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NEW FUNGAL RECORDS ON *MORUS ALBA* FROM FAISALABAD, PAKISTAN I.

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Abstract

Five fungi have been reported on *Morus alba* from Faisalabad, Pakistan. Three of them viz., *Tetracoccosporium aerium, Gliomastix novae–zelandiae* and *Septoria cytisi,* have been reported for the first time from Pakistan. Ahmad (1969) reported *Pseudocercospora mori* as *Cercospora mori* from Faisalabad and *Lasiodiplodia undulata* was also reported on *Morus alba* from Faisalabad, Pakistan.

Introduction

This study is a part of the project to study the fungal plant associationxx of District Faisalabad, Pakistan. Trees, shrubs, herbs, climbers and plants of wild and cultivated nature of Faisalabad are being thoroughly studied for the fungal association. In the present study *Morus alba* (Mulberry) is the focal plant.

Mulberry is an economically important plant that belongs to the family *Moraceae.* It is native to China. Four species of *Morus*. viz., *Morus alba* L., *M. nigra* L., *M. serrata* Roxb., *M. lavigata* have been reported from Pakistan. (Ghafoor, 1985) which are cultivated throughout Pakistan. Leaves of *Morus alba*, the white Mulberry are used as food for the silk-producing larvae. Other plant parts are used in making paper, pesticides, furniture, musical instruments, sculptures, sport goods and medicines (Khan, 1989). Pollen grains of *Morus nigra* cause allergic asthma, hence its cultivation is banned in Islamabad area.

Prior to this study, 84 fungi have been recorded on *Morus* spp. from Pakistan, (Ahmad, 1956, 1969; Ahmad *et al*., 1997; Khan, 1955, 1956, 1960; Khan and Bokhari, 1970; Qureshi & Ahmad, 1971; Qureshi & Jamal, 1972; Beg, 1973; Beg *et al*., 1974; Ghafoor & Khan, 1976; Mirza & Qureshi, 1978). During the present study three additions have been made to this list.

Materials and Methods

Diseased samples of *Morus alba* plant part were collected from selected plantation sites of District Faisalabad and Gujranwala. The samples were categorized as (a) wet and (b) dry. The dry samples were put in polyethylene bags and tagged with date, place of collection collector's name and sealed. Dry samples were kept in low temperature incubator (Sanyo MDF-U53V) at -40˚C for 24 hours. Herbarium sheets were made. Insecticide powder Mortein Coopex Reckitt Benckiser was also put in them to protect the samples from damage by insects. Herbarium sheets were kept in Mycological herbarium at the Department of Botany, GC University, Faisalabad for future reference. Wet samples were kept within blotting papers to absorb moisture and papers were changed daily to make them completely dry. The rest of the treatment is similar to dry samples.

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For isolation of fungi, diseased specimens were surface sterilized either by 0.1% Mercuric chloride or Hydrogen peroxide, for 1-10 seconds then rinsed with distilled water. 2% potato dextrose agar (PDA) medium was autoclaved (Astell CE 0353, England) at 121^{o}C 15lb/inch^{2} for 15 minutes and was poured into sterilized Petri plates. Samples were observed under dissecting microscope to select infected parts and a small piece was taken by sterilized needle/scalpel and transferred under aseptic conditions to the sterilized Petri plates containing 15 mL 2% PDA. Inoculated Petri plates were kept in incubator set at 25-30˚C (KD Binder CB 210#06-02897 Germany) for few days for fungal growth and germination. The plates were observed regularly for pure fungal growth and if it was contaminated, the process was repeated till the pure culture of fungus was obtained. Slants were also made from culture for stocks for further reference.

Infected specimens or isolated fungal culture were used for identification. Specimens were directly observed under stereo microscope and a small piece of selected part was taken from the host tissue on glass slide with the help of sterilized needle. The material was spread over slide by needle and stained with cotton blue (for hyaline spores) and mounted in lactophenol and studied under optical microscope (SWIFT M 7000 D Japan) equipped with objectives of different magnifications (4x, 10x, 40x and 100x). Slides were also prepared from fungal culture. Small piece from the periphery of fungal colony was taken aseptically with the help of a needle and put on slide, mycelia were spread by needle, stained with cotton blue and mounted in lactophenol. Measurements of fungi were recorded and photographs were taken by digital camera (Sony T.20, 8.1 megapixel). Identification of fungi was carried out after consulting pertinent literature (Chupp, 1953; Ellis, 1971, 1976; Carmichael *et al.*, 1980; Sutton,1980; Punithalinghum,1980; Ahmad *et al*., 1997; Kirk, 2009)

Results and Discussion

1. *Tetracoccosporium aerium* Misra & Srivastava. (1976). *Mycotaxon* 4: 276-278. [Fig. 1 (A-D)]

Mycelium superficial, brown, septate, consisting of sparsely branched hyphae, 1-2 µm wide, brown, septate, stroma, setae and hyphopodia absent. Conidiophores olivaceous brown to pale brown, smooth, loosely branched, septate, branches usually at right angles to main axis and to each other, 5-25 x 2-3 µm. Conidiogenous cells integrated, hologenous, monoblastic. 5-10 x 1.5-2 µm. Conidia solitary, acrogenous, dark brown, verruculose, (minutely echinulate), 4- celled, divided cruciately by septa at right angles to one another and constricted at the septa, 10-12 x 11-13µm.

The fungus found on bark of *Morus alba* is characterized by having 4- celled conidia with two septa at right angle to each others. Fungus under study was identified as *Tetracoccosporium aerium.* This species was describbed by Misra & Srivastava in 1976.

Five species have been described in the genus *Tetracoccosporium* viz., ***T. paxianum*** Szabo, *Hedwigia* 44: 77 (1905); ***T. aerium*** Misra & Srivastava; ***T. cupulatum*** P. R. Rao & D. Rao; ***T. Saccharii*** Stev; and ***T. qaudratum*** (Cooke) Wiltshire (Kirk, 2009). Lindau (1910) transferred *Tetracoccosporium paxianum* Szabo to S*temphyliuum paxianum* (Szabo) Lindau and *Tetracoccosporium saccharii* Stevenson was transferred to *Dictyoarthrinium saccharii* (Stevenson) Damon by Damon (1953).

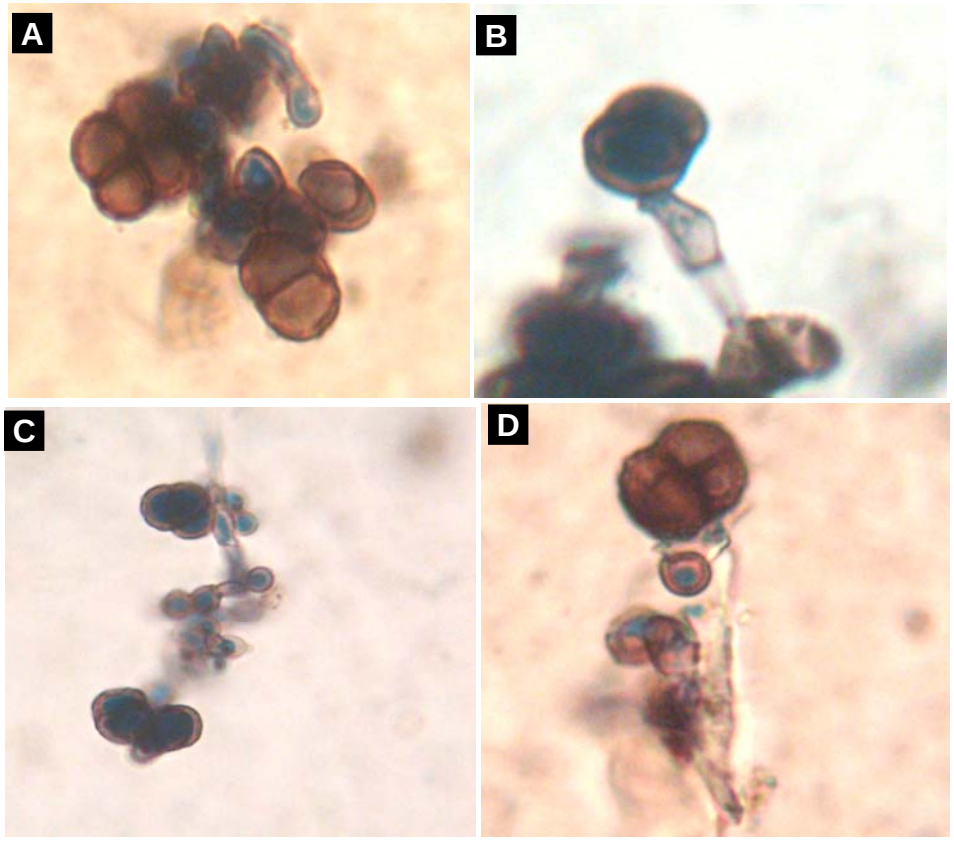

Fig. 1(A-D). *Tetracoccosporium aerium* (A) Four-celled mature conidia 1000x (B) Conidiophore with conidia 1000x (C) Conidiophore at right angle to each other 1000x (D) thick- walled mature conidia 1000x.

We believe that *T. paxianum* should not be transferred to *Stemphylium* since the characteristic feature of S*temphylium* "dark bulbous regions" in conidiophores are not present in figures drawn by Ellis (1971) and Carmichael *et al*., (1980), for illustrations of *Tetracoccosporium paxianum*. However, in the present study it was compared with the fungus under study and other *Tetracocosporium* spp. ***T. paxianum*** differs in having bigger, thick- walled and verrucose conidia (12-18x 11-18 µm) than *Tetracoccosporium aerium* (8-12 µm in diam., in face view and 6-8 µm thick). Furthermore, conidiogenous cells are ampulliform in ***T. paxianum*** and are longer and becoming narrow towards tips in *T aerium.* Similarly *T. quaternatum* also differs in having bigger conidia (20 µm in diam.) and constricted at septal wall than ***T. paxianum*** (12-18 x 11-13 µm) and *T. aerium* (8-12 in diam. in face view and 6-8 µm thick).

Genus *Tetracoccosporium* was not reported from Pakistan (Ahmad *et al.,* 1997). Therefore *T. aerium* is also a new fungal record to Pakistan and *Morus alba* is also a new host of it from Faisalabad, Pakistan.

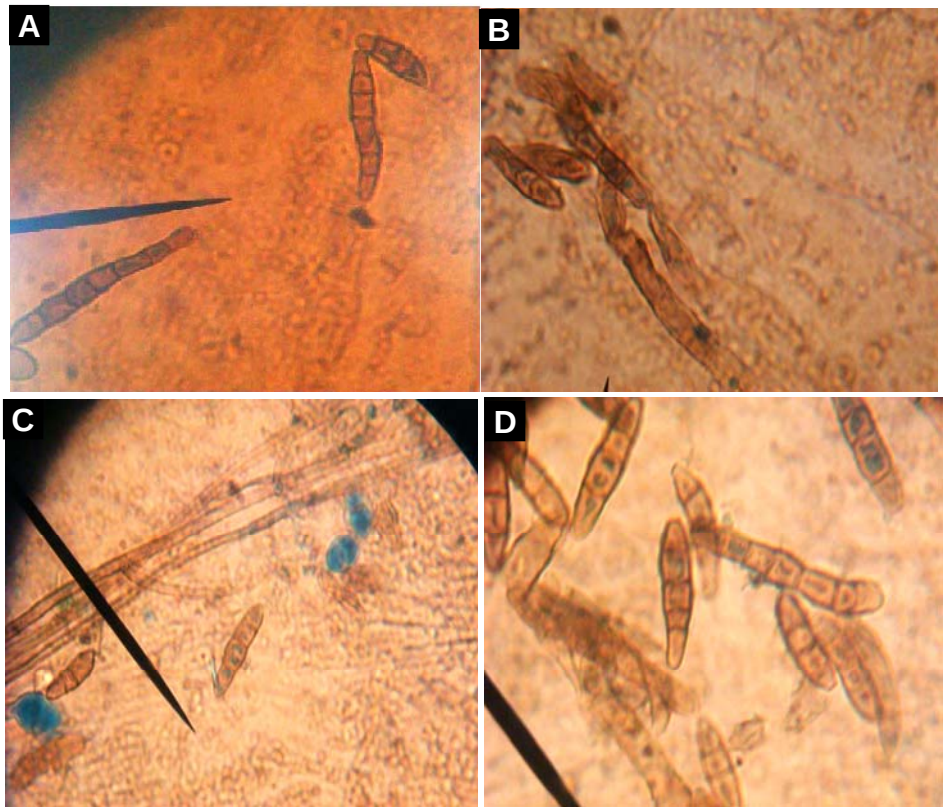

Fig. 2(A-D). *Pseudocercospora mori* (A) Conidia 1000x (B) Conidiophore with conidia 1000x (C) Mycelium 400x (D) Conidia 1000x.

Specimen examined

Tetracoccosporium aerium Misra & Srivastava on the bark of *Morus alba*; Govt. College University Faisalabad; 25 July, 2007; S.Q. Abbas & R. Ayesha, G.C.U.F.M.H. # 9.

2. *Pseudocercospora mori* (Hara) Deighton. (1976) *Mycol. Pap*., 140: 148. [Fig. 2. (A-D)] = *Cercospora mori* Hara *Journal of the Sericultural Association of Japan*, 27(no. 314): 227 (1918).

Colonies mostly on the lower surface of leaves, grey to black, round and angular. Mycelium embedded to the natural substrate. Hyphae branched, septate, pale brown. Conidiogenous cells integrated, terminal, often monoblastic and extending the entire length. Conidiophores arising from stromata and emerging through stomata, pale to mild brown, 110 x 5 µm. Conidia thick walled, pale to mild brown, 2-9 septate, mostly (5 or 6 septa) 30-40 x 4-5 µm, at the base 3-5 µm wide.

Pseudocercospora mori (Hara) Deighton the grey leaf spot pathogen of Mulberry has been previously reported from USA, Congo, Japan, Taiwan, China. (Chupp, 1953, Dighton, 1976); from India (Sriavastava *et al*., 1978) and from Australia, on *Morus alba* and *Morus nigra* (Grice *et al*., 2006). The fungus was identified with the help of

description and illustration (Hsieh & Gho, 1990; Babu *et al*., 2002). Hsieh & Gho (1990) reported Conidiophore 20-90 x 3-5 µm and conidia 20-80 x 3.5µm., and Grice *et al*., (2006) reported conidiophore 1-4(7) septate and 30-67µm. and conidia 1-5(7) septate and 18-61 x 3-4.5µm. The fungus found on *Morus alba* closely resembles with *Pseudocercospora mori* but differs from *P. vitis* which has 6-8 septate and more longer and wider conidia 35-95 x 6-8 µm.

The fungus found on the leaves of *Morus alba* having (2-8) transverse septa and 30-40 x 4-5 µm conidia, It belongs to the genus *Pseudocercospora* because conidia are oblong, obclavate, transversely septate and conico-truncate at the base. It closely resembled with *Pseudocercospora mori*.

Previouly only *Pseudocercospora vitis* (Lev.) Speg., had been reported on leaves of *Vitis vinifera* from Tandojam, Pakistan. (Mirza & Qureshi, 1978; Ahmad *et al*., 1997). *Pseudocercospora mori* has already been recorded on *Morus alba* from Faisalabad by Ahmad (1969) as *cercospora mori,* which was not correct so it is described as *Pseudocercospora mori*.

Specimen examined

Pseudocercospora mori on the leaves of Morus *alba*; Gujranwala 20 October, 2007; R. Ayesha and S.Q. Abbas G.C.U.F.M.H # 8

3. *Gliomastix novae - zelandiae* Hughes & Dickinson., *N.Z. J. Bot*., 6:108 (1968). [Fig. 3 (A- [C)]

=*Acremonium novae-zelandiae* (Hughes & Dickinson). Gams, *Cephalosporium-artige Schimmelpilze (Stuttgart)*: 93 (1971).

= *Sagrahamala novae-zelandiae* (Hughes & Dickinson) Subram., *Curr. Sci*., 41(2): 49 (1972).

Colonies effuse dark olive to black. Mycelium branched, septate. Conidiophores up to 50 µm long, arising from a runner like structure, and usually pale brown with a massive black deposit at the apex forming a cupulate collarette, 3-4 µm thick tapering to about 2 µm just below the collarette. Conidiogenous cells terminal, hologenous, stationary (phialidic). Conidia catenate, ellipsoidal or doliform, apex and base truncate, mid to dark brown, smooth 7-10.5 x 3.5-4 µm.

In the present study a fungus was also found on *Morus alba* which belongs to the genus *Gliomastix* because conidiophores arising from a runner like structure. Conidia catenate or aggregated in slimy heads, semi endogenous, simple, unseptate, pigmented, smooth, doliform, ellipsoidal, apex and base truncate.

On comparison of the fungus under study with closely related *Gliomastix* spp., it was found that the characters of the fungus under study closely resembles with *Gliomastix novae–zelandiae*. It differs from *G. cerealis* in having bigger conidia than *G. cerealis* (3-5 x 2-3.5 µm) and *G. musicola* (5-7 x 2-3.5 µm). Furthermore, the conidia of *Gliomastix novae–zelandiae* are ellipsoidal while that of *G. cerealis a*nd *G. musicola* are pyriform to doliform. Conidiophores of *G. novae–zelandiae* possesses a cupulate collerate at the apex while it is absent in *G. cerealis* and *G. musicola.*

Gliomastix cerealis (Karst) Dickinson was reported by Matsushima (1993) from soil of Alpine trees, Mealow Mt. Gilipur, Nangaperbat, Pakistan.

In the present study *G. novae-zelandiae* is reported for the first time from Faisalabad, Pakistan, on *Morus alba*.

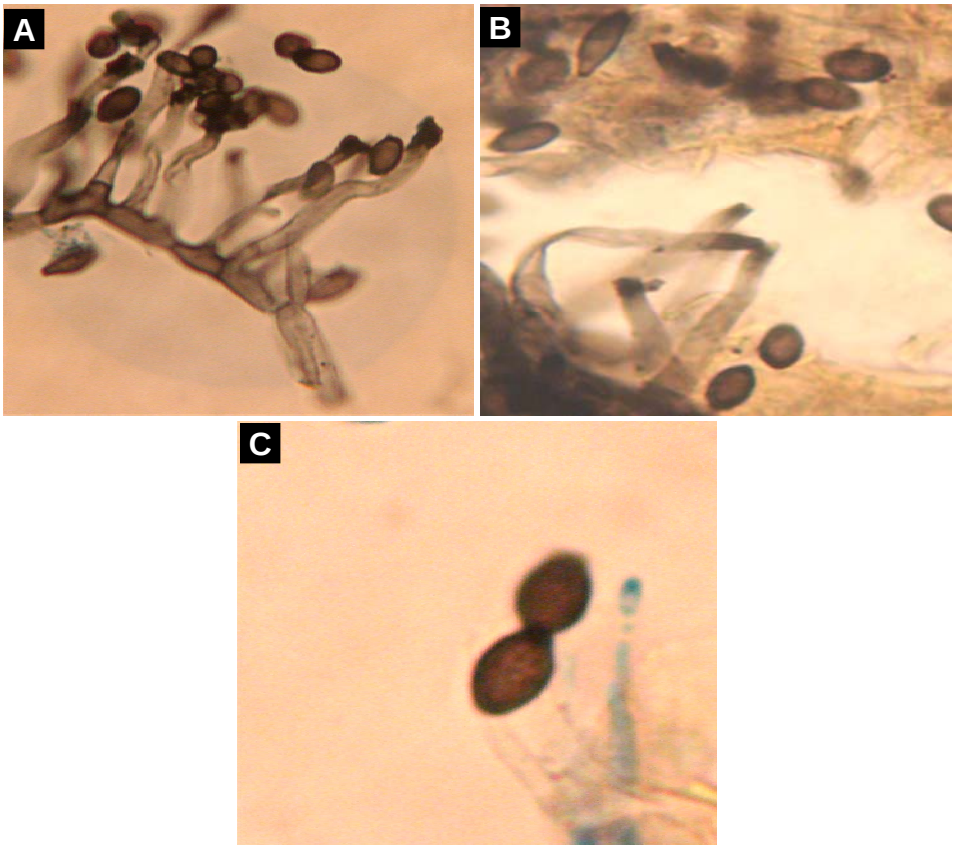

Fig. 3(A-C). *Gliomastix novae – zelandiae* (A-C). (A) Conidiophore with a runner like structure 1000x (B) Conidiophore with a black collarete at the apex with conidia 1000x (C) Conidial attachment 1000x.

Specimen examined

Gliomastix novae - zelandiae on the bark of *Morus alba*; Govt. College University Faisalabad; 13 May, 2007; S.Q.Abbas and R. Ayesha, G.C.U.F.M.H # 5.

4. *Lasiodiplodia undulata* (Berk. & Curt.) Abbas, Sutton, Ghaffar & Abbas. *Pak. J. Bot*., 36: 209-218. [Fig. 4 (A-C)].
=*Botryodiplodia theobromae* Pat., *Bull. Soc. mycol. Fr*., 8: 136 (1892)
=*Lasiodiplodia theobromae* (Pat.) Griff. & Maubl., *Bull. trimest. Soc. Mycol. Fr*., 25:57 (1909)
=*Diplodia theobromae* (pat.) Nowell, *Disease of crop plants in the Lesser Antilles*: 158 (1923)
=*Lasiodiplodia tubericola* Ell. & Ev. Apud Clendenin, *Bot. Gaz.* 21: 92 (1896)
=*Diplodia tubericola* (Ell. & Ev.) Taubenh. *Amer. J. Bot*., 2: 328 (1915)
=*Botryodiplodia tubericola* (Ell. & Ev.)Petrak, *Annls Mycol*., 21: 332 (1923)

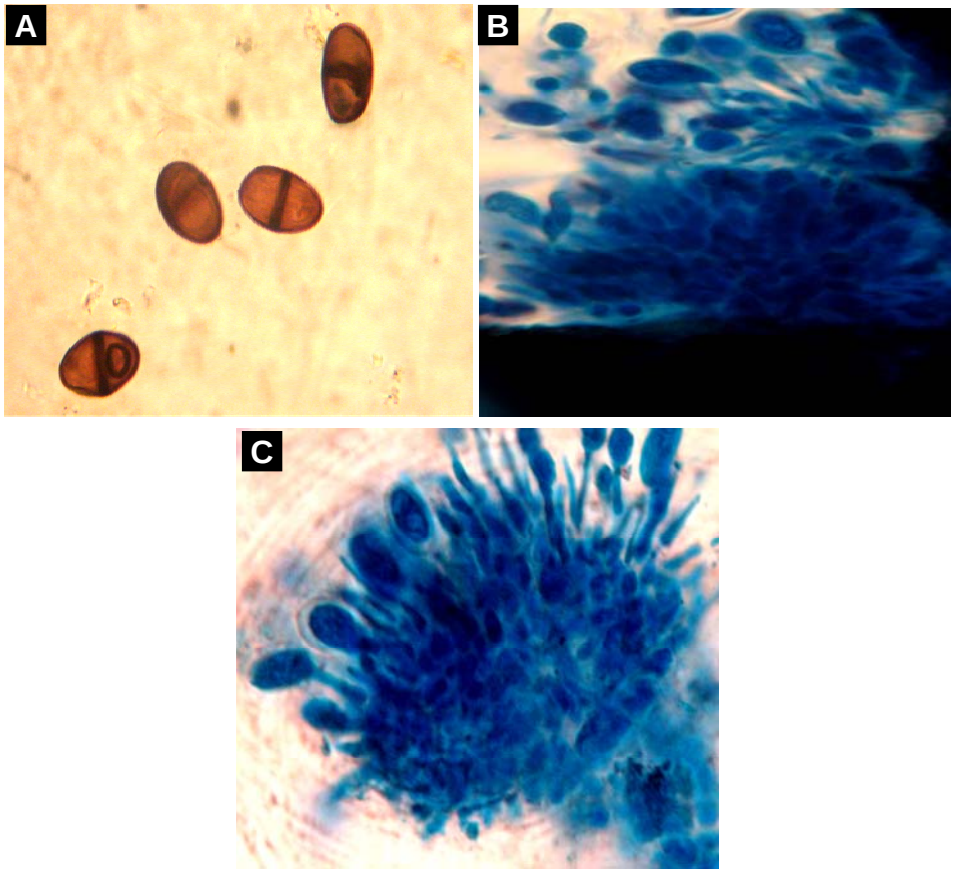

Fig. 4(A-C). *Lasiodiplodia undulata* (A) Mature conidia 1000x (B) Conidia released from conidiomata 1000x (C) Conidia with conidiogenous cells 1000x.

A fungus was found on *Morus alba* and identified as *Lasiodiplodia undulata* after consulting (Sutton, 1980; Punithalinghum, 1980; Abbas *et al*., 2004; Kirk, 2009).

Mycelium immersed, branched, septate, dark chocolate brown. Conidiomata eustromatic, immersed separate and aggregated and confluent, globose, 300 µm in diameter. Ostiole absent. Conidiophores absent. Conidiogenous cells hologenous stationary, non proliferating, discrete, cylindrical, hyaline, smooth 5-15 x 1.5-3 µm. Conidia acrogenous, hyaline and thin walled when young, later becoming thick walled, dark brown with longitudinal striations, finally developing a median septum, 20 x 12 µm. paraphysis, hyaline, cylindrical, septate.

Genus *Lasiopdiplodia* has 17 species Kirk (2009). According to Sutton (1980) correct name of *Botryodiplodia theobrome* was *Lasiodiplodia theobromae* but Abbas *et al*., (2004) when reassesing the *Sphaeropsis undulata* Berk. & Curt, pointed out that there was an earlier name *Sphaeropsis undulata* Berk. & Curt aviable for *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl. Therefore, a new combination *Lasiodiplodia undulata* (Berk. & Curt.) Abbas, Sutton, Ghaffar & Abbas (2004) was proposed.

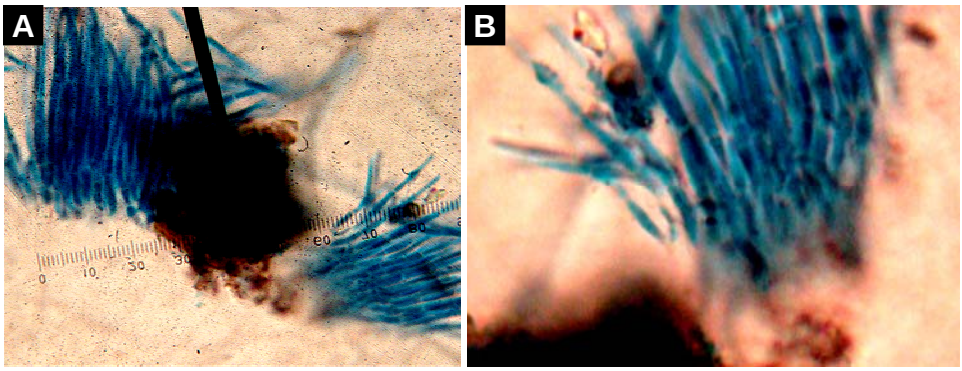

Fig. 5(A-B). *Septoria cytisi* (A) Mycelium, Conidia and conidiogenous cells 1000x (B) 4 septate conidia with conidiogenous cells.1000x.

In Pakistan *Lasiodiplodia undulata* (as *Botryodiplodia theobromae*) has been reported to cause diseases, in 38 different host plants belonging to different families viz., On *Bignonia* sp., *Capparis decidua, Withania somnifera, Citrus aurantium, Dalbergia sisoo, Euphorbia tricaulli, Gossypium neglectum, Gossypium* sp., *Ipomoea carnea, Cosmos sulphureus*, *Mimosa rubicaulis, Psidium guajava, Pandanus adoratissimus, Arachis hypogaea, Citrus aurantifolia, Ficus palmata, Ipomoea gossypioides, Melia azedarach, Moringa oleifolia, Morus alba, Prosopois juliflora, Prosopis spicigera, Helianthus annuus, Mangifera indica, Manihot utillissima, Vinca rosea. Argyrea* sp., *Bauhinia variegata, Albizia lebbeck, Aloe barbadensis, Althurium andraeanum, Borassus flabellifer*, *Erythrina indica, Nerium indicum, Pedilanthus tithymaloides, Ziziphus mauritiana* and *Broussonetia papyrifera*, from Lahore, Changa Manga, Ladhar (Sheikhupura); Tandojam; Bimber and Karachi (Ahmad, 1962, 1968, 1972; Ahmad & Arshad, 1972; Ghaffar & Abbas, 1972; Ghaffar & Kafi, 1968; Khan & Kamal, 1968).

In the present study, *Lasiodiplodia undulata* is also reported on *Morus alba* from Faisalabad, Pakistan.

Specimen examined

Lasiodiplodia undulata on the branches of *Morus alba*; Govt. College University Faisalabad; 19 May, 2007; S.Q. Abbas and Rubab Ayesha G.C.U.F.M.H # 12.

5. *Septoria cytisi* Desm. *Ann. Sci. Nat. Ser*., 3, 8: 24 (1847). [Fig. 5 (A -B)]

Mycelium immersed, branched, septate, pale brown. Conidiomata pycnidial, immersed, globose, brown, 150-175µm. Ostiole single, circular, central. Conidiophores absent. Conidiogenous cells 9-15.5 x 3-5.5 µm, hologenous and stationary. Conidia hyaline, transversely septate (4-6 septate) 58-93 x 4-5.5 µm, tapered gradually towards the obtuse end and base truncate.

Fungus under study was compared with different species of *Septoria*. It closely resembles with *Septoria cytisi* (Sutton, 1980, Kirk, 2009). It has hyaline transversely 4-6 septate conidia, tapering gradually towards the apex and base truncate. It differs from *S. empetri* having sparsely guttulate conidia and from *S. crateriformis* where conidia have small guttulae at each end. Similarly it was different from *S. olivae, S. avellanae* and *S. microsperma* possessing large filliform, sigmoid, guttulate and curved conidia. *S. cytisi* can also be distinguished from *S. pinea* having euseptate conidia.

Twenty-four species of *Septoria* have been reported from Pakistan; Ahmad *et al*., (1997). *Septoria cytisi* is recorted for the first time on *Morus alba* from Faisalabad, Pakistan.

Specimen examined

Septoria cytisi on the bark of *Morus alba*; Govt. College University Faisalabad; 13 May, 2007; S.Q.Abbas and R. Ayesha, G.C.U.M.H # 13.

Acknowledgements

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