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Natural Dye from *Nerium oleander* L. and Dyeing of Cotton Fabrics Using Different Mordants with Evaluation of Colour Fastness, Strength and Antifungal Properties

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ABSTRACT: Globally, there is growing demand in natural colourants from plants in the food, cosmeceutical and pharmaceutical fields. Generally, among the various categories of secondary metabolites, carotenoids, anthocyanins, and phenols are significant plant-based pigments. Coloured pigments possess remarkable anthocyanin related to anti-oxidant, anti-inflammatory and anti-metastatic features. The major objective of this work was to elucidate the coloured pigments from *Nerium oleander* L. ornamental flowering shrub and its dying properties in cotton fabrics. Natural dye from flower methanolic extract of *Nerium oleander* was extracted. Dying experiment on bleached (H_2O_2) cotton fabric with density of 140 g / square meter was used. Pre, simultaneous and post -mordanting action of cotton fabric samples were carried out using 1-3 % of various metallic salts such as ferrous sulphate, potassium dichromate, stannous chloride, potassium aluminium sulfate. Color strength of the dyed samples for different dyeing condition was assessed by means of K/S value. Best color strength was yielded for the samples dyed with *N. oleander* which were mordanted with Potassium aluminium sulfate and FeSO4. Color fastness of the selected dyed samples to water, washing, perspiration and rubbing were also ideal. In all cases mordanted samples with Potassium aluminium sulfate and $FeSO_4$ exhibited best results with simultaneous or post mordanting respectively. This results justifies designing a sustainable technology for mobilizing the usage of *N. oleander* for coloration of cotton fabrics. Fungicidal potential against *A. flavus*, *A. niger*, *F. moniliforme* and wash analysis also yielded significant results. The exquisite assessment of all tested properties of colored samples evolves this technology can be commercially implemented by considering large scale trails.

KEYWORDS: Natural colourant, flower extract, *Nerium oleander*, mordanting agents, K/S Value, color fastness, fungicidal.

INTRODUCTION

Natural dyes, extracted from plants, animals etc. shows minimum environmental impact and used not only in the coloration of textiles but also in the food ingredients and cosmetics[1,2,3]. The use of natural dyes by man was reduced by the invention of synthetic colorants in the 19th Century. Increased demand of synthetic dyes in the textile and other industries is due to its high aesthetic value and the colouring strength and cost efficiency. In the 20th Century, various studies on synthetic dyes were carried out in their conservation[4,5] .Besides their various uses in the textiles and other industries synthetic pigments contribute many problems also. Synthetic dyes cause toxicity and carcinogenicity in the human body. As a result of this human impacts reduce their use and increases the demand of natural pigments [6,7]. Regarding food colour industry trends, the use of natural food pigments has increased in foods and beverages as substitutes for their synthetic counterparts. This is mainly due to the highly awareness of the environmental threats and the potential side-effects of the chemicals that are used in the synthesis of food colorants [8].

Anthocyanins (in Greek anthos means flower, and kyanos means blue) , the natural water-soluble and non-toxic pigments widely existing in plant kingdom is a group of phenolic compounds imparting orange to blue colours to the plants [9]. Flavonoids, carotenoids, and chlorophylls are the major pigments that provide various colouration to flowers and fruits. Of which flavonoids are the most significant flower colour pigments, especially the anthocyanins [10]. Anthocyanins are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium or flavylium salts. The differences in the position of glycosidic bond binding, the type and number of sugars, the number of hydroxyl groups, the type and number of aromatic acids made the anthocyanin in six major groups namely, cyanidin(Cy), petunidin(Pt), peonidin(Pn), pelargonidin (Pg), delphinidin(Dp), and malvidin(Mv)[11]. The

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sugar-free counterparts of anthocyanins is anthocyanidins having sugar and acyl conjugates represent anthocyanins [12]. Of the six major anthocyanins the three non-methylated anthocyanidins Cyanidin ,delphinidin and pelargonidin are the most widespread in nature and being present in 80% of pigmented leaves, 69% of fruits and 50% of flowers. Based on the several studies the most widespread anthocyanin is cyanidin 3-glucoside and it is estimated that more than 400 anthocyanins have been present in nature.

Present study focuses on the extraction of natural dye from the methanolic extract of dark red flower of *Nerium oleander* L. *N. oleander* is an evergreen shrub or small tree in Apocynaceae family [13,14]. The Apocynaceae family is one of the largest families with 550 genera, has around 5,100 plant species included in the order Gentianales and subclass Asteridae being found with greater diversity including tropical trees, shrubs, herbs, stem succulents and vines [15,16]. Currently, anthocyanin is extracted not only from flowers but also from fruits, vegetables, coloured grains, by-products of fruit and vegetable processing etc.[17]. Colouring properties of anthocyanin is widely exploited in food and beverage industries [18,19].A wide variety of products using anthocyanin such as dairy products , low-pH beverages , solid food matrices *etc*.[20,21,22,23].

MATERIALS AND METHODS

Plant material

Fresh flowers of *Nerium oleander* were collected from the natural habitats of Kollam, Thiruvananthapuram district, Kerala.

Extraction of crude dye liquor

Dye extracts were carried from flowers of *N. oleander* by adopting standard alcoholic method. Natural dried and pulverized flowers (200g) were soaked in methanol for 30 minutes. The mixtures were gently heated to 40˚C and maintained for 30 min later the temperature was increased to between 60˚C and 90˚C to boil and it was maintained at the boiling temperature for 1 h to yield a crude dye extract. The extract was made to stand for 30min at ambient temperature and then filtered. The filtrate (dye liquor) was then immediately used for dyeing.

Pre-mordanting of cotton fabrics with flower extract of *Nerium oleander* and metallic salts:

H_2O_2 bleached cotton fabrics were subjected to pre-mordanting i.e., mordanted prior to dyeing using 1-3 % of any one of the chemical mordants like ferrous sulphate, potassium dichromate, stannous chloride, potassium aluminium sulfate followed by the methanolic extract of *N. oleander*, at 60^0C for 30 min with material-to-liquor ratio of 1:20.

Simultaneous mordanting of cotton with flower extract of *Nerium oleander* and metallic salts:

Bleached cotton were treated with the methanolic extract of *N. oleander* and metal salts simultaneously, using 1-3 % of any one of the chemical mordants like ferrous sulphate, potassium dichromate, stannous chloride, potassium aluminium sulfate at 60^0C for 30 min with material-to-liquor ratio of 1:20.

Post-mordanting of cotton with flower extract of *Nerium oleander* and metallic salts:

Bleached cotton was dyed with dye extract. The wetted out cotton samples were dipped into different dye baths containing required amount of dye extract and water. After 10 min required amount of sodium sulphate was added. After 20 minutes required amount of sodium chloride was added. The dyeing was carried out for one hour at 50^0C. The unwashed dyed samples were taken out, squeezed and used for treatment with metal salts. The dyed cotton samples were treated with different metal salts using 1-3 % of any one of the chemical mordants like ferrous sulphate, potassium dichromate, stannous chloride, potassium aluminium sulfate at 60^0C for 30 min with material-to-liquor ratio of 1:20.

In all the three methods, after the dyeing was over, the dyed samples were subjected to soaping with 2 g / l soap solution at 50^0C for 10 min, followed by repeated water wash and drying under sun.

Determination of surface colour strength (K/S value)

The K/S value of the dyed cotton was determined by measuring the surface reflectance of the samples using a computer-aided Macbeth 2020 plus reflectance spectrophotometer, using Kubelka Munk equation with the help of software:

$K/S = (1 - R\lambda_{max})^2 / 2 R\lambda_{max} = \alpha Cd$ where K is the coefficient of absorption; S the coefficient of scattering; Cd, the concentration of the due and $R\lambda_{max}$ the surface reflectance value of the sample at a particular wavelength, where maximum absorption occurs for a particular dye/colour component.

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Evaluation of colour fastness:

Color fastness properties were evaluated for the cotton dyed fabric following the standard protocols. Color fastness to wash, rubbing (dry and wet), water, perspiration, was accessed by using grey scale of color change and staining according to ISO 105 C04 (AATCC.2013), ISO 105 X12 (AATCC.2013), ISO 105 E01(AATCC.2008), ISO 105 E04(AATCC.2006) respectively.

Analysis of the fungicidal feature of dyed cotton fabrics

The antifungal activity of the dyed cotton fabrics against pathogenic fungi such as *A. flavus, A. niger, F. moniliforme* was analyzed quantitatively [24]. 5 ± 0.1 cm diameter circular discs of freshly dyed (flower methanolic extract of *N. oleander*) cotton fabrics were placed in PDA plates and sterilized for 15 min at 121°C. Spore suspensions (1000 µL) of each fungus were added to the center of fabric discs and incubated for 24 h at 37±1^{0}C. Test solutions of flower methanolic extract of *N. oleander* were made through ten-fold serial dilutions. A fixed volume of each dilution (100 µl) was inoculated on PDA plates and the plates were incubated at 37±1^{0}C for 24 hours. Untreated circular fabric discs of the same dimension were taken as control. Radial diameter in mm of fungal growth on the agar plates (control & treated) was measured and the % of reduction in the fungal growth was calculated using the following formula:

R (%) = A-B/A x 100

Where R = Reduction in fungal growth; A = Fungal growth on the control (untreated fabrics), and B = Fungal growth on the treated fabrics.

Wash durability

The treated fabrics were kept in a beaker containing a standard detergent solution with only 2% on the weight of the fabric [25,26]. The washed fabrics were tested for the retention of antibacterial activity for the second, fourth and sixth laundry washes using AATCC 147 method and the zone of inhibition (mm) was recorded.

Statistical analysis

The data obtained from the studies were represented as Mean ± SD using the Graphpad Instat version 3.05 and Microsoft Excel 2010 were used for statistical analysis.

RESULTS AND DISCUSSION

Natural colorants are eco-friendly and biodegradable as these are obtained from natural sources such as flowers and other parts of the plant body. The plant based dye has been widely used for a long period and increased consumer importance in the usage of textile industries.

Dyeing with cotton fabrics

Bleached cotton fabrics mordanted with varying concentration of mordants have been subsequently dyed by pre, simultaneous and post-mordanting protocols. All the dyed fabrics have been assessed for their colour strength (K/ S) value and were narrated in the Table 1. Surface colour strength by ferrous sulphate was 1.82, 2.61, 2.45 with pre-mordanting, simultaneous mordanting and post-mordanting respectively. Similar values with potassium dichromate were 1.44, 1.56, and 1.70; stannous chloride was 1.61, 1.70, and 1.94 respectively. Potassium aluminium sulfate also displayed optimal values (1.89, 2.71 and 2.64). Interestingly, all the mordant displayed maximal values with post-mordanting method, meanwhile ferrous sulphate and potassium aluminium sulfate with simultaneous mordanting.

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Table I**:** Surface colour strength of dyed cotton fabrics after pre, simultaneous and post –mordanting protocols by using 1-3% mordant doses K/S(λ=420 nm)

Mordant Concentration (%)	Mordants	Pre-mordanting	Simultaneous mordanting	Post-mordanting
1%	Ferrous sulphate	1.75	2.08	2.14
2%		1.79	2.23	2.45
3%		1.82	2.61	2.45
1%	Potassium dichromate	1.27	1. 36	1.53
2%		1.32	1.48	1.65
3%		1.44	1.56	1.70
1%	Stannous chloride	1.48	1.77	1.88
2%		1.52	1.69	1.92
3%		1.61	1.70	1.94
1%	Potassium aluminium sulfate	1.78	2.33	2.27
2%		1.87	2.45	2.36
3%		1.89	2.71	2.64

All the dyed cotton fabrics was evaluated for their colour fastness behaviour to washing, rubbing and exposed to light and perspiration (acidic and alkaline) and were recorded in the Table II. All the treated cotton fabrics subjected to light which show fairly good (4) to light fastness and excellent grade to washing fastness. Further, all the treated cotton fabrics showed no colour staining to washing fastness. The colour change to dry and wet rubbing for cotton fabrics was excellent (5 and 4-5). There was marginal colour staining except for simultaneous mordanting where it was negligible staining (4-5). Potassium dichromate mordant on cotton showed low perspiration values under acidic and alkaline conditions (3) under different mordanting methods.

Table II: Colourfastness of dyed cotton fabrics with selective mordants using pre, simultaneous and post–mordanting methods

Mordants	Method of mordanting	Mordant conc (%)	Washing		Rubbing		Sun light	Perspiration			
					Dry	Wet		Acidic		Alkaline	
			CC	CS	CC	CS		CC	CS	CC	CS
Ferrous sulphate	Pre-mordanting	1 2 3	5 5 5	4 4 5	5 5 5	5 5 5	4 4 4	5 5 4	5 5 5	5 5 5	5 5 5
	Simultaneous mordanting	1 2 3	5 5 5	4 -5 4 -5 4	5 5 5	5 4-5 5	4 4 4	5 5 5	5 5 4	5 5 5	5 5 5
	Postmordanting	1 2 3	5 5 5	4 -5 4 -5 4	5 5 5	5 5 5	4 4 4	5 5 5	5 5 5	5 5 5	5 5 5
Potassium dichromate	Pre-mordanting	1 2 3	5 5 5	4 4 4	5 5 5		4 4 4	3 3 3	5 5 5	3 3 3	3 3 3
	Simultaneous mordanting	1 2 3	5 5 5	4 4 4	5 5 5		4 4 4	3 3 3	5 5 5	3 3 3	3 3

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	Postmordanting	1 2 3	5 5 5	4 4 4	5 5 5		4 4 4	4 3 3	5 5 5	3 3 3	4 3 3
Stannous chloride	Pre-mordanting	1 2 3	5 5 5	4 4 4	5 5 5		4 4 4	4 4 4	5 5 5	4 4 4	5 5 5
	Simultaneous mordanting	1 2 3	5 5 5	4 4 4	5 5 5		4 4 4	4 4 4	5 5 5	4 4 4	5 5 5
	Postmordanting	1 2 3	5 5 5	4 4 4	5 5 5		4 4 4	4 4 4	5 5 5	4 4 4	5 5 5
Potassium aluminium sulfate	Pre-mordanting	1 2 3	5 5 5	4 4 4	5 5 5		4 4 4	4 4 4	5 5 5	4 4 4	5 5 5
	Simultaneous mordanting	1 2 3	5 5 5	4 4 4	5 5 5		4 4 4	4 4 4	5 5 5	4 4 4	5 5 5
	Postmordanting	1 2 3	5 5 5	4 4 4	5 5 5		4 4 4	4 4 4	5 5 5	4 4 4	5 5 5
Control			4 -5	4 -5	4	5	5	5	5	4	4

The perspiration fastness grades ranged from 4 to 5 for the fabrics in acidic and alkaline conditions. There was no colour staining (5) for the fabrics in both acidic and alkaline conditions. From the table it is possible to speculate that among the three mordanting methods, simultaneous mordanting yielded good results (K/S value) when compared to other mordanting system with ferrous sulphate. However, for the other mordants analyzed it was post mordanting was ideal than others.

It was noticed that among differently mordanted bleached cotton subsequently dyed with 5 % methanol flower extract of *N. oleander* with 3 % ferrous sulphate by simultaneous mordanting rendered the fabric relatively higher K/S value (~2.61) when compared to other mordanting protocols. The application of 3 % stannous chloride and copper sulphate by simultaneous mordanting method rendered 1.94 K/S value.

Among all the mordants analyzed, the increase in K/S value was found to be maximal for ferrous sulphate mordant due to the inherent colour of ferrous sulphate salts. This is more predominant on cotton. The increase in colour strength K/S values after pre, simultaneous and post-mordanting with selective mordants (1-3%) on cotton fabrics were in the following sequence: Potassium aluminium sulfate > Ferrous sulphate > Stannous chloride > Potassium dichromate.

Remarkable K/S value to a different extent after pre, simultaneous and post-mordanting may be because of the changes in scattering due to the chemical interaction between fibres and metallic salts along with the additional inherent colour input of the corresponding mordants.

Thus, based on the dyeing results, the simultaneous mordanting with 3 % FeSO4 + methanol flower extract of *N. oleander* and 3 % Potassium aluminium sulfate or stannous chloride + flower extract of *N. oleander* were found to be more prospective i.e., yielded higher degree of increase in surface colour strength. The observed highest K/S value indicates the synergistic intensification of colour yield may be due to higher absorption and fixation of the dye by the complex formed between the Fe/ Al salts and the glycosidic bonds in the fabrics.

The dyes extracted from the stem bark of *Albizia coriaria* yielded diverse color shades with multiple mordants[27]. The cotton fabrics dyed without the use of mordants exhibited optimal wash fastness of 4-5, ideal dry and wet rub fastness of 5, and a moderate light fastness of 4. Kumaresan., *et al.* evaluated the dye from *Achras sapota* and observed that the application of 3 % of $Al_2(SO_4)_3$

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and 3 % of $FeSO_4$ displayed better mordanting system[28]. The surface colour strength indicates that the 60 min dyeing time, 60°C dyeing temperature, 1:20 material-to-liquor ratio, 3 % mordant concentration, 5 % dye concentration and 5g /1 common salt were the optimum values with minor differences among the different silk and cotton fabric mordant system analyzed. Thy study also visualized the effect of myrobolan and metallic mordants. The mordant dyeing on plain weave cotton and silk fabrics with dye reported from *Albizia coriaria, Vitellaria paradoxa, Morinda lucida* and *Harungana madagascarensis*[29]. Mordant dyeing under optimized conditions enhanced color strengths and fastness on cotton and silk fabrics. Silk fabrics required heating (80˚C to 95˚C) for 1hr 30 mins than cotton to achieve colou strengths. The fastness varied from 3 to excellent 5 with cotton giving better fastness. However, silk recorded better color strength 110 to 260 in term of k/s values. Dye from *M. lucida* was superior on both fabrics and with both mordants. Study results of Chhipa., *et al*. (2017) reflected that peanut pod dye was highly suitable for Cotton material with ferrous mordant.[30] The pre-mordant with 10 and 15% of dye were ideal for Cotton material. The dyeing quality of silk fabrics with lac versus mordants like potassium aluminium sulfate, stannous chloride, ferrous sulfate and sodium chloride with simultaneous mordanting using pad-dry and pad-batch protocol on colour characteristics has already reported[31]. The data revealed that silk fabric dyed without and with stannous chloride and sodium chloride mordant yielded shade of light pink, while those with aluminum potassium sulfate pink shade. Light grey shades by adding ferrous sulfate mordant. The washing fastness property showed insignificant level, while light fastness was satisfactory. Nasim-Uz-Zaman., *et al*. (2018) analyzed the color strength, color fastness, water washing, perspiration and rubbing of *Bixa orellana* mordanted with $CuSO_4$[32] .The mordanted samples exhibited ideal results. *Ficus cunia* with diverse mordants and different mordanting protocols were also studied[33] .The natural dye yielded wide array of shades using different natural and synthetic mordants. The washing, rubbing and light fastness features were good to excellent fastness grades.

Fungicidal potential of dyed cotton fabrics

Many herbals have been evaluated as a source of natural colourant having potentiality to impart functional features to textiles such as microbicidal, insecticidal deodorizing, and UV guarding effect, besides imparting attractive colours. Due to the occurrence of potent secondary metabolites especially anthocyanins, phenols offer microbicidal properties, hygienic, and therapeutic roles. Thus, the colourants with functional properties have emerged as a high priority area in textile finishing and a key factor for protective clothing.

Fungicidal feature of dyed cotton fabrics exhibited optimal reduction of fungal growth in *A. flavus* followed by *A. niger*. Dyed cotton fabrics with *F. moniliforme* showed lesser growth reduction among the tested fungal species (Table III). 84.5% reduction of fungal growth (%) was seen with *A. flavus* at 5% colourant. Meanwhile, 50% reduction of fungal growth (%) was noticed with *F. moniliforme* at the similar dose.

The fungicidal assays suggest an exciting opportunity for the textile materials dyed with natural dye that can be useful in developing protective clothing to protect users against common infections caused by pathogens. The minimum inhibitory concentrations of dye from *Barleria prionitis* against tested fungi such as *Aspergillus niger, A. flavus, A. parasiticus, Fusarium moniliforme* and *Penicillium canescens* were ranged between 22.50-23.50 µg/mL[34]. Dyed silk, wool, and cotton fabrics also showed remarkable antifungal efficacy against the tested fungi. Dyed silk fabrics exhibited the maximum growth reduction followed by wool and cotton. The logwood dye in combination with alum and ferric chloride mordants inhibited the fungal growth of *Fusarium solani* and *Penicilium decumbens* are also studied[35]. On the other hand, treatment with cochineal dye alone and in combination with mordants was not effective against *Fusarium solani* and *Penicilium decumbens*. The data displayed an increase in inhibition % when dyeing cotton tissue was dyed with rhubarb dye by 6.95%, while reverse trend was noticed when the polyester fabric treated with rhubarb dye by 8.21%.

Natural colourant from aerial parts of *Perilla frutescens* was employed against the fabrics inoculated with pathogenic fungi viz., *Aspergillus niger, Aspergillus flavus, Fusarium moniliforme, Fusarium solani,* and *Penicillium decumbens* and was evaluated by agar-well diffusion method. The MIC of the natural dye against each of the test fungi was determined by broth dilution method. Dyed silk, wool and cotton fabrics were also evaluated for antifungal activity by standard method. The natural dye showed antifungal activity against all the five test fungi in a concentration dependent manner. The treatment dose of 1000µg/ml recorded the highest growth reduction in all the test fungi, nearly at par with the positive control. The MICs of natural dye against the test fungi ranged

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from 32.39 to 36.50µg/ml. All kinds of dyed fabrics also showed remarkable antifungal efficacy against the test fungi. Dyed wool fabric exhibited the maximum growth reduction followed by silk and cotton [36].

Table III: Antifungal activity of dyed cotton fabrics against tested fungi

Concentration	Fungicidal potential of dyed fabrics against tested fungi Reduction in fungal growth (%)		
	A. flavus	*A. niger*	*F. moniliforme*
2.5 mg/ml	70.2	62.4	43.3
5 mg/ml	84.5	75	50

Wash durability

Cotton fabrics treated with *N.oleander* extract managed to maintain an approximately 60.3% of its initial antifungal features after the 8th wash in *A. flavus* with an average zone of inhibition of 13 mm (Fig. 1). However, *F. moniliforme* retained about 36.5 % of its initial antifungal features .Similar values for *A. niger* was 51% only. During 16th wash the values were reduced to 12, 8 and 4% respectively among the species. The effect of mordant salts on antibacterial activity of wool fabric dyed with pomegranate and walnut shell extracts also recorded[37]. They recorded significant tannin content act as a natural mordant which strengthen the dye attachment to the fabric and thus, resulted in the higher color strength of the natural dye into the fabric. These data strengthen and supports the results obtained from current study and thus explains why *N.oleander* extract treated cotton fabric able to retain optimal levels of it initial antifungal powers even after 8th washing.

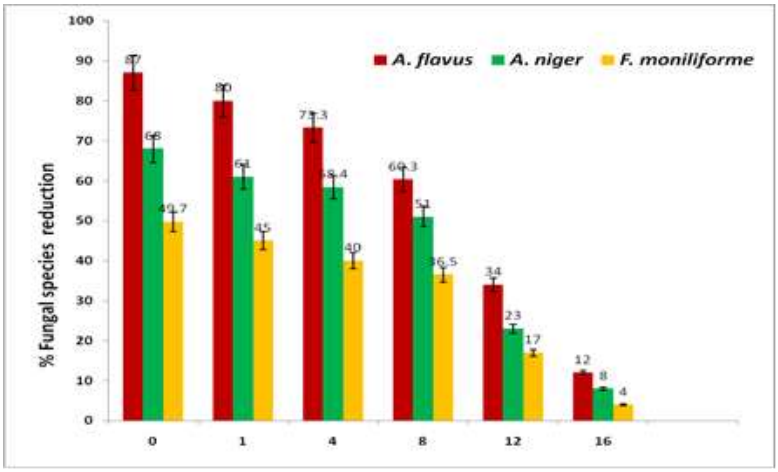

Figure 1: Number of wash cycles on cotton fabrics dyed with *N.oleander* flower extract

CONCLUSION

The present work showed that, natural colourants extracted from the flowers of *N.oleander* can be used as a dye for cotton fabrics. Optimal colour fastness can be obtained by incorporating a mordant ferrous sulphate in simultaneous mordanting technique. Stannous chloride, copper sulphate, potassium dichromate yielded moderate results. Similarly, the mordanted fabric yielded the best colour fastness property to washing, perspiration and daylight. Fungicidal and wash durability results also were remarkable. The overall data suggest that *N.oleander* may be a good source of natural dye with functional properties and can be commercialized in dyeing and protective finishing of different kinds of textile fabrics.

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