INTERNATIONAL JOURNAL FOR INNOVATIVE RESEARCH IN MULTIDISCIPLINARY FIELD
ISSN(O): 2455-0620 [Impact Factor: 9.47]
Monthly, Peer-Reviewed, Refereed, Indexed Journal with IC Value : 86.87
Volume - 11, Issue - 07, July - 2025

DOIs:10.2015/IJIRMF/202507020 --:-- Research Paper / Article / Review

A comparative study on certain enzymes in flowers of Rangoon creeper *(Combretum indicum)* at different stages of maturity

[1, 2]Janaki Subramanyan, [1,3]Veena Beri, [1]Barha Sajid, [1]Charvi Khanna, [1]Durriya Samad, [1]Nishika, [1]Rishav Mangar

[1]D.S. Kothari Centre for Research and Innovation in Science Education, Miranda House, University of Delhi, Delhi, India
[2] Former Professor, Department of Botany, Miranda House, University of Delhi, Delhi, India
[3] Assistant Professor, Department of Botany, Miranda House, University of Delhi, Delhi, India
Email –[2] janaki.subramanyan@gmail.com, [3] veena.beri@mirandahouse.ac.in (Corresponding Author)

Abstract: *The flowers of Combretum indicum are with white corolla at anthesis and gradually turn deep pink passing through the pale pink and intermediate pink stages. The principal anthocyanin in the petals is cyanidin 3-O-glucoside. The plant is dichogamous and self-incompatible and the flowers attract different pollinators at the different colour stages. In the present study catalase, oxidase and peroxidase activities have been compared using aqueous extracts prepared from the corolla at the four identified colour stages. Catalase activity was highest in the white stage and decreased and remained nearly the same at the following stages. This showed that catalase had an important role as an antioxidant enzyme in detoxification at the initial stage of flower development. Oxidase activity was maximum at the intermediate pink stage and marginally less at the remaining stages. Interestingly, peroxidase activity was not detected at any developmental stage. We suggest that the cells are protected from oxidative damage primarily by the enzymatic antioxidant catalase at the white stage, and by oxidase and anthocyanin, a nonenzymatic antioxidant, at the later developmental stages thus ensuring that the flower is maintained till pollination.*

Key Words: *Combretum indicum, catalase, oxidase, peroxidase, anthocyanin, antioxidant.*

1. INTRODUCTION:

Combretum indicum (L.) DeFilipps (syn. *Quisqualis indica* L., family Combretaceae), commonly called the Rangoon creeper or Burma creeper, is a fast-growing woody climber widely distributed in tropical and subtropical regions. The buds open out into flowers with white petals post-sunset and on the next day turn pink and finally red; however, the flowers continue to remain on the plant till senescence [1]. *C. indicum* is dichogamous and self-incompatible, and the flowers attract different sets of pollinators at their different colour stages; the combined effect of floral colour change and pattern of both nectar secretion and release of scent ensure reproductive success [2]. The colour transformation in *C. indicum* flowers is because of the gradual accumulation of anthocyanins, particularly cyanidin 3-O-glucoside [1].
Following pollination, flowers senesce. Floral senescence is controlled by ethylene and abscisic acid; and the degradation of the macromolecules in the senescing floral part occurs and the products are transferred to the ovary, floral buds and other sinks [3, 4]. During floral senescence, it has been reported that the petals show both increased levels of free radicals and hydrogen peroxide (H_2O_2), and accumulation of the products of lipid peroxidation [3]. Reactive oxygen species (ROS) includes free radicals such as superoxide and hydroxyl, non-radicals (e.g., singlet oxygen) and H_2O_2. The ROS cause oxidative damage to the cell [5]. Nevertheless, up to a certain level the reactive oxygen species (ROS) also activate cellular responses to detoxify the ROS and protect the cell from damage: nonenzymatic antioxidants, e.g., anthocyanins, anthoxanthins, betalains and carotenoids, as well as antioxidant enzymes such as catalase, guaiacol peroxidase and superoxide dismutase are able to detoxify the ROS [5]. Floral senescence is accompanied by increase in ROS levels, and concomitant increase in the ROS scavenging enzymes such as superoxide dismutase, catalase and peroxidase (e.g. ascorbate peroxidase) in order to protect the cells from the damaging effects of ROS; however, at the last phase of senescence the activity of ROS scavenging enzymes decreases [6, 4].

Hydrogen peroxide, a stable and toxic molecule is formed by oxidases and peroxidases, and during electron transport in photosynthesis and respiration, and during photorespiration in peroxisomes [7]. Catalase degrades and detoxifies H_2O_2 to form water and oxygen. Peroxidases use H_2O_2 to oxidize substrates. Polyphenol oxidases oxidize phenolic compounds, which are compartmentalized in the vacuoles, to quinones. The quinones later polymerize to form brown melanin. Based on the substrate acted upon, polyphenol oxidases are classified as tyrosinase, catechol oxidase and laccase [8]. Oxidases protect plants from biotic stress, and probably also from abiotic stress [8, 9]. In the present investigation a comparison of the activities of catalase, oxidase and peroxidase in *C. indicum* petals at four stages of flower maturity has been undertaken to understand the role of the enzymes as the flowers progress in their development and the corolla changes from white to deep pink.

2. MATERIALS AND METHODS:

2.1 Plant material:

The inflorescences of *Combretum indicum* were plucked from the climbers growing in Sri Aurobindo Park in Sadik Nagar, Delhi, between 07:00 and 09:00 a.m. during June-July 2024, the period of our study. The inflorescences were placed in a polybag with a moist cloth and brought to the laboratory where the cut ends of the inflorescences were kept dipped in a beaker containing water. Flowers at the four developmental stages white, pale pink, intermediate pink, and deep pink were picked from the inflorescences, segregated and used in the study (Figure 1).

Figure 1: The four stages of maturity of *C. indicum* flowers. From left to right: Flowers with white, pale pink, intermediate pink, and deep pink corolla.

2.2 Preparation of enzyme extracts:

An extract of fresh petals was prepared by homogenizing 2 g of the petals in 30 mL distilled water using a motor and pestle. The homogenate was filtered through muslin cloth. The filtrate was used immediately as the crude enzyme extract.

2.3 Detection of catalase, oxidase and peroxidase activities:

The substrate used was H_2O_2 (1%, v/v) for the detection of catalase activity [10, 11]. The experiment was set up as given in Table 1. The oxygen evolved was recorded 4 minutes from incubation. For the detection of oxidase activity, catechol (1%) was used as the substrate [10]. The intensity of brown colour that developed 15 minutes from incubation was recorded visually by giving arbitrary plus (+) marks (Table 2). For the detection of peroxidase activity, in addition to the substrate catechol (1%), H_2O_2 (1%, v/v) was added as the source of oxygen [12];and the intensity of brown colour developed was recorded 15 minutes from incubation by giving plus (+) marks (Table 3). Control reaction mixtures with no enzyme, with no substrate, and with boiled and cooled enzyme were maintained for all the three enzymes. In case of peroxidase, an additional control reaction mixture with the enzyme and catechol but without H_2O_2 was also set. The activities of the three enzymes were studied at the four stages of flower maturity. All experiments were conducted three times and the average values were taken for interpreting the results.

3. RESULTS AND DISCUSSION:

The bud opens out into a flower with white corolla. The flower remains on the plant and does not abscise. However, the corolla changes its colour and starts turning pink. The anthocyanin pigment synthesis increases and as the pigment accumulates the corolla gradually passes through the pale pink, intermediate pink and finally reaches the deep pink stage (Figure1). The principal anthocyanin is cyanidin 3-O-glucoside [1].

3.1 Catalase activity:

Table 1: Detection of catalase activity in the corolla of *C. indicum* flowers at different stages of maturity*.

Corolla colour	Enzyme extract (mL)	H_2O_2 (mL)	Initial volume (mL)	Final volume (mL)	Average O_2 evolved** (mL)
White	2	2	4	5	1.0
Pale pink	2	2	4	4.4	0.4
Intermediate pink	2	2	4	4.3	0.3
Deep pink	2	2	4	4.4	0.4

* Catalase activity was not detected in all the control tubes at all stages of flower maturity.

**Average of three replicates.

Catalase activity was observed at all four stages of flower maturity, i.e., white, pale pink, intermediate pink, and deep pink colour of the corolla (Table 1, Figure 2 A, B, Figure 3). The catalase activity was highest at the white stage and decreased in the subsequent stages. However, catalase activity was nearly the same in pale pink, intermediate pink and deep pink corollas. No catalase activity was observed in the control reaction mixtures (Figure 2 A, B). The boiled and cooled enzymes showed no activity because boiling denatured the enzyme. The result suggests that catalase plays an important role in managing oxidative stress during early flower development preventing the accumulation of ROS. In a study on *Bougainvillea glabra* and *Delonix regia*, which are drought-tolerant plants, it has been reported that the fresh white flowers of *B. glabra* showed more catalase activity than senescing flowers; however, fresh flowers of *D. regia* lacked catalase activity whereas the senescing flowers had a low catalase activity [13]. Leaves also usually show a decrease in catalase activity with senescence. In both attached and detached leaves of rice, catalase activity decreased with the progress of senescence, and phenolics accumulated in the detached leaves but not in the attached leaves [14]. During senescence catalase activity decreased in tobacco leaves but in increased in wheat and barley leaves [14].
The senescing flowers of both *B. glabra* and *D. regia* contained more chlorophyll a, chlorophyll b, and carotenoids than the fresh flowers; betacyanin was absent in the bracts of fresh flowers but present in senescing flowers in *B. glabra*, and senescing flowers of *D. regia* had more anthocyanin than fresh flowers [13]. Carotenoids, anthocyanins and betacyanin are antioxidants [5]. The gradual synthesis of anthocyanins in the petals as the flowers turn pink in *C. indicum* is not only an attractant for pollinators but also a protection as an antioxidant. It is quite likely that the antioxidant role of protection provided by catalase in the young flower at the white petal stage is partially taken over by anthocyanin as the flowers mature. The aqueous extract of flowers of *C. indicum* has been shown to contain a high concentration of quercetin, a flavonoid [15] and an extract of *C. indicum* leaves in 50% ethanol had a high total phenolic content and showed good antioxidant property [16]. In fact, all parts of *C. indicum* have good antioxidant activity which is in support of the plant's use in traditional medicine [15]. The extracts of leaves and flowers of *C. indicum* have been shown to inhibit free radical formation which proves their antioxidant property [17]. The antioxidant enzymes superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, glutathione peroxidase, and dehydroascorbate reductase decrease oxidative stress and improve anthocyanin accumulation [18]. A study on flower buds of apple showed that the activities of superoxide dismutase and catalase were low in the dormant buds; however, the enzyme activities increased 2- to 5-times during bud swelling. But with the onset of bud break the activity of superoxide dismutase decreased sharply, although the level of catalase remained high during flower bud development [6]. *Azadirachta indica* and *Murraya koenigii* are drought-tolerant trees, and the increase in catalaseactivity in the mature fruits compared to the corresponding young fruits is a response to tolerate the oxidative stress and water deficit stress during fruit ripening [13].

3.2 Oxidase activity:

Oxidase activity was present at all the four stages of flower maturity. Maximum oxidase activity was observed at the intermediate pink stage (+5). The enzyme activity was good (+4) at the white and deep pink stages (Table 2, Figure 2 C, D, Figure 3). The control without the enzyme did not show browning of the reaction mixture. The control with the enzyme but no substrate showed oxidase activity at all four stages of flower maturity (Figure 2 C, D). However, the activity was less than the corresponding experimental reaction mixtures. This showed that the enzyme extract contained internal substrate/s that came in contact with oxidase because the cells had been crushed. The control with boiled and cooled enzyme also showed some activity at all four stages of flower maturity. This is because oxidase had already begun acting on the internal substrate/s in the time interval between enzyme extraction and enzyme boiling (Table 2). Therefore, the brown colour developed in the experimental reaction mixtures is contributed by the products formed from oxidase acting on both the added substrate catechol and the internal substrate/s.

Flowers of *C. indicum* are rich in polyphenols and flavonoids [17] and contain a high concentration of quercetin, a flavonoid [15]. Also, anthocyanins which are flavonoid pigments are present in the extracts of pale pink, intermediate pink and deep pink corollas. Maximum antioxidant potential and hence free-radical scavenging capacity has been detected in the methanolic extract of the petals of *C. indicum* at the red stage [1]. Therefore, the corolla extracts contain abundant internal substrates that have got released from the cell vacuole when the petals were crushed and came in contact with the polyphenol oxidases resulting in the enzymatic reaction. Polyphenol oxidases are believed to be present in the cytoplasm and in plastids [8].Owing to the good antioxidant and anti-tyrosinase action shown by the leaves of *C. indicum,* it has been suggested that an effective natural cosmetic for treating hyperpigmentation could be developed using the leaves [16].In a study on rice leaves, it has been reported that polyphenol oxidase activity increased with the progress of senescence in both attached and detached leaves [14]. Fresh flowers of *B. glabra* had more oxidase activity than senescing flowers whereas the oxidase activity was identical in fresh and senescing flowers of *D. regia* [13]. Increase in oxidase activity in mature fruits compared to young fruits has been reported in the fruits of *A. indica* and *M. koenigii* [13]. Oxidases also function as protective enzymes helping plants endure stressful conditions [8, 9]. Hence, it is the oxidase and the antioxidants together that protect the cells from oxidative damage.

Table 2:Detection of oxidase activity in the corolla of *C. indicum* flowers at different stages of maturity.

Corolla colour	Enzyme extract (mL)	Catechol (mL)	Distilled water (mL)	Intensity of brown colour (+) *
White	2	2	-	+4
Control 1	-	2	2	-
Control 2	2	-	2	+2
Control 3	2 (B & C)	2	-	+1
Pale pink	2	2	-	+3
Control 1	-	2	2	-
Control 2	2	-	2	+1
Control 3	2 (B & C)	2	-	+2
Intermediate pink	2	2	-	+5
Control 1	-	2	2	-
Control 2	2	-	2	+2
Control 3	2 (B & C)	2	-	+3
Deep pink	2	2	-	+4
Control 1	-	2	2	-
Control 2	2	-	2	+3
Control 3	2 (B & C)	2	-	+2

*Average of three replicates. B & C: Boiled and cooled enzyme extract.

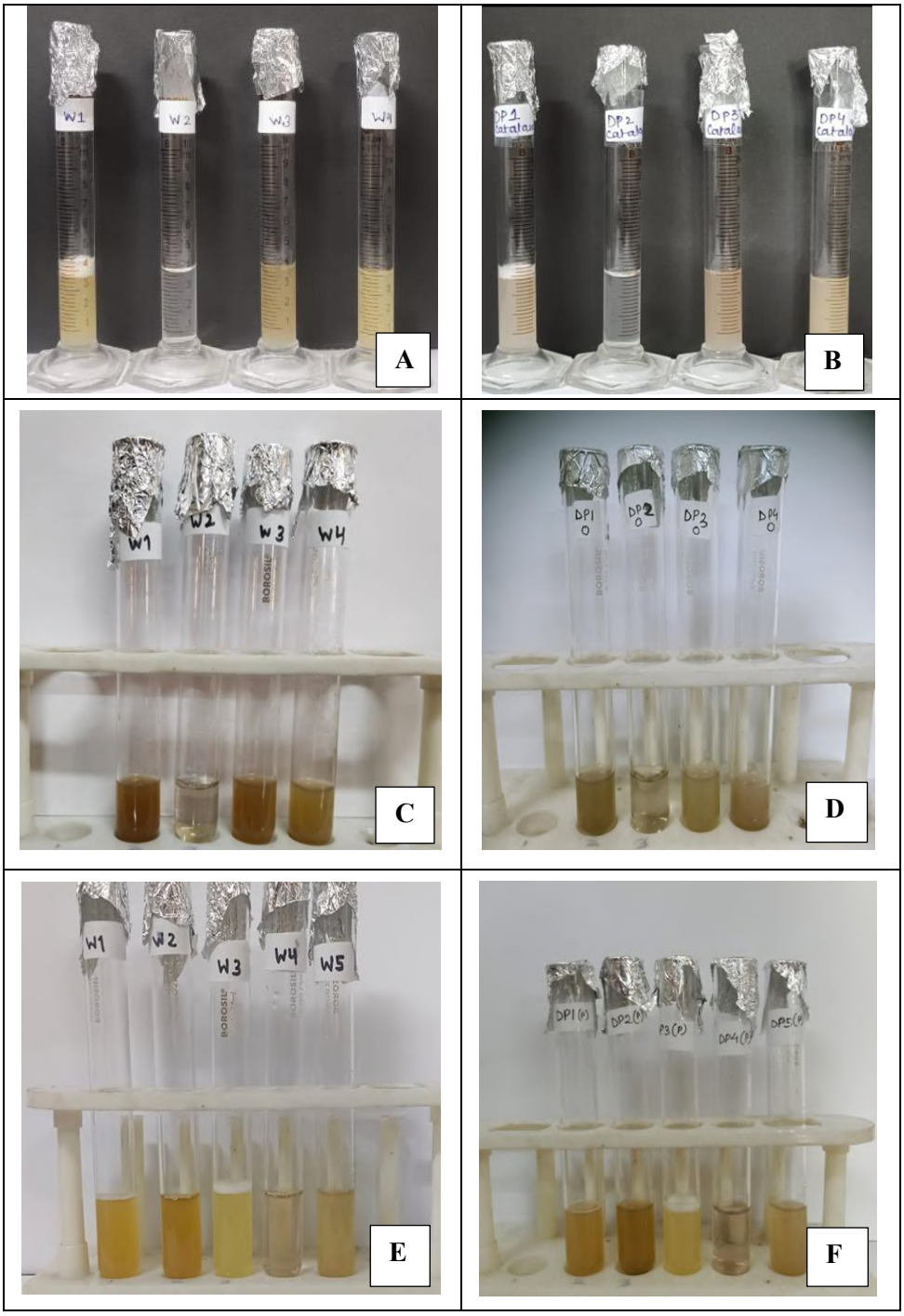

Figure 2: Catalase, oxidase and peroxidase activities in the corolla of *C. indicum* flowers at the white and deep pink stages of maturity. In A-D, the measuring cylinder/ test tube at the extreme left is the experimental one containing both enzyme and substrate; the second from the left is the control without enzyme; the third from the left is the control without substrate; and the fourth is the control with boiled and cooled enzyme and substrate. A, B. Catalase activity at the white and deep pink stages, respectively. C, D. Oxidase activity at the white and deep pink stages, respectively. E, F. Peroxidase activity at the white and deep pink stages, respectively. The test tube at the extreme left is the experimental one containing enzyme, catechol and H_2O_2; the second from the left is the control containing enzyme and catechol (without H_2O_2); the third from the left is the control containing enzyme and H_2O_2 (without catechol); the fourth is the control without enzyme but has catechol and H_2O_2; and the fifth is the control with boiled and cooled enzyme, catechol and H_2O_2.

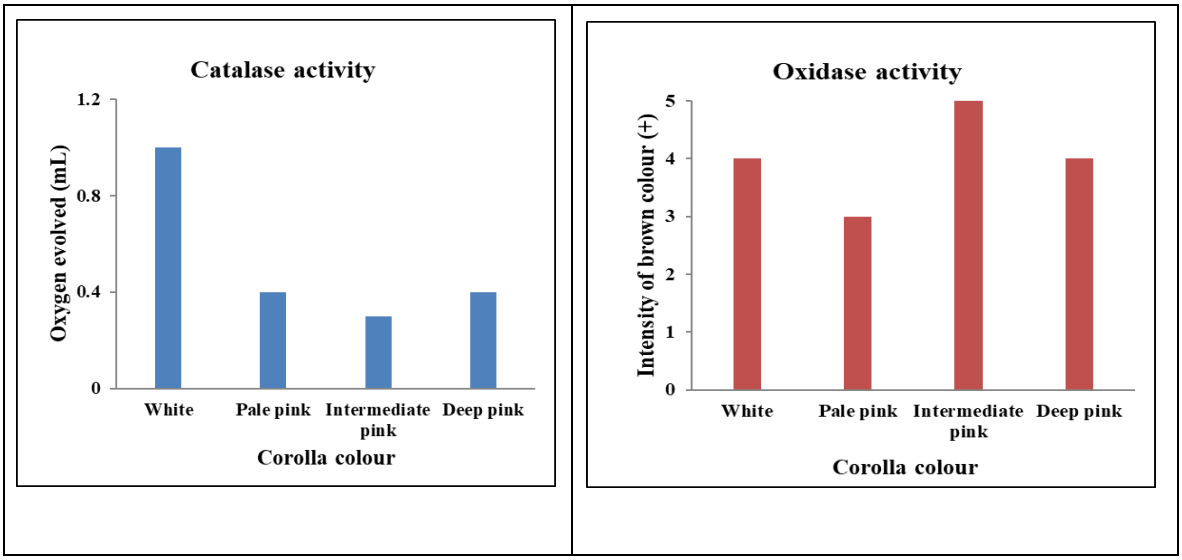

Figure 3.Catalase (left) and oxidase (right) activities in the corolla of *C. indicum* flowers at different stages of maturity.

3.3 Peroxidase activity:

Table 3: Comparison of oxidase and peroxidase activity in the corolla of *C. indicum* flowers at different stages of maturity. The control tubes with enzyme and catechol represent oxidase activity, whereas the experimental tubes with enzyme, catechol and H_2O_2represent peroxidase activity.

Corolla colour	Intensity of brown colour (+)*	
	Oxidase	Peroxidase
White	+6	+5
Pale pink	+6	+4
Intermediate pink	+6	+4
Deep pink	+6	+4

*Average of three replicates.

Interestingly, at all four stages of flower maturity, the controls without H_2O_2showed a more intense brown colour than the corresponding experimental tubes with H_2O_2 (Figure 2 E, F, Table 3.). This showed that whatever brown colour developed was only because of oxidase activity. If peroxidase activity had been present, the intensity of brown colour in the experimental tubes with enzyme, catechol and H_2O_2should have been more intense than in the control tubes with only catechol and without H_2O_2 (Table 3). Based on the results, it is concluded that peroxidase activity is absent at the four stages of flower maturity. However, providing anaerobic conditions to the reaction mixtures and increasing the period of incubation will help in arriving at a better conclusion. It is suggested that the concentration of catalase in the petals is sufficient to utilize H_2O_2 formed and peroxidase, if present, is present at a very low concentration at all stages of flower maturity. In apple, the peroxidase activity was low in dormant flower buds and but increased significantly at bud swelling; however, at bud break the activity again decreased [6]. Kar & Mishra [14] reported an increase in peroxidase activity when attached and detached leaves of rice senesced.

4. CONCLUSION:

Catalase and oxidase activities were present but peroxidase activity was absent at all the four stages of floral maturity. Catalase activity was maximum at the white stage whereas oxidase activity was maximum at the intermediate pink stage of the corolla. It can be concluded that it is the cumulative effect of the antioxidant enzyme catalase, stress-protection enzyme oxidase, and nonenzymatic antioxidants importantly anthocyanin that helps the flowers manage oxidative stress and maintain the flowers till pollination occurs.

Acknowledgements:
We sincerely thank Professor Bijayalaxmi Nanda, Principal, Miranda House, and Professor Monika Tomar, Coordinator, DS Kothari Centre for Research and Innovation in Science Education, Miranda House, for organizing the Summer Internship Programme Flavour of Research: Investigative Projects in Multidisciplinary Contexts in 2024 under which projects could be undertaken by students during the summer vacations. The laboratory staff of the Department of Botany were very supportive and we thank them.

REFERENCES:

1. Ghissing, U., Goswami, A., & Mitra, A. (2023). Temporal accumulation of pigments duringcolourtransformationfrom white to red in *Combretum indicum* (L.) DeFilipps (syn. *Quisqualis indica* L.) flowers. *Natural Product Research,37*(3), 529-533. DOI: 10.1080/14786419.2021.1984467
2. Yan, J., Wang, G., Sui, Y., Wang, M.,& Zhang, L.(2016). Pollinator responses to floral colour change, nectar, and scent promote reproductive fitness in *Quisqualis indica* (Combretaceae). *Scientific Reports*,*6*(1), 1-10.http://www.nature.com/scientificreports| 6:24408 | DOI: 10.1038/srep24408
3. Ahmad, S.S.,and Tahir, I. (2016). How and why of flower senescence: understanding from models to ornamentals. *Plant Physiology Reports,21*(4), 446-456.
4. Cavaiuolo, M., Cocetta, G.,& Ferrante, A. (2013). The antioxidants changes in ornamental flowers during development and senescence.*Antioxidants,2*, 132-155.DOI:10.3390/antiox2030132
5. Subramanyan, J., Rajeshwari, Thakur, K., Jandial, M., &Ritika. (2023): A comparative study of oxidative stress biomarkers in leaflets of neem and curry-leaf tree. *Zeichen Journal,9*(5),158-167.
6. Abassi, N.A., Kushad, M.M.,& Endress, A.G. (1998). Active oxygen-scavenging enzymes activities in developing apple flowers and fruits. *Scientia Horticulturae,74*(3), 183-194.
7. Mhamdi, A., Queval, G., Chaouch, S., Vanderauwera, S., Breusegem, F.V.,&Noctor, N. (2010).Catalase function in plants: a focus on Arabidopsis mutants as stress-mimic models. *Journal of Experimental Botany,61*(15),4197-4220.
8. Zhang, S. (2023).Recent advances of polyphenol oxidases in plants. *Molecules,28*(5):2158. https://doi.org/10.3390/molecules28052158
9. Boeckx, T., Winters, A.L., Webb, J.K.,& Kingston-Smith, A.H. (2015).Polyphenol oxidase in leaves: is there any significanceto the chloroplastic localization? *Journal of Experimental Botany*,*66*(12), 3571-3579.
10. Machlis, L.,&Torrey, J.G.(1956).*Plants in action: a laboratory manual of plant physiology* (pp. 112-114). San Francisco: W.H. Freeman and Company.
11. Bajracharya,D.(1999). *Experiments in plant physiology*(pp. 109-110). New Delhi: Narosa Publishing House.
12. Kochhar, S.L.,& Gujral, S.K. (2012).*Comprehensive practical plant physiology* (p. 124). Delhi: Macmillan Publishers India Ltd.
13. Subramanyan, J., Rajeshwari, Patel, D.,&Sharma, P. (2023). A comparative study of oxidative stress biomarkers in flowers and fruits during senescence. *International Journal of Botany Studies,8*(7), 11-17.
14. Kar, M.,& Mishra, D. (1976).Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence.*Plant Physiology,57*(2), 315-319. https://doi.org/10.1104/pp.57.2.315
15. Shah, A., Khan, Z., Saleem, S.,& Farid, S. (2019). Antioxidant activity of an ethnobotanically important plant *Quisqualis indica* Linn. *Pakistan Journal of Pharmaceutical Sciences*, *32*(1), 95.
16. Thanaseelan, T., Hidayu, D.N., Sahirah, N.,&Rajaletchumy, V.K. (2024).Evaluation of antioxidant and anti-tyrosinase activities of surfactant and alcohol extracted *Combretum indicum* leaves. *Materials Today Proceedings,107*, 224-230. https://doi.org/10.1016/j.matpr.2023.10.077
17. Dutta, A., Biswas, S., Biswas, M., Ghosh, P., Ghosh, C., Das, S.,&Chatterjee, S. (2019). Phytochemical screening, antioxidant and antimicrobial activity of leaf, stem and flower of Rangoon creeper: a comparative study. *Journal of Medicinal Plants Studies,7*(2), 123-130.
18. Sharma, H., Sharma, P., Kumar, A., Chawla, N., & Dhatt, A.S. (2024). Multifaceted regulation of anthocyaninbiosynthesis in plants: a comprehensive review. *Journal of Plant Growth Regulation,43*, 3048-3062.https://doi.org/10.1007/s00344-024-11306-x