Natural thalli and cultured mycobiont of *Usnea ghattensis* G. Awasthi- A potential source of purple dye yielding lichen from India

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*Usnea ghattensis* G. Awasthi and its cultured mycobiont were used to extract dye for silk fibre, using ammonia fermentation, boiling water and cow urine methods. Both natural thalli as well as lichen cultured mycobiont yield a Purple dye from ammonia fermentation method, while the other two methods produced light shade of colours. The dyed silk threads were evaluated for fastness properties by some treatments like effect of sunlight and detergent. Both natural lichen and cultured mycobiont dyes yield fast colour to the fibre.

**Keywords:** Lichen, Dye, *Usnea ghattensis*, Secondary metabolite, Cow urine, India.

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**Introduction**

The lichens produce unique secondary metabolites mostly not known in other plant groups. The lichen chemicals exhibit excellent dyeing properties and used as dye stuff from the ancient time. The litmus dye from *Roccella* species is the oldest lichen dye known in the world. In India lichen grow luxuriantly in the Himalayas and higher altitudes of Western Ghats. So far more than 2300 species of lichens are known from India. The studies on lichens regarding their use in conducting biomonitoring and biodeterioration studies together with taxonomic studies are available in India. However, poor records of use of lichens as dye stuff from India are available. In the present study the lichen dye from one of the most common endemic fruticose lichen *Usnea ghattensis* G. Awasthi has been attempted. The species is epiphytic and grows luxuriantly on different trees and shrubs in Mahabaleshwar area of Western Ghats.

Apart from the natural thalli the mycobiont of the species cultured in laboratory condition was also used for extraction of dyes in ammonia, boiling water and cow urine.

**Materials and Methods**

*Usnea ghattensis* G. Awasthi is dark brown fruticose lichen endemic to Northern Western Ghats of the country. It grows on bark and can be easily identified by its erect-bushy thallus with usnic acid as major secondary metabolite. The surface of the thallus of *U. ghattensis* is papillate and pseudocyphellate when seen under microscope. *U. ghattensis* has terminal apothecia (fruiting body) with ciliate margins.

**Collection of lichen samples**

The lichen samples were collected and studied, morpho-anatomically, chemically and identified at the species level with the help of relevant keys using a Labomed stereomicroscope and Leica DM 500 light microscope. The lichen substances were identified with the help of thin-layer chromatography (TLC) following the method of Elix et al (Ref 4) (1993) and Orange et al (Ref 5). The lichen sample was sorted, cleaned of substratum and dried. The voucher specimen was deposited at the lichen herbarium (LWG), National Botanical Research Institute (NBRI), Lucknow, India.

**Natural lichen thalli**

Dried sample of lichen *Usnea ghattensis* was ground to powdered form with the help of mortar and pestle and weighed. The powdered lichen sample was used for dyeing.

**Mycobiont culture**

The colonies of mycobiont were obtained using discharged spore method. Thalli bearing fruiting bodies (ascocarps) were washed in a turbulent flow of
tap water in order to remove as much surface contamination as possible. Individual ascomata were cut off and attached to the inside of the petriplate lids with the help of petroleum jelly. Petriplates containing Potato Dextrose Agar (having compositions per 1000 mL as Potato: 200 g, dextrose: 20 g, Agar: 15 g and pH 5.6) were then inverted over the lids and ascospores were allowed to discharge onto the agar medium. Plates were incubated at 20°C in BOD incubator and observations were recorded periodically over 3 to 5 months period.

Cultured mycelial mat from 150 days old culture (mycobiont) were used for dye preparation. Mycobiont was removed by the help of water bath at 80°C and dried with Lyophilizer for 12 h. Obtained biomass was crushed using a mortar and pestle and the powdered mycobiont was used for dye preparation.

Silk fibre used
Silk fibre (5 g) used for dyeing were washed in distil water and rinsed thoroughly before being placed in the dye solution.

Extraction of dye from natural thalli and mycobiont

Ammonia fermentation method (AFM), Boiling Water Method (BWM) and Cow Urine method (CUM) were used for dyeing the silk fibre. The dye extractions and dyeing were performed in Erlenmeyer flasks, covered with a double layer of parafilm (except during heating). The natural lichen and cultured mycobiont to silk ratio was 1:1. The silk fiber was dyed at two dyeing temperatures e.g. room temperature, 88°C. pH was adjusted according to the method employed and no mordents were added.

Ammonia fermentation method (AFM)
Added 5 g lichen/mycobiont with ammonium hydroxide solution (1:10, one part NH₄OH and 10 parts H₂O) in an Erlenmeyer flask and store for one month. Filtered the extract using Whatman’s filter paper no-4 in a Buchner funnel. Adjusted the pH of dye and add 5 g of silk.

Boiling water method (BWM)
5 g lichen material or powdered lichen mycobiont to distilled water heat it till boiling maintain at simmer for 1h. Strained the dye bath liquid into clean container and repeated the process at least two times or more until the desired colour is obtained. Adjusted the pH of the dye. After that immersed pre-soaked silk thread in dye bath and slowly heated the dye bath at maximum 88°C for 2 h. Turned off heat and cooled the skein in dye bath for 2 h after that removed and rinsed it in cold water.

Cow urine method (CUM)
Covered 5 g dry crushed lichen or mycobiont with cow urine in a flask. Stirred the vat content vigorously to incorporate oxygen and then replaced the lid with parafilm. Stirred the vat content daily. Maintained the dye content at room temperature for sufficient time to develop orchil. Through a strainer poured dye into non-reactive container (stainless steel or glass container). Adjusted the pH of dye and added 5 g of silk for dyeing.

Evaluation of fastness properties
To check whether the dye is colour fast, the silk threads were exposed to direct sun for 3 days and were washed with detergent. The fastness property of the dyed silk fibres from both, the natural thallus as well as from the mycobiont were evaluated using method used by Kumar and Singh (2012).9

Results and Discussion
Both natural lichen and cultured mycobiont yielded different shades of colour to the silk (Plates 1A-I). Out of the three methods purple colored dye was extracted from Ammonia fermentation method. The colour names used were those matching Ridgway colours (1912). Colour of the silk thread dyed with natural lichen thalli by AFM was Blanc’s violet while the colour with mycobiont was veronica purple. The boiling water method (BWM) with natural lichen thalli impart clear yellow green colour to the silk fiber while the mycobiont yield light yellow green. In cow urine method the natural thalli produced light viridine colour to the silk while no colour was obtained through cultured mycobiont. The ammonia fermentation method (AFM) is the best preferred method to extract purple dye from Usnea ghattensis. Results of fastness properties of dyed silk fibres are given in Table 1. The silk fibres dyed using AFM and CUM showed better fastness properties than silk dyed using BWM. It is well known that mycobiont in axenic cultures retain the capacity to biosynthesize secondary products found in the lichenized state and the metabolites produced in the greatest abundance might differ from those found in the lichen. Similarly in the present study Usnea ghattensis produced usnic acid and unknown triterpene between Rf classes 3-4 in natural thalli, while only usnic acid
Plate 1(a-i)—a. Thalli of *Usnea ghattensis* G. Awasthi; b. Cultured mycobiont of *Usnea ghattensis*; c. Dyed silk thread from natural thalli (ammonia fermentation method); d. Dyed silk thread from natural thalli (boiling water method); e. Dyed silk thread from natural thalli (Cow urine method); f. (1-3). Silk thread without dye (as control); g. Dyed silk thread from mycobiont (ammonia fermentation method); h. Dyed silk thread from mycobiont (boiling water method); i. Dyed silk thread from mycobiont (Cow urine method)
Table 1—Fastness properties of silk fibres dyed from natural thalli and cultured mycobiont of *U. ghattensis* G. Awasthi using AFM, BWM and CUM

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<tr>
<th>Treatments</th>
<th>Fastness property of silk dyed from natural thalli of <em>U. ghattensis</em></th>
<th>Fastness property of silk dyed from mycobiont of <em>U. ghattensis</em></th>
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<tr>
<td></td>
<td>AFM</td>
<td>BWM</td>
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<td>Effect of sunlight</td>
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<td>Effect of washing with detergent</td>
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was spotted in cultured mycobiont. The use of mycobiont can overcome the disadvantage of natural lichen extracts for industrialization of their metabolites because of their much faster growth and larger production in culture than the natural thalli.

**Conclusion**

From the present study it could be concluded that the secondary metabolites in mycobiont culture of *Usnea ghattensis* are potential source of unique purple dye for dying silk and other fibers.

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**References**