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Effect of fungi on dyes used in making Nigerian tie and dye cloths ('Adire') and shelf-life determination

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Abstract

Dye quality and fastness properties could only be guaranteed for a finite period of time on shelf. In this study, the shelf-life of natural indigo dye and indigo VAT dye was studied for 360 days. Physico-chemical parameters monitored includes; total viable count, viscosity, pH, specific gravity, absorbance and transmittance from 360 days. Fungi isolated from the dyes on shelf were *Achaetomium strumarium*, *Aspergillus niger*, *Aspergillus flavus*, *Duitina rugosa*, *Penicillium citrinum* and *Peyronellaea prosopidis*. The fungal population count was monitored monthly throughout the shelf-life study period. Multiple linear regression model was used to predict the shelf-life of the dyes from the growth data of the isolated organisms and the physico-chemical parameters. The total fungal viable counts ranged from 1.267×10^5-3.213×10^5 spores ml^{-1} for indigo VAT dye and 1.453×10^5-3.347×10^5 spores ml^{-1} for natural indigo dye over the 12 months study period. The changes in the physico-chemical parameters ranged from pH 12.94±0.00-10.39±0.01 and 12.54±0.00-10.01±0.01, specific gravity 1.279±0.00-1.106±0.005 and 1.445±0.00-1.176±0.001, viscosity 1.288±0.00-1.215±0.001 cSt and 1.283±0.00-1.176±0.001 cSt, optical density 1.627±0.002-3.323±0.001 and 1.705±0.001-3.315±0.002, transmittance 5.573±0.003-2.652±0.001 and 6.124±0.002-2.108±0.001 for indigo VAT dye and natural indigo dye respectively. The percentage dye exhaustion of the shelf-life products determined after the study period peaked at 88±0.001 and 90±0.001 % for indigo VAT dye and natural indigo dye respectively. The shelf-life were found to be 17 months and 10 months for indigo VAT dye and natural indigo dye respectively. The synthetic indigo VAT dye has a higher shelf-life than the natural indigo dye. Imported indigo VAT dye is however recommended based on its longer shelf-life. The results further buttressed the trend where textile dyers in Nigeria and indeed the West African region largely employ imported indigo VAT dye than locally produced natural indigo dye.

Keywords: Shelf-life; Nigerian tie and dye cloths 'Adire'; indigo VAT dye; natural indigo

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Introduction

Dyes (either natural or synthetic) can last for a finite or specified period of time if stored properly. Amongst vat dyes, indigo is commonly used for the manufacture of denim. Over time on the shelf, there is an observable change in colour, quality and occasionally quantity of the dyes which lead to decrease in their economic value (Bankole et al., 2017). The change in colour causes decrease in the quality and shelf-life of the dyes. Efforts geared at controlling the effect of fungi and change in the dyes physico-chemical

parameters on shelf has not been fully explored. Dyes are versatile chemicals which are employed for several purposes in textiles, leathers, plastics, cosmetics, pulp, paper making, food and pharmaceutical industries, agricultural research, light-harvesting arrays, desizing, scouring, bleaching, finishing, photo-electrochemical cells and hair colouring (Kiran et al., 2009; Aksakal and Ucun, 2010).

Fungi are ubiquitous and thrive pathogenically on most natural and synthetic substrates. The advent of synthetic dyes brought about tremendous decline in the use of natural dyes because the existing natural dyes failed to fulfil market demands (Singh and Purohit, 2012). Thousands of synthetic dye varieties have since been prepared and used in place of traditional natural dyes (Zollinger, 2003). The limitations to the use of natural dyes in textile dyeing are colour yield, standardization, complexity of dyeing process, limited shades, blending problems and inadequate fastness properties (Sachan and Kapoor, 2005). The use of natural dyes became less pronounced recently with the discovery of reactive textile dyes.

Sporulation of fungi on dyes synergistically with other factors causes decrease in the shelf-life of the dyes used in making Nigerian native cloths. The effect that changes in the physico-chemical parameters (optical density, pH, Specific gravity, Transmittance and Viscosity) brings about in the quality, economic value and shelf-life of the dyes has also not been fully explored. Dyes are well preserved over a long period of time if the manufacturer's instructions are adhered to strictly but their quality and stability depend on the concentration, exposure to moisture and light, pH, heat and microbial infestation. Information on the shelf-life of dyes is quite elusive to researchers, textile practitioners and industry players. The dye decolorization potential of fungi makes significant impact on the quality and shelf-life of the dyes over a period of time. Shelf-life of dyes depends on the degradation mechanism of the dye constituents. Physico-chemical parameters of products are mostly used as variables in shelf-life determination through mathematical modelling (Azanha, 2005). Prediction of shelf-life through mathematical modelling of changes in product characteristics that depletes over time. The study will provide accurate data and information on the effect of fungi and changes in the physico-chemical parameters of the dyes used in the Nigerian tie and dye cloths. The shelf-life of synthetic indigo VAT dye was higher than the natural indigo dye produced locally. This study has established that fungi grow on dyes which causes their gradual depletion of colour, economic value, quality and fastness properties on shelf. The findings in this study further established that the sporulation of fungi along with changes in the physicochemical parameters led to a decrease in shelf-life of the dye. This probably might have contributed to the loss of natural indigo dye's widespread utilization, frequency of use, commercial relevance and market value in today's world of tie and dye practice. Hence this present study is to evaluate the effect of fungi on the shelf-life of indigo dyes and their exhaustion properties.

Materials and Methods
Chemicals
The indigo VAT dye was purchased from DyStar Company, Frankfurt, Germany while natural indigo dye was kindly donated by Nike Arts Gallery, Osogbo, Osun State, Nigeria which is a foremost tie and dye manufacturing. They were collected in sterile airtight plastic can and stoppered before experimental usage.

Description of the indigo dye
Indigo dye belongs to the VAT class of dyes with Molecular weight: 262.26 g $mol^{-1;}$ Molecular formula: $C_{16}H_{10}N_2O_2$ and chemical structure (Figure 1).
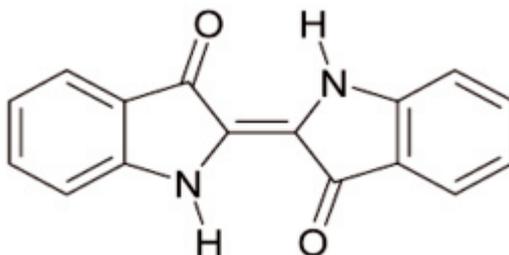

Figure 1: Chemical structure of indigo dye

Isolation and identification of fungi from Indigo VAT and Natural indigo dyes
Aliquots were prepared by seeding 1 ml of the 500 mg L^{-1} of the concentrations of the dyes in sterilized 10 ml test tubes containing 9 ml of sterile distilled water. The test tubes containing the mixture was shaken for homogenization and the contents were serially diluted in ten (10) folds. 1 ml aliquots of 10^{-2}, 10^{-6} and 10^{-8} (representing low to medium and high dilutions) were then

plated on already prepared plates containing sterile solidified potato dextrose agar medium which were then incubated at room temperature (30 °C) with daily inspections for growth of fungal colonies and re-isolation till a pure isolate is obtained. This was done repeatedly on a monthly basis for the 12 months period of shelf-life study, estimation and prediction. Neubauer Improved Counting Chamber system (Haemocytometer) was used in taking monthly measurement of fungi growth on the dyes (APHA, 2005). The 23S ribosomal ribonucleic acid (rRNA) analyses were contracted out to Laragen Incorporated, USA. Basic Local Alignment Search Tool (BLAST) query was conducted with previous submissions obtainable in the GenBank database. Submission of the molecular sequence data was done at the GenBank using the BankIt tool (Bankole et al., 2017).

FTIR characterization of natural indigo dyestuff and indigo VAT dye at day 0 and day 360.
The method of Dolores et al. (2008) was adopted. UV–visible spectrum analysis of the Natural Indigo dye was carried out immediately after production using Shimadzu UV spectrophotometer (Shimadzu UV 1600, Japan) with dilution and changes in its absorption spectrum of the dyes in the visible range (200–800 nm) were recorded. Fourier Transform Infrared Spectroscopy (FTIR) spectrum of natural indigo dyestuff and indigo VAT dye was carried out using FTIR model800 spectrophotometer (Shimadzu, Japan) (Olukanni et al., 2010). The samples (control at day 0 and 360 days metabolites) were previously mixed with Infrared (IR) grade Potassium Bromide (KBr) in the ratio 10:90, fixed in sample holder before scans were done.

PHYSICO-CHEMICAL PARAMETERS MONITORING
pH
The method of Obidi et al. (2010) was used. The pH of the dyes was measured and monitored with the aid of Hanna pH meter (Hanna pH Meter 290, Hanna Instruments, Rhode Island, USA). The meter was calibrated and standardized with sterile distilled water (pH 7.00) and phosphate buffer (pH 4.00).

Kinematic Viscosity
Kinematic viscosity of the various prepared dye concentrations was determined using a glass capillary tubular viscometer (Capirograph Toyoseikl Seisaku-Sho Ltd., Ibaraki, Japan) as described by Rammohan and Yassen (2003) and by following Japanese Pharmacopeia specifications. The dye sample was allowed to flow through an outlet tube (measuring tube which is narrowed into a capillary tube above the outlet). Two annular reference marks on the measuring tubes were used. The time (in seconds) it took each sample meniscus to drop from the upper to the lower reference mark was taken manually with a stop-watch and recorded accordingly in Centistokes (cSt). The viscosity was then calculated by multiplying the measured time by the viscometer calibration factor CF, 0.0009418 at room temperature (30 ^{0}C).

Viscosity = Time (in seconds) × CF

Where CF= Viscometer Calibration Factor=0.0009418.

Specific gravity
Specific gravity was determined using a pycnometer (Ohwoavworhua and Adelakun, 2005) with slight modification and in accordance with Japanese Pharmacopeia specifications. A sterile dried and emptied pycnometer of 50 ml capacity was weighed and the weight recorded as W_1. The pycnometer was then filled with water and weighed. The weight was recorded as W_2. From the 1 litre formulations of different dyes sample, 50 ml was transferred into the pycnometer. The pycnometer and its dye content were weighed and the weight was recorded as W_3. The pycnometer was shaken many times to allow all trapped air within to be expelled when filled with either the dye sample or sterile distilled water. Specific gravity was then calculated and recorded in triplicates using the formula:

$$SG = \frac{W2 - W1}{W2 - W1}$$

Spore count
The method of Gupta (2010) was used to count the spores on observation of full growth within 48-72 hours of fungal cultures. The spore suspension was prepared by adding 5 ml of distilled water to each of the plates (in triplicates) and shaken well so that the spores were dislodged and homogenized. Spore lumps were dislodged using a sterile scalpel. 1 ml was then taken with the use of a sterile pipette and mixed with 2 % Trypan blue in a sterile test tube. The coverslip was then fixed on the Neubauer Improved Counting Chamber system (Superior Marienfeld Laboratory Glassware, Germany). Ten microliters (10µl) aliquot of the spore suspension mixture with Trypan blue was placed beside the coverslip of the chamber and allowed to flow by capillary action for 2 minutes until the stop of flow. The Trypan blue added was to differentiate and count only the viable cells in the suspension. The chamber was then placed under a light microscope for enumeration of the number of

spores under ×10 objective magnification and the numbers of spores in 5 squares were counted. The spore count was monitored in each of the conical flasks of different concentrations (in triplicates) set of shelf-life determination and recorded accordingly. This was done on monthly basis for the 12 months period of shelf-life study. The number of spores ml^{-1} was calculated using the formula:

Absorbance and Transmittance

The method of Obidi et al. (2010) was employed. The absorbance was monitored and recorded at a wavelength of 600 nm with a Spectrophotometer (UNICO 1200 Series, United Products and Instruments Incorporated, USA). The spectrophotometer was standardized by adjusting it to read 100 % light transmittance with 5 ml distilled water at a wavelength pre-set at 600 nm. Five (5 ml) of diluted dye samples of different concentrations were poured in the cuvette and then placed in the spectrophotometer. The top scale readings represented the absorbance taken on a monthly basis. The absorbance and transmittance for each of the dye concentrations under shelf-life study was done in triplicates.

SHELF-LIFE STUDY

Multiple linear regression model of Bankole et al., 2017 was employed in the shelf-life study with slight modifications. 500 mg L^{-1} concentrations each of natural indigo dye and indigo VAT dye solutions were prepared and stocked in twelve (12) different well corked, airtight Erlenmeyer flasks for each of the dye formulations. The formulations of each of the concentration of the dyes were done in triplicates. Physico-chemical parameters (Specific gravity, Transmittance, Absorbance, pH and Viscosity) and spore count were then observed, monitored and recorded (in triplicates) in each flask on a monthly basis for twelve (12) months.

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + U_T$$

Eqn. 1

Where β_0 is the regression intercept, $\beta_1 - \beta_6$ are regression parameter estimates (at $0 - 360$ days) $X_1 - X_6$ (Specific gravity, Transmittance, Absorbance, pH and Viscosity) are the regression coefficients of the dye under shelf-life study, Y_i (the Dependent Variable) is the time (Days), U_T is the error term.

$$Y_i = \beta_0 + \beta_1(Absorbance) + \beta_2(pH) + \beta_3(Specific\ gravity) + \beta_4(Spore\ count) + \beta_5(Transmittance) + \beta_6(Viscosity) + U_T$$

Eqn. 2

DETERMINATION OF DYE PERCENTAGE EXHAUSTION

Hussain et al (2004) method was used in the determination of the dye percentage exhaustion of cotton fabric (E %) and calculated thus:

$$\% \text{ Dye Exhaustion} = \frac{A_o - A_t \times 100}{A_o}$$

where A_0 = Absorbance taken before the dyeing process at maximum wavelength (λ_{max} = 620 nm), A_t = Absorbance taken during the dyeing process {at time (t=20, 40, 60, 80, 100 and 120 minutes)}. The dyeing of cotton fabric and dye exhaustion percentage was carried using the control dye on day 0 and the 360 days shelf-life product of each of the dyes.

FASTNESS PROPERTIES ASSESSMENT OF THE DYES AND THEIR SHELF-LIFE PRODUCTS
Fastness to washing and rubbing

This was done using the method of Sahoo et al. (2012). The dimension of the dyed fabric was 5 cm X 4 cm. Each dyed piece was placed between two pieces of undyed cotton and nylon fabrics of the same dimension. Stitching was done around the edges to make a composite specimen. Both fabrics were rubbed by hand vigorously against one another. The specimen was later withdrawn, rinsed and air-dried. Greyscale was used in assessing the change in colour and the staining of the adjacent undyed cloth.

Fastness to light

The dyed fabrics were cut into strips. The strips were later mounted on a black cardboard paper. This was achieved using the method of Haruna et al. (2013). The portions (half) portions of the fabric strips were covered to block illumination to that portion. The specimens were exposed to natural sunlight and fluorescent lamp light for 14 days. The strips were later removed. Greyscale was deployed in the assessment of fading of the dyed fabric strips.

Fastness to perspiration

Acidic and alkaline perspiration solutions were prepared to assess the fastness properties. Dyed and undyed fabric strips were immersed separately in alkaline and acidic solutions using liquor ratio of 20:1. The specimens were left in the solutions for 30 minutes at room temperature after which the specimens were brought out, drained and placed in between two glass slides for four hours. They were later dried upon removal. Geometric greyscale was used in assessing the colour change of the dyed fabrics and degree of staining of the adjacent white fabrics. This was achieved using the method of Haruna et al. (2013).

Results and Discussion
Phenotypic and molecular characterization of the isolates

Five isolates were obtained from both dyes during the shelf-life study period (Table 1). The isolates were documented in the GenBank with Accession Numbers

Table 1: Fungi isolated during the shelf-life study period

Isolates	Achaetomium Strumarium	Aspergillus flavus	Aspergillus niger	Duitina rugosa	Penicillium citrinum	Peyronellaea prosopidis
Dye						
Indigo VAT	+	+	+	+	+	+
Natural Indigo	+	+	+	+	+	+

+ = Present

FTIR CHARACTERIZATION OF THE DYES (CONTROL) AND THEIR 360 DAYS METABOLITE

In the 360 days shelf-life metabolite (Figure 2), it could be observed vividly that the broad peak at 3443.06 cm^{-1} indicated the formation of more secondary alcohol molecules. It is also obvious that there is the formation of carboxylic acids being represented by sharp absorbance peaks at 1641.48 cm^{-1} and 1685.54 cm^{-1}. On further observation of the IR spectra of both the control and 360 days shelf life metabolite of the Indigo VAT dye, it showed clearly that there has been a removal of the basic functional groups from 6 peaks to 4 peaks and from 11 peaks to 10 peaks in all.
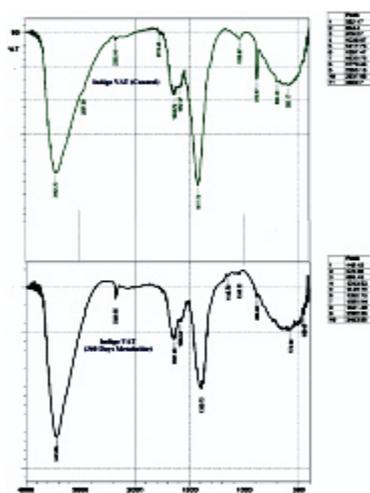

Figure 2: FT-IR Characterization of the Indigo VAT Dye (Control) and 360 Days metabolite

In the 360 days shelf-life metabolite (Figure 3), it could be observed vividly that the broad peak at 3444.98 cm^{-1} indicated the formation of more secondary alcohol molecules, The corresponding carbonyl group which depicted in the control (C-O stretching) the primary and secondary alcohol functional groups by the sharp absorbance at 1072.46 cm^{-1} and 1126.47 cm^{-1} respectively has been removed. On further observation of the IR spectra of both the Control and 360 days shelf-life metabolite of the Natural indigo dye, it showed clearly that there has been a removal of the basic functional groups from 5 peaks to 4 peaks and from 22 peaks to 9 peaks in all.
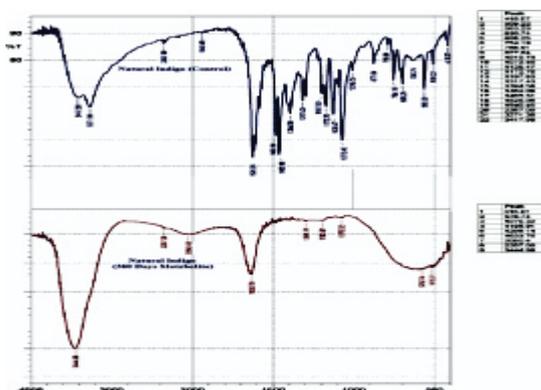

Figure 3: FT-IR Characterization of the Natural Indigo dye (Control) and 360 Days metabolite

PHYSICO-CHEMICAL PARAMETERS MONITORING

The total fungal viable counts were 1.267×10^5-3.213×10^5 spores ml^{-1} for indigo VAT dye and 1.453×10^5 -3.347×10^5 spores ml^{-1} for natural indigo dye. The changes in the physico-chemical parameters ranged from pH 12.94 ± 0.00-10.39 ± 0.01 and 12.54 ± 0.00-10.01 ± 0.01, specific gravity 1.279 ± 0.00-1.106 ± 0.005 and 1.445 ± 0.00-1.176 ± 0.001, viscosity 1.288 ± 0.00-1.215 ± 0.001 cSt and 1.283 ± 0.00-1.176 ± 0.001 cSt, absorbance 0.545 ± 0.002-0.956 ± 0.001 and 0.705 ± 0.001-0.989 ± 0.002, transmittance 1.573 ± 0.002-0.558 ± 0.02 and 1.673 ± 0.02-1.165 ± 0.02 for indigo VAT dye and natural indigo dye respectively. It is also noteworthy that the basicity of all the dyes increases with increasing concentration. A remarkable steady decrease was observed in the specific gravity of all the dye solutions studied and across different concentrations of the dyes. The decrease in specific gravity is attributable to the activities of fungi and changes in other physico-chemical parameters which cause the disintegration of intermolecular forces responsible for the covalent bonds and cohesion of the dye molecules. This in turn causes reduction in the weight of the solution. The increase in number of spores is attributable to the growth and sporulation of fungi on all the dyes. The increase is also as a result of the fact that all the fungi isolated ab initio grows and thrive well in near neutral, neutral pH medium to a high acidic medium.

PERCENTAGE DYE EXHAUSTION

The percentage dye exhaustion rate recorded for both indigo VAT dye (control) and shelf-life product (day 360) was 83 % and 88 % at 120 hours respectively (Table 3). The percentage dye exhaustion rate recorded for both natural indigo dye (control) and shelf-life product (day 360) was 88 % and 90 % at 100 hours respectively (Table 3). The percentage dye exhaustion rate recorded by shelf-life product of the dye was significantly greater than the control dye. The higher exhaustion percentage rate of the dyes shelf-life product recorded might not be unconnected with the fact that there has been dissociation and depletion of the bonding structures of the dye and the intermolecular forces of the solution making it possible for the cotton fabric to exhaust them at a very fast rate than the control dyes

Table 3. Percentage dye exhaustion of the indigo VAT dye and natural indigo dye and the shelf-life product.

Time (hr)	Indigo VAT dye (%)		Natural indigo Dye (%)	
	Control	Test	Control	Test
20	22 ± 0.12	25 ± 0.27	43 ± 0.55	55 ± 0.11
40	63 ± 0.57	65 ± 0.32	62 ± 0.17	63 ± 0.23
60	70 ± 0.19	72 ± 0.78	75 ± 0.25	78 ± 0.13
80	75 ± 0.52	80 ± 0.15	83 ± 0.31	85 ± 0.72
100	80 ± 0.17	86 ± 0.67	88 ± 0.57	90 ± 0.32
120	83 ± 0.14	88 ± 0.29	61 ± 0.42	63 ± 0.22

It was evident from the result of the assessments presented on Table 4 that on the cotton fabric, the control dye exhibited good to excellent fastness test to washing, rubbing, light and perspiration but very poor to poor to fair fastness was recorded on dyeing the cotton fabric with the shelf-life product.

Table 4. Fastness properties assessment of the dyes.

Dye	Wash fastness Colour change	Stain on cotton	Perspiration fastness Colour change	Stain on cotton	Rubbing fastness Wet	Dry	Light fastness
Indigo VAT							
Control	4	4	4/5	4	4/5	4	3
Shelf-life Product	1	2	2/3	3	3	2	2
Natural Indigo							
Control	3	3	3	4	4	3	3
Shelf-life product	3	2	2/3	3	3/4	2	2

Greyscale ratings for washing fastness test: 5-Excellent; 4-Good, 3- Fair 2-Poor, 1-Very poor

Greyscale ratings for staining: 1-Much change, 2-Considerable change, 3-Noticeable change, 4-Slight change and 5-Negligible change

Greyscale ratings for light fastness test: 1- very poor; 2- poor; 3- moderate; 4- fairly good; 5- good; 6- very good; 7-excellent; 8-outstanding

Conclusion

Changes occur in quality and fastness properties of the dyes, indigo VAT (synthetic) and natural indigo on shelf as a result of infestation by microbes (fungi), light penetration and other environmental conditions. These changes which seems unnoticeable physically affects the dye fastness, light fastness, wash fastness, rubbing fastness and perspiration fastness. Hence, this study was designed to scientifically predict a determinate shelf-life of dyes used in Nigerian tie and dye cloths ('Adire'). This would further provide information and educate textile dye practitioners on the length of time the quality of the dyes can be guaranteed on shelf.

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