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Using black mulberry extracts as an alternative biological dye for endophytic fungi staining

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Abstract: Fungi microbiomes can be studied using a several approaches. Most entail using chemical dyes to stain typical morphological traits, which can be hazardous to both the environment and the health of users. In order to use safe alternative dyes for fungi microorganisms' studies, the objective of the present work was to find new alternative dyes that would help us study the biodiversity of root microbiomes in their natural biofilm. Roots of seven different species were harvested: *Poa* spp., *Genista aspalatoid*, *Cistus libanotis*, *Thymelaea tartonraira*, *Juniperus oxycedrus*, *Cupressus dupreziana*, and *Pinus halpensis*. Following blanching the roots using a modified Philips and Hayman's (1970) procedure, we stained the various endophytic microbiome structures with black mulberry dye and compare this dye's effectiveness to that of two types dyes; natural and synthetic (Curcuma and methylene blue). Results revealed that the best staining was obtained with Black mulberry, the roots' species' endophytic fungal structures were transparent and stained with a brown sparkle hue. The rate of colonization of these fungi is very high in the herbaceous species. A new shape of sporangium was found in the T. tartonraira specie. This procedure of staining is successful, effective and offers safer alternatives to the harmful chemical's dyes used in traditional staining methods.

Keywords: Algeria, dyes, black mulberry, fungi, roots.

Introduction

Until the discovery of the first synthetic dyes in 1856, the only colored pigments available to humans were (and therefore) natural dyes (Hamdy and Hassabo, 2021). Dyes are substances used to add color to textiles, fabrics, leather, and other materials. They are colored compounds that form chemical bonds with the substrate to which they are applied [1].

Classifying colorants derived from natural sources can be challenging due to their diverse inherent properties. These colorants come from various natural origins, which gives them a wide range of chemical compositions, impacting their properties, solubility, and stability in unique way [2]. In recent years, there has been a shift toward replacing synthetic colorants with natural alternatives, largely driven by the growing consumer demand for natural products [3].

Morus nigra L. (M. nigra), is belonging to the Moraceae family, the tribe Moracea, and the genus Morus [4]. The white, black, and red mulberries are the species of the Morus genus that are most often grown [5,6]. It originates from Southwestern Asia and has been cultivated across Europe and throughout the Mediterranean region for hundreds of years [7].

Mulberry plants are cultivated to provide nourishment for silkworms and as raw materials for producing jams, marmalades, vinegars, juices, wines, and cosmetics [8]. The leaves and fruits of M. nigra have shown various pharmacological properties, including antinociceptive [9], anti-inflammatory [10], antimicrobial [11] , anti-melanogenic [12], antidiabetic [13] and anticancer activities [14] .

Mulberry fruit contains numerous chemical compounds, one of which is a benzopyran derivative pigment known as anthocyanins and flavonoids. Anthocyanins are often used as red dyes in the field of cosmetics [15] .

Concerns over the harmful effects of synthetic dyes have driven many scientists to explore eco-friendly natural colorants for use in morphological studies and microorganism identification [16]. Naturally derived pigments, available in a range of hues and tones, are currently utilized for coloring textiles, food materials, and have various biomedical applications [17]. The application of natural dyes from plants for coloring of diverse biological tissues from an alternative source will cut the cost of purchasing the synthetic dye and diminish their impact on humans and the environment [18].

With growing public awareness around eco-preservation, safety, and health, there has been a revolution in textile research and development focused on sustainable, non-toxic, and environmentally friendly bio-based colorants [17].

According to [19], synthetic dyes threats human health. For example, the waste color issue of the textile industry is very harmful to the environment and most of it is discarded into water [1]. However, over 80% of the wastewater generated that contains dyes is frequently dumped untreated into streams or used straight for irrigation, which has a negative effect on ecosystems and public health [20]. Many articles related to the removal of color from wastewater: [21, 22, 23, 24, 25, 26, and 27]. However, very few of technologies to remove color from solution are used in water treatment works thus reflecting the complexity of the problem [28].

Laboratory activities generally use synthetic dyes which are expensive and can be damaged the researchers. Therefore, the main objective of this research was to study the properties of mulberry fruit pigments as a natural dye for their use in the field of microbiological staining. We compared the effectiveness of this dye with two other dyes, a natural dye; Curcuma and a synthetic dye; Methylene Blue. In this work we will try to color the endophytic fungi in their natural habitat; roots, which differentiated our work from previous research focused on the culture of these fungi in Petri dishes, after staining them to observe their morphological structures.

Materials and Methods

Study area

The current study focuses on the province of Djelfa, located in north-eastern Algeria. In this area (Figure 1), two sites were chosen: Djalaliya (site 1) and Senelba forest (site 2). The main features of these sites are shown in Table 1.

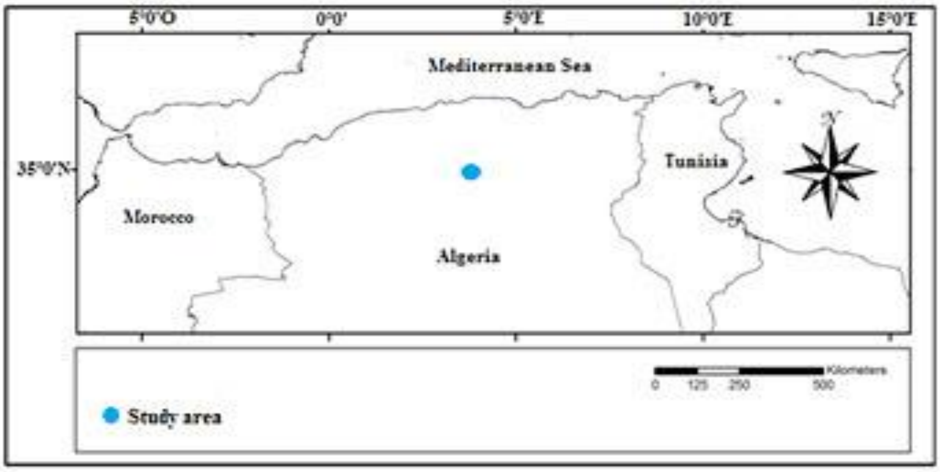

Fig. 1. Situation of study area.

Table 1. Determination of the bioclimatic levels of the study sites.

	Latitude	Longitude	Altitude(m)	M (°C)	m (°C)	P (mm/an)	Q_3	Bioclimatic levels
Site 1	34°44'N	3°8'E	1037	35,52	-0,44	280,55	28,03	Arid (cold winter)
Site 2	34°53'N	3°03'E	1363	32,91	-0,63	344,7	35,25	Semiarid (cold winter)

Samples material

Fine roots (≈0.5 mm) of five different subjects for each species across various vegetation layers were randomly sampled. These layers include the ground layer (Poa spp., Genista aspalatoid, Cistus libanotis, and Thymelaea tartonraira), small shrub layer (Juniperus oxycedrus), medium shrub layer (Cupressus dupreziana), and the understorey layer (Pinus halpensis). One species, Genista aspalatoid, was taken from site 1, while the remaining species were collected from site 2. Root samples were packed in plastic bags and transported to the laboratory.

Dye preparation

About 50 g of C. longa rhizomes were acquired from the Djelfa, Algeria, local market for the Curcuma dye. After being sliced into little pieces and cleaned with distilled water, they were dried. After that, they were ground and put through a 20 µm filter in order to produce a fine powder.

For black mulberry dye; approximately 200 g of fresh fruits of Black mulberry plants were harvested and rinsed by water. These fruits have been cleaned well. They were divided into small pieces. Afterwards, they are dried in an oven at 120°C for about 10 minutes; we let them cool down. They are then ground into a fine powder and stored in a sterile bottle in a cool and dry place.

Staining Technique

The harvested roots are gently washed to remove adhering soil particles. Five roots per individual were randomly severed from plants collected from the site. The method used to clean and blanch the roots is that of [29]. The roots are cut into small fragments, about 1 cm, and placed in a potassium hydroxide (KOH) solution at 10 % for Pinus halpensis, Juniperius oycesdrus, and Cupressus dupreziana, and at 5 % for the other species. They are then placed in the oven at 90°C for 1 hour. To remove the remaining pigments, the roots are transferred to a 10 % solution of H_2O_2 at 90°C for 20 minutes until they turn white. The blanched roots were then rinsed several times with water and set up in a 3 % lactic acid bath for few minutes (≈3 minutes).

In this study, we grouped our blanched root samples into four groups, group 1: the roots are colored by black mulberry, group 2: the roots were colored by Curcuma, group 3: the roots were colored by methylene blue and for group 4: the roots were not colored (witness experience). in this experiment we used black mulberry and Curcuma as natural dyes and methylene blue as synthetic dye.

blanched roots were transferred to three test tubes of; 5% black mulberry solution (tube A), 1% Curcuma solution (tube B) and 0.1% methylene blue (tube C). Tubes were placed in the oven at 90°C for 30 minutes (tube C), and for 45 minutes (tube A and B). After removing the roots from the dye solution, they were rinsed with tap water and placed in a Petri dish containing 87% glycerol.

Results and Discussion

Staining of microbial cells is a very important procedure in microbial identification. Cells need to be fixed and stained to increase contrast, and to study morphological structures under microscope [16].

Roots fragments were collected from different species during the spring season, crushed, blanched, stained and observed under the light microscope at different magnifications. Our results with the three dye (black mulberry (BM), methylene blue (MB) and curcuma) reveal that, following an acceptable clearing time and staining, the approach given yields satisfactory staining results for the black mulberry and methylene blue (Fig.02). The clarifying time for root tissues should be adjusted based on the plant species being investigated. Roots fragments collected from different species during the spring season, crushed, blanched, stained and observed under the light microscope at different magnifications.

According to the morphological shapes characteristics stained by BM and MB, the structures belong to the fungi phylum. We observed an endophytic fungus in the first root group stained with BM (Fig.02A), and a mycorrhizal fungus with systematic features including vesicles and arbuscules in the third root group stained with MB (Fig.02B). However, in the second roots group stained with curcuma dye, (Fig.02C), the presence of structures belonging to the two types of fungi listed below was not clearly established by the microscopic observations. Except for the Glomeromycota's mycorrhizal fungi, root endophytes have not gotten much attention in the literature. Although knowledge of the identity and diversity of foliar endophytic fungus communities in temperate and tropical regions is growing [30,31]. The communities that live in roots are sometimes inadequately described; species are frequently grouped together under labels like "fine endophytes" or "dark septate"[32] .

The comparison between the BM and MB, show as to understand that the use of BM staining technic increased the likelihood of correctly identifying the structures of endophytic fungi, because the use of MB allowed the staining of the mycorrhizal fungi by blue color while the rest of dye obscures the view. The derived dye from black mulberry was utilized as an alternative dye to identify and differentiate various brain sections and nervous tissue cells [33]. It was used to identify sperm cells, which were visible in red on a pale pink background (V). It was also utilized to identify and differentiate between different parasites (Fasciola sp.) [34] .

We were particularly interested by the endophytic fungus in this study, and our findings showed that they looked good while dyed with black mulberry. Distinct fungal structures found in roots species, including hyaline hyphae and sporangium, were found and the majority of these structures are located on the surface of the roots. This fungus group was abundant in Poa spp. and T. tartonraira, moderate in G. aspalatoid, C. libanotis, J. oxycedrus, and C. dupreziana, and infrequent in P. helpensis. According to [35] there is a high degree of host selectivity herbaceous, and the notable variations in fungal endophyte plant colonization strongly imply that only a few species should be regarded as ubiquitous.

By displaying the structures found in the roots, techniques for viewing fungi in roots have been developed as quick tests with the best material staining, ensuring the accuracy of the microscopic assessment of the degree of colonization of the roots [36] .

Anthocyanins have a wide range of biological activities including: redox/ antioxidant, anti-inflammatory, antimicrobial, anti-carcinogenic, fostering eye health, neuroprotective, prevention of LDL oxidation, improvement of capillary stability, supporting collagen, and increasing intercellular levels of vitamin C [37]. They are also active substances soluble in water and can bind to glycoproteins from bacteria. [25, 38, 39].

A variety of factors contribute to anthocyanin stability, including intermolecular and intramolecular complexation. According to [40], as a weak acid, anthocyanins become deprotonated with increasing pH, which can increase electron polarization, resulting in a variety of colors such as red, purple, and blue.

Microscopic examinations of roots, showed us a various morphological structure. Morphological identification of these structures reveals that this structure belongs to fungi microorganisms; endophytic type due to the presence of some typical structures of these microorganisms, such as hyphae, sporangium, sporangiophores, Columella and chlamydospores. These structures are present in all roots of; Poa spp., Genista aspalatoid, Cistus libanotis, Thymelaea tartonraira, Juniperus oxycedrus, Cupressus dupreziana, except for Pinus halepensis, this species contained only trace amounts of these microorganisms. These structures are:

-Hyphae; they are abundant, hyaline, with different diameters; Fin hyaline hyphae (±1µm) were abundant, with some being wide and thick. (±10µm), coenocytic and containing or not structures such as sporangium, columella at their tips (Fig. 3).

-Sporangiophores; has different pathways, branched, Abundant on the surface of the roots (Fig. 3).

-Sporangium; warty and having various and irregulars' shapes, located both outside and inside the roots, the most abundant form is the form of coffee beans (Fig. 4A), it found in all the species. Two other new forms (Fig. 4B/C) have also been found only in Thymelaea tartonraira.

-Sporangiospores; are found in the cortical parenchyma of the roots (Fig. 4D-F), where they are clustered into grape clusters or scattered separately.

-Columella; They are plentiful and globose in shape, and they appear near the end of the sporangiophore.

-Chlamydospore; present, have irregular shape, consisting of widened hyphal branches.

Regarding these seven species' root endophytic fungus, no research has been done. There are other works in the other part of these species were studied like twigs, leaves and needles [41, 42] .The majority present data concerning the distribution and abundance of endophytes in asymptomatic leaf tissue. Many fungal endophytes are highly plant-specific, which accounts for their vast diversity [43]. According to [44], Black mulberry extract could be a potential raw material for herbal medicine, especially anticaries which has antibacterial activity, it was used to stain Streptococcus bacteria glycoproteins found in dental plaque. In this study, we can see the staining of glycoproteins in the various parts of fungi. (Figure 3, 4 and 5).

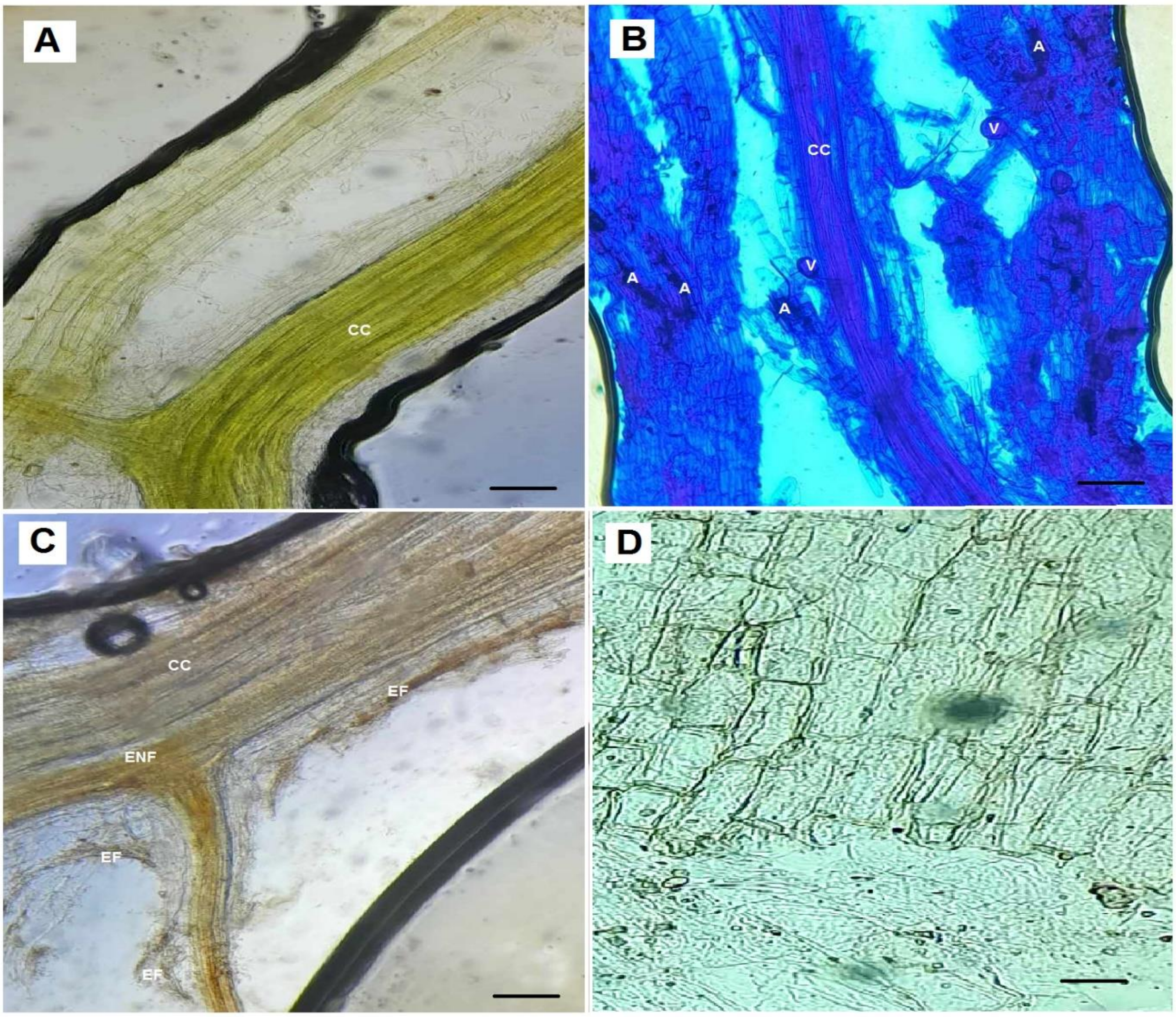

Fig. 2. Microscopic observations of roots. A; roots staining with Curcuma dye, B; roots staining with methylene blue, C; roots staining with black berry and D; roots not staining. Scale bars = 90 µm (A, B and C), scale bars = 20 µm(D). A, B and C: (GX10), D: (GX40).C: central cylinder, V: vesicle, A: arbuscule, ENF and EF: endophytic fungi.

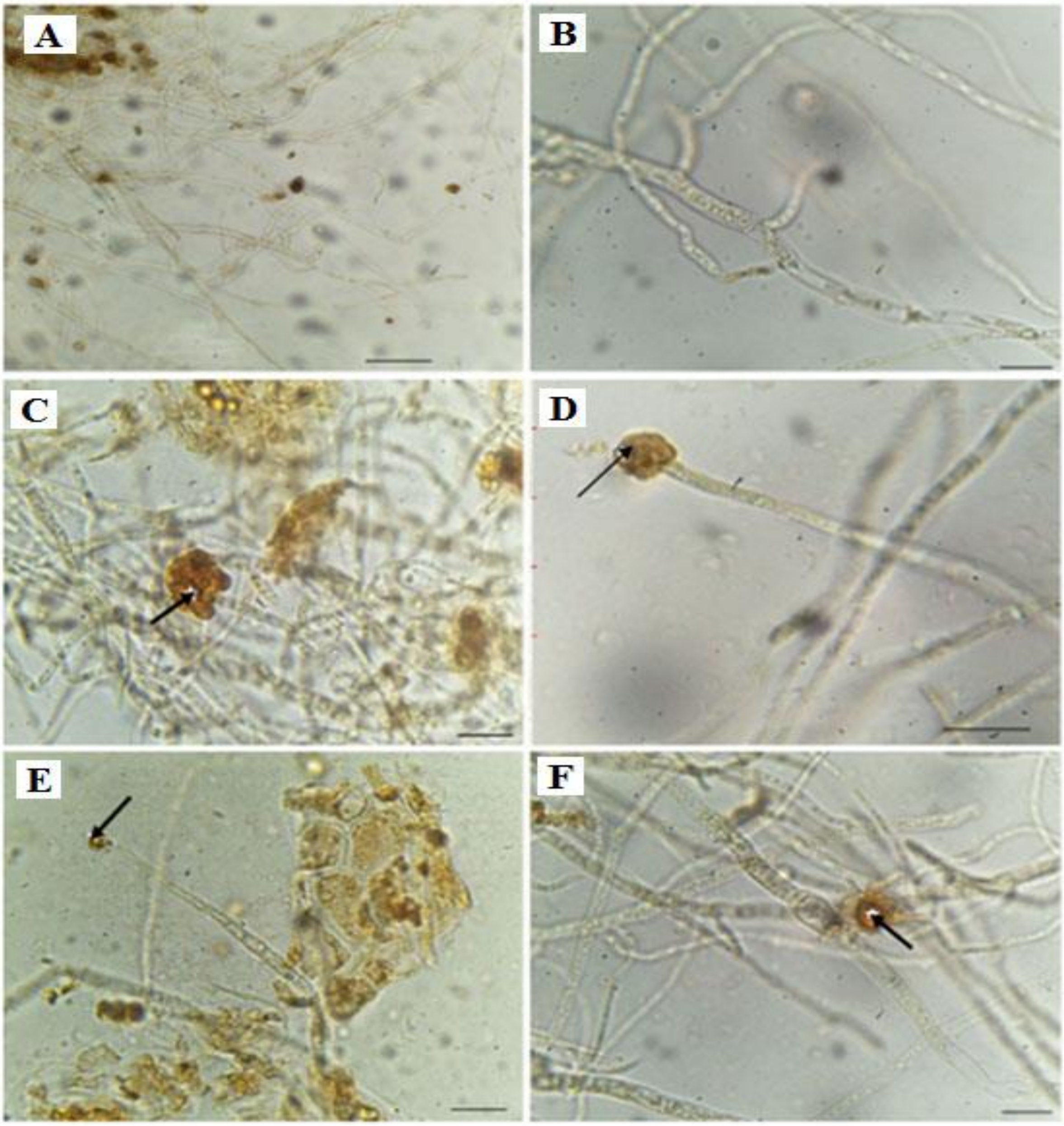

Fig. 3. Morphology characteristics of endophytic fungi observed by in Thymelaea tartonraira roots: A-D et F and Juniperus oxycedrus : E. A: Sporangiophores with irregular basitonous branching pattern. B: sporangiophores (arrow) with different size and sympodial branching pattern (hyalines hyphae).C: Sporangiophore, a small with intact sporangium and a taller one where the sporangium already released the sporangiospores. D: unmatured sporangium. E: Tip of sporangiophore without columella(arrow). F: Chlamydospore (arrow). Scale bars = 20 µm. A: (GX10), B-F: (GX40).

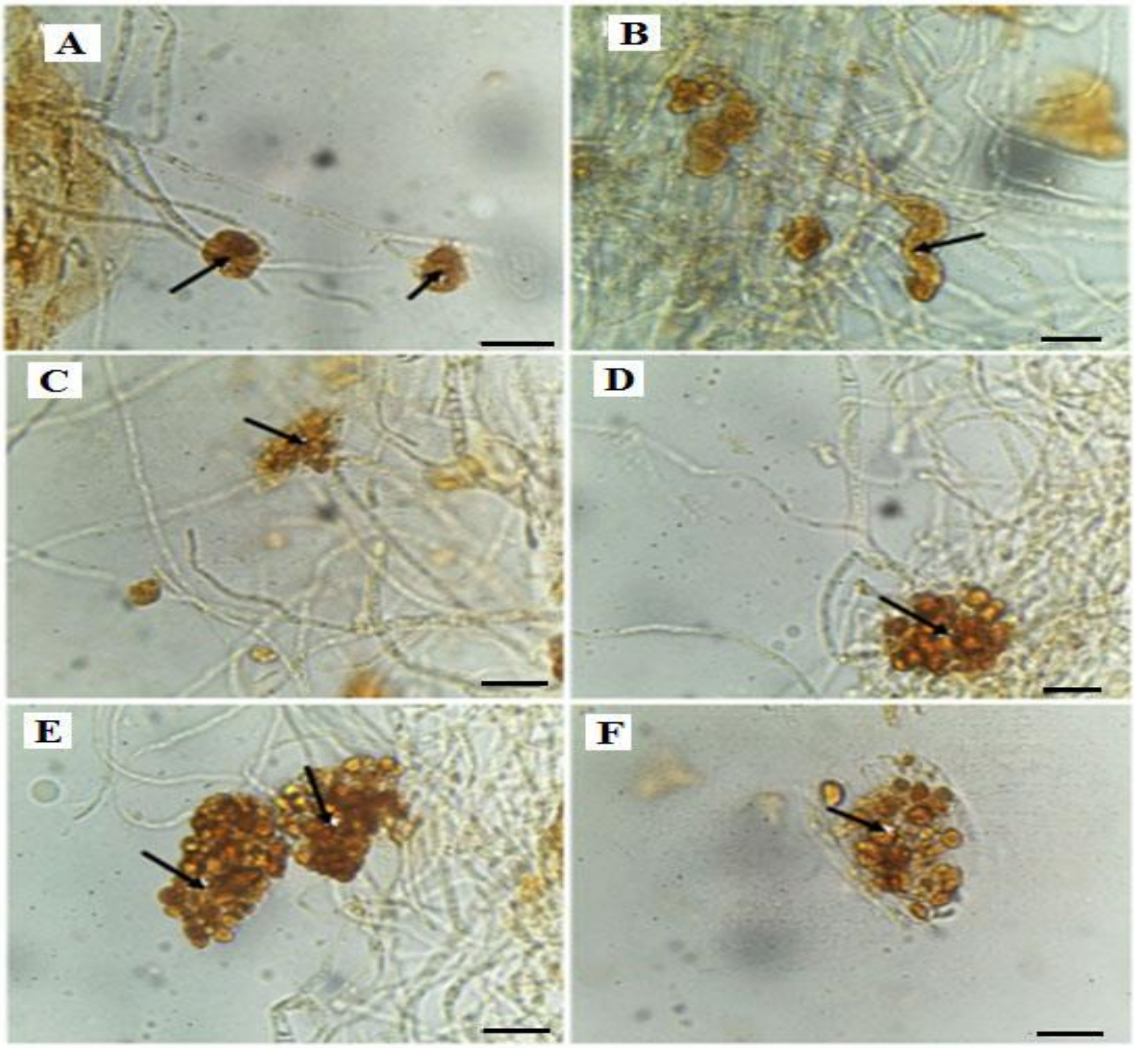

Fig. 4. Morphology characteristics of Sporangium observed in Thymelaea tartonraira roots. A, B and C: Various shapes of unmatured sporangium (arrow) at tip of sporangiophore. D and E: Multi-spored sporangium attached to the sporangiophore. F: Multi-spored sporangium (arrow) detached to the sporangiophore. Scale bars = 20 µm. (GX40).

According to the characteristics of fungi structures and shapes of sporangia, these fungi belong to Mortierella genus. Currently, Mortierella is a member of the family Mortierellaceae, order Mortierellales, subphy-lum Mortierellomycotina [45, 46]. About 100 species of Mortierella have been described to date [47]. This genus's members are distinguished by the development of a mostly coenocytic, albeit irregularly septate, mycelium.Simple or multibranched, sporangiophores terminate in sporangia and, on occasion, in aswelling at the base. Sporangia are globose, containing one, a few, or many spores. Mortierella species are commonly isolated from freshwater, soil, dead or dying plant tissues, and animal feces [48, 49, 50, 51, 52, and 53].

It has been demonstrated that some individuals in the order Mortierellales predominate in fungal communities found in naturally occurring environments [54].

Recent studies on the soil microbiota on a global scale reported Mortierella spp. to be important members of the soil core microbial community [55,56]. Members of Mortierellaceae are reported to produce polyunsaturated fatty acids and arachidonic acid, which are crucial for several biological functions in mammalians [57], and are widely used for many commercial purposes such as biofuel production (Du et al. 2018). In this study, we found a lot of structures belong the two genera; Linnemannia and Mortierella. There are still many undescribed species of Mortierellaceae [58], in this study a new shape of sporangium was found in T. tartonraira (Figure. 3B), it could a new specie. This requires different approaches in order to be able to identify it.

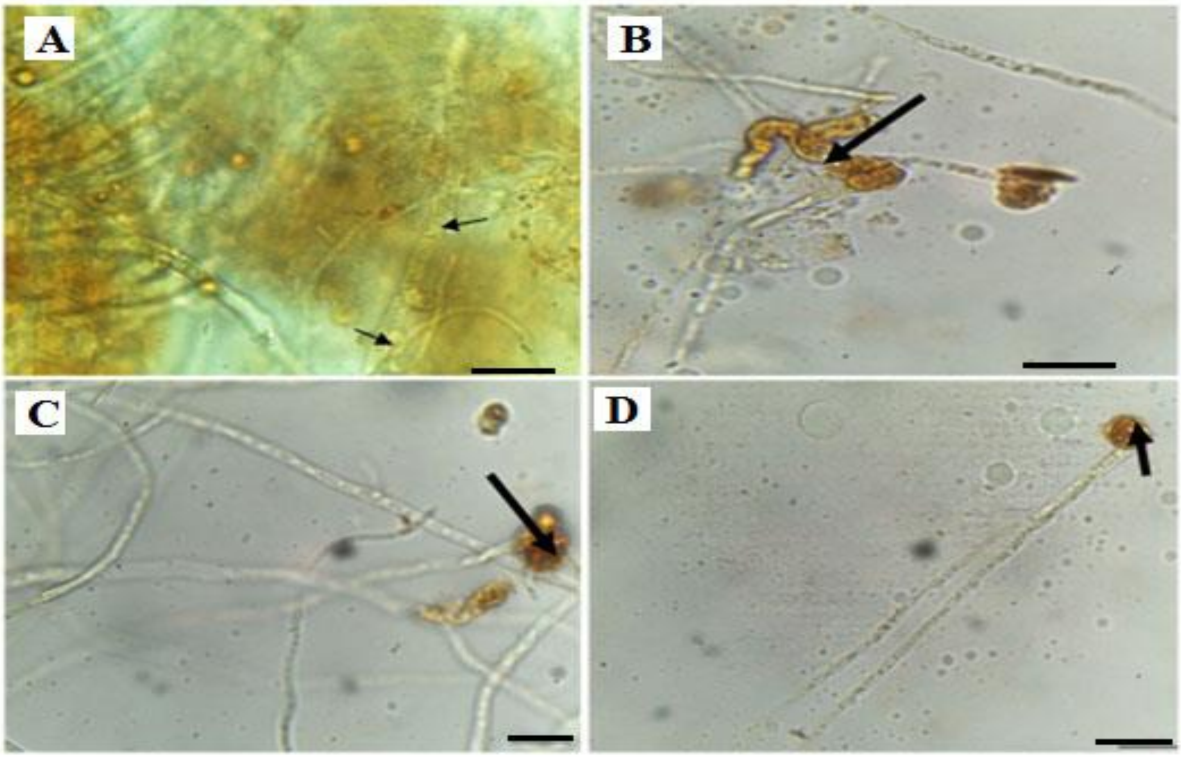

Fig. 5. Sexual and Asexual morphology of endophytic fungi observed in Thymelaea tartonraira (B-D) and Juniperus oxycedrus (A) roots: A: formation of chlamydospores, B: sexual morphology, C: Typical chlamydospore with hyphal outgrowings, D: Germination of sporangiospore. Scale bars = 20 µm. A: (GX40).

Conclusions

In conclusion Black mulberry can be a good eco-friendly dye to stain endophytic fungi. This procedure offers safer alternatives to the toxic, harmful, and occasionally cancerous chemicals used in traditional staining methods. It is critical to identify microorganisms and research biodiversity, but the most important aspect is to utilize reagents that are safe for both human health and the environment.

More experimental implementations should be focused to take on new technologies for producing natural colorants as a compatible as well as eco-safe alternative with synthetic colorants in different spheres of our life to make a greener world.

The new morphological structures discovered in our samples need the use of genetic tools to identify this endophytic fungus species in addition to morphological methods.

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Conflict of interest: If necessary, authors should describe any potential conflicts of interest.

References

1. Garg, R. K., Garg, K., Gupta, N., Chopra, V., & Gupta, A. (2022). COVID-19 Vaccine Behaviour among People Attending a Tertiary Care Center, Punjab, India. JOURNAL OF CLINICAL AND DIAGNOSTIC RESEARCH, 16(3), LC26-LC32.
2. Sigurdson, G. T., Tang, P., & Giusti, M. M. (2017). Natural colorants: Food colorants from natural sources. Annual review of food science and technology, 8(1), 261-280.
3. Torres, F. A. E., Zaccarim, B. R., de Lencastre Novaes, L. C., Jozala, A. F., Santos, C. A. D., Teixeira, M. F. S., & Santos-Ebinuma, V. C. (2016). Natural colorants from filamentous fungi. Applied microbiology and biotechnology, 100, 2511-2521.https://doi.org/10.1007/s00253-015-7274-x.
4. Ozrenk, K., Gazioglu, S. R. I., Erdinc, C., Guleryuz, M., & Aykanat, A. (2010). Molecular characterization of mulberry germplasm from Eastern Anatolia. African Journal of Biotechnology, 9(1).
5. Datta, R. K. (2002). Mulberry cultivation and utilization in India.
6. Özgen, M., Serçe, S., & Kaya, C. (2009). Phytochemical and antioxidant properties of anthocyanin-rich Morus nigra and Morus rubra fruits. Scientia horticulturae, 119(3), 275-279.
7. Fahad Hussain, F. H., Zohaib Rana, Z. R., Hassan Shafique, H. S., Arif Malik, A. M., & Zahid Hussain, Z. H. (2017). Phytopharmacological potential of different species of Morus alba and their bioactive phytochemicals: a review. https://doi.org/10.1016/j.apjtb.2017.09.015.
8. Lim SungHo, L. S., & Choi ChangIk, C. C. (2019). Pharmacological properties of Morus nigra L.(black mulberry) as a promising nutraceutical resource.https://doi.org/10.3390/nu11020437.
9. Chen, H., Yu, W., Chen, G., Meng, S., Xiang, Z., & He, N. (2017). Antinociceptive and antibacterial properties of anthocyanins and flavonols from fruits of black and non-black mulberries. Molecules, 23(1), 4.https://doi.org/10.3390/molecules23010004.
10. Chen Hu, C. H., Pu JunSong, P. J., Liu Dan, L. D., Yu WanSha, Y. W., Shao YunYing, S. Y., Yang GuangWei, Y. G., ... & He NingJia, H. N. (2016). Anti-inflammatory and antinociceptive properties of flavonoids from the fruits of black mulberry (Morus nigra L.).https://doi.org/10.1371/journal.pone.0153080.
11. Tahir, L., Aslam, A., & Ahmed, S. (2017). Antibacterial activities of Diospyros blancoi, Phoenix dactylifera and Morus nigra against dental caries causing pathogens: An in vitro study. Pakistan Journal of Pharmaceutical Sciences, 30(1).
12. Koyu, H., Kazan, A., Demir, S., Haznedaroglu, M. Z., & Yesil-Celiktas, O. (2018). Optimization of microwave assisted extraction of Morus nigra L. fruits maximizing tyrosinase inhibitory activity with isolation of bioactive constituents. Food chemistry, 248, 183-191. https://doi.org/10.1016/j.foodchem.2017.12.049.

13. Alkhalidy, H., Wang, Y., & Liu, D. (2018). Dietary flavonoids in the prevention of T2D: An overview. Nutrients, 10(4), 438.https://doi.org/10.3390/nu10040438.
14. Çakıroğlu, E., Uysal, T., Koçal, G. Ç., Aygenli, F., Baran, G., & Baskın, Y. (2017). The role of Morus nigra extract and its active compounds as drug candidate on human colorectal adenocarcinoma cell line HT-29. International Journal of Clinical Oncology and Cancer Research, 2(1), 10-14.doi: 10.11648/j.ijcocr.20170201.13.
15. de Albuquerque Veloso Machado, M., Roberts, B., Wong, B. L. H., van Kessel, R., & Mossialos, E. (2021). The relationship between the COVID-19 pandemic and vaccine hesitancy: a scoping review of literature until August 2021. Frontiers in public health, 9, 747787.
16. Sutradhar, P., & Bhattacharya, C. (2021). Use of the natural pigments of red beet root pomace (Beta vulgaris L.) to develop a mycological stain: An eco-friendly alternative substitute. Journal of Scientific Research, 65(3), 73-77. https://doi:10.37398/JSR.2021.650309
17. Yusuf, M., Shabbir, M., & Mohammad, F. (2017). Natural colorants: Historical, processing and sustainable prospects. Natural products and bioprospecting, 7, 123-145.https://doi.org/10.1007/s13659-017-0119-9.
18. Hartika, G., Zulharmita, Z., & Asra, R. (2021). Utilization of natural dyes substances for histological staining: a review. Asian Journal of Pharmaceutical Research and Development, 9(1), 149-158.https://doi.org/10.22270/ajprd.v9i1.925.
19. Dalalibera, A., Vilela, P. B., Vieira, T., Becegato, V. A., & Paulino, A. T. (2020). Removal and selective separation of synthetic dyes from water using a polyacrylic acid-based hydrogel: characterization, isotherm, kinetic, and thermodynamic data. Journal of Environmental Chemical Engineering, 8(5), 104465.
20. Lin, J., Ye, W., Xie, M., Seo, D. H., Luo, J., Wan, Y., & Van der Bruggen, B. (2023). Environmental impacts and remediation of dye-containing wastewater. Nature Reviews Earth & Environment, 4(11), 785-803.
21. Ali, H. (2010). Biodegradation of synthetic dyes—a review. Water, Air, & Soil Pollution, 213, 251-273.
22. Rafatullah, M., Sulaiman, O., Hashim, R., & Ahmad, A. (2010). Adsorption of methylene blue on low-cost adsorbents: a review. Journal of hazardous materials, 177(1-3), 70-80.
23. Baig, N., Sajid, M., & Saleh, T. A. (2019). Graphene-based adsorbents for the removal of toxic organic pollutants: a review. Journal of Environmental Management, 244, 370-382
24. Mokif, L. A. (2019). Removal methods of synthetic dyes from industrial wastewater: a review. Mesopotamia Environmental Journal (mesop. environ. j) ISSN: 2410-2598, 5(1), 23-40.
25. Liu, J., Zhou, H., Song, L., Yang, Z., Qiu, M., Wang, J., & Shi, S. (2021). Anthocyanins: Promising natural products with diverse pharmacological activities. Molecules, 26(13), 3807.https://doi.org/10.3390/molecules26133807.
26. Loulidi, I., Boukhlifi, F., Ouchabi, M., Amar, A., Jabri, M., Kali, A., ... & Aziz, F. (2020). Adsorption of crystal violet onto an agricultural waste residue: kinetics, isotherm, thermodynamics, and mechanism of adsorption. Sci World J. 2020; 2020: 1–9.
27. Sarkar, P., & Dey, A. (2021). Phycoremediation–An emerging technique for dye abatement: An overview. Process Safety and Environmental Protection, 147, 214-225.
28. Keharia, H., & Madamwar, D. (2003). Bioremediation concepts for treatment of dye containing wastewater: a review.
29. Phillips J.M. & Hayman D.S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society, 55, 1, P158
30. Arnold, A.E. & Lutzoni, F. (2007) Diversity and host range of foliar endo-phytes: are tropical leaves biodiversity hotspots? Ecology, 88, 541–549
31. Eschen, R., Hunt, S., Mykura, C., Gange, A.C. & Sutton, B.C. (2010). Thefoliar endophytic fungal community composition in Cirsium arvense isaffected by mycorrhizal colonization and soil nutrient content. Fungal Biology, 114, 991–998
32. Addy, H.D., Piercey, M.M. & Currah, R.S. (2005) Microfungal endophytes inroots. Canadian Journal of Botany, 83, 1–13.
33. Tousson, E. M., & Al-Behbehani, B. (2010). Black Mulberries (Morus nigra) as a natural dye for nervous tissues staining. The Egyptian Journal of Experimental Biology (Zoology), 6(1), 159-164.

34. Tousson, E., & Al-Behbehani, B. (2011). Black mulberries (Morus nigra) as a natural dye for animal tissues staining. Animal Biology,61(1), 49-56 https://doi.org/10.1163/157075511X554419
35. Wearn, J. A., Sutton, B. C., Morley, N. J., & Gange, A. C. (2012). Species and organ specificity of fungal endophytes in herbaceous grassland plants. Journal of Ecology, 100(5), 1085-1092.
36. Brundrett, M.C. (2004) Diversity and Classification of Mycorrhizal Associations. Biological Reviews, 78, 473-495. http://dx.doi.org/10.1017/S1464793103006316
37. Mansour, R., Yusuf, M. (2018). Handbook of Renewable Materials for Coloration and Finishing, 75:102.
38. Oancea, S. A. (2021). Review of the Current Knowledge of Thermal Stability of Anthocyanins and Approaches to Their Stabilization to Heat. Antioxidants 2021, 10, 1337. https://doi.org/10.3390/antiox10091337
39. Arruda, H. S., Silva, E. K., Peixoto Araujo, N. M., Pereira, G. A., Pastore, G. M., & Marostica Junior, M. R. (2021). Anthocyanins recovered from agri-food by-products using innovative processes: Trends, challenges, and perspectives for their application in food systems. Molecules, 26(9), 2632.https://doi.org/10.3390/molecules26092632.
40. Houghton, A., Appelhagen, I., & Martin, C. (2021). Natural Blues: Structure Meets Function in Anthocyanins. Plants 2021, 10, 726.https://doi.org/10.3390/plants10040726.
41. Qadri, M., Rajput, R., Abdin, M. Z., Vishwakarma, R. A., & Riyaz-Ul-Hassan, S. (2014). Diversity, molecular phylogeny, and bioactive potential of fungal endophytes associated with the Himalayan blue pine (Pinus wallichiana). Microbial ecology, 67, 877-887.https://doi.org/10.1007/s00248-014-0379-4.
42. Sanz-Ros, A. V., Müller, M. M., San Martín, R., & Diez, J. J. (2015). Fungal endophytic communities on twigs of fast and slow growing Scots pine (Pinus sylvestris L.) in northern Spain. Fungal biology, 119(10), 870-883.https://doi.org/10.1016/j.funbio.2015.06.008.
43. Wehner, J., Powell, J. R., Muller, L. A., Caruso, T., Veresoglou, S. D., Hempel, S., & Rillig, M. C. (2014). Determinants of root-associated fungal communities within A steraceae in a semi-arid grassland. Journal of Ecology, 102(2), 425-436. https://doi.org/10.1111/1365-2745.12197.
44. Budiman, A., & Aulifa, D. L. (2020). Antibacterial activity and mode of action of Black Mulberry (Morus nigra) fruits extract against Streptococcus mutans. Pharmacognosy Journal, 12(6s). https://doi:10.5530/pj.2020.12.233.
45. Coemans, E. (1863). Quelques hyphomycetes nouveaux.
46. Hibbett, D. S., Binder, M., Bischoff, J. F., Blackwell, M., Cannon, P. F., Eriksson, O. E., ... & Zhang, N. (2007). A higher-level phylogenetic classification of the Fungi. Mycological research, 111(5), 509-547
47. Wagner, L., Stielow, B., Hoffmann, K., Petkovits, T., Papp, T., Vágvölgyi, C., ... & Voigt, K. (2013). A comprehensive molecular phylogeny of the Mortierellales (Mortierellomycotina) based on nuclear ribosomal DNA. Persoonia-Molecular Phylogeny and Evolution of Fungi, 30(1), 77-93.
48. Linnemann, G. (1941). Die Mucorineen-Gattung Mortierella Coemans. Pflanzenforschung, Heft 23.
49. Chien, C. Y., Kuhlman, E. G., & Gams, W. (1974). Zygospores in two Mortierella species with “stylospores”. Mycologia, 66(1), 114-121.
50. Kirk, P. M. (1997). Mortierella elongata. IMI Descr Fungi Bact, 1303, 1-2.
51. Degawa, Y., & Gams, W. (2004). A new species of Mortierella, and an associated sporangiiferous mycoparasite in a new genus, Nothadelphia. Stud. Mycol, 50(2), 567-572.
52. Hyde, K. D., Tennakoon, D. S., Jeewon, R., Bhat, D. J., Maharachchikumbura, S. S., Rossi, W., ... & Doilom, M. (2019). Fungal diversity notes 1036–1150: taxonomic and phylogenetic contributions on genera and species of fungal taxa. Fungal diversity, 96, 1-242.
53. Ariyawansa, H. A., Hyde, K. D., Jayasiri, S. C., Buyck, B., Chethana, K. T., Dai, D. Q., ... & Hernawati. (2015). Fungal diversity notes 111–252—taxonomic and phylogenetic contributions to fungal taxa. Fungal diversity, 75, 27-274.
54. Uehling, J., Gryganskyi, A., Hameed, K., Tschaplinski, T., Misztal, P. K., Wu, S., ... & Bonito, G. (2017). Comparative genomics of Mortierella elongata and its bacterial endosymbiont Mycoavidus cysteinexigens. Environmental microbiology, 19(8), 2964-2983.
55. Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N. S., Wijesundera, R., ... & Abarenkov, K. (2014). Global diversity and geography of soil fungi. science, 346(6213), 1256688. https://doi:10.1126/science.1256688.

56. Zhang, T., Wang, Z., Lv, X., Li, Y., & Zhuang, L. (2019). High-throughput sequencing reveals the diversity and community structure of rhizosphere fungi of Ferula Sinkiangensis at different soil depths. Scientific reports, 9(1), 6558.. https://doi.org/10.1038/s41598-019-43110-z.

57. Yadav, D. R., Kim, S. W., Babu, A. G., Adhikari, M., Kim, C., Lee, H. B., & Lee, Y. S. (2014). First report of Mortierella alpina (Mortierellaceae, Zygomycota) isolated from crop field soil in Korea. Mycobiology, 42(4), 401-404. https://doi.org/10.5941/MYCO.2014.42.4.401.
58. Telagathoti, A., Probst, M., Mandolini, E., & Peintner, U. (2022). Mortierellaceae from subalpine and alpine habitats: new species of Entomortierella, Linnemannia, Mortierella, Podila and Tyroliella gen. nov. Studies in Mycology, 103(1), 25-58. https://doi.org/10.3114/sim.2022.103.02.