

NEW FUNGAL RECORDS ON *MORUS ALBA* FROM FAISALABAD PAKISTAN II

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Abstract

Cunninghamella echinulata (Maturchat) Thaxter, *Graphium putredinis* (Corda) Hughes., *Memmoniella echinulata* (Riv.) Galloway, *Curvularia lunata* (Wakker) Boedijn and *Drechslera* state of *Cochliobolus spicifer* Nelson are first time reported on *Morus alba* from Faisalabad Pakistan.

Introduction

Previously 84 fungi have been recorded on *Morus spp.* from Pakistan (Ahmad 1956, 1969, Ahmad *et al.*, 1997, Khan 1955, 1956, 1960, Khan & Bokhari 1970, Qureshi & Ahmad 1971, Ghafoor & Khan 1976, Mirza & Qureshi 1978, Ahmad *et al.*, 1997). In continuation of the study of fungal flora of the District Faisalabad on *Morus alba*, Abbas *et al.*, (2010) reported 5 more fungi viz., *Tetracoccoporium aerium*, *Pseudocercospora mori*, *Gliomastix novae-zelandiae*, *Septoria cytisi* and *Lasiodiplodia undulata* on *Morus alba* and thus the number of recorded fungi become 99. In the present study five more fungi were reported on *Morus alba* from Pakistan. This study is a part of a project entitled "survey and surveillance of fungal association to flora of District Faisalabad" funded by HEC Pakistan.

Materials and Methods

Materials and methods are the same as described by Abbas *et al.*, (2010).

Identification upto species level were carried out after consulting (Ahmad *et al.*, (1997), Carmichael *et al.*, (1980), Ellis (1971, 1976), Kirk (2009), Mirza *et al.*, (1979), Morris (1963), Sivanesan (1987).

Result and Discussion

In the present study a fungus was found on the dead twigs of *Morus alba* specimen G.C.U.M.H # 1 and it was identified as *Cunninghamella echinulata* (Maturchat) Thaxter

1. *Cunninghamella echinulata* (Maturchat) Thaxter. *Rhodora*, **5**: 98(1903). Fig1 (A-C)

=*Actinocephalum japonicum* Saito, *Bot. Mag.*, Tokyo **19**: 36 (1905)

=*Cunninghamella africana* Matr., *Annls mycol.*, **1**(1/2): 47 (1903)

=*Cunninghamella bainieri* Naumov, *Opred. Mukor.* Edn **2**: 108 (1935)

=*Cunninghamella blakesleeana* var. *verticillata* (F.S. Paine) Bajjal & B.S. Mehrotra
Sydowia, **33**: 10 (1980)

=*Cunninghamella dalmatica* Pišpek, *Acta Bot. Inst. Bot., Zagreb*, **4**: 100 (1929)

=*Cunninghamella echinata* Pišpek, *Acta Bot. Inst. Bot., Zagreb*, **4**: 100 (1929)

=*Cunninghamella echinulata* (Thaxt.) Thaxt., *Rhodora*, **5**: 98 (1903)

=*Cunninghamella echinulata* var. *verticillata* (F.S. Paine) R.Y. Zheng & G.Q. Chen, *Mycosystema*, **8-9**: 7 (1996)

=*Cunninghamella elegans* var. *chibaensis* Kuwab. & Hoshino, in Hoshino, Takano & Kuwabara, *Techn. Bull. Fac. Hort. Chiba univ.*, **17**: 37 (1969)

=*Cunninghamella japonica* (Saito) S. Ito, *Mycol. Fl. Japan* 1 (Phycomycetes): 304 (1936)

=*Cunninghamella ramosa* Pispek, *Acta Bot. Inst. Bot.*, Zagreb, **4**: 102 (1929)

Description of the fungus identified: Colonies on PDA white, rapidly growing, growth luxuriant at 30°C, mycelium well branched and coenocytic, rhizoids well-developed, present inside the substrate, branched, hyaline, smooth, 1.8 - 16.8 x 1 - 2 µm. conidiophores erect, branched and collapsing, branches ending into vesicles bearing conidia on short sterigmata, branches never strictly at right angles, variable in length, 420 x 14 µm. with an apical large vesicle and a cluster of lateral smaller vesicles, 2-9 in number. Vesicles globose, subglobose, hyaline, smooth to slightly rough bearing short sterigmata for conidial attachment, 40 - 50 µm in diameter (Fig. 1B). Conidia of only one type, mostly globose, subglobose, hyaline to light yellow, 10 -17 µm in diameter, echinulations prominent, 2 - 4 µm (Fig. 1C).

The fungus under study was compared with different *Cunninghamella* spp., and it closely resembled with *C. echinulata* in vesicle and conidial morphology and measurement. In *C. echinulata* vesicle are 24-49.3µm diam. and 40-50 µm diam., in the fungus under study. Conidia in *C. echinulata* are 5.5-18.7 µm diam., globose and 7.36-25 x 7.2-70-8 µm ovoid to club shaped whereas conidia are 10-17µm diam., in the fungus under study. Conidiophore differs in both in length, it is 4.8-22.4 µm in length in *C. echinulata* and 16-42 µm in fungus under study, further more lateral vesicle are absent. Therefore, the fungus under study was identified as *C. echinulata*. It differed from *C. ramosa* which had smooth walled conidia and also differed from *C. elegans* and *C. phaeospora* where conidia are shortly echinulated and small, conidia in *C. elegans* are 4.6-18.7 µm diam. and in *C. phaeospora* 7-15.5 µm diam.

Four species of *Cunninghamella* have been reported from Pakistan viz., 1) *C. elegans* (According to Mirza *et al.*, (1979) and Zycha *et al.*, (1969). *C. blakesleeana* is synonym of *C. elegans*. On soil, from Quetta, Baluchistan, Faisalabad, Khanewal (Mirza & Qureshi 1978; Mahmood & Mirza, 1972), on dung of different animals and plant substrate (Mahmood & Mirza, 1972, Mirza *et al.*, 1979), in soil from Karachi, on deer dung Lahore, on cow dung Faisalabad (Rizvi, 1966; Ahmad, 1969), 2) *C. echinulata* (Maturchot) Thaxter was reported in soil from Karachi (Ghaffar *et al.*, 1971), in soil and dung from Lahore and Mianwali (Mirza *et al.*, 1979) on *Sonchus rouxburghii* from Gatwala (Faisalabad), on goat dung from Lahore (Chaudhari & Sachar 1934 'as *C. verticellata*'), (Ahmad, 1956; Hussain *et al.*, 1966; Qureshi 1966; Ahmad, 1967; Ghaffar *et al.*, 1971; Mirza *et al.*, 1979; Rizvi, 1966; (as *C. echinata*), 3) *C. microspora* (Rivolta) Matrouchot was reported by Mirza & Qureshi (1978) but not mentioned in their monograph "Mucorales of Pakistan". (Mirza *et al.*, 1979), 4. *C. phaeospora* Boeddiijn, was reported in soil from Punjab, in soil, from Faisalabad, soil and from rat dung, Multan, Piranwal, Muzaffargrah, Kot adu soil and dung (Mirza *et al.*, 1979; Ahmad, 1997).

In the present study *Cunninghamella echinulata* is reported on the dead branches of *Morus alba*; a new host record to this fungus from Faisalabad, Pakistan.

Specimen examined: *C. echinulata* on dead twigs of *Morus alba*; specimen G.C.U.M.H # 1. Govt. College University, Faisalabad; 25 May, 2007; S.Q. Abbas & R. Ayesha.

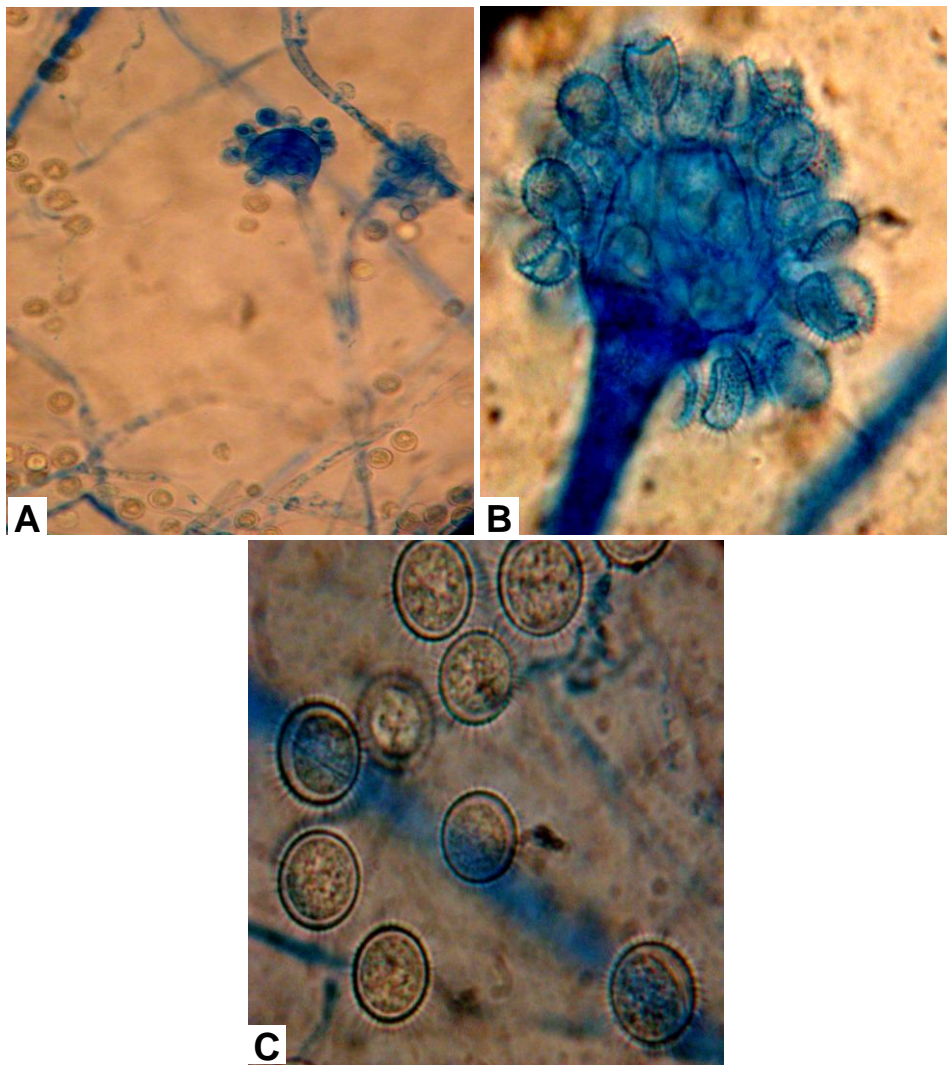


Fig. 1. (A-C) *Cunninghamhamella echinulata* (A) Conidiophore with vesicles 400x (B) Vesicle with short sterigmata on which conidia are attached 1000X. (C) Conidia with echinulations 1000x

In the present studies another fungus was also found on the bark of *Morus alba*. Specimen G.C.U.M.H # 6, and identified as *Graphium putredinis* (Corda) Hughes

2. *Graphium putredinis* (Corda) Hughes *Can. J. Bot.*, 1958. Fig: 2 (A-D)

Description of fungus identified: Mycelium black, immersed, branched, septate. Synnemata dark brown, becoming pale towards the apex, 250 x 1 5-30 μm , individual threads, 1-2 μm wide, hogenous and progressive (sensu Hennebert & Sutton, 1994); Conidiogenous cells subulate, sub-hyaline, 50-70 x 1-2 μm . Conidia hyaline, unseptate cylindrical to ellipsoidal obtuse at the apex and truncate at the base with two guttules, actually it is the stained cytoplasm which was giving the impression of (single septum) 5-7 x 2-2.5 μm .

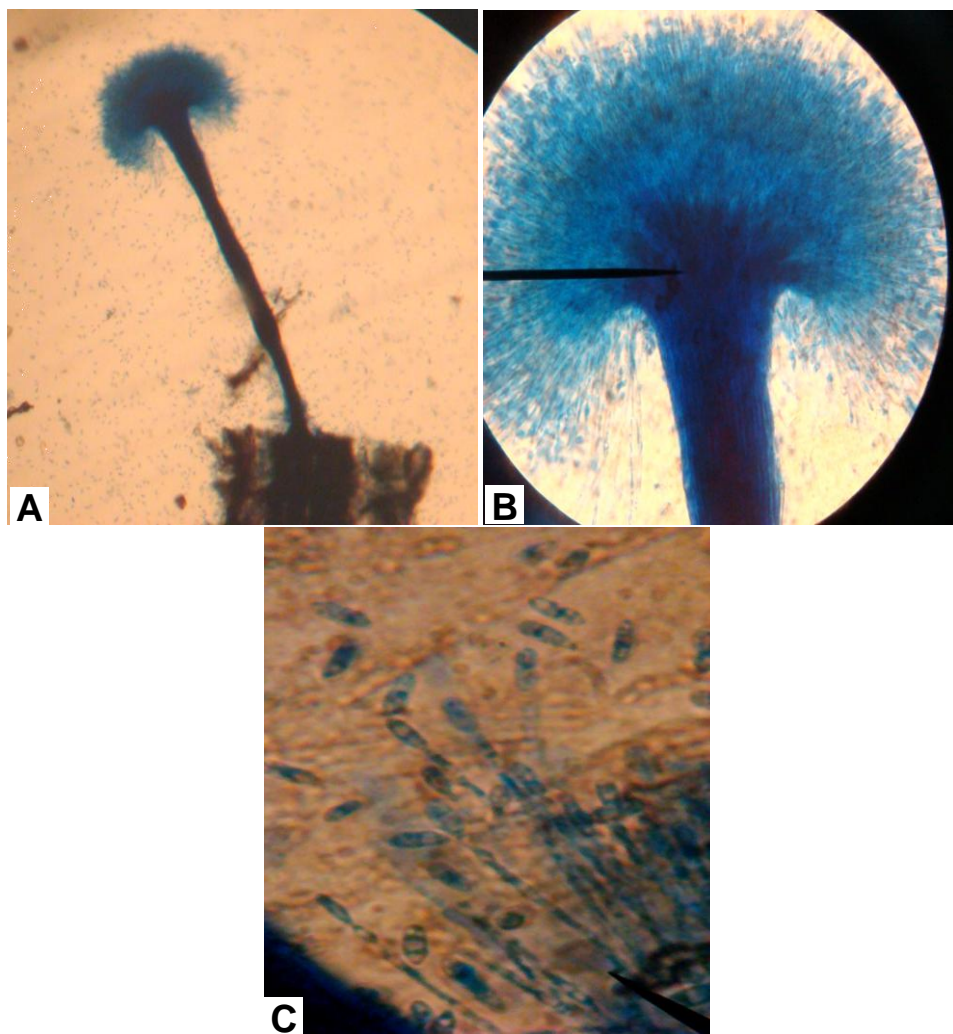


Fig. 2. (A-C) *Graphium putredinis* (A) Synnema 100x (B) Apical region of the synnema 1000x (C) Conidiophore with conidia 1000x.

Graphium putredinis was characterized by in having synnemata upto 1000 μm long and 40 μm wide and conidia, ellipsoidal to cuneiform with two guttules 5-11 x 2-4 μm . The fungus under study closely resembles with *Graphium putredinis* in having similar synnemata in shape and size (250-15-30 μm) and conidia hyaline, unseptate, cylindrical to ellipsoidal, obtuse at the apex and truncate at the base with two guttules 5-7 x 2-2.5 μm . This also resembles with *Graphium penicillioides* in having synnematosous conidiomata and unseptate cylindrical to cunniform and hyaline conidia but differs in the shape of synnemat head which is penicillous in *G. penicillioides* and dome shaped in *G. putredinis*. Furthermore conidia are smaller in *G. penicillioides* (4-7x1-2 μm) and bigger in *G. putredinis* (5-11x2-4 μm). This also differs from *G. calicioides* which has much longer synnemata (5000 μm) and very small conidia (1.5-3 x 1-2 μm).

Previously from Pakistan only two species were reported viz., 1) *G. paradoxum* Sacc., was reported on dung from Lahore, (Ginia, 1936; Ahmad, 1956) and 2) *G. penicillioides* Corda., on grass land soil from Mt. Gilipur, Nangaparbat, (Matsushima, 1993).

In the present study *G. putredinis* is reported for the first time from Pakistan on *M. alba* which is a new host record of the fungus from Faisalabad Pakistan.

Specimen examined: *G. putredinis* on the bark of *M. alba*; Govt. College University Faisalabad; Pakistan. 25 July, 2007; S.Q. Abbas & R. Ayesha, G.C.U.M.H # 6.

In the present studies another fungus was found on bark of *Morus alba* specimen G.C.U.M.H #3 It belonged to the genus *Curvularia* and identified as *Curvularia lunata* (Wakker) Boedijn after consulting Ellis (1971, 1976); Sivanesan (1987).

3) *Curvularia lunata* (Wakker) Boedijn, *Bull. Jard. Bot. Burtensz*, **3**, 13: 127 (1933) Fig. 3 (A-B)

= *Curvularia lunatus* Nelson & Hassis, *Mycologia*, **56**: 316 (1964)

= *Pseudocochliobolus lunatus* (Nelson & Hassis) Tsuda, Ueyama & Nishihara, *Mycologia*, **69**: 1118 (1977)

= *Acrothecium lunatum* Wakker, in Wakker & Went, *Die Ziekten van het Suikerriet op java*: 196 (1898)

= *Helminthosporium caryopsidum* Sacc. *Annls mycol.*, **12**: 313 (1914)

= *Helminthosporium sudanensis* Cif. & Frag. *Bol. R. soc. Espana Hist. Nat.*, **26**: 497 (1926)

= *Curvularia caryopsidum* (Sacc.) S.C. Teng, *Fungi of China*, 760 (1963)

Description of the fungus identified: Colonies effuse, black, velvety. *Mycelium* septate, well developed, well branched, blackish brown. Conidiophore macronematous, mononematous, straight, clavate, 250 x 5-7 µm. Conidiogenous cells polytretic, integrated (Fig. 3A). Conidia acropleurogenous, simple, often curved, middle cell much broader, clavate, brown, apical cell light colour, basal cell sub hyaline, 3 transversely septate, hilum not prominent, 18 - 26.5 x 8 - 16 µm (Fig. 3B).

The fungus under study was compared with species of *Curvularia* spp. Comparison was based on various aspects like conidiogenous cells, conidiophores and conidial shape, size, colour and septa. Genus *Curvularia* can easily be grouped in three categories depending upon the number of septa.

1. 5-septate conidia. *C. segnegalensis*, *C. richardii*, *C. stapeliae*

2. 4-septate conidia. *C. catenulata*, *C. prasadii*, *C. protuberata*, *C. geniculata*

3. 3-septate conidia. *C. deightonii*, *C. borrieriae*, *C. harveyi*, *C. leonsis*, *C. uncinata*, *C. verruciformis*, *C. ovoida*, *C. eragrostidis*, *C. brachyspora*, *C. intermedia*, *C. cymbopogonis*, *C. comoriensis*, *C. crepinii*, *C. trifolii*, *C. trifolii f.sp. gladioli*, *C. lunata*, *C. lunata* var. *aeries*, *C. geniculata*, *C. affinis*, *C. falla*.

Curvularia lunata and fungus under study has three septate conidia without protuberant hilum, smooth, middle septum not median, asymmetrical, some conidial cell always mid dark brown, curved. Conidia in under study fungus are 18 - 26.5 x 8 - 16 µm., and in *C. lunata* conidia are (20-32 x 8-16 µm).

All the characters of the fungus under study closely resembled with *C. lunata*. Therefore, the fungus was identified as *C. lunata*. Thirteen species of *Curvularia* including *C. lunata* were reported from Pakistan (Ahmad *et al.*, 1997). *C. lunata* was found on various substrates. On soil and seed of *Plantago ovata*, on leaves of *Glycine max*, *Linum usitatissimum*, *Cucumis sativa*, *Legenaria vulgaris* fruits, *Momordica charantia*, *Spinacea oleracea*, on seeds of *Hordeum vulgare*, *Pennisetum typhoides*, *Zea mays*, from Lahore, Faisalabad and Karachi (Qureshi, 1966; Rizvi, 1966; Khan & Kamal, 1968; Hussain & Ahmad, 1971; Mirza & Qureshi, 1978; Ahmad *et al.*, 1997). *M. alba* is a new host record of *Curvularia lunata* from Faisalabad, Pakistan.

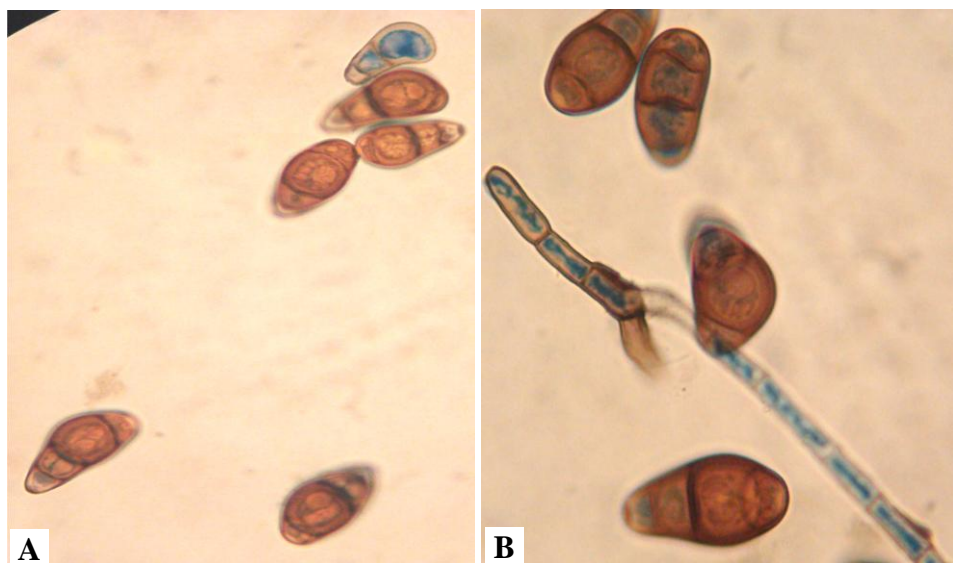


Fig. 3. (A–B): *Curvularia lunata* (A) Conidia 400 x (B) conidia and conidiophore 1000 x.

Specimen examined: *Curvularia lunata* on the bark of *M. alba*; Govt College University Faisalabad; 25 July, 2007; S.Q.Abbas & R. Ayesha, G.C.U.F.M.H. # 3.

In the present study a fungus was found on bark of *Morus alba* specimen. G.C.U.F.M.H. 4. It belonged to genus *Drechslera* and identified as *Drechslera* state of *Cochliobolus spicifer* Nelson after consulting literature. (Ellis, 1971, 1976; Sivanesan, 1987).

4. *Drechslera* state of *Cochliobolus spicifer* Nelson, *Mycologia*, 56: 198. (1964) Fig. 4A & 5B

= *Brachycladium spiciferum* Bainier, *Bull. trimest. Soc. mycol. Fr.*, **24**: 81-82. (1908)

= *Curvularia spicifera* (Bainier) Boedijn, *Bull. Jard. bot. Buitenz.* 3, **13** (1): 127(1933)

= *Helminthosporium spiciferum* (Bainier) Nicot, *Ost .bot. Z.*, **100**: 482(1953).

Description of fungus identified: Colonies effuse, grey, hairy. Mycelium well developed well branched, septate, chocolate brown. Conidiogenous cells polytretic, integrated, terminal and cicatrized. Conidiophores solitary or in small groups, flexuous, repeatedly geniculate with numerous well defined scars, mid to dark brown, up to 280 µm long and 4–9 µm thick (Fig. 4A). Conidia straight, oblong or cylindrical, rounded at the ends, when mature golden brown except for a small area just above the dark scar which remains hyaline or very pale, smooth, 3 pseudoseptate, 20–40 x 9–14 µm (mostly 30–36 x 11–3 µm), hilum 2–3 µm wide.

The fungus on *M. alba* from Faisalabad was identified as *Drechslera* state of *Cochliobolus spicifer*, because it shared all the characteristics of *Drechslera* state of *Cochliobolus spicifer*. Conidiogenous cells solitary, repeatedly geniculate with well defined numerous scars and 3-septate, straight, cylindrical, conidia round at both ends, golden brown, have a hyaline small area just above the dark scar. It has 3-septate conidia, and was also different from *D. bromi*, *D. catenaria*, *D. fugax*, *D. graminea*, *D. nobleae*, *D. teres*,

and *D. tritici-repentis* in having 5-10 septate conidia, and *D. campanulata*, *D. dactylidis*, *D. poae*, *D. siccanis* differ in having 0-2 septate conidia, it was also distinguished from *D. andersenii* and *D. dictyoides* which have 12-15 septate conidia.

Drechslera state of *Cochliobolus spicifer* resembled with *D. papendorfii*, *D. australiensis* and *D. hawaiiensis* in having 3-septate curved conidia. However, conidia in *D.* state of *Cochliobolus spicifer* are straight and are curved in *Drechslera papendorfii*, furthermore, *Drechslera* state of *Cochliobolus spicifer* differed from *D. australiensis* in having dark brown conidia and conidiophore, with prominent black conidial scars. Similarly *D.* state of *Cochliobolus spicifer* differed from *D. hawaiiensis* where the conidia have narrow endings and shorter conidiophore (up to 120 μm) than that of *D.* state of *Cochliobolus spicifer* (up to 280 μm).

Four species of *Drechslera* have been reported from Pakistan (Ahmad *et al.*, 1997).

1) *Drechslera hawaiiensis* (Dugn.) Subram & Jain. has been reported on seeds of *Phaseolus radiatus*, *Cajanus indicus*, *Cyamopsis psoralioides*, *Sesamum esculanta*, *Brassica campestris*, *Lens esculantum*, *Cicer arietinum*, *Sorghum vulgare*, *Pennisetum typhoides*, *Plantago ovata*, *Linum usitatissimum*, from Karachi (Hussain & Ahmad, 1971). 2) *D. cynodontis* (Marignoni) Subram & Jani has been reported from soil of Banjasa; (Matsushima, 1993). 3) *D. sorokiana* Sacc., has been reported from soil of a cultivated field from Jarshing, Nangaparbat; (Matsushima, 1993). 4) *D. spicifera* (Drechsler.) V. Arx. has been reported from the soil of coniferous forest from Matiltan, Swat (Matsushima, 1993).

Drechslera state of *Cochliobolus spicifer* is reported for the first time from Pakistan on *M. alba*.

Specimen examined: *Drechslera* state of *Cochliobolus spicifer* on the bark of *M. alba*; Govt. College University Faisalabad; 13 May, 2007; S.Q.Abbas & R. Ayesha G.C.U.F.M.H. 4.

In the present studies a fungus was also found on the dead branches of *Morus alba* specimen G.C.U.F.M.H. # 7. It belonged to the genus *Memmoniella* and identified as *Memmoniella echinulata* (Riv.) Galloway, after consulting literature (Ellis, 1971, 1976; Charmichael *et al.*, 1980)

5. *Memmoniella echinulata* (Riv.) Galloway, *Trans. Br. mycol. Soc.*, 18: 63-166 (1933) Fig. 5A.

=*Cephalotrichum echinatum* (Rivolta) Toro, (1932)

=*Haplographium echinatum* (Rivolta) Sacc. *Syll. fung.*, 4: 307 (1886)

=*Penicillium echinatum* Rivolta, 45 (1873)

=*Stachybotrys echinata* (Rivolta) G. Sm., *Trans. Br. mycol. Soc.*, 45: 392 (1962)

Description of fungus identified: Colonies effuse, black, powdery on natural substrate. Mycelium immersed or partly superficial, septate, branched. Stroma none. Setae, swollen at the apex, pale brown to mid brown, smooth, covered in part with dark granules. 48 - 95 \times 3.5-4.8 μm . Conidiogenous cells hologenous and stationary, discrete, in groups almost 8 in number at the apex of the conidiophore, cylindrical, usually with a small opening and with no collarete 8-10 \times 3.5- 4 μm . Conidia catenate, acrogenous, simple, spherical, dark brown, minutely verrucose, 5.2 μm in diameter (Fig. 5A)



Fig. 4. (A-B): *Dreschlera* state of *Cochliobolus spicifer* (A) Mycelium, conidia and conidiophore with scars 400x (B) Conidia with 3 prominent pseudosepta 1000x.

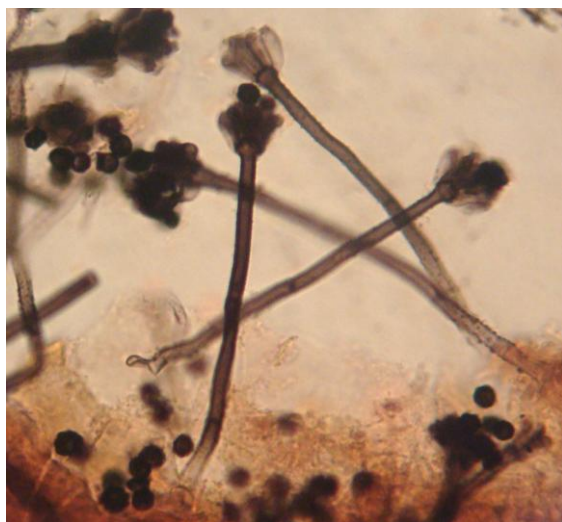


Fig. 5 (A). *Memmoniella echinulata* conidia, conidiophore, conidiogenous cells.1000x.

Fungus under study was compared with different *Memmoniella* spp. Measurements of its conidia, conidiogenous cells and conidiophores matched with *Memmoniella echinulata*. Conidia verrucose, conidiogenous cells hologenous and stationary and 8 in number. Conidiophore $48-95 \times 3.5-4.8 \mu\text{m}$. It differed from *M. levispora* whose conidia were smooth, however, it also resembled with *M. subsimplex* in having verrucose conidia, however it can be distinguished from *M. subsimplex* due to difference in the size of conidia and conidiophore. *Memmoniella echinulata* has smaller conidia ($3.5-5 \mu\text{m}$) and conidiophore ($48-95 \times 3.5 - 4.8 \mu\text{m}$) than *M. subsimplex* which has longer conidia ($6-9 \mu\text{m}$) and conidiophores ($150-180 \mu\text{m}$).

Previously *Memmoniella echinulata* (Rivolta) Galloway has been reported from Lahore, on cow dung (Ahmad, 1962), and on stem of *Salvadora persica*, from Karachi; (Ghaffar & Abbas, 1972).

In the present studies *Memmoniella echinulata* is reported for the first time on *M. alba* from Faisalabad, Pakistan.

Specimen examined: *Memmoniella echinulata* on the bark of *M. alba*; Govt. College University Faisalabad; 13 May, 2007; S.Q.Abbas & R. Ayesha, G.C.U.F.M.H. # 7.

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