ORIGINAL RESEARCH PAPER
Volume - 14 | Issue - 10 | October - 2025 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

EFFECTS OF CHEMICAL DYES USED IN TEXTILE AND FOOD INDUSTRIES ON *IN VITRO* POLLEN GERMINATION OF *CATHARANTHUS ROSEUS* (L.) G. DON. L.

Botany

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ABSTRACT

Catharanthus roseus (L.) G. Don. (periwinkle) is a common garden plant from the family Apocynaceae. It is also useful as a medicinal plant for the treatment of cancer nervous system and muscular pain. The chemical dyes are widely used synthetic colorant for textile, food and paper manufacturing industries. Such synthetic dyes are hazardous for human health and polluting environment including soil and water bodies. In present study, the effects of various coloured food and textile dyes were observed at various concentrations (20-1000 ppm) on in *vitro* pollen germination of *Catharanthus roseus* (L.) G. Don. Observations were made after various time intervals of 2-48 hrs using Stereomicroscope, Compound microscope and Scanning Electron Microscope (SEM). The study showed that the percentage of pollen germination and length of pollen tube get reduced in all the treatments when compared to the control, suggesting the hazardous effects of chemical colouring agents at cellular level.

KEYWORDS

Textile Dye, Food Dye, Pollen Germination, Catharanthus

INTRODUCTION

Azo dyes are used globally as colour additives in food, drug, plastic, cosmetics, paints, textile, leather and paper industries (Coultate et al, 2018) as these are cheaper alternatives of plant based natural dyes. More than 1500 different types of azo dyes are available in the market (Bruna and Maria, 2013). It has been shown that these azo dyes are carcinogenic and toxic to the environment. Effects of hazardous and toxic heavy metals were studied earlier on Pollen germination (Muradoglu et al, 2017).

Catharanthus roseus (L.) G. Don. is a source of drugs vincristine and vinblastine (Moudi et al, 2013). Earlier, protocol for pollen germination of *Catharanthus roseus* (L.) G. Don. was reported (Bandyopa and Mukherjee, 1977).

MATERIAL AND METHOD

Pollen used in this experiment was collected from freshly opened flowers of *Catharanthus roseus* (L.) G. Don. during early morning hours. Optimization for required media for Pollen germination was carried out. Out of various sucrose concentrations (4, 8, 12, 15, 18 %) and at various time intervals, the highest rate of pollen germination was achieved with 15% of sucrose with 50 mg of boric acid solution after 2 hours. This concentration was used further as control. Best suited media for pollen germination was utilized as control (15 % of Sucrose with 50 ppm Boric acid solution) to study the effect of Acid Red 119 type of azo dye, Food colours 999 (green, yellow and red) as well as textile dyes (green, yellow and red) at various concentrations (20, 40, 60, 80, 100, 200, 300, 400, 500 and 1000 ppm) on Pollen germination.

The rate of pollen germination and length of pollen tube were measured at different time intervals (1/2, 1, 2, 3, 4, 24, 48, 72 hrs) using compound microscope with ocular micrometer for Red textile dye AR 119. The data for various other coloured food and textile dyes was compared and shown here for 2 hr time intervals. Moreover, Observations were made using stereomicroscope and SEM for the same for further investigation to observe changes in the structure of wall material. Data for percentage pollen germination and the length of pollen tube was analyzed for each treatment and the data was represented through mean and mean + standard error, correspondingly for 100-500 ppm concentrations of various food and textile dyes.

RESULT AND DISCUSSION

In Vitro pollen germination was achieved only after 2 h. After 2-4 hrs, percentage of pollen germination was highest in 60 ppm of Red textile dye AR 119.; which was higher by 379.7 % over control (Table 1). Similarly, after 2 h; maximum length of pollen tube was found in the treatment of 500 ppm; which was higher by control over 14.5 % (Table 2). However, after 24 h; disintegration was seen in pollen tube and minimum pollen tube length was seen for the same treatment. After 24 h, curvature in pollen tube growth was observed after 2 hrs in the treatment of 100, 200 and 300 ppm. Similarly, after 24 h, disintegration of pollen tube started in 200 and 300 ppm (Fig. 1-3). Moreover, pollen tube turned spiral in growth for 300 ppm onwards. After 48 h, disintegration of pollen tube was observed in the treatment of 100 ppm and onwards (Table 1-4). In addition to this, higher the concentration, more disintegration of pollen tube was observed after 48 hrs. After 72 hrs, there was a complete disintegration in pollen tube for each of the dye treatments.

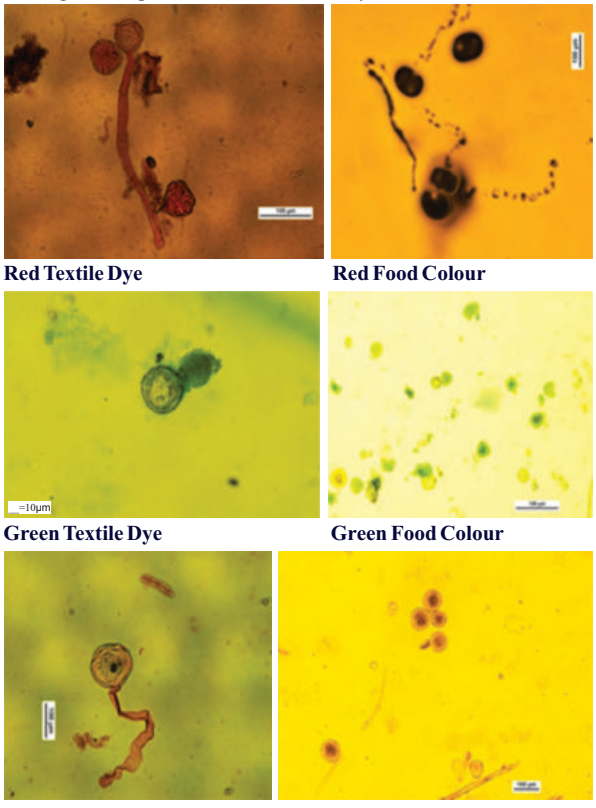


Fig.1. Stereomicroscopic images of pollen germination after 2 hrs in 500 ppm of red, green and yellow textile dyes and food colours (Horizontal bar=100 µm)

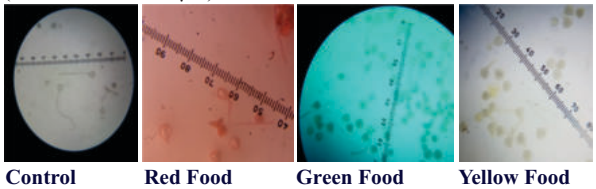
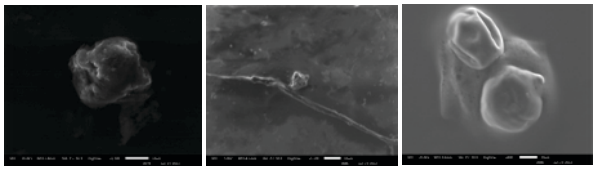
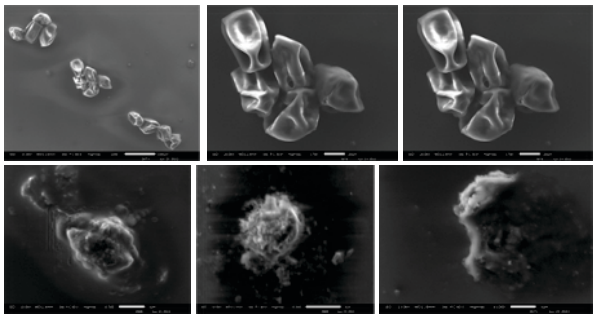


Fig. 2. Compound microscopic images for pollen germination of Catharantus in various food Colouring dyes at 500 ppm after 2 hrs

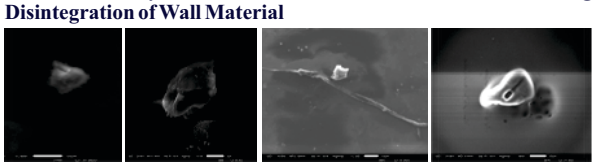
PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr



Red Food Colour



Red Textile Dye Ar 119 Initial and After 2 hrs Showing Disintegration of Wall Material



Yellow Food Yellow Textile Green Food Green Textile

Fig. 3. SEM images for for pollen germination of Catharantus in various coloured dyes treatments at 500 ppm after 2-4 hrs showing degradation of wall material releasing cytoplasmic content from germinated pollengrains

Earlier, effect of azo dye was studied on respiration of plants in an artificial pond system. Moreover, algal growth was shown to get prohibited due to azo dyes in waste waters. Microalgae play an important role in both the ecological and economical manners. The negative effects of such chemical dyes were found in fishes and their predators, damaging gills, liver, kidney, gut and cells. Toxic impact of textile dyes were studied on human health showing inactivation of certain enzymes causing skin and eye irritation, allergy, asthama, central nervous system and interfering ovulation and spermatogenesis (Rania et al, 2022). Mutagenic potential of azo dye causing cancer (Piatkowska et al, 2018). The carcinogenic effect of azo dye in human was studied in hepatoma cells and human lymphocytes. It was found that these dyes have ability to increase the frequency of micronuclei and mutagenic activity at chromosome level (Will et al, 2016).

Such carcinogenic azo textile dyes are widely used across the world and the affluent from textile industries; which are released in water bodies, polluting soil and rivers which resulted in depletion of biodiversity and deterioration of human health. Every year, cancer patients have been shown to get increased at alarming rate in such scenario as well. Physical, electrochemical and fungal assisted degradation of such chemical dyes for bioremediation of waste water have been proposed at pilot scale time to time by many investigators (Rania et al, 2022).

However, use of plant based eco-friendly and non-hazardous dying substances could be the more appropriate solution, as even though it seems that chemical dyes are cheaper economically, but in long run the way we have lost biodiversity and human lives, causing soil degradation and polluted rivers in millions of acres, and the way we need to spent millions of dollars per annum for conservation of biodiversity, management of soil and cleaning of river projects; thus suggesting that utilization of chemical dye should be considered as very costly affair, and not a cheaper alternative as it was found to be hazardous for the growth.

Table 1: Effect of azo dye AR 119 (red coloured textile) on *in vitro* pollen germination of *Catharanthus roseus* (L.) G. Don. at 20-1000 ppm level after 2 hrs

	Control	Treatment (ppm)				
		20	60	80	100	1000
Percentage Germination (%)	15.16	30.76	72.73	27.78	41.67	40
Length of pollen tube (µm)	15.57+ 1.04	2.4+ 0.48	1.1+ 0.33	2.84+ 0.44	1.34+ 0.29	3.0+ o.58

Table 2: Effect of azo dye AR 119 (Red coloured textile) on *in vitro* pollen germination of *Catharanthus roseus* (L.) G. Don. at 100 to 500 ppm level after different time intervals

(2 hrs)	Control	Treatment (ppm)				
		100	200	300	400	500
Percentage Germination (%)	15.16	50	44.44	100	6.98	17.95
Length of pollen tube (µm)	15.57+ 1.04	1+ 0.0	5.2+ 0.98	9.36+ 0.71	4+ 0.0	17.83+ 1.09

(24 hrs)	Control	Treatment (ppm)				
		100	200	300	400	500
Percentage Germination (%)	63.11	18.18	50	50	35	10
Length of pollen tube (µm)	21	17+ 15.16	15+ 0.59	5+ 1.88	12+ 1.03	1

(48 hrs)	Control	Treatment (ppm)				
		100	200	300	400	500
Percentage Germination (%)	63	52.94	50	50	50	10
Length of pollen tube (µm)	22.67+ 2.52	9+ 2.08	17.5+ 2.3	14.67+ 0.91	1.5+ 0.59	1

Table 3: Effect of green coloured chemical dye on *in vitro* pollen germination of *Catharanthus roseus* L. after 2 hrs

green coloured Textile dye	Control	Treatment (ppm)				
		100	200	300	400	500
Percentage Germination (%)	30.68+ 3.64	4.83+ 1.44	2.77+ 1.12	3.20+ 1.22	0.68+ 0.56	6.64+ 1.41
Length of pollen tube (µm)	17.43+ 1.59	8.16+ 1.36	4+ 0.99	4.33+ 0.93	9.88+ 1.59	7.83+ 1.13

green coloured Food dye	Control	Treatment (ppm)				
		100	200	300	400	500
Percentage Germination (%)	90.48+ 1.53	72.22+ 1.68	96+ 0.71	81.60+ 0.4	16.66+ 1.27	40.83+ 1.48
Length of pollen tube (µm)	23.3+ 1.38	8.8+ 1.25	7.5+ 1.54	14.48+ 1.85	2.72+ 0.87	9.2+ 1.57

Table 4: Effect of yellow coloured chemical dye on *in vitro* pollen germination of *Catharanthus roseus* L. after 2 hrs

Yellow coloured Textile dye	Control	Treatment (ppm)				
		100	200	300	400	500
Percentage Germination (%)	72.91+ 2.28	68.3+ 1.79	75.47+ 1.24	81.68+ 1.91	17.27+ 1.73	20.10+ 2.52
Length of pollen tube (µm)	18+ 0.99	12+ 2.05	30+ 1.82	13.7+ 0.95	5.16+ 0.62	5.6+ 0.6

Yellow coloured Food dye	Control	Treatment (ppm)				
		100	200	300	400	500
Percentage Germination (%)	72.91+ 2.28	95.8+ 1.55	55.09+ 2	97.33+ 1.53	15.05+ 0.86	67.05+ 2.1
Length of pollen tube (µm)	18+ 0.99	16.6+ 1.21	25.14+ 1.31	30+ 1.48	13.33+ 0.87	18.5+ 0.45

Acknowledgment

Authors would like to thank the Director, Physical Research Laboratory (PRL), Ahmedabad for their assistance and help for providing SEM images.

Funding Agency

The research was not funded by any agency.

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