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EXPRESSION OF DEFENSE-RELATED ENZYMES IN WILT RESISTANT AND SUSCEPTIBLE VARIETIES OF *CHRYSANTHEMUM MORIFOLIUM* RAMAT

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ABSTRACT
Wilt induced by *Fusarium* sp. is among the most devastating diseases that restrict the productivity and commercial production of chrysanthemum (*Chrysanthemum morifolium* Ramat.). Comprehending the biochemical pathways that govern host resistance is crucial for the development of resistant varieties and the formulation of efficient disease management methods. This study aimed to examine the biochemical responses linked to wilt resistance in two distinct chrysanthemum varieties, Cent Yellow and Champagne Orange, after inoculation with a virulent *Fusarium* sp. isolate. Total protein content, peroxidase (POX), catalase (CAT), polyphenol oxidase (PPO) activities, and total phenol content were assessed at 0, 24, 48, 72, and 96 hours post inoculation (hpi), in conjunction with their corresponding uninoculated controls. The resistant variety demonstrated a gradual increase in total protein content during the trial period, while the susceptible variety exhibited a consistent decrease. The activities of POX and CAT markedly rose in both varieties post-inoculation; however, the induction was more pronounced, prolonged, and elevated in the resistant variety. Polyphenol oxidase activity was constantly high in the resistant variety, but it exhibited only a temporary increase followed by a decrease in the susceptible variety. The total phenol content increased upon pathogen exposure, with the resistant variety consistently exhibiting significantly greater phenolic levels than the susceptible variety over the observation period. The findings indicate that augmented antioxidant enzyme activities, heightened protein accumulation, and higher phenolic content jointly foster resistance to *Fusarium* wilt in chrysanthemum. Consequently, POX, CAT, PPO, total protein, and total phenol content may function as dependable biochemical indicators for evaluating chrysanthemum germplasm for wilt resistance and facilitate future resistance breeding initiatives.

KEYWORDS: Chrysanthemum; *Fusarium* wilt; peroxidase; catalase; polyphenol oxidase; total phenol; disease resistance

INTRODUCTION

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) is one of the most important commercial flower crops grown worldwide for loose flowers, cut flowers and pot plants, valued for its wide range of colours, shapes and long vase life. Its cultivation, however, is seriously constrained by several fungal, bacterial and viral diseases, among which wilt caused by *Fusarium* sp. is one of the most destructive, often resulting in sudden wilting, vascular discolouration and collapse of susceptible plants, leading to considerable yield and quality losses (Singh and Kumar 2014).

Plants respond to pathogen invasion through a coordinated set of biochemical defenses that include changes in total protein content and the induction of antioxidant and phenol-oxidising enzymes such as peroxidase (POX), catalase (CAT) and polyphenol oxidase (PPO), together with the accumulation of phenolic compounds at the site of infection (Zeyad et al. 2022; Sowmya et al. 2025). Peroxidase contributes to the generation of reactive oxygen species, cross-linking of cell wall structural proteins and lignification, thereby restricting pathogen spread (Zeyad et al. 2022), while catalase regulates the levels of hydrogen peroxide generated during the oxidative burst that follows infection (Sun et al. 2022). Polyphenol oxidase catalyses the oxidation of phenolic compounds to highly reactive and fungitoxic quinones, and elevated PPO activity has repeatedly been associated with resistant host-pathogen combinations in *Fusarium*-challenged crops, including coriander and faba bean (Sowmya et al. 2025; Zhang et al. 2022).

In chrysanthemum specifically, POX, PPO and phenylalanine ammonia-lyase have been reported to accumulate large amounts of phenolic compounds as part of the early defense response against *Fusarium oxysporum* infection (Miao et al. 2023), and increased polyphenol oxidase, phenylalanine ammonia-lyase and antioxidant enzyme (catalase, peroxidase, superoxide dismutase) activity has similarly been documented following *Fusarium solani* infection of chrysanthemum cv. 'Guangyu' (Liu et al. 2024). Despite this, comparative time-course information on total protein, POX, CAT, PPO and total phenol content between wilt-resistant and wilt-susceptible chrysanthemum varieties remains limited.

This study was conducted to characterize and compare the biochemical defensive responses of two chrysanthemum varieties, Cent Yellow and Champagne Orange, based on the differential expression of *Fusarium* wilt symptoms under field circumstances, where Cent Yellow displayed more disease severity than Champagne Orange. The investigation sought to clarify the defense mechanisms engaged in the host response to *Fusarium* infection by assessing total protein and total phenol levels, as well as the activities of peroxidase (POX), catalase (CAT), and polyphenol oxidase (PPO) after inoculation with a virulent *Fusarium* isolate.

MATERIALS AND METHODS

2.1. Plant material and inoculation

Two chrysanthemum varieties with contrasting reaction to wilt, namely Cent yellow and Champagne Orange were used in the

study. Healthy, uniform rooted cuttings of both varieties were raised in pots under controlled conditions and challenge-inoculated with a virulent isolate of *Fusarium* sp. An equal number of uninoculated plants of each variety were maintained as controls. Samples for biochemical analysis were collected at 0, 24, 48, 72 and 96 hours post inoculation (hpi) from both inoculated and control plants of each variety, with three replications per treatment at each time interval.

2.2. Enzyme extraction

Fresh plant tissue was homogenised in an ice-cold mortar and pestle with the appropriate ice-cold extraction buffer for each assay. The homogenate was centrifuged in a cooling centrifuge and the clear supernatant was used as the source of crude enzyme extract for all subsequent estimations. All extraction and assay steps were carried out at 0–4°C unless stated otherwise.

2.3. Total protein content

Total soluble protein content of the extract was estimated by the method of Lowry et al. (1951) using bovine serum albumin (BSA) as the standard, with colour development read at 660 nm on a UV-Vis spectrophotometer. Protein content was expressed as mg g^{-1} fresh weight and was also used to express the specific activity of POX, CAT and PPO.

2.4. Peroxidase (POX) activity

Peroxidase activity was assayed following Hammerschmidt et al. (1982) using guaiacol/pyrogallol as the hydrogen donor in the presence of hydrogen peroxide. The increase in absorbance was recorded at fixed intervals (0, 30 and 60 s) at 420–470 nm, and enzyme activity was expressed as specific activity (change in absorbance min^{-1} mg^{-1} protein).

2.5. Catalase (CAT) activity

Catalase activity was estimated following the method of Aebi (1984), based on the rate of decomposition of hydrogen peroxide monitored spectrophotometrically at fixed time intervals (0, 30 and 60 s). Activity was expressed as specific activity (units min^{-1} mg^{-1} protein).

2.6. Polyphenol oxidase (PPO) activity

Polyphenol oxidase activity was determined by the method of Mayer et al. (1966) using catechol as the substrate. The increase in absorbance due to enzymatic oxidation of catechol was recorded at fixed intervals (0, 30 and 60 s), and activity was expressed as specific activity (units min^{-1} mg^{-1} protein).

2.7. Total phenol content

Total phenol content was estimated following the Folin-Ciocalteu based procedure of Malik and Singh (1980). Absorbance of the developed colour was recorded spectrophotometrically, and phenol content was calculated from a standard curve prepared using catechol, and expressed as mg per 100 g of plant sample.

2.8. Experimental design and statistical analysis

The experiment was executed using a Completely Randomized Design (CRD) with three replications for each treatment combination. The experimental variables included two chrysanthemum types (Cent Yellow and Champagne Orange), two treatments (inoculated and uninoculated control), and five sample intervals (0, 24, 48, 72, and 96 hours post-inoculation). The biochemical data, specifically total protein, peroxidase (POX), catalase (CAT), polyphenol oxidase (PPO), and total phenol content, were obtained from three independent biological replicates and presented as mean ± standard error (SE).

RESULTS

3.1. Total protein content

Total protein content showed contrasting trends between the two varieties following inoculation (Figure 1). In Cent Yellow, protein content declined progressively from 1.75 mg g^{-1} at 0 hpi to 1.08 mg g^{-1} at 96 hpi in inoculated plants (a 38.2% reduction), against a comparatively smaller decline of 33.7% in the uninoculated control. In Champagne Orange, protein content increased steadily from 1.93 to 3.04 mg g^{-1} (57.6% increase) in inoculated plants, exceeding the increase recorded in its control (47.7%). The decline in protein content in the inoculated susceptible variety, as against the increase in the inoculated resistant variety, suggests accelerated protein degradation in Cent Yellow and enhanced defense-related protein synthesis in Champagne Orange.

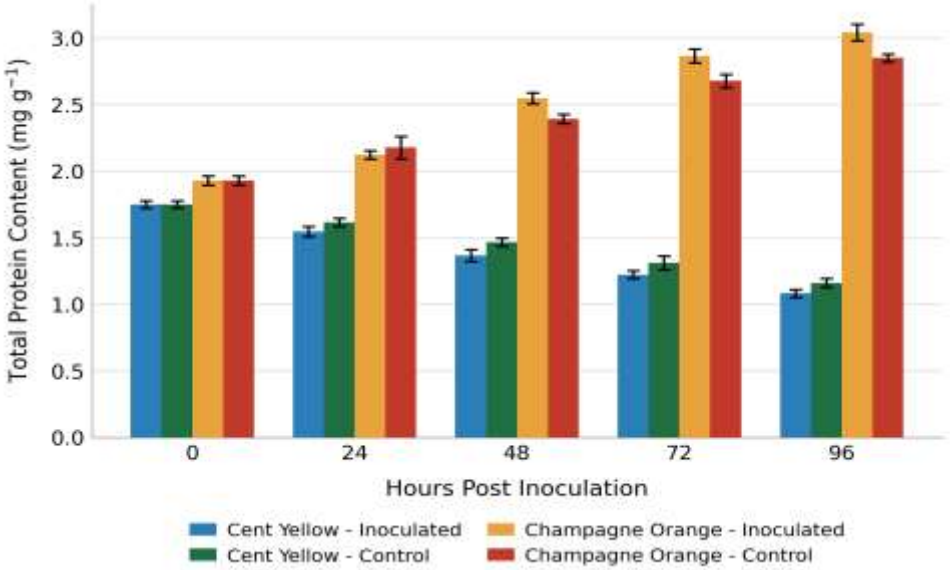

Figure 1. Total protein content in Cent Yellow (susceptible) and Champagne Orange (resistant) chrysanthemum varieties, inoculated and control, over 0–96 hpi.

3.2. Peroxidase (POX) activity

Peroxidase specific activity increased in both varieties after inoculation but followed distinct patterns (Figure 2). In Cent Yellow, POX activity in inoculated plants rose sharply from 0.54 to a peak of 1.03 units mg^{-1} protein at 48 hpi, thereafter declining to 0.64 units at 96 hpi, while the control showed a more gradual, sustained rise (0.42 to 0.69 units). In Champagne Orange, POX activity in inoculated plants increased continuously throughout the period, from 0.51 to 1.06 units mg^{-1} protein (a 106% increase), consistently exceeding the control at every time point. The sustained rise in the resistant variety, as opposed to the transient peak-and-decline pattern in the susceptible variety, indicates a more prolonged oxidative defense response in Champagne Orange.

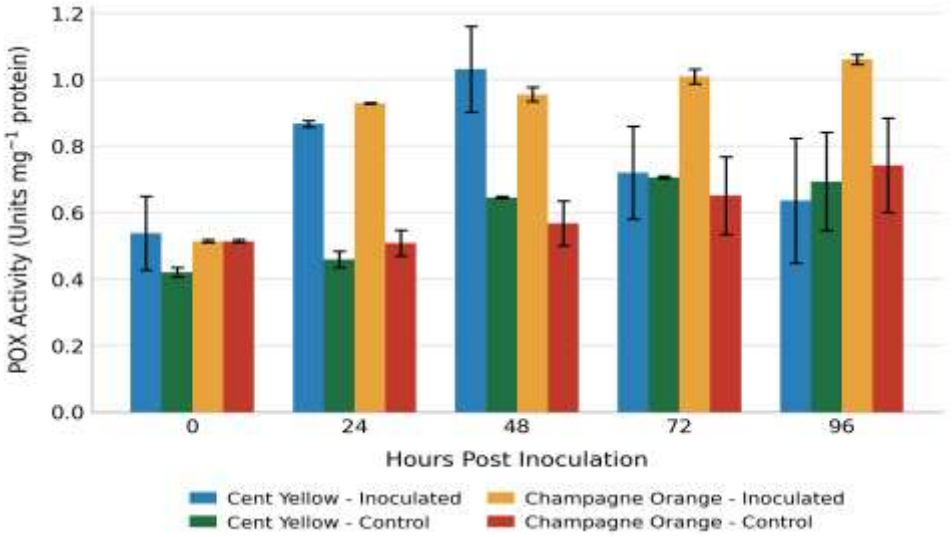

Figure 2. Peroxidase (POX) specific activity in Cent Yellow and Champagne Orange chrysanthemum varieties, inoculated and control, over 0–96 hpi.

3.3. Catalase (CAT) activity

Catalase activity increased markedly in inoculated plants of both varieties relative to their respective controls (Figure 3). In Cent Yellow, CAT activity rose from 0.28 to 0.64 units mg^{-1} protein (126% increase) in inoculated plants, while the control remained largely unchanged (0.15 to 0.16 units). Champagne Orange showed an even greater induction, rising from 0.30 to 0.74 units mg^{-1} protein (147% increase) in inoculated plants, whereas its control declined slightly over time. The stronger and more consistent catalase induction in Champagne Orange suggests more efficient scavenging of reactive oxygen species in the resistant variety.

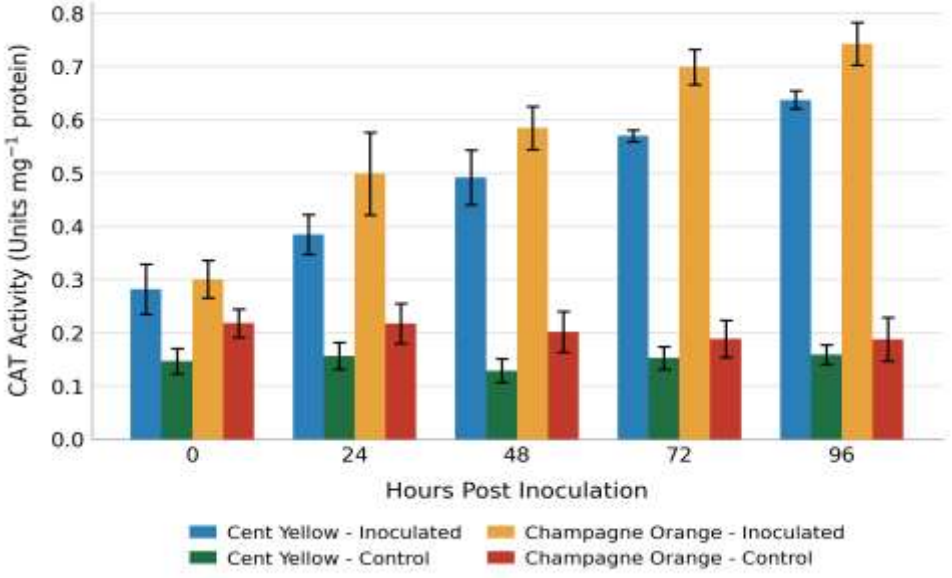

Figure 3. Catalase (CAT) specific activity in Cent Yellow and Champagne Orange chrysanthemum varieties, inoculated and control, over 0–96 hpi.

3.4. Polyphenol oxidase (PPO) activity

PPO activity displayed the most divergent pattern among the parameters studied (Figure 4). In Cent Yellow, inoculated plants showed an early peak in activity at 24 hpi (1.81 units mg^{-1} protein), followed by a steady decline to 0.94 units at 96 hpi, while the control rose from 1.24 to a peak of 1.64 units mg^{-1} protein at 72 hpi before declining slightly to 1.47 units by 96 hpi. In Champagne Orange, inoculated plants maintained relatively stable activity through 72 hpi (1.51 to 1.65 units mg^{-1} protein) before rising further to 1.96 units mg^{-1} protein at 96 hpi (an overall 29.6% increase), while the control declined steadily from 1.51 to 0.98 units. The early, transient spike followed by a collapse of PPO activity in inoculated Cent Yellow, as against the steady overall rise sustained by Champagne Orange through 96 hpi, may reflect an overwhelmed defense response in the susceptible

variety and a sustained phenol-oxidising capacity supporting resistance in Champagne Orange.

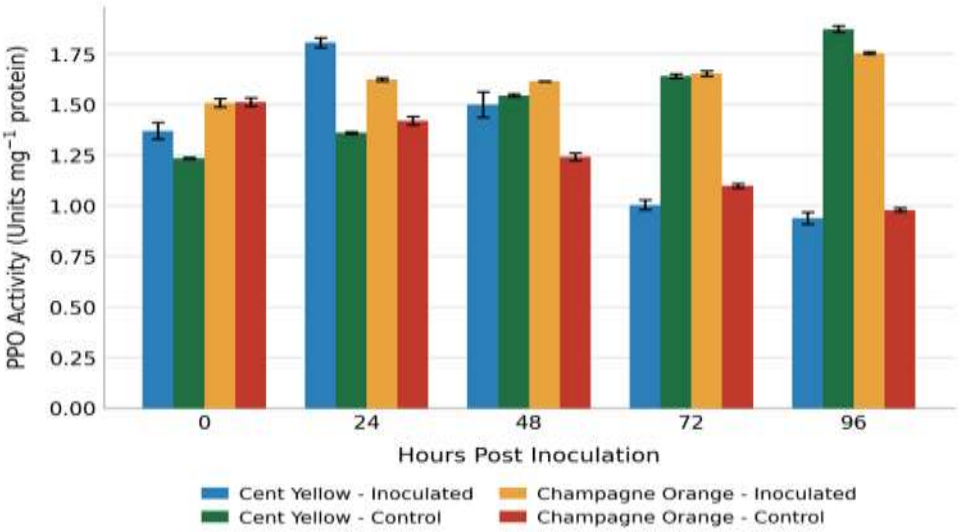

Figure 4. Polyphenol oxidase (PPO) specific activity in Cent Yellow and Champagne Orange chrysanthemum varieties, inoculated and control, over 0–96 hpi.

3.5. Total phenol content

Total phenol content increased in all treatments over the 96 hpi period, though the magnitude of the increase varied considerably between varieties (Figure 5). Cent Yellow inoculated plants showed the largest relative increase (19.7 to 86.8 mg 100 g^{-1}, a 340% rise), compared with a 268% rise in its control. Champagne Orange inoculated plants increased from 255.4 to 362.3 mg 100 g^{-1} (42% rise), while its control changed only marginally (7%). Although the relative percentage increase was higher in Cent Yellow, the absolute phenol content of Champagne Orange remained several-fold higher than that of Cent Yellow at every time point, consistent with a constitutively higher baseline phenolic defense in the resistant variety.

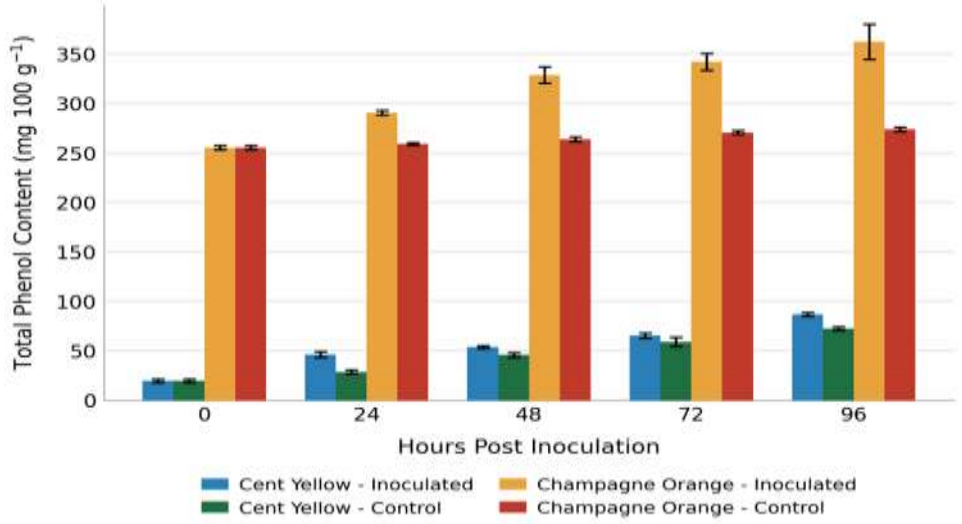

Figure 5. Total phenol content in Cent Yellow and Champagne Orange chrysanthemum varieties, inoculated and control, over 0–96 hpi.

DISCUSSION

The present study demonstrates a clear divergence in the biochemical defense response of wilt-resistant (Champagne Orange) and wilt-susceptible (Cent Yellow) chrysanthemum varieties following *Fusarium* inoculation. The steady increase in total protein content in the resistant variety, in contrast to the progressive decline observed in the susceptible variety, points towards active synthesis of defense-related proteins in Champagne Orange, consistent with reports of a resistance-associated rise in total soluble protein and pathogenesis-related protein content in *Fusarium*-challenged Cucumis melo genotypes (Jain et al. 2023), as opposed to pathogen-induced protein degradation in Cent Yellow.

Peroxidase catalyses the generation of reactive oxygen species that create a toxic environment for the invading pathogen and also restricts pathogen spread by promoting cross-linking and lignification of the host cell wall (Sun et al. 2022). The sustained rise in POX activity in inoculated Champagne Orange plants throughout the observation period, compared with the transient peak-and-decline pattern in Cent Yellow, suggests that a prolonged peroxidase response is associated with resistance, which is consistent with earlier reports of increased and sustained peroxidase activity in resistant genotypes of pearl millet against blast (Singh et al. 2020), in coriander against *Fusarium* wilt (Sowmya et al. 2025), and in chickpea primed against *Fusarium* wilt (Zeyad et al. 2022).

Catalase regulates the levels of hydrogen peroxide generated during the oxidative burst that follows pathogen attack, and its

activity has been reported to increase more prominently in resistant than in susceptible host-pathogen combinations (Zeyad et al. 2022; Sowmya et al. 2025). In the present study, the greater and more consistent induction of CAT activity in inoculated Champagne Orange plants compared with Cent Yellow suggests a more efficient antioxidant system operating in the resistant variety, allowing it to regulate reactive oxygen species without extensive cellular damage, a pattern broadly consistent with the antioxidant role attributed to catalase in *Fusarium*-challenged cucumber (Sun et al. 2022).

Polyphenol oxidase oxidises phenolic compounds to highly reactive, fungitoxic quinones and forms an additional line of induced defense in wounded and pathogen-challenged plants. The steady, overall rise in PPO activity maintained by Champagne Orange through 96 hpi, in contrast to the early spike and subsequent collapse observed in inoculated Cent Yellow, indicates that a sustained rather than merely a strong initial PPO response may be more important for effective resistance. Similar associations between higher and sustained PPO activity and disease resistance have been reported in pearl millet against blast (Singh et al. 2020) and in coriander challenged with *Fusarium* wilt, where PPO, peroxidase, superoxide dismutase and total phenol content were consistently higher in resistant genotypes (Sowmya et al. 2025), and are broadly consistent with the induction of PPO alongside other defense enzymes reported in *Fusarium*-infected chrysanthemum cv. 'Guangyu' (Liu et al. 2024).

The markedly higher absolute total phenol content maintained by Champagne Orange relative to Cent Yellow at every time point, despite a smaller relative percentage increase, suggests that a higher constitutive phenolic pool, rather than the magnitude of induction alone, contributes to resistance. Phenolic compounds not only exert direct antifungal toxicity but also serve as substrates for POX- and PPO-mediated oxidation to quinones, and their accumulation has been reported to be closely linked with resistance in several *Fusarium*-challenged host-pathogen systems, including coriander (Sowmya et al. 2025) and faba bean (Zhang et al. 2022), as well as chrysanthemum's own response to *Fusarium oxysporum* infection, where POX, PPO and phenylalanine ammonia-lyase were found to drive the accumulation of phenolics as part of the early defense response (Miao et al. 2023).

Taken together, these results suggest that resistance to wilt in the Champagne Orange variety is associated with a more prolonged induction of POX and CAT activity, a stable rather than transient PPO response, and a higher constitutive phenolic and protein pool, all of which likely act in combination to restrict pathogen establishment and spread. These biochemical parameters, particularly the sustained pattern of enzyme induction rather than the peak magnitude alone, may serve as useful markers for screening chrysanthemum germplasm for wilt resistance.

REFERENCE

1. Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology*, 105, 121–126.
2. Chandrasekaran, M., Belachew, S. T., Yoon, E., & Chun, S. C. (2017). Expression of β-1,3-glucanase (GLU) and phenylalanine ammonia-lyase (PAL) genes and their enzymes in tomato plants induced after treatment with *Bacillus subtilis CBR05 against Xanthomonas campestris pv. vesicatoria. Journal of General Plant Pathology,* 83(1), 7–13.
3. Hammerschmidt, R., Nuckles, E. M., & Kuc, J. (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium. Physiological Plant Pathology*, 20(1), 73–82.
4. Jain, C., Gupta, S., Sharma, S. P., Sangha, M. K., Kalia, A., & Sarao, N. K. (2023). Effect of *Fusarium* wilt on proteinaceous content and pathogenesis-related proteins in *Cucumis melo. Biologia*, 78(11), 3329–3338.
5. Liu, L., Jin, Y., Chen, M., Lian, H., Liu, Y., Yin, Q., & Wang, H. (2024). A study of soil-borne *Fusarium* wilt in continuous cropping chrysanthemum cultivar 'Guangyu' in Henan, China. *Journal of Fungi*, 10(1), Article 14.
6. Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). *Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry*, 193(1), 265–275.
7. Malik, C. P., & Singh, M. B. (1980). *Plant enzymology and histo-enzymology*. Kalyani Publishers.
8. Mayer, A. M., Harel, E., & Shaul, R. B. (1966). *Assay of catechol oxidase: A critical comparison of methods. Phytochemistry*, 5(4), 783–789.
9. Miao, W., Yang, Y., Wu, M., Huang, G., Ge, L., Liu, Y., Guan, Z., Chen, S., Fang, W., Chen, F., & Zhao, S. (2023). Potential pathways and genes expressed in chrysanthemum in response to early *Fusarium oxysporum* infection. *BMC Plant Biology*, 23, Article 312.
10. Singh, P. K., & Kumar, V. (2014). *Fusarium* wilt of chrysanthemum: Problems and prospects. *Plant Pathology & Quarantine,* 4(1), 34–44.
11. Singh, S., Pushpavathi, B., Madhavan, S., & Sharma, R. (2020). Expression of defense-related enzymes in blast (Magnaporthe grisea [Herbert] Barr) resistant and susceptible genotypes of pearl millet (Pennisetum glaucum [L.] R. Br.). *Archives of Phytopathology and Plant Protection*. https://doi.org/10.1080/03235408.2020.1853915
12. Sowmya, M., Giridhar, K., Priya, B. T., Lakshmi, T. V., & Kalpana, M. (2025). Biochemical, histological and molecular investigations of coriander genotypes against *Fusarium* wilt. *Plant Science Today,* 11(Special Issue 3).
13. Sun, S., Yang, Z., Song, Z., Wang, N., Guo, N., Niu, J., Liu, A., Bai, B., Ahammed, G. J., & Chen, S. (2022). Silicon enhances plant resistance to *Fusarium* wilt by promoting antioxidant potential and photosynthetic capacity in cucumber (Cucumis sativus L.). *Frontiers in Plant Science*, 13, Article 1011859.
14. Zeyad, M. T., Tiwari, P., Ansari, W. A., Kumar, S. C., Kumar, M., Chakdar, H., Srivastava, A. K., Singh, U. B., & Saxena, A. K. (2022). Bio-priming with a consortium of Streptomyces araujoniae strains modulates defense response in chickpea against *Fusarium* wilt. *Frontiers in Microbiology*, 13, Article 998546.
15. Zhang, C., Ou, X., Wang, J., Wang, Z., Du, W., Zhao,J., & Han, Y. (2022). Antifungal peptide P852 controls *Fusarium* wilt in faba bean (Vicia faba L.) by promoting antioxidant defense and isoquinoline alkaloid, betaine, and arginine biosyntheses. *Antioxidants*, 11(9), Article 1767.