(1):325-330. http://dx.doi.org/10.21276/ap.2021.10.1.36

Costa, C.; Teodoro, M.; Giambò, F.; Catania, S.; Vivarelli, S. and Fenga, C. (2022). Assessment of mancozeb exposure, absorbed dose, and oxidative damage in greenhouse farmers. International Journal of Environmental Research and Public Health, **19**(17):10486.

Sabagh, E.L.; Islam, A.; Hossain, M.S.; Iqbal, A.; Mubeen, M.A., Waleed, M. and Abdelhamid, M.T. (2022). Phytohormones as growth regulators during abiotic stress tolerance in plants. Frontiers in Agronomy, **4**:765068.

Fatma, F.; Kamal, A. and Srivastava, A. (2018) A. Exogenous application of salicylic acid mitigates the toxic effect of pesticides in *V. radiata* (L.) Wilczek. Journal of Plant Growth Regulation, **37**:1185-94

Gupta, P.; Singh, R.; Malhotra, S.; Boora, K.S. and Singal, H.R. (2010). Characterization of seed storage proteins in high protein genotypes of cowpea (*Vigna unguiculata* (L.) Walp.). Physiology and Molecular Biology of Plants, **16**:53-58.

Hadi, S. and Al-Lami, H.F.D. (2024). Glutathione-s-transferase enzymes in plants, a review article. Web of Agriculture: Journal of Agriculture and Biological Sciences, **2**(5):10-14.

Iram, S. and Bilal, B. (2024). Dynamics of plant growth promoting fungi in biotic and abiotic stress tolerance of leguminous crops. In Microbial Technology for Agro-Ecosystems, **42**:333-359

Kaur, R.; Singh, D. and Kumari, A. (2023). Pesticide residues degradation strategies in soil and water: A review. Int. J. Environ. Sci. Technol., **20**:3537-3560.

Khan, M.A.; Ammar, M.H.; Migdadi, H.M.; El-Harty, E.H.; Osman, M.A.; Farooq, M. and Alghamdi, S.S. (2015). Comparative nutritional profiles of various *V. faba* and chickpea genotypes. International Journal of Agriculture and Biology, **17**(3):449-457.

Mano, J.; Sakai, H.; Koeduka, T.; Ishibashi, A.; Morita, C.; Biswas, M.S.; Muneuchi, H. and Kitajima, S. (2016). Acrolein-detoxifying isozymes of glutathione transferase in plants. Planta, **245**(2):255-264.

Salman, M.; Prabha, D.; Grace, S.; Ahmad, G; Naik, S. B. and Rashid, M. M. (2022). Minimizing the allelopathy of *Solanum nigrum* L. in green gram *V. radiata* (L.) Wilczek through *Pseudomonas fluorescens*. Ann. Phytomed., **11**(1):687-693.

Monika, and Kidwai, M.K. (2017) Effect of Mancozeb on Mustard (*Brassica juncea* L.): An *in vitro* study. Tropical Plant Research, **4**(1):55-61.

Muslu, S.; Kasapoðlu, A.G.; Gines, E.; Aygören, A.S.; Yiðider, E.; Ilhan, E. and Aydýn, M. (2024). Genome-wide analysis of glutathione S-transferase gene family in *P. vulgaris* under drought and salinity stress. Plant Molecular Biology Reporter, **42**(1):57-76.

Nahar, K.; Rahman, M.; Hasanuzzaman, M.; Rahman, A.; Fujita, M. and Suzuki, T. (2016). cadmium stress tolerance in *V. radiata* (*V. radiate L.*) seedlings. Environmental Science and Pollution Research International, **23**(21):21206-21218.

Parcharidou, E. (2024). The role of glutathione transferases in herbicide detoxification: A genome-wide study on flufenacet resistant black-grass. Reviews, pp:411-423.

Saidaiah, P.; Rao, P.S.; Geetha, A.; Kumar, A.K. and Kamala, V. (2021). Assessment of genetic diversity based on principal component analysis in vegetable cowpea (*Vigna unguiculata* (L.) Walp.) germplasm. Ann. Phytomed., **10**(1):20814. http://dx.doi.org/10.21276/ap.2021.10.1.22

Sehr, E.M.; Agusti, J.; Lehner, R.; Farmer, E.E.; Schwarz, M. and Greb, T. (2010). Analysis of secondary growth in the Arabidopsis shoot reveals a positive role of jasmonate signalling in cambium formation. The Plant Journal, **63**(5):811-22

Sehrawat, N.; Yadav, M.; Sharma, A.K.; Kumar, S.; Singh, M.; Kumar, V. and Singh, R. (2021). Mung bean (*V. radiata* L. Wilczek) as functional food, agronomic importance and breeding approach for development of climate resilience: Current status and future perspectives. Asian J. Biol. Life Sci., **10**(1):87-92.

Sengupta, K. (2018). *V. radiata* (Green gram). In: Forage Crops of the World, Apple Academic Press. **2**:159-174.

Sharma, A.; Kumar, V.; Yuan, H.; Kanwar, M.K.; Bhardwaj, R.; Thukral, A.K. and Zheng, B. (2018) Jasmonic acid seed treatment stimulates insecticide detoxification in *Brassica juncea* L. Frontiers in Plant Science, **9**:415669

Singh, A.K.; Bhardwaj, R. and Singh, I.S. (2014) Assessment of nutritional quality of developed *V. faba* (*Vicia faba* L.) lines. Journal of Agric., **1**:96-10.1

Sirhindi, G.; Mir, M.A.; Abd-Allah, E.F.; Ahmad, P. and Gucel, S. (2016). Jasmonic acid modulates the physiobiochemical attributes, antioxidant enzyme activity, and gene expression in *Glycine max* under nickel toxicity. Frontiers in Plant Science, **7**:591.

Kurella, S.; Addepally, U. and Umakanth, A.V. (2022). Bioactive components and beneficial nutritive properties of *V. radiata* from Indian cultivars. Ann. Phytomed., **11**(1):606-612.

USDA (US Dept. of Agriculture). (2021). Food Data Central (Nutrient Database). Retrieved from https://fdc.nal.usda.gov/

Valente, I.M.; Maia, M.R.G; Malushi, N.; Oliveira, H.M.; Papa, L. and Rodrigues, J.A. (2018). Cabrita ARJ Profiling of phenolic compounds and antioxidant properties of European varieties and cultivars of *Vicia faba* L. pods. Photochemistry, **152**:223–229.

Feng, W.; Lindner, L.; Robbins, N.E. and Dinneny, J.E. (2016) Growing out of Stress: The role of cell and organ-Scale growth control in plant water-stress responses, The Plant Cell, **28**:1769-1782.

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