

The present research delves into the production of natural yellowish brown azaphilone dye using *Aspergillus niger* through fermentation, focusing on optimizing various parameters for enhanced dye synthesis. The research started with the cultivation and characterization of *Aspergillus niger* on Potato Dextrose Agar medium. Six different culture media were explored, including synthetic and sustainable media, to determine optimal conditions for dye synthesis. Color shift of the reaction mixture from original colour to yellowish brown confirmed the presence of dye. UV-VIS spectroscopy exhibited azaphilone dye formation at 450nm. Static and shaking conditions were compared, with static proving more conducive to higher dye yields. Fed-batch fermentation emerged as a favorable approach than batch fermentation. The impact of incubation time on dye production was examined, revealing utmost production after 28 days. Likewise, varying temperatures, pH levels and inoculum percentages influence dye synthesis. Best production of yellowish brown dye that is 6% (v/v) was attained in Yeast extract peptone broth medium optimized at 30°C, pH 7 and 3% (v/v) spore suspension at static state. Thin layer chromatography showed single spots having Rf values of 1.2 and 1.4 respectively. GCMS analyses confirmed the presence of Azaphilone dye in *Aspergillus niger* culture filtrate. Application of dye was also performed on pretreated cotton cloth ultimately. These findings contribute to a deeper understanding of optimizing fungal dye production for sustainable and eco-friendly applications, particularly in the realm of textile coloration. This is the first time study reported on the production of azaphilone dye by *Aspergillus niger* in YEPB according to best of our knowledge.