

## A NEW SPECIES OF *TIAROSPORELLA AZADIRICHTA* AND NEW FUNGAL RECORDS ON *AZADIRACHTA INDICA* FROM PAKISTAN

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### Abstract

A new species of *Tiarospora azadirachta* has been described on *Azadirachta indica* and some new fungal records viz., *Diplozythiella bambusina*, *Ulocladium chartarum*, *Cladosporium nigrellum*, *Cladosporium oxysporum*, *Didymostilbe coffeae*, *Muellerella pygmaea*, *Lasiodiplodia paraphysaria*, *Monochaetina terminalae*, *Trimmatostroma* sp., and *Epidermophyton floccosum* are reported on *Azadirachta indica* for the first time from Pakistan.

### Introduction

*Azadirachta indica* (as *Azadirachta Juss*, *Melia azadirachta*) belong to the family *Meliaceae*. Neem is its common name. It is important due to its commercial and medicinal value. *Azadirachta indica* is also an important plant for its potential of anti-fungal, anti-bacterial and insecticidal activity. Decline of trees due to fungi are increasing tremendously in Pakistan especially in Punjab and Sindh (Javed *et al.*, 2004; Khanzada *et al.*, 2011; Fateh *et al.*, 2011; Rheman *et al.*, 2011; Farooq *et al.*, 2011).

Sixteen fungi has been reported on *Azadirachta indica* from Pakistan viz., *Fuligo septica* var. *septica* (L.) Wiggers on trunk of *Azadirachta indica*; *Hypocreopsis macrostoma* (Berk. & Curt.) Mueller on bark of *Azadirachta indica* (as *Melia azadirachta*); *Pleospora pellita* (Fr.) Rab, on stem of *Melia azadirachta*; *Eutypa lundibunda* Sacc., on branches of *Azadirachta indica* (as *Melia azadirachta*); *Eutypella russedes* (Berk. & Br.) Berl, on dead branches of *Azadirachta indica* (*Melia azadirachta*); *Ganoderma lucidum* (Curtis) Karst. (as *Fomes lucidus*), on *Azadirachta indica* (Neem); *Polyporus ostreiformis* Berk., causes wood rot in *Azadirachta indica* (as *Melia azadirachta*); *Fomes senex* Nees et Mont., causes white wood rot of *Azadirachta indica*, *Alternaria tenuissima* (Nees. ex. Fr.) Wiltshire, causes leaf spot of *Azadirachta indica*; *Cercospora subsessilis* Syd., causes leaf spot of *Azadirachta indica*, *Cercospora meliae* Ell. & Ev., causes leaf spot on *Azadirachta indica*, *Oidium* spp., cause powdery mildew in *Azadirachta indica*; *Botryodiplodia azedarachta* (Ell. & Ev.) Ahmad; causes die back of *Azadirachta indica*; *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl (as *Botryodiplodia theobromae* Pat., on dead branches of *Azadirachta indica*; *Diplodia meliae* Sacc. & Roum., on leaf rachis of *Azadirachta indica*; *Dothiorella meliae* Ahmad on leaf rachis of *Azadirachta indica*; Ahmad, 1956, 1962, 1969, 1978; Ahmad & Arshad, 1972; Ahmad *et al.*, 1997; Ghaffar & Kafi, 1968; Ghaffar *et al.*, 1971; Ghaffar & Abbas, 1972; Ghafoor & Khan, 1976; Khan, 1952, 1969, 1989; Khan & Kamal 1963, 1968 Kamal & Mughal, 1968; Malik & Khan, 1944.

It is very surprising that only 16 fungi have been reported from Pakistan on *Azadirachta indica* a widely cultivated and wildy grown tree. Why it is so? Whether this plant is neglected for fungal screening? or it is its antifungal, antibacterial and anti-insecticidal activity that play a role to protect this plant from pathogens. For this

reason a detailed survey of the plant for fungi was under taken in this study.

### Materials and Methods

Samples of *Azadirachta indica* were collected from the different areas of District Faisalabad and Gojra. The different areas were G.C. University, Faisalabad, Agriculture University Faisalabad, Gutwala forest (park) Faisalabad, Tandlianwala and Gojra City. Methods and materials are the same as described Abbas *et al.*, (2010). Identification up to species level were carried out after consulting (Morris, 1963; Ellis, 1971, 1976; Carmichael *et al.*, 1980; Sutton, 1980; Ahmad, 1978; Ahmad *et al.*, 1997; Abbas *et al.*, 2004; Kirk, 2012).

### Results and Discussion

The fungus on *Azadirachta indica* specimen G.C.U.F. Mycol. H. # 40 was identified as a *Diplozythiella bambusina* Died.

*Diplozythiella bambusina* Died., *Annls mycol.* 14: 215 (1916); Sutton, *The Coelomycetes*:58-61 (1980)

**Description of the fungus under study:** Conidiomata eustromatic, ostiolate, dark brown to black, 152-190  $\mu$ m. Conidiophores absent. Conidiogenous cells hyaline, enterogenous. Conidia uniseptate, hyaline, cylindrical, rounded at ends, constricted at septa, 3.8-11.4  $\times$  1.26-1.50  $\mu$ m Fig. 1(A-E).

*Diplozythiella* Died is a monotypic genus based on *Diplozythiella bambusina* Died. Fungus under study on *Azadirachta indica* was compared with the description of *D. bambusina* and found that the fungus under study completely resembled with *D. bambusina*, Therefore, the fungus on *A. indica* is identified as *D. bambusina*. Genus *Diplozythiella* has not been previously reported from Pakistan (Ahmad *et al.*, 1997). It is an addition to fungal flora of Pakistan and *Azadirachta indica* is also a new host of *Diplozythiella bambusina* from Pakistan.

**Specimen examined:** *Diplozythiella bambusina* on bark of *Azadirachta indica*; Pensara Road Gojra, Pakistan; 22 April, 07; G.C.U.F. Mycol. H. # 40: S. Qaiser Abbas & Nabila Iftikhar.

The fungus on G.C.U.M.H.No.42 is described as *Tiarosporella azadarichta* sp. nov.

***Tiarosporella azadarichta* sp. nov.**

**Description of the fungus:** Colony blackish grey appearance on natural sample. Conidiomata pycnidial, separate, globose, thick walled and dark brown, 150 µm in diameter, ostiole absent. Conidiogenous cells hyaline, (16.5-21 × 3.5µm). Conidia aseptate, cylindrical, hyaline 12.25-16.5 (mostly 14µm) × 3.5µm and covered with gelatinous sheath which form cap like structure on apical side of conidia Fig. 2(A-D).

**Latin description:** Conidiomata pycnidiala, separatata, globosa, unilocularia 150µm dia., ostiola absentia, conidiophora non observa, cellulae conidiogenae hyalinae (16.5-21 × 3.5 µm). Conidia aseptata, hyalinae., cylindrica, mucilaginatae enclosa, ad apicem capitata 12.25-16.5 (14µm) × 3.5 µm.

Holotypus-*Tiarosporella azadarichta* in ramis emortuis *Azadirachta indica* Green Town Gojra Pakistan; 3 May, 07; S. Qaiser Abbas & Nabila Iftikhar G.C.U.F. Mycol. H. # 42.

*Tiarosporella* Höhn., and *Tiarospora* Sacc. & March are two genera, which closely resemble with the fungus found on *Azadirachta indica*. Resemblance lies in that both genera, have pycnidial conidiomata, dark brown, separate, globose, thick walled, of textura angularis and ostiole central, circular and papillate. Conidiophores absent. Conidiogenous cells hogenous and non-proliferating, discrete, lageniform, hyaline and smooth. However they differ from each other in some respect. *Tiarosporella* has aseptate conidia and *Tiarospora* has uniseptate conidia. The fungus under study on *A. indica* has aseptate, cylindrical, hyaline conidia with gelatinous sheath, therefore it belongs to the genus *Tiarosporella*.

*Tiarosporella azadarichta* resembles with *Tiarosporella graminis* (Pirozynski & Shoemaker) Nag Raj, in having straight, cylindrical, ellipsoideo-fusiform conidia with apical appendages but *T. azadarichta* differs from *T. graminis* (Pirozynski & Shoemaker) Nag Raj in size of conidiogenous cells and conidia. Conidiogenous cells of *T. graminis* are smaller and less wider (12-15×1.5-2.5µm) than *T. azadarichta* (16.5-21×3.5µm), whereas conidia in *T. graminis* are longer and wider (20-29.5×7-9µm) than *T. azadarichta* (12.25-16.5 (mostly 14×3.5µm). Similarly in *T. graminis* var. *karoo* conidiogenous cells are smaller and less wider (12-18×1.5-2.5µm) than *T. azadarichta* (16.5-21×3.5µm). Conidiomata of *T. abietis* Whitney, Reid & Pirozynski, *T. parka* (Berk. & Br.) Whitney, Reid & Pirozynski and *T. pseudotsugae* Whiting, Reid & Pirozynski are larger and wider (550-600µm in diam.) than *T. azadarichta* (150-190µm in diam.). Furthermore in *T. tritici* Sutton & Marasas, conidiomata are slightly bigger and wider (200µm in diam.) than *T. azadarichta* whereas conidia in *T. tritici* are oval to fusiform, straight 29-38 × 12-17µm., more longer and wider conidia than *T. azadarichta* (16.5-21×3.5µm).

*T. madreya* (Subram. & Ramakr.) Nag Raj, closely resembled *T. azadarichta* in having slightly bigger conidiomata (200µm) than this fungus (150-190µm).

Similarly the conidiogenous cells of *T. madreya* are more smaller and less wider (8-10×2.5-4µm) than *T. azadarichta* (16.5-21×3.5µm) and conidia are straight, cylindrical, much longer and wider (19-27×4.5-6µm) than *T. azadarichta* [12.25-16.5µm (mostly 14µm) × 3.5µm]. It is clear that *T. madreya* is near to *T. azadarichta*, but conidia of *T. azadarichta* are smaller and less wider, therefore the under study fungus is described as a new species.

Previously the genus *Tiarosporella* has not been reported from Pakistan (Ahmad *et al.*, 1997). The genus *Tiarosporella* is a new addition to the fungal flora of Pakistan and *Tiarosporella azadarichta* is a new species described from Gojra, Pakistan.

**Specimen examined:** *Tiarosporella azadarichta* from dead branch of *Azadirachta indica*; Green Town Gojra Pakistan; 3 May, 07; G.C.U.F. Mycol. H. # 42; S. Qaiser Abbas & Nabila Iftikhar.

The fungus on *Azadirachta indica* specimen G.C.U.F.MH. # 34 is identified as *Ulocladium chartarum* (Preuss) Simmons.

*Ulocladium chartarum* (Preuss) Simmons, *Mycologia*, 59: 88-90 (1967).

**Description of the fungus under study:** Colonies effuse and black. Mycelium, superficial, black brown. Conidiophores dark brown, branched, morphologically different from vegetative hyphae, 30-52.5×3.5-7µm. Conidiogenous cells olivaceous brown, cylindrical, 7×3.5µm. Conidia broadly ellipsoidal or inversely ovoid, brown 1-5 oblique and transverse septa, beak not present, thick walled, echinulated, 11.4-34.2×7.6-15.2µm Fig. 3(A-G).

Genus *Ulocladium* and *Alternaria* show close resemblance with each other but differ in conidial attachment to conidiophores. In *Alternaria* conidia are attached from their broader side while in *Ulocladium* conidia are attached from their narrow side.

*Ulocladium chartarum* (Preuss) Simmons, *U. consortile* (Thüm.) Simmons and *U. alternarie* (Cooke) Simmons are characterized in having 1-5 transverse septa and several longitudinal or oblique septa, whereas *U. botrytis* Preuss has 1-3 transverse septa. *U. atrum* Preuss., and *U. oudamansii* Simmons, have 3-5 transverse septa. *U. clamydosporum* Mouch has 2-7 transverse and several longitudinal and oblique septa.

The fungus present on *A. indica* has 1-5 transverse septa and several longitudinal and oblique septa. These characters are common in 3 species of *Ulocladium* viz., *U. chartarum*, *U. consortile* and *U. alternarie*. However in *U. chartarum* (Preuss) Simmons conidiophores are 50 × 5-7µm, conidia (18-38 × 11-20µm) in chains of 2-10 with small or false beaks. Whereas *U. consortile* and *U. alternarie* differ from *U. chartarum* in that conidia are without beaks and are not in chains (16-34 × 10-15µm). In *U. consortile* conidiophores are (60 × 4-5µm), whereas in *U. alternarie* conidiophores are 100 × 4-7µm and conidia are 18-35 × 15-20 µm, that is smaller than *U. chartarum*. In the fungus under study, conidial lower limit range 11.4-34.2 × 7.6-15.2µm is lower than *U. alternarie* 18-35 × 15-20µm and *U. consortile* 16-34 × 10-15µm and conidia are less wider than *U. alternarie* and *U. consortile*.

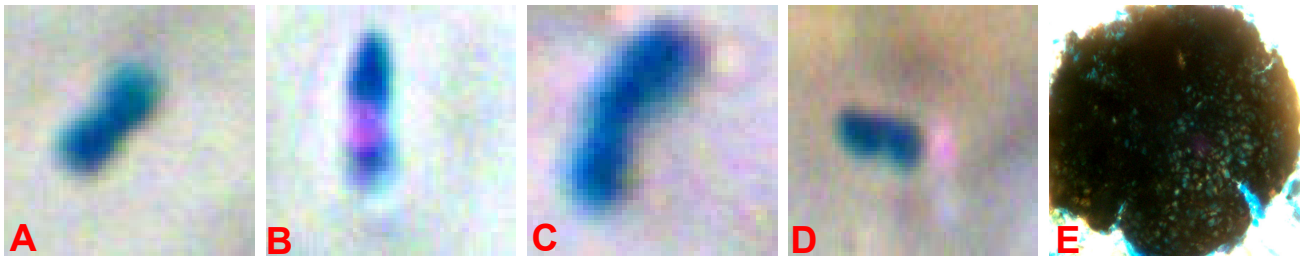


Fig. 1(A-E). *Diplozytheilla bambusina*: A, B, C & D. Conidia.1000X; E. conidiomata.400X.

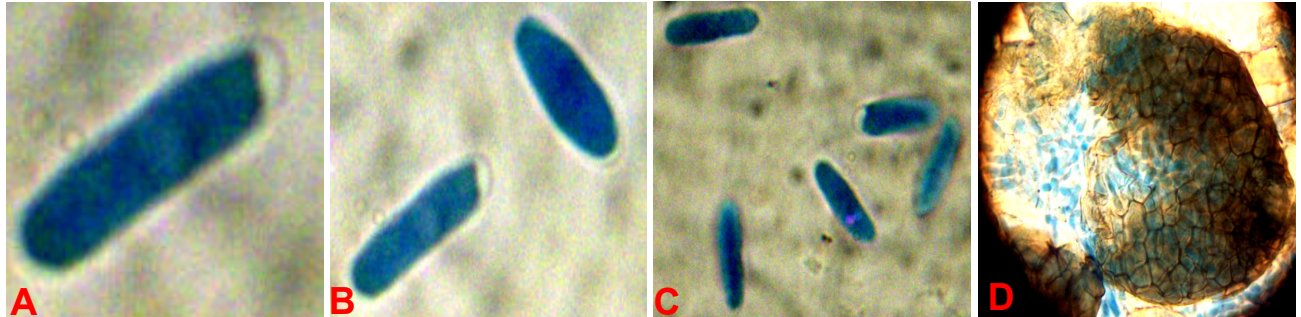


Fig. 2(A-D). *Tiarosporella azadarichta* A. Conidia with mucilaginous cap. 1000X; B. Conidia with gelatinous sheath.1000X; C. Conidia.1000X; D. Conidiomata.400X.

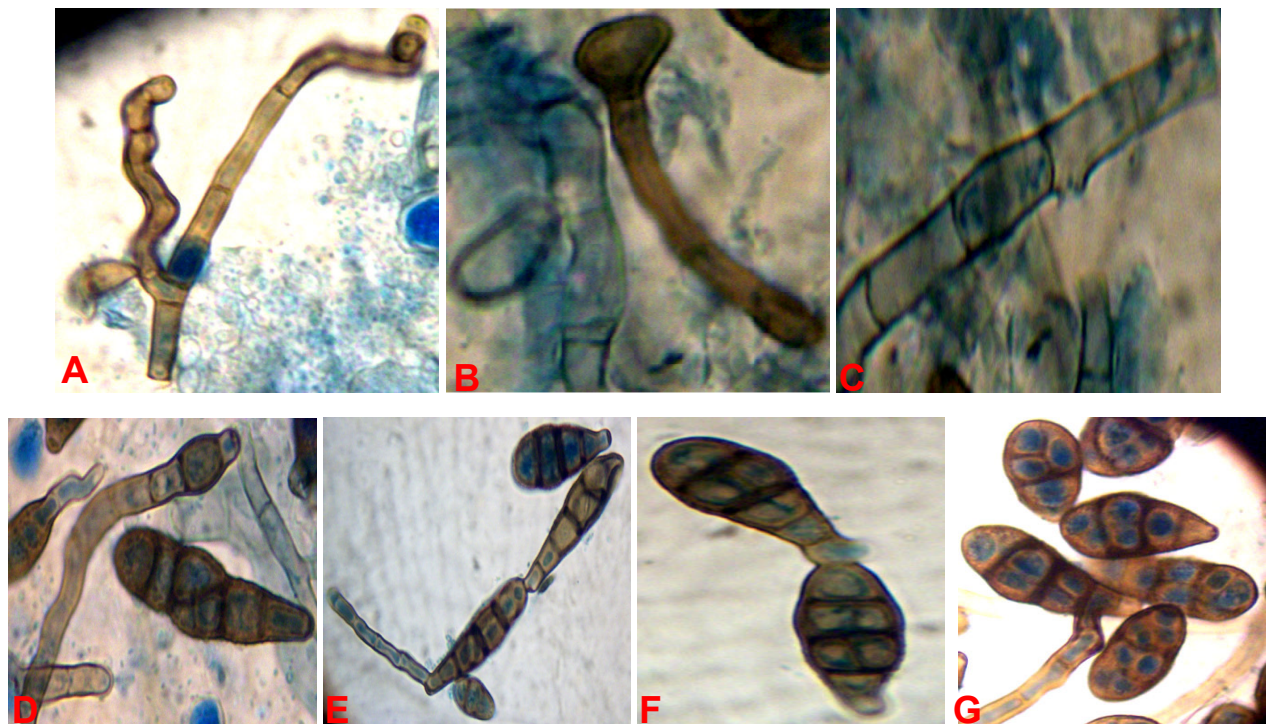


Fig. 3(A-G). *Ulocladium chartarum*: A. Conidiophores with conidial attachment; B. Conidiophore with unseptate conidia (400X); C. Conidiophore (1000X). D. Attachment of conidia; E. Chain of 3 conidia; F. Chain of 2 conidia; G. Conidia with variable septa number (D, F, G, 1000X; E, 400X).

The fungus under study completely resembled with *Ulocladium chartarum* except some minor differences in conidial dimension, therefore, it is identified as *Ulocladium chartarum*.

Three *Ulocladium* spp., viz., *Ulocladium botrytis* Preuss, *U. atrum* Preuss., *U. consortiale* (Thüm.) Simmons, have been reported from Pakistan (Ahmad, 1969; Nisa *et al.*, 1971; Matsushima, 1993).

*U. chartarum* has not been previously reported from Pakistan (Ahmad *et al.*, 1997). *U. chartarum* is an addition to fungal flora of Pakistan and *Azadirachta indica* is a new host record of *U. chartarum*.

**Specimen examined:** *Ulocladium chartarum* Preuss, on branches of *A. indica*; Green town Gojra Pakistan; 29 April, 07; G.C.U.F. M.H. # 34; S.Q. Abbas and Nabila Iftikhar.

The fungus on *Azadirachta indica* specimen G.C.U.FMH. # 37 is identified as *Cladosporium nigrellum* Ellis & Everh.

***Cladosporium nigrellum*** Ellis & Everh., *Proc. Acad. N. Sci. Phalid.* 1893: 463 (1894).

**Description of the fungus under study:** Mycelium septate, superficial, pale brown. Conidiophores septate, brown, scars present 230-250×5-9µm. Conidia cylindrical to oval, attached at apex as well as at lateral sides of conidiophores, 0-3 septate, 11.4-16 × 3.8-8µm Fig. 4(A-E).

The fungus under study on *Azadirachta indica* has 0-3 septate conidia. *Cladosporium nigrellum* Ellis & Everh, *C. apicale* Berk. & Brown, *C. uredinicola* Speg., *C. macrocarpum* Preuss, *C. variable* (Cooke) de Vries, and *C. brassicae* (Ellis & Barthol.) Ellis have 0-3 septate conidia. In spite of similarity in having 0-3 septate conidia, however there are sufficient differences among them are found, which separate them from one another.

*Cladosporium brassicae* have shorter conidiophores (150×6-9µm) with terminal and intercalary swellings of 10-12µm. than fungus under study where conidiophores are 230-250×5-9µm; and without terminal and intercalary swelling. Similarly *C. apicale* also have wider conidiophores 260×6-8µm than fungus under study 230-250×5-9µm whereas *C. variable* have long conidiophores 350×6-8µm than fungus under study which has conidiophores of 230-250×5-9µm and *Cladosporium macrocarpum* differs from fungus under study in having longer and less wider conidiophores 300×4-8µm with terminal and intercalary swellings of 9-11µm diameter. Furthermore *Cladosporium uredinicola* also differs from under study fungus in having longer and less wider conidiophores 300×3-5µm than the under study fungus where conidiophores are 230-250×5-9µm. Furthermore conidiophores in *C. nigrellum* are wavy, smooth, reddish brown, septate, 250 × 5-9µm; Conidia cylindrical, narrowing at the ends, lemon shaped, in simple or branched chains, smooth, light brown, 5-15×4-7µm. After comparison it is concluded that the under study fungus on *Azadirachta indica* closely resembled with *C. nigrellum*, hence it is identified as *C. nigrellum*.

The fungus on *A. indica* identified as *C. nigrellum* has not been previously reported from Pakistan (Ahmad *et al.*, 1997) and it is a new report on *A. indica* from Gojra Pakistan.

**Specimen examined:** *Cladosporium nigrellum* from leaves of *Azadirachta indica*; Green Town Gojra Pakistan; 10 September, 07; G.C.U.F. Mycol. H. # 37; S. Qaiser Abbas & Nabila Iftikhar.

The fungus on *Azadirachta indica* specimen G.C.U.FMH. # 36 is identified as *Cladosporium oxysporum* Berk. & Curt

***Cladosporium oxysporum*** Berk. & Curt., (1868); Ellis. More Dematiaceous Hyphomycetes, CAB(IMI), Kew, Surrey, England, pp. 507, (1976).

**Description of the fungus under study:** Mycelium superficial, light brown, septate. Conidiophore olivaceous brown, macronematous, 152-268×3.8µm, chlamydospores present, 7.6 µm. Conidia sub rounded, ellipsoidal to cylindrical, 0-1 septate, 10-19×3.8-5.7µm, solitary or in

chains. Chains consist of 3-4 conidia (Fig. 4). Colony on *Azadirachta indica* has green appearance Fig. 5(A-E).

*Cladosporium orchidearum* Cooke & Masee, *C. brassicae* (Ellis & Barthol.) Ellis, *C. uredinicola* Speg., *C. nigrellum* Ellis & Everh., *C. macrocarpum* Preuss and *C. variable* (Cooke) de Vries have 0-1 septate conidia and differ from under study fungus which has 0-3 septate conidia. However, *Cladosporium oxysporum* Berk. & Curt., *C. herbarum* (Pers.) Link ex Gray, *C. colocasiae* Sawada, *C. orchids* E. A. Ellis & M. B. Ellis, *C. gallicola* Sutton, *C. aecidiicola* Thüm, and *Cladosporium tenuissimum* Cooke have 0-1 septate conidia and resemble with under study fungus in this regard, however there are some differences which separate each from other *Cladosporium gallicola* has longer and wider conidiophores (250×5-9µm) than under study fungus (152-268×3.8µm). Similarly *C. aecidiicola* (100×4-6µm) and *Cladosporium orchids* (100×3-8µm) have shorter and slightly wider conidiophores than under study fungus (152-268×3.8µm). Further more *C. tenuissimum* has much longer conidiophores (800×3-6µm) than under study fungus (152-268×3.8µm) and *C. herbarum* has conidia, smaller and wider (5-23×3-8µm) than under study fungus (10-19×3.8-5.7µm).

Fungus under study completely resembles with *Cladosporium oxysporum* where conidiophores can easily be differentiated from vegetative hyphae, straight or slightly wavy, swollen nodes near the apex and upto 500 µm long and 3-5 µm wide with terminal and intercalary swellings (6-8 µm). Conidia arising from terminal swellings which later become smooth, intercalary, forming short simple and branched chains, (5-30×3-6µm). Fungus on *Azadirachta indica* shows its complete resemblance with *C. oxysporum*, therefore it is identified as *C. oxysporum*.

Previously *Cladosporium oxysporum* Berk. & Curt., has been also recorded on 25 different plants belonging to different families from Pakistan (Ahmad *et al.*, 1997). However it is first time recorded on *Azadirachta indica* from Gojra Pakistan.

**Specimen examined:** *Cladosporium oxysporum* on leaves of *Azadirachta indica*; Quaid.e.Azam School, Gojra, Pakistan; 19 September, 07; G.C.U.F. Mycol. H. # 36 S. Qaiser Abbas & Nabila Iftikhar.

The fungus on *Azadirachta indica* specimen G.C.U.FMH. # 38 is identified as *Didymostilbe coffeae* Henn.

***Didymostilbe coffeae*** Henn. *Hedwigia*, 41: 148 (1902).

**Description of the fungus under study:** Mycelium septate, branched. Synnemata capitate, 816×38µm, apex broad, 49.4-68.4µm; hyaline, thick and steriated. Conidiophores 19-27×3.8µm. Conidia uniseptate, obovoid to cylindrical, apex and base obtuse 4.6-6.5×3.04-3.8µm. Fig. 6(A-G).

Under study fungus found on *Azadirachta indica* resembles with genera *Didymobotryum* Sacc., and *Didymostilbe* Hennings. Both genera have tall cylindrical stipe. Conidia uniseptate, oblong or cylindrical but the difference between the two genera is that in *Didymobotryum* conidia are dry and in *Didymostilbe* conidia were produced in mucoid mass. The fungus isolated from *Azadirachta indica* (Neem) closely resemble with genus *Didymostilbe*. The fungus from *A. indica* was identified as *Didymostilbe coffeae* Henn., after consulting Morris, (1963); Ellis (1971, 1976).

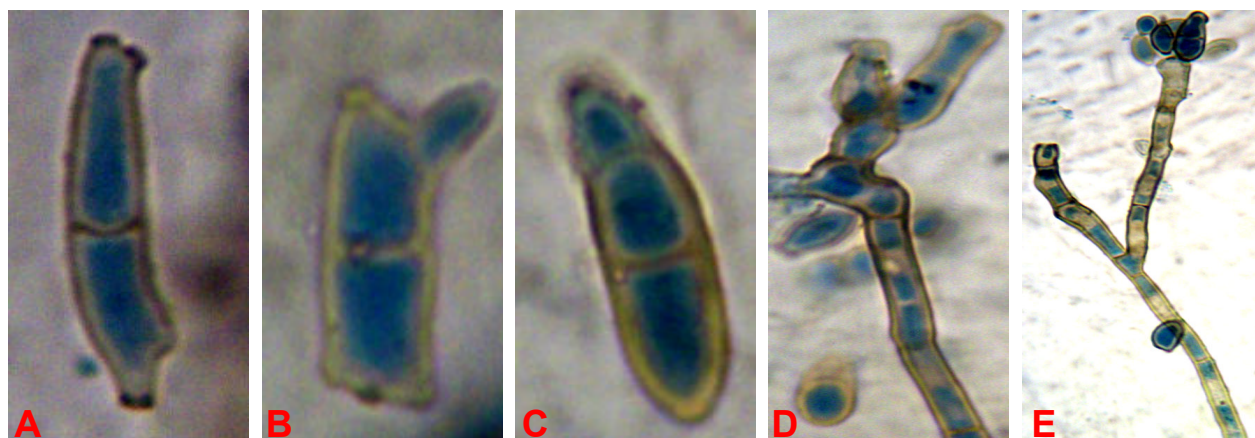


Fig. 4(A-C). *Cladosporium nigrellum*: A. uniseptate conidia with prominent scars; B. uniseptate conidia; C. biseptate conidia; (A, B, C, 1000X). D & E. Conidial attachment (D & E, 400X).

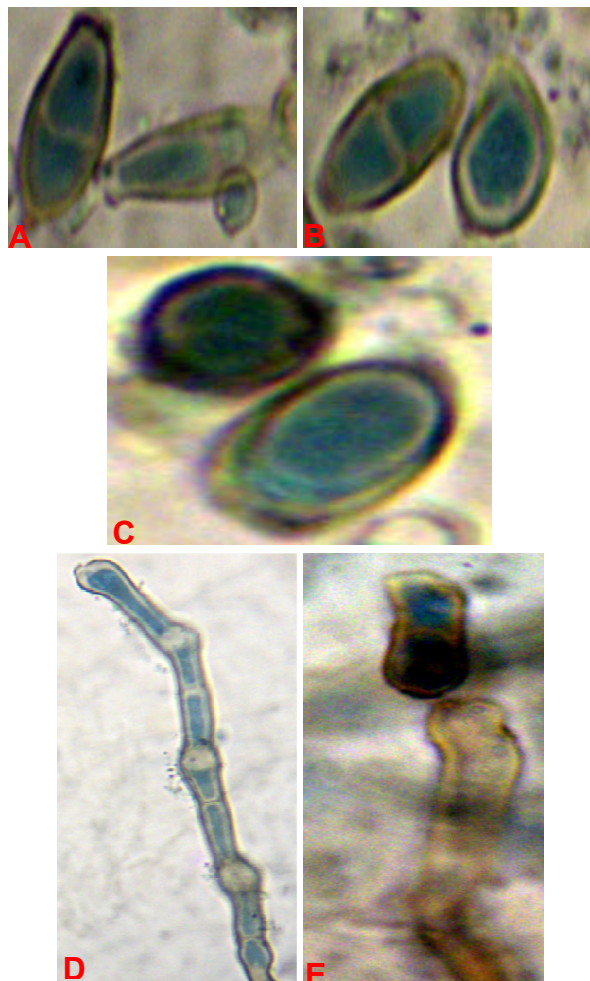


Fig. 5. *Cladosporium oxysporum*. A, B & C. aseptate & uniseptate conidia (A, B, C 1000X). D & E. Conidiophores with conidial attachment (D400X; E1000X).

Previously *Didymostilbe coprophila* has been reported on dung from Faisalabad Pakistan (Mirza & Qureshi, 1970). *D. coffeae* is a new fungal record from Pakistan and *Azadirachta indica* is a new host for this fungus from Faisalabad, Pakistan.

**Specimen examined:** *D. coffeae* from bark of *Azadirachta indica*; Tandlianwala district Faisalabad Pakistan; 15 September, 07; G.C.U.F. Mycol. H. # 38; S.Q. Abbas & Nabila Iftikhar.

Fungus on *Azadirachta indica* specimen G.C.U.F.MH. # 39 is identified as *Muellerella pygmaea* (Körb.) D. Hawksworth *Muellerella pygmaea* (Körb.) D. Hawksworth. *Bot. Notiser*, 132(3): 289 (1979)

=*Eendococcus pygmaeus* (Körb.) Th. Fr.

=*Microthelia ecatonospora* Anzi, *Atti Soc. ital. Sci. nat. (Modena)*, 9: 256 (1866)

=*Microthelia pygmaea* (Körb.) Körb., (1855)

=*Muellerella pygmaea* (Körb.) D. Hawksw., *Bot. Notiser*, 132(3): 289 (1979) var. *pygmaea*

=*Mycoporum pygmaeum* (Körb.) Jatta, (1900)

=*Pyrenula pygmaea* (Körb.) Tuck., *Gen. lich. (Amherst)*: 272 (1872)

=*Sychnogonia pygmaea* (Körb.) Trevis., (1860)

=*Tichothecium pygmaeum* Körb., *Parerga lichenol. (Breslau)*: 467 (1865); Ahmad, *Ascomycetes of Pakistan*, part 1. *Biological society of Pakistan*, monograph 137(1978).

=*Tichothecium pygmaeum* var. *ecatonosporum* Anzi & G. Winter (1885)

=*Tichothecium pygmaeum* Körb., *Parerga lichenol. (Breslau)*: 467 (1865) var. *pygmaeum*

**Description of the fungus under study:** Ascocarp flask-shaped, dark brown, superficial, ostiolate with colorless appendages on ostioler region, 425.5-485.5µm. Asci clavate, 28-35×10.5-14µm. Ascospores hyaline, many, oval to ellipsoidal, uniseptate, 7×3.5µm. Fig. 7(A-E).

This fungus *Muellerella pygmaea* (Körb.) D. Hawksworth was reported by Ahmad (1978) and Ahmad *et al.*, (1997) as *Tichothecium pygmaeum* from Changa Manga Pakistan on *Salvadora oleoides*. Hawksworth (1979) replaced it to *Muellerella pygmaea* (Körb.) Hawksworth.

Fungus under study completely resembled with *Muellerella pygmaea* (Körb.) Hawksw (syn. *T. pygmaeum* Körb) in having ascocarp of flask-shaped, dark brown, superficial and ostiolate, 425.5-485.5 µm; appendages hyaline at ostioler region. Asci clavate, 28-35×10.5-14µm. Ascospores hyaline, many, uniseptate, oval to ellipsoidal, 7×3.5µm *Azadirachta indica* is a new host for *Muellerella pygmaea* (Körb.) D. Hawksw from Gojra Pakistan.

**Specimen examined:** *Muellerella pygmaea* (Körb.) D. Hawksw as *Tichothecium pygmaeum* on bark of *Azadirachta indica*; Bilal park Gojra Pakistan; 20 April, 07; S.Q. Abbas & Nabila Iftikhar. G.C.U.F. Mycol. H. # 39.

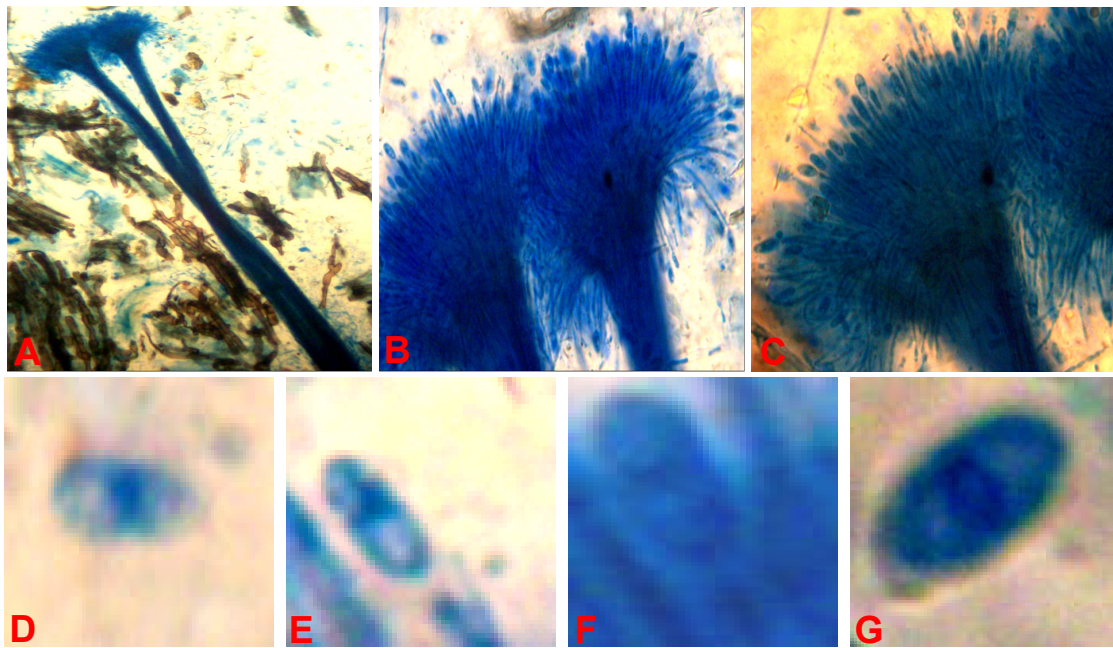


Fig. 6(A-G). *Didymostibe coffeae*: A synnemata (60X); B,C capitates head of synnemata 9400X); D,E,F,G uniseptate conidia (1000X).

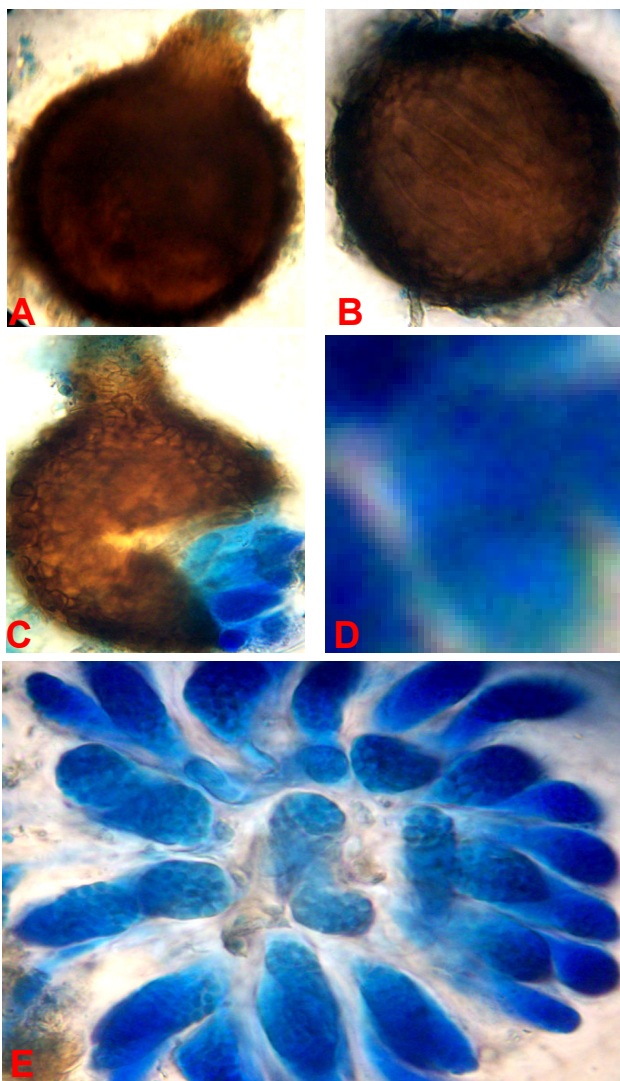


Fig. 7. *Muellerella pygmaea*: A. Ascocarp 400 X; B. Ascocarp with asci 400 X; C. Crushed ascocarp. 400X; D. Ascospores. (1000X). E. Asci.1000X.

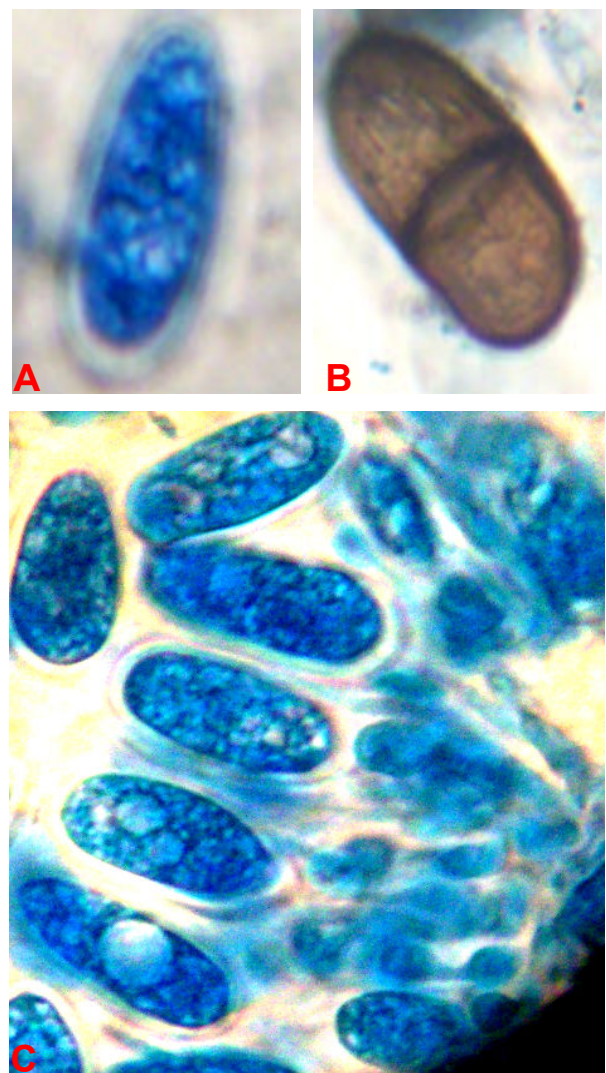


Fig. 8(A-C). *Lasiodiplodia paraphysaria*: A. Hyaline conidia. 1000X; B. Mature conidia.1000X; C. Conidia with conidiogenous cells.1000X.

Fungus found on *Azadirachta indica* specimen GCUF#42 is identified as *Lasiodiplodia paraphysaria* (Sacc) Keissl.

***Lasiodiplodia paraphysaria*** (Sacc.) Keissl., *Beih. bot. Zbl.*, Abt. 2 36: 314 (1918)  
=*Diplodia paraphysaria* Sacc., *Bull. Soc. R. Bot. Belg.*, 35: 130 (1896)

**Description of the fungus under study:** Brownish black patches on the bark of *Azadirachta indica*. Conidiomata pycnidial, non ostiolate, dark brown, thick-walled, 296  $\mu$ m. Conidiophore absent. Conidiogenous cells hyaline, unseptate, thin walled, 9-9.5 $\times$ 3.8 $\mu$ m. Immature conidia hyaline, aseptate, oval and thickwalled, at maturity conidia become brown, uni-septate, thick walled with longitudinal striations, from apex to base 30-33.4 $\times$ 14-16 $\mu$ m Fig. 8(A-C).

Sutton (1980) was of the opinion that correct name of *Botryodiplodia theobromae* was *Lasiodiplodia theobromae*.

However, Punithalingum (1980) dealt it as *Botryodiplodia theobromae* in his monograph. Abbas *et al.*, (2004) when reassessing the *Sphaeropsis undulata*, they pointed out that *Sphaeropsis undulata* Berk & Curt., was an earlier name for *Lasiodiplodia theobromae*.

After 2004, work on *Lasiodiplodia* was carried out both morphological as well on DNA finger printing and sequence (Pavlic *et al.*, 2004; Burgess *et al.*, 2006; Damm *et al.*, 2007; Alves *et al.*, 2008); Abdollahzadeh *et al.*, (2010) dealt 14 species of *Lasiodiplodia*. Abdollahzadeh *et al.*, (2010) was of the opinion that conidial dimension of *Botryodiplodia theobromae* never exceed 30  $\mu$ m in length and 16  $\mu$ m in width, while the conidial length in *Lasiodiplodia undulata* are up to 32 $\mu$ m and width is up to 19.2 $\mu$ m therefore they consider that both species are separate taxa.

Total species of *Lasiodiplodia* spp., are tabulated with reference to conidial measurement (Abdollahzadeh *et al.*, 2010).

Name of species	Conidial measurement ( $\mu$ m)	Reference
<i>L. abnormis</i>	25-28 $\times$ 13-15	Saccardo (1913)
<i>L. citricola</i>	22.5-26.6 $\times$ 13.6-17.2	Abdollahzadeh <i>et al.</i> , (2010)
<i>L. crassispota</i>	27-30 $\times$ 14-17	Burgess <i>et al.</i> , (2006)
<i>L. fiorii</i>	24-26 $\times$ 12-15	Saccardo (1913)
<i>L. gilanensis</i>	28.6-33.4 $\times$ 15.6-17.6	Abdollahzadeh <i>et al.</i> , (2010)
<i>L. gonubiensis</i>	32-36 $\times$ 16-18.5	Pavlic <i>et al.</i> , 2004
<i>L. hormozganensis</i>	19.6-23.4 $\times$ 11.7-13.3	Abdollahzadeh <i>et al.</i> , (2010)
<i>L. iraniensis</i>	18.7-22.7 $\times$ 12.1-13.9	Abdollahzadeh <i>et al.</i> , (2010)
<i>L. margaritacea</i>	14-17 $\times$ 11-12	Pavlic <i>et al.</i> , (2008)
<i>L. paraphysaria</i>	30-32 $\times$ 15-16	Saccardo (1913)
<b>Fungus under study</b>	30-33.4 $\times$ 14-16 $\mu$ m	<b>Present study</b>
<i>L. parva</i>	18.3-22.1 $\times$ 10.7-12.3	Alves <i>et al.</i> , (2008)
<i>L. plurivora</i>	26.7-32.5 $\times$ 14.4-16.7	Damm <i>et al.</i> , (2007)
<i>L. pseudotheobromae</i>	25.5-30.5 $\times$ 14.8-17.2	Alves <i>et al.</i> , (2008)
	21.7-26.3 $\times$ 13.4-14.8	Abdollahzadeh <i>et al.</i> , (2010)
<i>L. ricinii</i>	16-19 $\times$ 10-11	Saccardo (1913)
<i>L. rubropurpurea</i>	24-33 $\times$ 13-17	Burgess <i>et al.</i> , (2006)
<i>L. theobromae</i>	23.6-28.8 $\times$ 13-15.4	Alves <i>et al.</i> , (2008)
	22.4-24.2 $\times$ 12.9-14.3	Abdollahzadeh <i>et al.</i> , (2010)
<i>L. thomasiana</i>	28-30 $\times$ 11-12	Saccardo (1913)
<i>L. undulate</i>	20-32 $\times$ 13.5-19.2	Abbas <i>et al.</i> , (2004)
<i>L. venezuelensis</i>	26-33 $\times$ 12-15	Burgess <i>et al.</i> , (2006)

Fungus under study differs from the following *Lasiodiplodia* spp., in having bigger conidia viz., *L. abnormis* (25-28 $\times$ 13-15  $\mu$ m), *L. citricola* (22.5-26.6 $\times$ 13.6-17.2  $\mu$ m), *L. crassispota* (27-30  $\times$  14-17  $\mu$ m), *L. fiorii* (24-26  $\times$  12-15  $\mu$ m), *L. hormozganensis* (19.6-23.4  $\times$  11.7-13.3  $\mu$ m), *L. iraniensis* (18.7-22.7  $\times$  12.1-13.9  $\mu$ m), *L. margaritacea* (14-17  $\times$  11-12  $\mu$ m), *L. parva* (18.3-22.1  $\times$  10.7-12.3  $\mu$ m), *L. pseudotheobromae* [(25.5-30.5 $\times$  14.8-17.2  $\mu$ m, Alves *et al.*, 2008). (21.7-26.3  $\times$  13.4-14.8, Abdollahzadeh *et al.*, 2010)], *L. ricinii* (16-19  $\times$  10-11  $\mu$ m); *L. theobromae* [ ( 23.6-28.8  $\times$  13-15.4  $\mu$ m; Alves *et al.*, 2008), (22.4-24.2  $\times$  12.9-14.3  $\mu$ m, Abdollahzadeh *et al.*, 2010)]. However *L. gonubiensis* (32-36  $\times$  16-18.5  $\mu$ m) has more longer and wider conidia than the fungus under study. *L. gilanensis* (28.6-33.4  $\times$  15.6-17.6  $\mu$ m ), *L. thomasiana* (28-30  $\times$  11-12  $\mu$ m) and *L. undulata* (20-32  $\times$  13.5-19.2  $\mu$ m) differ from the fungus under study by having smaller conidia, since their upper limit of conidial length is near or less than the lower length range of fungus under study.

Further more conidial width of *L. undulate* is more wider than the conidial width of fungus under study.

Fungus under study (30-33.4  $\times$  14-16 $\mu$ m) more closely resembles with *L. paraphysaria* (30-32  $\times$  15-16  $\mu$ m), therefore it is identified as *Lasiodiplodia paraphysaria* *Lasiodiplodia paraphysaria* is for the first time reported from Gojra, Pakistan.

**Specimen examined:** *Lasiodiplodia paraphysaria* on bark of *Azadirachta indica*; 305 J.B. Gojra, Pakistan; 10 April, 07; S. Qaiser Abbas & Nabila Iftikhar G.C.U.F. Mycol.H. # 42.

Fungus found on *Azadirachta indica* specimen # G.C.U.F. Mycol. H. # 44 is identified as *Monochaetium terminalae* (Bat. & Bezerra) Muthumary, Abbas & Sutton.

***Monochaetium terminalae*** (Bat. & Bezerra) Muthumary, Abbas & Sutton, *Trans. Br. Mycol. Soc.*, 87(1): 103-108, (1986).

**Description of the fungus under study:** Conidiomata eustromatic, globose, dark brown with rough surface, 114µm. Conidiogenous cells hyaline, cylindrical, hogenous,  $5-7 \times 2\mu\text{m}$ . Conidia 3-euseptate, ellipsoidal, hyaline, simple appendages or beak on both sides,  $20.9-26.6 \times 3.8\mu\text{m}$ . The length of basal appendage 2-2.85µm, the length of terminal appendage 5.7-7.6µm. Fig. 9(A-C).

Genus *Monochaetia* (Sacc.) Allesch. (1902) and *Monochaetinu* Muthumary, Abbas & Sutton (1986) show close resemblance with under study fungus. and have already been reported from Pakistan. Genus *Monochaetia* (Sacc.) Allesch. is characterized by acervular conidiomata and 4-euseptate conidia. While *Monochaetinu* Muthumary et al., (1986) has eustromatic conidiomata and 3-euseptate conidia. The under study fungus completely matches with

*Monochaetinu terminalae* in having similarity in conidiomata, conidiogenous cells, conidiophores and conidial measurements. Therefore the fungus under study was identified as *Monochaetinu terminalae*.

Previously *Monochaetinu terminalae* was reported from Pakistan on stem of *Capparis decidua* and *Mimosa hamata*, Muthumary et al., (1986). *Azadirachta indica* is an addition to host list of *Monochaetinu terminalae* from Faisalabad Pakistan.

#### Specimen examined

*Monochaetinu terminalae* on bark of *Azadirachta indica*; G.C.University Faisalabad Pakistan; 26 April, 07; S. Qaiser Abbas & Nabila Iftikhar G.C.U.F. Mycol. H. # 44.

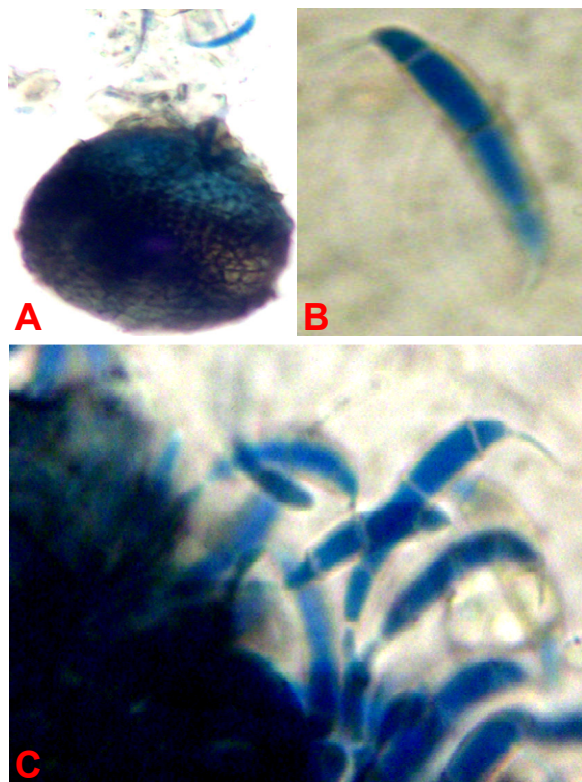


Fig. 9(A-C). *Monochaetinu terminalae*: A. Pycnidium.100X; B. Conidium.1000X; C. Conidia with conidiogenous cells.1000X.

Fungus found on *Azadirachta indica* specimen # G.C.U.F. Mycol. H. # 35 is identified as *Trimmatostroma* sp.

**Description of the fungus under study:** Colonies dark brownish black on natural substrate. Stroma large, thick and brown. Conidiophore micromenatou slightly different from vegetative hyphae, unbranched, straight or wavy, pale brown and verrucose,  $21-23 \times 6-7\mu\text{m}$ . Conidiogenous cells terminal and cylindrical,  $9-10 \times 7\mu\text{m}$ . Conidia thick walled, highly variable in size and shape, 1-several transverse and oblique septa, brown,  $45.6-182.4 \times 7.6-15.2\mu\text{m}$ . Fig. 10(A-E).

*Trimmatostroma scutellare* (Berk. & Br.) Ellis., *T. eriodictyonis* (Dearn. & Barthol.) Ellis., and *T. macowanii* (Sacc.) Ellis., differ from *T. salicis* Corda, and *T. betulinum* (Corda) Hughes by forming conidia in

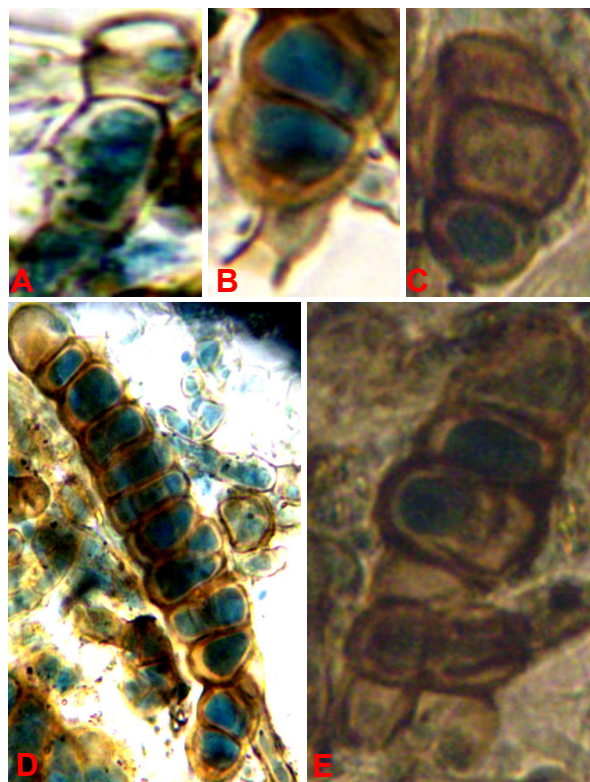


Fig. 10(A-E). *Trimmatostroma* sp. A. Conidiogenous cell; B. uniseptate conidium; C. biseptate conidium. (A, B, C, 1000X), D. Conidial attachment; E. Conidia. (D & E, 1000X).

simple and branched chains of variable in shape with 1 - several transverse and longitudinal or oblique septa. The size of conidia of *Trimmatostroma scutellare* (Berk. & Br.) Ellis., *T. eriodictyonis* (Dearn. & Barthol.) Ellis., and *T. macowanii* (Sacc.) Ellis also differ from the conidia of the fungus under study in having smaller conidia. Conidia are  $10-30 \times 8-25\mu\text{m}$  in *T. scutellare* ;  $14-50 \times 7-26\mu\text{m}$  in *T. eriodictyonis* and  $8-20 \times 5-9\mu\text{m}$  in *T. macowanii*, whereas conidia of under study fungus are  $45.6-182.4 \times 7.6-15.2\mu\text{m}$ . Furthermore *T. salicis* and *T. betulinum* have solitary conidium, often forming fork like structure, clavate and smooth or slightly verrucose and obtuse at the ends but both species differ in conidial size. In *T. salicis* conidia are  $12-38 \times 4-10\mu\text{m}$  and conidia in *T. betulinum*, are  $5-20 \times 5-14\mu\text{m}$ . Therefore it is concluded that the species under study does not match with any



species of *Trimmatostroma* and looks a new species, will be published in some where else.

Previously *Trimmatostroma betulinum* (Corda) Hughes., has been reported on dead branches of unknown host; Ahmad (1977) and *Trimmatostroma myrti* (Lind.) Hughes on dead branches of unknown host; Ahmad (1977) and from Khanspur Streams, Dadar Streams from Pakistan (Iqbal & Bhutty, 1979, 1980; Iqbal & Shahbaz, 1990).

*Azadirachta indica* is a new host record of *Trimmatostroma* sp., from Faisalabad Pakistan.

#### Specimen examined

*Trimmatostroma* sp., on branch of *Azadirachta indica*; Samanabad Gojra Pakistan; 25 April, 07; G.C.U.F. Mycol.H. # 35; S.Q. Abbas & Nabila Iftikhar..

Fungus found on *Azadirachta indica* specimen # G.C.U.F. Mycol. H. # 43. *Epidermophyton floccosum* (Harz) Langeron et Miloch

*Epidermophyton floccosum* (Harz) Langeron et Miloch., (1930); Beneke, E. S. Medical mycology laboratory manual, pp. 66, (1962).

**Description of the fungus under study:** Colonies white in color when grow on natural substratum. Mycelium superficial, thin, long and branched. Conidiophores hyaline and branched. Macro-conidia found, 3 septate, clavate, 10.5-17.5×3.5-7µm. No microconidia are found.

*Microsporium* Velen., and *Epidermophyton* Sabourd are two closely related hyphomycetous genera, resemble each other in many aspects. Both have velvety colonies, grow rapidly on medium with tan to brown in color. Hyphae septate, large. Macroconidia hyaline, multicellular, transversely septate, thick or thin walled. However they also differ from each other. In genus *Microsporium*, microconidia may appear on short hyphae. Macroconidia fusiform, often have an annular frill. Chlamydo-spores may present a fungal colony of buff to brown in color while in *Epidermophyton* colony color changes from buff to white at maturity. Macroconidia smooth and clavate-shaped with obtuse ends. They are found singly or in clusters, Fig. 11(A-C).

Characteristics of under study fungus completely matched with *Epidermophyton floccosum* (Harz) Langeron & Miloch.

*Epidermophyton floccosum* is generally human pathogen recorded from the world (Kazmi, 2004, Bundu & Pavihiran 2002; Macit, 2005; Mohmoudabadi, 1997 and from Pakistan (Khan & Anwar, 1969, Dilnawaz & Naseer, 2001; Hussain *et al.*, 1994; Thebo *et al.*, 2006). Recently a project on Dermatophytes of District Faisalabad was carried out collaboration of Department of Botany, G.C. University Faisalabad and Department of Dermatology. HO Faisalabd where one hundred and seventy nine patients were studied for dermatophytic infection. In 11 patients *Epidermophyton floccosum* was isolated (7-13%). This is a high % of occurrence of it a there is a big question that how this pathogen perpetuate in this area? Is this soil borne or borne in other substrate? The isolation from Neem tree is further alaraming to the mycologist working on plant pathogenic fungi. Generally it is supposed that they are non pathogenic to animals and human being.

However the fungus found on G.C.U.F. Mycol. H. # 43. identified as *Epidermophyton floccosum* on Neem tree is first report in this connection from Faisalabad, Pakistan.

#### Specimen examined

*Epidermophyton floccosum* on bark of *Azadirachta indica*; G.C. University Campus Alama Iqbal Road, Faisalabad, Pakistan; 27 April, 07; G.C.U.F. Mycol. H. # 43; S. Qaiser Abbass & Nabila Iftikhar.

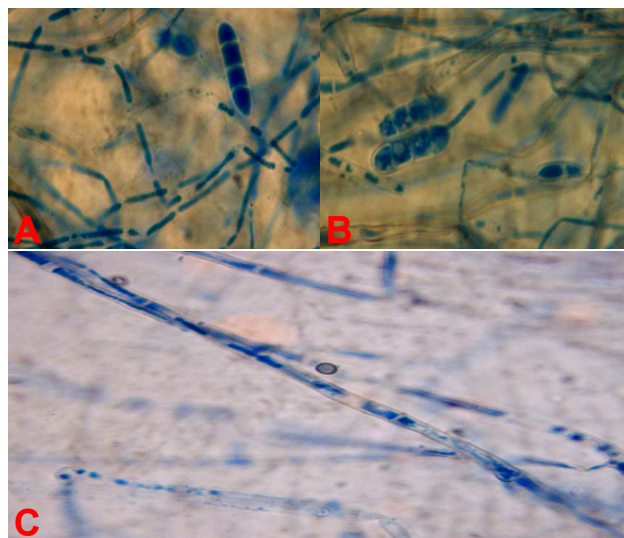


Fig. 11(A-C). *Epidermophyton floccosum*: A. triseptate conidium.; B. Conidia attached with conidiophore. C. Mycelium (A,B,C ;1000X.)

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