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STORAGE STUDIES OF NATURAL FLOWER PETAL INK

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ABSTRACT The present investigation aimed to determinate the effect of microbial growth on different inks prepared from various flower petals under different durations of boiling and to study their colour retention when written on paper with natural petal ink. Only two treatments, T_{10} and T_{35} , demonstrated the ability to write on both 50 and 70 GSM papers and these were evaluated. The results revealed that all flower petals boiled for 45 minutes showed no microbial growth. Among these, rose petals exhibited high consistency with a low quantity of ink. Treatments T_{10} and T_{35} , when written on 50 and 70 GSM paper, displayed slight colour changes during the 30 to 90-day storage period, while maintaining colour retention and showing no microbial growth.

Key words : Natural ink, Petal ink, Storage studies, Colour retention, Microbes.

Introduction

Globally, flower events hold cultural and religious significance, but they also generate a substantial amount of flower waste, particularly from temples, festivals, and markets. In India, approximately 40% of flower production is wasted (Waghmode *et al.*, 2018). Repurposing this flower waste into useful products, such as natural inks, offers a sustainable waste management solution and potential revenue. Historically, plant-based natural dyes were widely used, but the mass production of synthetic dyes reduced their use (Gilbert and Cooke, 2001). However, due to their sustainability and biodegradability, natural dyes/ inks are regaining interest due to environmental regulations and a growing awareness of health and environmental protection.

Creating natural flower inks is a sustainable alternative to synthetic inks and an environmentally responsible way to produce vibrant colours that connect us to the natural world. The heavy reliance on non-renewable synthetic resources in modern ink production poses risks to human health and the environment, including headaches, skin irritation, and neurological damage. Despite the long history of ink production, issues like deinking digitally printed paper intended for recycling persist. Adopting natural dyes and inks can help reduce these hazards and promote more sustainable and healthful ink production.

During storage, boiled inks can be affected by microbial growth, leading to spoilage characterized by unpleasant odors and off-colours. This microbial growth can alter the colour quality and reduce the viscosity or consistency of natural petal inks. Therefore, understanding and controlling microbial growth in natural petal inks is crucial for maintaining their quality and longevity.

Materials and Methods

The present investigation was carried out at the AICRP on Fruits, Dr. YSRHU- Citrus Research Station, Tirupati, located in the Rayalaseema region of Andhra Pradesh during the years 2022-2024. The station is situated at an elevation of 162 meters above mean sea level (MSL), at 13°65′ North latitude and 79°42′1″ East

longitude. The experiment was laid out in a factorial completely randomized design, replicated twice with two factors. Factor one included seven types of flowers: yellow chrysanthemum, red rose, orange marigold, white tuberose, pink lotus, purple orchids and blue clitoria. Factor two included five boiling durations: 10, 15, 20, 30 and 45 minutes.

Petal ink was extracted and filled into ballpoint pens, which were then used to write on 50 and 70 GSM papers in a previous study. In this study, the written papers and extracted dyes/inks were tested during a storage period of 30 to 90 days and observations were recorded to assess microbial growth and colour retention after 30, 60 and 90 days interval.

Quantity of dye extract (ml) from different flowers

Flower petals weighing 250 grams were added to 1000 millilitres of water and boiled for the specified duration. The resulting filtered flower extract was then collected and measured using a beaker.

Colour retention of the ink extract after writing on the paper (50 and 70 GSM Paper)

The colour retention of the written papers was tested after 30, 60, and 90 days of storage using the RHS (CIE lab $D65/10^{\circ}$) colour chart.

Microbial count (cfu/ ml)

To determinate the microbial count present in the petal ink, the serial dilution procedure was followed, and the count was measured in Colony Forming Units per milliliter (cfu/ ml). For fungal assessment, serial dilutions were carried out up to a 10^{-4} dilution.

Results and Discussion

Quantity of ink extract (ml) from different flowers

The imparted data delineated that the different coloured flower petals, boiling timings and their interaction had significant persuasion on quantity of dye extract and it is conspicuously rendered in Table 1.

Among the various flowers, significantly maximum quantity of dye extract was recorded in clitoria flower petals (F_7 , 579.1 ml), which was followed by tuberose (F_4 , 552.6ml), while the minimum quantity recorded in lotus flower petals (F_5 , 430.0 ml), which was on par with rose flower petals (F_7 , 438.5ml).

Among different boiling timings, 10 minutes boiling period resulted in maximum quantity of dye extract (B_1 , 770.71 ml) followed by 15 minutes (B_2 , 671.4 ml) and minimum quantity of dye extract was documented for 45 minutes (B_5 , 87.4 ml) and all the treatments were found to be significant.

 Table 1 : Quantity of different dye extracts (ml) as influenced by various boiling times.

Flowers (F)		Boil	ing time	e (B)		
11000015(1)	B ₁	B ₂	B ₃	B ₄	B ₅	Mean
\mathbf{F}_{1}	765.0	660.0	562.5	455.0	90.0	506.5
\mathbf{F}_{2}	700.0	627.5	510.0	305.0	50.0	438.5
F ₃	795.0	670.0	605.0	457.5	105.0	526.5
F ₄	845.0	717.5	635.0	460.0	105.5	552.6
F ₅	700.0	627.0	497.5	270.0	55.5	430.0
F ₆	720.0	642.5	552.5	370.0	80.0	473.0
F ₇	870.0	755.0	675.0	470.0	125.5	579.1
Mean	770.7	671.4	576.8	398.2	87.4	
Comparing Means		SEm(±))	(CD @5%	6
Flowers (F)		3.18			9.18	
Boiling time (B)		2.69			7.75	
F × B		7.12			20.52	
F ₁ - Chrysanth	emum	F ₅ - L	otus	B	= 10 mi	nutes
F_2 - Rose		$F_6 - C$	rchids	B ₂	=15 min	nutes
F_3 - Marigold		$F_7 - C$	litoria	B ₃	= 20 min	nutes
F_4 - Tuberose				B ₄	$= 30 \mathrm{mm}$	nutes
				B,	$= 45 \mathrm{mm}$	nutes



Fig. 1 a: Impact of different petal inks on colour retention after 30 days of written on 50 GSM paper. F_2 -Rose, F_7 - Clitoria; B_5 = 45 minutes.

Quantity of dye extract marked significantly among interactions. Maximum dye extract of 870 ml was noted in the interaction of Clitoria petals boiled for 10 minutes (F_7B_1) , which was followed bytuberose boiled for 10 minutes $(F_4B_1, 845 \text{ ml})$ and minimal quantity was obtained from rose with 45 minutes of time $(F_2B_5, 50.0 \text{ ml})$, which was on par with lotus boiled for 45 minutes $(F_5B_5, 55.5 \text{ ml})$.

It is speculated that interaction of clitoria petals for 10 minutes yields great amount of dye during the Table 2 : Microbial count (Total Fungal Count, cfu/ ml) of different extracted dyes at various boiling times after30 and 90 days of storage.

									Boiling	time (B)								
Flowers (F)		30 di	ays (× 1	0 ⁴ cfu/r	nl)			60) days (×	10 ⁻⁴ cfu/m	l)			90	days (×]	10 ⁻⁴ cfu/m	(1	
	\mathbf{B}_{1}	\mathbf{B}_2	B3	B₄	B,	Mean	B	\mathbf{B}_2	B ₃	\mathbf{B}_4	B,	Mean	\mathbf{B}_{1}	\mathbf{B}_2	\mathbf{B}_{3}	\mathbf{B}^{\dagger}	B,	Mean
F ₁	1	I	'		1		2.640 (1.907)	2.090 (1.756)	0 (1.000)	0 (1.000)	0 (1.000)	0.946 (1.333)	3.030 (2.007)	2.450 (1.856)	1.750 (1.657)	0.560 (1.249)	0 (1.000)	1.558 (1.554)
\mathbf{F}_{2}	1	1	1	1	1		2.650 (1.910)	2.150 (1.775)	0 (1.000)	0 (1.000)	0 (1.000)	0.960 (1.337)	3.130 (2.031)	2.560 (1.886)	1.650 (1.629)	0 (1.000)	0 (1.000)	1.468 (1.509)
F ₃	1	1	1		1	1	2.640 (1.908)	2.280 (1.809)	0 (1.000)	0 (1.000)	0 (1.000)	0.984 (1.343)	3.070 (2.016)	2.640 (1.908)	1.820 (1.678)	0.82 (1.349)	0 (1.000)	1.670 (1.590)
F.	1	I	1		1	1	2.670 (1.914)	2.380 (1.838)	0 (1.000)	0 (1.000)	0 (1.000)	1.010 (1.350)	3.150 (2.036)	2.750 (1.936)	1.980 (1.726)	1.03 (1.423)	0 (1.000)	1.782 (1.624)
F.	1	1	1		1	1	2.630 (1.904)	2.350 (1.829)	0 (1.000)	0 (1.000)	0 (1.000)	0.996 (1.347)	3.020 (2.005)	2.700 (1.933)	1.640 (1.623)	0 (1.000)	0 (1.000)	1.472 (1.512)
Ъ	1	1	1	1	1	1	2.620 (1.904)	2.360 (1.832)	0 (1.000)	0 (1.000)	0 (1.000)	0.996 (1.347)	2.990 (1.997)	2.720 (1.927)	1.690 (1.639)	0 (1.000)	0 (1.000)	1.480 (1.513)
F,	1	1	1		1		2.660 (1.913)	2.390 (1.841)	0 (1.000)	0 (1.000)	0 (1.000)	1.01 (1.351)	3.130 (2.033)	2.770 (1.942)	2.000 (1.732)	1.580 (1.605)	0 (1.000)	1.896 (1.662)
Mean	1	1	1	1	1	1	2.640 (1.909)	2.290 (1.811)	0 (1.000)	0 (1.000)	0 (1.000)		3.070 (2.018)	2.660 (1.913)	1.790 (1.669)	0.570 (1.232)	0 (1.000)	
Comparing Means			SEm	(Ŧ)	CD	5%		SEm(±)			CD @5%			SEm (±)			CD @5%	
Flowers (F)			I		'			0.004			0.012			0.002			0.006	
Boiling time (B)					'			0.004			0.011			0.002			0.005	
$\mathbf{F} \times \mathbf{B}$								0.010			0.028			0.005			0.013	
F ₁ -Chrysanthem Values in parent	um, F_2 -l hesis ar	Rose, F ₃ e square	-Marigo e root tr	ld, F ₄ -T ansform	uberose. 1ed valu	, F ₅ -Lotu tes.	Is, F_6 -Orc	shids, F_7 -	Clitoria;	$B_{1} = 10 m$	inutes, B	2 = 15 min	nutes, B ₃ =	= 20 minu	Ites, $B_4 = 3$	30 minute	$s, B_5 = 45$	minutes

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Fig. 1(b) : Impact of different petal inks on colour retention after 30 days of written on 70 GSM paper. F_2 -Rose, F_7 - Clitoria, B_5 = 45 minutes.



Fig. 1(c) : Impact of different petal inks on colour retention after 60 days of written on 50 GSM paper. F_2 -Rose, F_7 - Clitoria; B_5 = 45 minutes.

investigation. This might be due to small petals with less sugars and minutes boiling time. Petals with more sugar content extracted less amount of ink and this may be due to the increased consistency of the dye.

Colour retention of the ink extract after writing on the paper (50 and 70 GSM Paper)

Data illustrated in Figs. 1 (a) and 1 (b) indicated the values of ink colour on 50 GSM and 70 GSM papers after 30 days of storage period. The results graphically depicted the L, a, b values (RHS colour chart) of 50 GSM paper as 49, 31, -11 for T_{10} (Rose petals boiled for 45 minutes) and 57, -3, -34 for T_{35} (Clitoria petals boiled for 45 minutes) whereas on 70 GSM paper L, a, b values as 45, 33, -5 for T_{10} , the L, a, b values of T_{35} as 55, -2,-36.

The data shown in Figs. 1 (c) and 1 (d) represented the L, a, b values of ink colour on 50 GSM and 70 GSM papers after a storage period of 60 days. For 50 GSM paper, the L, a, b values for T_{10} (Rose petals boiled for 45 minutes) were 54, 25, -6, while for T_{35} (Clitoria petals boiled for 45 minutes), the values were 59, -2, -31 respectively. On 70 GSM paper, the L, a, b values for T_{10}



Fig. 1(d) :Impact of different petal inks on colour retention after 60 days of written on 70 GSM paper. F_2 -Rose, F_7 -Clitoria; B_5 = 45 minutes.



Fig. 1(e) :Impact of different petal inks on colour retention after 90 days of written on 50 GSM paper. F_2 -Rose, F_7 - Clitoria; B_5 = 45 minutes.

were 53, 26, -3 and for T_{35} were 57, -1, -33, respectively.

The data in Figs. 1 (e) and 1 (f) represented the ink colour values on 50 GSM and 70 GSM papers after 90 days of storage. For the 50 GSM paper, the L, a, b values were 60, 12, -5 for T_{10} (Rose petals boiled for 45 minutes) and 60, -1, -30 for T_{35} (Clitoria petals boiled for 45 minutes). For the 70 GSM paper, the L, a, b values were 58, 13, -1 for T_{10} and 59, -1, -30 for T_{35} .

The above findings related to colour retention of petal ink on 50 and 70 GSM papers. Among two papers the colour intensity is more in 70 GSM compared to 50 GSM papers. This might be due to quality of the paper. During the storage period from 30 to 90 days, colour slightly reduced on both the papers *i.e.*, lightness (L) value increased and their respective colour (a and b) values decreased. Compared to clitoria petal ink, rose petal ink on papers was degrade the more colour.

Microbial count (cfu/ ml)

Among interaction effect, highest fungal count (2.670 $\times 10^{-4}$) was recorded for T₁₆ (tuberose petals boiled for 10 minutes), whereas T₂ (chrysanthemum petals boiled



Fig. 1(f) : Impact of different petal inks on colour retention after 90 days of written on 70 GSM paper. F_2 -Rose, F_7 -Clitoria, B_5 =45 minutes.

for 15 minutes) showed lowest Total Fungal Count (TFC) (2.090 × 10⁻⁴), on the dyesafter 60 days of investigation. After 90 days of analysis, the maximum fungal count (3.150 × 10⁻⁴) was found in T₁₆ (Tuberose petals boiled for 10 minutes) and minimum count (0.560 x 10⁻⁴) was reported in T₄ (chrysanthemum petals boiled for 30 minutes (Table 2).

The findings indicate that shorter boiling times with higher moisture content in the ink lead to fungal infestation during storage. In contrast, longer boiling times with lower moisture content (resulting in higher consistency) resulted in zero fungal infestation in the inks.

Conclusion

From the results, it can be concluded that flower petals boiled for 45 minutes produced dyes/inks with good consistency, excellent colour retention on written papers, and zero microbial growth. Among the treatments, T_{35} (Clitoria petals boiled for 45 minutes) was found to be more preferable compared to T_{10} (Rose petals boiled for 45 minutes) in terms of colour retention, as T_{35} showed very little colour change during the storage period. Using natural ink instead of synthetic ink not only ensures effective performance, but also protects the environment.

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