Dyeing Cellulose-Based-Fabrics Using Pigmentsproduced by Fungi

Mibey R. K[1] *and Makumba B.A.N* [†2]
[1]*Vice Chancellor Moi University P.O. Box 3900-30100 Eldoret, Kenya*
[2]*School of Biological and Physical Sciences Department of Botany Moi University P.O. Box 3900-30100 Eldoret, Kenya.*

Abstract

Various synthetic colouring agents have the potential of carcinogenicity and/or teratoge extracted from fruits, vegetables, seed roots and microorganisms and often called "biocolours" due to their

biological origin have proved to be safe and edible colouring agents. The microfungi *Fusarium oxysporum* f.sp. *phaseolicola* and *Hormoconis resinae* f.sp. *avellaneum*were isolated from soil samples collected on plots under the bean crop and aviation gasoline respectively. Pure colonies were obtained and cultured in Potato Dextrose Broth (PDB) and Czapek Dox Broth (CDB) at pH between 1 and 14 and temperature between 20°C and 35°C. Considerable pigmentation was observed at a temperature of 30°C and at pH between 5 and 14,

with the maximum yield at pH 7 and 30°C. Potato Dextrose Broth inoculated with *Fusarium oxysporum* f.sp. *phaseolicola* produced three pigments from viz: violet, purple and pink pigments whilst Czapek dox Broth produced a pastel yellow pigment. Both PDB and CDB inoculated with *Hormoconis resinae* f.sp. *avellaneum*produced a dark brown pigment. All the pigments produced by the microfungi firmly dyed cellulose-based fabrics.

Keywords: *Fusarium oxysporum, Hormoconis resinae,pigment, cellulose-based-fabric.*

Introduction

Demand for natural instead of synthetic pigments for colouring fabrics, foods and cosmetics is increasing (Babu and Shenolikar, 1995; Khanna and Singh, 1975). Natural colours are generally extracted from fruits, vegetables, roots and microorganisms and are often called "biocolours" because of their biological origin (Pattnaik *et al.,* 1997). Microbial pigments are a promising alternative to other colour additives extracted from vegetables or animals because they are considered natural, pose no seasonal production problems and show high productivity. Pigment producing microorganisms which include yeast, fungi, bacteria, micro algae that are quite common in nature have recently received focus as sources of natural pigments (Mapari *et al.,* 2005).

Many fungal species produce pigments. *Monascus* fungi (Shin *et al.,* 1998; Tseng *et al.,* 2000) producered or yellow pigments that are used as food colourants. *Hahella* produces red, prodigiosin (Nakashima *et al.,*2005), *Ashbya* produces yellow riboflavin (Stahmenn *et al.,* 2001), *Phoma* produces orange aza-anthraquinone (Birch *et al.,* 1964) and *Chromobacterium* produces blue violacein

(Rettori and Durán, 1998). Several species of *Fusarium* isolated from their natural habitats often produce pigments both on solid and liquid broth (Balmas *et al.,* 2000; Leslie and Summerell, 2006). The ascomycete *Hormoconis resinae* (syn *Amorphotheca resinae*) Parbery (1969) produces a dark brown pigment (De Vries, 1952; Tan, 1972). This study seeks to find out whether pigments produced by *Fusarium* and *Hormoconis* species can be used to dye cellulose-based fabrics *in vitro*

Materials and Methods

Collection of materials

a) Soil samples

Soil samples were obtained from the University of Eldoret Farm. Sampling was randomly done on plots under beans.

b) Aviation gasoline

Aviation gasoline (Avgas[1]) was obtained from reserves of the Moi University plane (Varsity Plane) on very stringent conditions that it will only be used for experimental purposes.

c) Cellulose-based-fabric

[1] Avgasis ahigh-octaneaviation fuelused to power manyaircraftandracing cars.*Avgas*is aportmanteauforaviation gasoline, as distinguished from *mogas* (motor gasoline), which is the everydaygasolineused incars. Some light aircraft also use automobile fuel instead of avgas.

Avgas is used in aircraft that havepistonorWankel engines.Gas turbinesare able to operate on avgas, but typically do not. Turbine and diesel enginesare designed to usekerosene-basedjet fuel.

Bleached cotton (cellulose-based) fabric article 002A was obtained from Rift Valley Textiles (Rivatex) East Africa Limited processing department for experimental dyeing.

Isolation of fungal species

a) Fusarium oxysporum f.sp. phaseolicola

Standard isolation methods were used. Three soil samples were randomly chosen from those availed from the sampling site from which 2 grams were weighed and transferred into separate clean 250 ml Erlenmeyer flasks containing 50 ml of Sterile Distilled Water (SDW) treated with Streptomycin sulphate (100µg/l) and Chloramphenical (50 µg/l).

The flasks were mounted onto a mechanical shaker and allowed to shake at a speed of 120 rpm for 30 minutes. After a short period of settling, 1 ml of soil extract was drawn using a sterile tip of a micropipette and spread on Potato Dextrose Agar (PDA) plates using a sterile drigalski spatula. This was done in triplicates for each flask. Plates were then incubated at 25°C and monitored for a period of seven days for the growth of characteristic pink/violet *Fusarium oxysporum* f.sp. *phaseolicola* colonies. Colonies of interest were aseptically subcultured onto fresh PDA plates and incubated at 25°C for 7 days to obtain pure cultures (Rabeendran *et al.,* 1998).

b) Hormoconis resinae f.sp. avellaneum

A volume of about 500ml of Avgas was placed in a 1 litre Erlenmeyer conical flask to which 50ml of water was added, corked and shaken by hand for 2 minutes. The water was meant to
contaminate the gasoline to encour fungus″. The mixturetandfor3dayswas. allowed t
After the settling period, 1 ml of the mixture was drawn using a sterile tip of a micropipette and spread on Potato Dextrose Agar (PDA), Czapek Dox Agar (CDA) and Bushnell—Hass Agar (BHA) plates using a sterile drigalski spatula. This was done in triplicates. Plates were then incubated at 30°C and monitored for a period of

seven days for the growth of characteristic brownish/hazel *Hormoconis resinae* f.sp. *avellaneum* colonies. Colonies of interest were aseptically sub-cultured onto fresh PDA plates and incubated at 30°C for 7 days to obtain pure cultures (Sheridan *et al.,* 1972).

Identification of fungal isolates using morphological features

a) Fusarium oxysporum f.sp. phaseolicola

This was done to ascertain that the purified fungus obtained on PDA was *Fusarium oxysporum* f.sp. *phaseolicola*. Morphological characteristics were determined under a compound microscope at total magnifications of 40X, 100X and finally 400X. The following were observed:

- The fungi had both macroconidia and microconidia,
- Macroconidia observed had an average of 3 septa for each; apical and basal cells were not too pronounced,
- Microconidia were generally non-septate, oval, kidney-shaped and produced on false phialide heads. The base of microconidia did not look truncated.
- Phialides were short and single i.e., monophialides,
- Cultures gave violet and pink colourations on growth media. Most areas of mycelial growth bore a white colour. (Balmas *et al.,* 2000; Leslie and Summerell, 2006)

All these characteristics confirmed that the isolates were *Fusarium oxysporum* and most likely *Fusarium oxysporum* f.sp. *phaseolicola* given that isolation was done from plots under the bean plant.

Plate 1a*: Top Petri dish view of Fusarium oxysporum.oxysporum isolates: Strain I & II*

Plate 1b: *Bottom view of Fusariumisolates: Strain I & II*

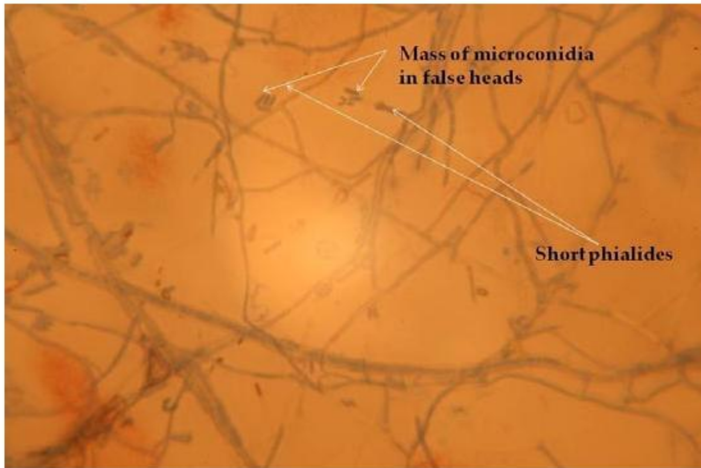

Plate 2: *Micrograph showing the morphology of Fusarium oxysporum at 400X*

b) Hormoconis resinae f.sp. avellaneum

Morphological characteristics observed under a compound microscope at total magnifications of 40X, 100X and finally 400X were as follows:

- The colonies put on a brown colour with a narrow white margin.
- The colonies had very little aerial mycelium only observed at the white narrow margin with profuse sporulation towards the centre of colonies.
- Samples observed under the microscope revealed long ellipsoidal conidia, some of which formed chains—ramoconidia. This description is similar to what was reported by Sheridan *et al.,* (1972) for *Hormocornis resinae* f.sp. *avellaneum*.
- Additional observations made were: Colonies produced concentric rings such as those observed in *Colletotrichum* spp.

A black tinge was observed from underneath. Grooves into the growth medium were also seen emanating from the centre of the colony to make them look like a wheel rim or the ancient ship steering wheel.

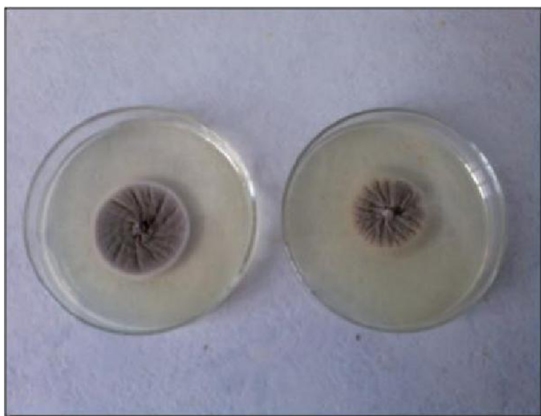

Plate 3a: *6-days-old Hormoconis resinae f.sp. avellaneum the colonies isolated from Avgas on PDA*

Plate 3b: *Up-close view of the colony from aboveplate. Note the radial grooves originating from thecentre to make the colony appear as a wheel rim*

Plate 3c: *Upclose view of the colony from below the plate. Note the black and cream sections of the colony.*

Plate 4: *Micrographs showing the morphology of Hormoconis resinae f.sp. avellaneum at 400X*

Fabric and fungal broth cultivation media preparations

Cotton fabric were washed with non-bleach soap, dried and cut into small pieces each weighing about 0.2 g /5g, able to fit in 250 ml / 1

litre Erlenmeyer flasks. The pieces were then wrapped with aluminium foil and autoclaved at $121^{O}C$ and 1 bar for 60 minutes.

Potato Dextrose Broth (PDB) and Czapek Dox Broth (CDB) were used to cultivate the fungi for pigment production. A volume of 100 ml / 400 ml of each broth was poured into four flasks for each medium and autoclaved at $121^{O}C$ at 1 bar for 15 minutes. The flasks were covered on their mouths with cotton wool and wrapped with aluminium foil.

Pigment production and fabric dyeing in culture

Fungal inocula were obtained by preparing plugs from cultures. This was done using a sterile 5mm diameter cork borer. Three plugs per conical flask of the two strains of *Fusarium oxysporum* f.sp. *phaseolicola* and *Hormocornis resinae* f.sp. *avellaneum* isolated were aseptically transferred into the sterile 100 ml/400 ml of PDB and CDB in 250 ml/1000 ml Erlenmeyer flasks (Asker and Ohta, 1999). Two pieces of sterile cotton fabrics (0.2g/5g) were then introduced. This was done in triplicates for each fungal strain in the two sets of experiments described below. Control flasks were not inoculated with the fungi.

In the first set of the experiments; flasks were incubated at different temperatures ranging from 20°C to 35°C (i.e., $20^{O}C$, $25^{O}C$, $30^{O}C$ and $35^{O}C$). The second set of experiments was carried out after establishing the optimal pigment production temperature. The pH of the media was varied from 1 to 14 (i.e., 1, 3, 5, 7, 9, 11, and 13) at the established optimal temperature. In both the sets, the flasks were incubated with shaking at 150 rpm for 7 days. All shake flask experiments were done in triplicate.

Testing dyed fabric (Fastness to washing)

A soap solution made up of 5 g of soap and 2 g of anhydrous sodium carbonate (Na_2CO_3) in 1 l of distilled water in a beaker free from fluorescent brightening agents was prepared and heated to $60^{O}C$. Specimens of dyed cotton fabrics were introduced and

scoured for 10 minutes. They were then removed, rinsed, wringed and dried (ISO, 1993).

Results

Effect of temperature on pigment production by the fungal isolates

There was a gradual and uniform increase in pigment production for both *Fusarium oxysporum* f.sp. *phaseolicola* and *Hormocornis resinae* f.sp. *avellaneum* with increase in temperature from 20°C to 30°C. At temperatures of 35°C, minimum pigment content was observed. Maximum pigment production was observed at 30°C which in this case was considered as the optimal temperature for pigment production.

Effect of pH on pigment production by the fungal isolates at 30°C

It was observed that maximum pigment production was attained at pH 7 for *Fusarium oxysporum* f.sp. *phaseolicola*. There was a gradual and uniform decrease in pigment production with increase in pH from 7 to 10. No growth or pigmentation was observed below pH 6 and above pH 10. In which case, the optimal pH conditions for *Fusarium oxysporum* f.sp. *phaseolicola* to produce pigments at 30°C was pH 7.

In the case of *Hormocornis resinae* f.sp. *avellaneum*, there was no visual differences in the amount of pigmentation at pH 5 and above. It therefore follows that the optimal pH range for pigment production by *Hormocornis resinae* f.sp. *avellaneum* at 30°C is pH 5—14.

Pigment colours and fabric dyeing

Conical flasks inoculated with *Fusarium oxysporum* f.sp. *phaseolicola* fungal strains produced three pigment colours: violet, purple and pink as observed in the PDB conical flasks by the fourth

day through the incubation period. CDB flasks produced a yellow shade by the fourth day (Figure 1a & b). The fabrics took up the colours of the pigments produced in the two broths (dye-baths) (Figures 2a-d). Conical flasks inoculated with *Hormocornis resinae* f.sp. *avellaneum* produced a dark brown pigment as observed in the PDB and CDB conical flasks by the third day through the incubation period (Figure 1c & d). The fabrics took up the colours of the pigment produced in the two broths (dye-baths) (Figures 2e-f).

Fig. 1a: *Conical flasks (250 ml)bearing different pigmentation by Fusarium oxysporum pigmentation after7 days of incubation in incubation in PDB at pH 7 and 30°C*

Fig. 1b: *Yellow/cream Fusarium oxysporum after7 days of CDB at 7 and 30°C. At the centre is the control flask.*

Fig. 1c: *Conical flasks (1 litre) inoculated with Hormoconisresinae showingpigmentation after7 days of incubation inPDB and CDB at pH 7 and 30°C. All media gave a dark-brown colouration.*

Figure 1d: *Up-close view of dyed cotton fabrics.*

Testing dyed fabric (Fastness to washing)

The fabric pieces showed no fading in the shade acquired during the dyeing process after carrying out the fastness to washing test. According to ISO (1993) geometric scale of 5 grades (Appendix 1), fastness in the fabrics gave grade 5.

Fig. 2a: *Fabric pieces dyed violet in PDB inoculated with Fusarium oxysporum on the left. Controls on the right.*

Figure 2d: *Fabric pieces dyed pink in PDB inoculated with Fusarium oxysporum on the left. Controls on the right.*

Figure 2c: *Fabric pieces dyed purple in PDB inoculated with Fusarium oxysporum on theleft.Controls on the right.*

Figure 2d: *Fabric pieces dyed pastel lemon yellowin CDBinoculated with Fusarium oxysporum on the left. Controls on the right.*

Fig. 2e: *Cotton fabric dyed in PDB inoculated with Hormoconisresinae on the right. Control on the left.*

Fig. 2f: *Cotton fabric dyed in CDB inoculated with Hormoconisresinae on the right. Control on the left.*

Discussion

The pigments produced by the fungi as evidenced in this study are well capable of dyeing cellulose-based fabrics *in vitro*.

In trying to optimize conditions for pigment production other than temperature and pH for the aerobic fungus, the volume of culture media used in the experiments set up was intentional. Experiments have revealed that broth used in shaker flasks should at least only occupy one fifth of the total volume of the shaker flask. This allows sufficient circulation of oxygen to be utilized by the organism being cultured. Increasing the volume of the medium beyond the one fifth mark decreases the amount of dissolved oxygen; thereby, making growth and pigment production by the cells decreased under these conditions (Asker and Ohta, 1999). Yokoyama and Miki (1995) reported that varying the medium volume controlled the growth of and carotenoid production by *Agrobacterium auranticum.*

By visual observation guided by the intensity of pigment within culture, the optimum growth and pigment production was realized when broth was inoculated and cultured at 30°C and pH 7 with

shaking at 150 rpm for 7 days for *Fusarium oxysporum* f.sp. *phaseolicola* whilst an optimal temperature of 30°C and a pH range of 5—14 with shaking at 150 rpm for 7 days was realized for *Hormocornis resinae* f.sp. *avellaneum*. The present study indicates that pigment production is influenced by physical factors such as temperature and pH of the culture medium. There should be many other factors, affecting pigmentation by the fungi such as shaker speed, source and concentration of nutrient components. Various broth media can be used to establish the best in terms of nutrient components. Chiba *et al* (2006), while producing a magenta pigment from *Phoma herbarum*established that addition of diverse fibres to the CDB used affected the colours of pigments produced. This was attributed to topographical cues the fungus in question responded to as it grew, prompting a new biochemical pathway. CDB was chosen in this study following reports by Tan (1972) that f.sp. *avellaneum* produces more pigment when the medium contains sodium nitrate. CDB has sodium nitrate as one of its constituents.

Quantitative methods to establish the authenticity of the visual observations made in this study while determining optimal physical conditions for pigment production and chemical analysis of the pigments produced such as High Performance Liquid Chromatography (HPLC), can be adopted. A thorough understanding of the regulation and pathway of pigment production will allow the development of defined bioprocesses for the enhanced production of the desired pigment.

Cellulose-based fabrics used in this study dyed with the pigments produced by the fungi in the two broth media used. Fastness to washing produced a good grade, an indication that the dyeing process was quite elaborate. Similar work has been performed by other workers such as Nagia and El-Mohamedy (2007) who dyed wool with natural anthraquinone dyes from *Fusarium oxysporum.*

Fungal pigments are secondary metabolites just like antibiotics are. Mass production of these pigments for commercial use can be made possible in the same way antibiotics are mass produced from fungi by deep-tank fermentations.

References

Asker D. and Y. Ohta (1999): Production of canthaxanthin by extremely halophilic bacteria, *J. Biosci. Bioeng*. 88(6), 617-621.

Babu, S. and I.S. Shenolikar (1995): Health and nutritional implications of food colors, *Indian J. Med. Res.,* 102, 245-249.

Balmas, V., Santori, A., and L. Corazza (2000): Le specie di *Fusarium* più comuni in Italia. *Giornale di Patologia delle Piante*, 10 (1).

Birch, A. J., Butler, D. N., and R. W. Rickards (1964): The structure of the aza-anthraquinone phomazarin. *TetrahedronLett.*, 28, 1853–1858.

Chiba, S., Tsuyoshi, N., Fudou, R., Ojika, M., Murakami, Y., Ogoma, Y., Oguchi, M., and S. Yamanaka (2006): Magenta pigment produced by fungus. *Journal of. General. Applied. Microbiology.*, 52, 201–207

De Vries GA (1952): *Contribution to the knowledge of the genus Cladosporium Link ex Fries.* Thesis, University of Utrecht.

International Standards Organization (1993): Grey Scale for Assessing Change in Colour, EN ISO 105-A02 / IUF 131 / VESLIC C 1210

Khanna S.K. and G.B. Singh (1975): Toxicity of commonly used food colors: A review, *J. Sci. Indian Res.,* 34, 631-635.

Leslie, J. F., and B. A. Summerell (2006): The *Fusarium* Laboratory Manual. Blackwell Publishing.

Mapari, S. A. S., Nielsen, K. F., Larsen, T. O., Frisvad, J. C., Meyer, A. S., and U. Thrane (2005): Exploring fungal biodiversity for the production of water-soluble pigments as potential natural food colorants. *Curr. Opin. Biotechnol.*, 16, 231–238.

Nagia F. A. and R. S. R. El-mohamedy (2007): Dyeing of wool with natural anthraquinone dyes from *Fusarium oxysporum*.

Dyes and pigments, 75, (3), pp. 550-555. Elsevier, Oxford, ROYAUME-UNI (1980) (Revue)

Nakashima, T., Kurachi, M., Kato, Y., Yamaguchi, K., and T. Oda (2005): Characterization of bacterium isolated from the sediment at coastal area of Omura Bay in Japan and several biological activities of pigment produced by this isolate. *Microbiol. Immunol.*, 49, 407–415.

Parbery., D. G (1969a): *Amorphotheca resinae* gen. Nov., sp. Nov.: The perfect state of *Cladosporium resinae. Australian Journal of Botany* 17:331 -357.

Parbery., D. G (1969b): The natural occurrence of *Cladosporium resinae. Trans. Br. mycol. Soc.* 53 (1): 15-23.

Pattnaik P., Roy U. and P. Jain (1997): Biocolours: New Generation Additives for Food, Indian Food Industry, 16(5), 21-32.

Rabeendran, N., Jones, E. E., and A. Stewart (1998): Isolation and in vitro screening of soil fungi for biological control of *Sclerotinia sclerotiorum*. *Microbial Control of Plant Pathogens*.

© 1998 New Zealand Plant Protection Society (Inc.) www.nzpps.org. *http://www.nzpps.org/terms_of_use.html*

Rettori, D. and N. Durán (1998): Production, extraction and purification of violacein: An antibiotic pigment produced by *Chromobacterium violaceum*. *World J. Microbiol. Biotechnol.*, 14, 685–688.

Sheridan, J. E., Tan Y. L. and Nelson J. (1972):Studies on the 'Kerosene Fungus' *Cladosporium resinae* (Lindau) De Vries — Part III. Morphology, Taxonomy and Physiology. Tuatara: 19, (3)

Shin, C. S., Kim, H. J., Kim, M. J., and J. Y. Ju (1998): Morphological change and enhanced pigment production of *Monascus* when cocultured with *Saccharomyces cerevisiae* or *Aspergillus oryzae*. *Biotechnol. Bioeng.*, 59, 576–581.

Stahmenn, K. P., Arst, H. N., Jr., Althöfer, H., Revuelta, J. L., Monschau, N., Schlüpen, C., Gätgens, C., Wiesenburg, A.,

and T. Schlösser (2001): Riboflavin, overproduced during sporulation of *Ashbya gossypii*, protects its hyaline spores against ultraviolet light. *Environ. Microbiol.*, 3, 545–550.

Tan Yeap Leong (1972): Studies on the morphology, physiology and taxonomy of the 'kerosene fungus' *Amorphotheca resinae* Parbery (Conidial state *Cladosporium resinae* (Lindau) de Vries). M.Sc. thesis, Victoria Univ. of Wellington.

Tseng, Y. Y., Chen, M. T., and C. F. Lin (2000): Growth, pigment production and protease activity of *Monascus purpureus* as affected by salt, sodium nitrite, polyphosphate and various sugars. *J. Appl. Microbiol.*, 88, 31–37.

Yokoyama, A. and W. Miki (1995): Composition and presumed biosynthetic pathway of carotenoids in the astaxanthin-producing bacterium *Agrobacterium aurantiacum*, FEMS Microbial. Lett., 128, 139-144.