



New Fungal Records on Guava (*Psidium guajava*) from Pakistan

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Abstract: *Rutola graminis* (Desm.) Crane & Schokn., *Cladosporium nigrellum* Ellis & Everh. and *Gliomastix* state that *Wallrotheilla subiculosa* Hohn. are reported for the first time on guava (*Psidium guajava* L.) from Pakistan. *Alternaria tenuissima* (Kunze ex Pers.) Wiltshire is also reported for the first time on guava from Faisalabad, Pakistan.

Keywords: *Rutola graminis* (Desm.) Crane & Schokn, *Cladosporium nigrellum* Ellis & Everh. *Gliomastix* state of *Wallrotheilla subiculosa* Hohn, *Alternaria tenuissima* (Kunze ex Pers.) Wiltshire

1. INTRODUCTION

In a project on survey and surveillance of fungal associations to the flora in district Faisalabad, Pakistan, a detailed survey of the area was carried out for guava (*Psidium guajava* L.; local name, *Amrood*).

Guava is a small tree or shrub, belonging to family *Myrtaceae*, about 6 m tall. In Pakistan guava is cultivated on more than 62.5 thousand hectares [11] and in the Punjab province, Pakistan on 50.3 thousand hectares; its annual production in the country is 421.3 thousand tones [9]. Guava fruit contains 0.7% protein, 11% carbohydrates and appreciable amounts of certain vitamin A, B1, B2 and C and some minerals [10]. The ripened fruit is eaten in fresh and is used in jams, juices and sauces. Guava may boost natural immunity and is thought to provide antioxidant protection for heart and against cancer.

Guava is grown worldwide and its growth and production is reported to be stressed by a number of diseases [24]. Among the diseases which attack guava plant, wilt is very destructive. This disease is

characterized by yellowing and browning of leaves and the tips of the twigs. Another important disease which is reported recently in Pakistan is anthracnose of guava, caused by *Gloeosporium psidii* which attacks all the above ground plant parts resulting in death of the branches. [25]. In India, guava decline due to fungal attack is the most serious and devastating disease, destroying thousands of trees annually, thus attaining the status of a national problem [27].

Twenty one (21) fungi have been reported to infect guava plants in Pakistan, viz., 1) *Phytophthora parasitica* in Faisalabad [12]; 2) *Capnodium sp.*, on guava in Karachi [18]; 3) *Glomerella cingulata* Stonem.) Spauld & Schrenk, on guava fruit in Karachi [20]; 4) *Alternaria alternata* on bark of guava in Pattoki and Sharqpur [17]; 5) *Alternaria tenuissima*. (Kunze ex Pers.) Wiltshire, on guava leaves in Karachi [18]; 6) *Cladosporium sp.* on leaves in Karachi [19] and on guava's twigs in Pattoki and Sharqpur [17]; 7) *Curvularia sp.* on tree branches in Pattoki and Sharqpur [17]; 8) *Fusarium solani* in Larkana, [11] ; 9) *Fusarium solani f. f. sp. psidi* in Faisalabad [12]; 10)

Fusarium oxysporum on tree branches in Pattoki and Sharqpur [17]; 11) *Fusarium oxysporum* f. sp. *psidi* in Faisalabad [12]; 12) *Gleosporium psidii*, on plant twigs of in Pakistan, [19]; 13) *Penicillium* sp. on tree bark in Pattoki and Sharqpur [17]; 14) *Pestalotiopsis brevista* Sacc. on leaves and fruit in Karachi [18,20]; 15) *Phomopsis* sp. on fruit in Karachi [18,20]; 16) *Phoma psidii* Ahmad on dead branches in Lahore and Karachi [3, 6, 18]; 17) *Diplodia psidii* Ahmad on tree branches in Bhawalpur [5, 7]; 18) *Stagnopsis psidii* on branches in Bhawalpur [5,7]; 19) *Pestalotia psidii* Pat., on dead twigs in Pakistan [26]; 20) *Lasiodiplodia undulata* (Pat). Abbas, Sutton, Ghaffar, and Abbas [1] (as *Botryodiplodia theobromae* Pat) on dead branches of *Psidium guajava* in Tandojam [21] and Faisalabad [12, 18]; and 21) *Polyporus* sp. on tree anches in Haripur (Hazara) [19].

2. MATERIALS AND METHODS

Methodology of the study was the same as described elsewhere [2]. Samples of infected *Psidium guajava* were collected from the different areas of Faisalabad city, i.e., G.C. University, Faisalabad; University of Agriculture, Faisalabad; and Sheikh Colony, Faisalabad. Identification of fungi, up to species level, was carried out [8, 13, 15, 16, 22].

3. RESULTS

The fungus found on *Psidium guajava*, specimen number 18 belonged to *Rutola graminis* (Desm.) Crane & Schokn.

1) *Rutola graminis* (Desm.) Crane & Schokn., *Can. J. Bot.*, **55** (24): 3015 (1978) (1977)

= *Torula graminis* Desm. *Annls Sci. nat.*, 11, 2: 72 1834.

Description of the Identified Fungus

Mycelium immersed. Cronate conidiogenous cells absent, Conidia brown, minutely verruculose, conidia in a long chain, sometimes branched chains which break up into 0, 1, 2, 3 or more segments. Conidia. 0-septate, almost spherical but often slightly broader than long, 4 - 4.8 x 4.2 - 5.8 μ m.

The fungus under study was compared with *Rutola graminis* (Desm.) Crane & Schokn. It

was observed that it closely resembled to *Rutola graminis*. Resemblence lies in conidial size and absence of cronate conidiogenous cells. In *Rutola graminis* conidia were 4-5 x 4-6 μ m, where as in the fungus under study conidia were 4 - 4.8 x 4.2 - 5.8 μ m, therefore fungus under study was identified as, *Rutola graminis* (Desm.) Crane & Schokn.

Crane & Schokn, 1978 [14] erected genus *Rutola* based on *Torula graminis* Desm. Crane & Schokn studied the type specimen of *Torula graminis* Desm. (1834), they observed that the distinguishing character "cronate conidiogenous cells" of genus *Torula* Persoon ex Fries was not present in *Torula graminis* Desm. Therefore they erected a new hyphomycetous genus *Ratula* Crane & Schokn based on *Torula graminis* Desm. This is a monotypic genus with only one species *Rutola graminis* (Desm.) Crane & Schokn.

In the present study, genus *Torula* was first time replaced in Pakistan. *Psidium guajava* is a new host record of *Rutola graminis* from Faisalabad, Pakistan.

Specimens Examined

Rutola graminis, on branches of *Psidium guajava*, collected from University of Agriculture, Faisalabad: August 19, 2007; S.Q. Abbas and Abida Perveen; G.C.U.F.M.H# 18.

The fungus found on *Psidium guajava*, specimen # 19, was identified as *Cladosporium nigrellum* Ellis Everh.

2) *Cladosporium nigrellum* Ellis Everh: 1894 (1893), *Proc. Acad. N. Sci. Philad.* : 463, Ellis More Dematiaceous Hyphomycetes: 329 (1976). Fig. 2 (A-B).

Description of the identified fungus: Mycelium brown, septate. Conidiophore septate, brown, 240 - 250 x 5 - 9.32 μ m. Conidia smooth, brown, conidia ellipsoidal, cylindrical, oval and 0-3 septate. 5.32-15.31 x 4- 7 μ m.

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. The fungus under study on *Psidium guajava* also has 0-3 septate conidia. In spite of

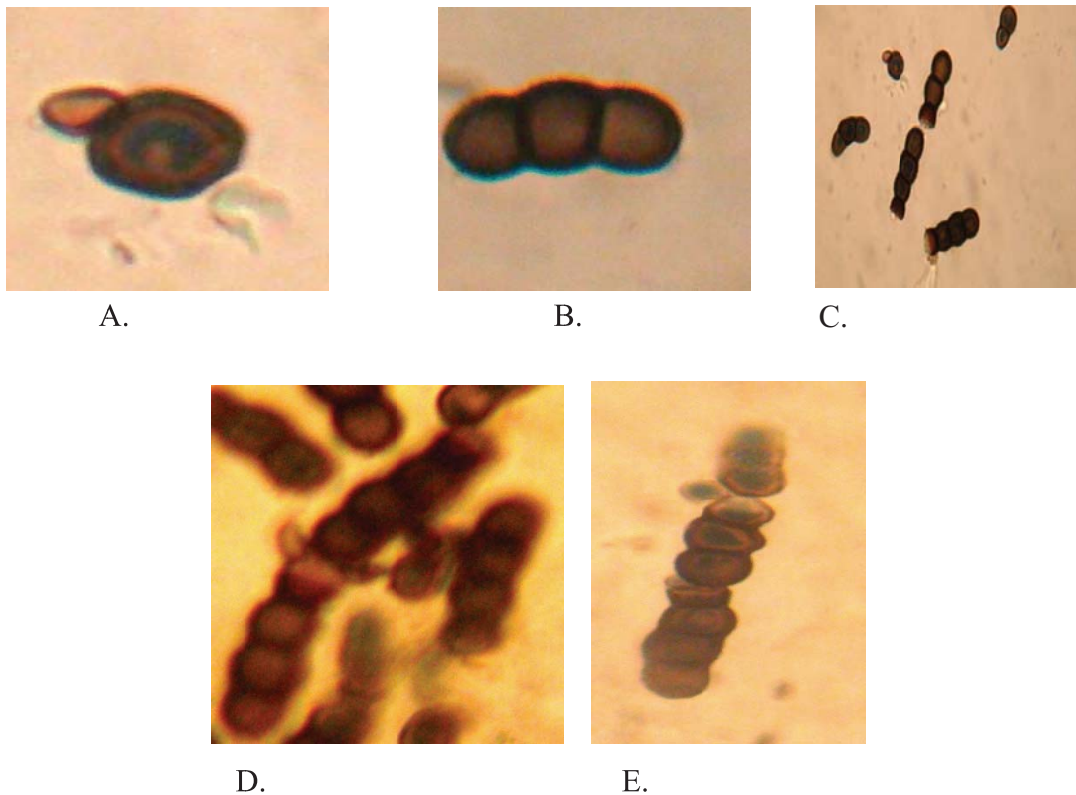


Fig. 1. *Rutola graminis* (A-E), A. Conidia 1000X, B. Conidia in a chain of three cells (400X), (C.D.E), Conidia in a chain of more than nine cells (1000X).

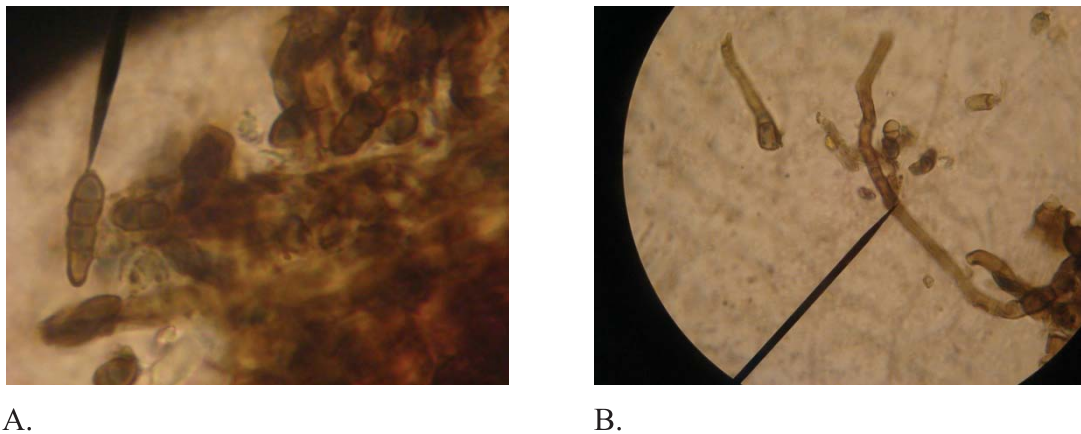


Fig. 2. (A-B): *Cladosporium nigrellum* A. 1-septate, 2-septate and 3-septate conidia 1000X, B. conidia and conidiophore 400X.

similarity in having 3 septate conidia, there are some differences among them; *C. brassicae* have shorter conidiophores $150 \times 6-9 \mu\text{m}$ with terminal and intercalary swellings of diameter $10-12 \mu\text{m}$ than the fungus under study which has conidiophores of $240 - 250 \times 5 - 9.32 \mu\text{m}$. Similarly *C. apicale* also have longer and wider conidiophores $260 \times 6 - 8 \mu\text{m}$ than the fungus under study $240 - 250 \times 5 - 9.32 \mu\text{m}$.

Whereas *C. variable* have longer conidiophores $350 \times 6 - 8 \mu\text{m}$ than the under study fungus. *C. macrocarpum* differs from under study fungus in having longer but less wider conidiophores $300 - 4 - 8 \mu\text{m}$ with terminal and intercalary swellings of diameter $9 - 11 \mu\text{m}$. Furthermore *C. uredinicola* also differs from under study fungus in having longer and less wider conidiophores $300 \times 3 - 5 \mu\text{m}$ than

the under study fungus where the conidiophores are of 240 - 250 x 5 - 9.32 μm . Conidiophores in *Cladosporium nigrellum* are wavy, smooth, reddish brown, septate, 240 - 250 x 5 - 9.32 μm . and conidia are lemon shaped to cylindrical, narrowing at the ends, , in simple or branched chains, smooth walled, light brown, 5 - 15 x 4 - 7 μm .

After comparative studies, it was concluded that *Cladosporium nigrellum* closely resembled with fungus under study found on *Psidium guajava*, from Faisalabad, Pakistan. Therefore, it is identified as *Cladosporium nigrellum*.

Eighteen (18) species of *Cladosporium* were identified from Pakistan; however, *Cladosporium nigrellum* was not previously reported from Pakistan [8].

In this report *Cladosporium nigrellum* is reported for the first time from Faisalabad, Pakistan. Further more, *Psidium guajava* is an addition to the list of hosts of *Cladosporium nigrellum* from Faisalabad, Pakistan.

Specimens Examined

Cladosporium nigrellum on the branches of *Psidium guajava*; Sheikh Colony, Faisalabad, August 21, 2007: S.Q.Abbas and Abida Perveen G.C.U.F.M.H. # 19.

The fungus found on branches of *Psidium guajava*; G.C.U.M.H.# 20 was *Gliomastix* state of *Wallrothiella subiculosa* Hohn.

3) *Gliomastix* state of *Wallrothiella subiculosa* Hohn., *Sber. Akad. Wiss. Wien*, Math. aturw. Kl., Abt. 1, 121: 381(1912), Ellis, *Dematiaceous Hyphomycetes* CAB, IMI Kew: 520, (1971) Fig. 3, (A-D).

Description of the Identified Fungus

Conidiophore branched, sometimes simple, usually brown in colour. The base of conidiophore is darker than the upper part, septate. Conidia light brown, oval, some times globose, smooth walled, 3.8-4.1-7.6 x 2.68-3.84 μm . Conidia formed endogenously from the tip of the conidiophore and aggregated in a mucilaginous sheath.

The fungus under study closely resembled with *Gliomastix* state of *Wallrothiella subiculosa*. The

surface and the shape of conidiophore of both were the same, the base of conidiophores are more darker than the upper region and septate. Further more the conidia of both are smooth and aggregated in slimy mass. Conidial length and breadth were also same. In *Gliomastix* state of *Wallrothiella subiculosa* conidia are (3-8 x 2-4. μm) and conidia in the fungus under study are (3.8- 7.6 x 2.68-3.84 μm), Therefore the fungus under study was identified as *Gliomastix* state of *Wallrothiella subiculosa*. Hohn.

The species under study was also compared with *Gliomastix murorum* Hughes, which resembles with *Gliomastix* state of *Wallrothiella subiculosa*. In both species conidia arising from the apex of conidiophores which act as conidiogenous cells, but differs that the conidiophores of *Gliomastix murorum* are not septate, while they are septate in *Gliomastix* state of *Wallrothiella subiculosa*. Conidia of *Gliomastix murorum* are smaller (2.5 - 5.5 x 2 - 4.5 μm) than the conidia of *Gliomastix* state of *Wallrothiella subiculosa*. (3.8 - 7.6 x 2. 68 - 3.84 μm).

Gliomastix state of *Wallrothiella subiculosa* also resembles with *Gliomastix cerealis*, *Gliomastix cereale* (Karst) Dickinson. In both species conidia formed from the tip of the conidiophore, which act as conidiogenous cells. Further more similarity also lies in having septate conidiophores. However they differ in that, conidial surface are verruculose in *Gliomastix cerealis* and smooth. in *Gliomastix* state of *Wallrothiella subiculosa*

Four species of *Gliomastix* have been reported from Pakistan on different hosts/substrate, but was not reported earlier on *Psidium guajava*, viz.: 1) *G. murorum* (Corda) Hughes; 2), *G. convoluta* (Harz) Mason; 3) *Gliomastix cerealis* (Kraus) Dickinson. 4) *G. luzulae* (Fucekl) Mason ex Hughes.

Correct name of *G. murorum* (Corda) Hughes is *Gliomastix murorum* (Corda). var. *murorum* Hughes and *G. convoluta* (Harz) Mason is the synonym of *Gliomastix murorum* (Corda). var. *murorum* Hughes; thus the reported species of *Gliomastix* remained three. 1) *Gliomastix cerealis* (Kraus) Dickinson from soil of Alpine meadow, Mt Gilpur (Nangaparbat) [23]; 2) *G. Gliomastix murorum* (Corda). var. *murorum* Hughes (as *G. Gliomastix murorum* (Corda) Hughes), from

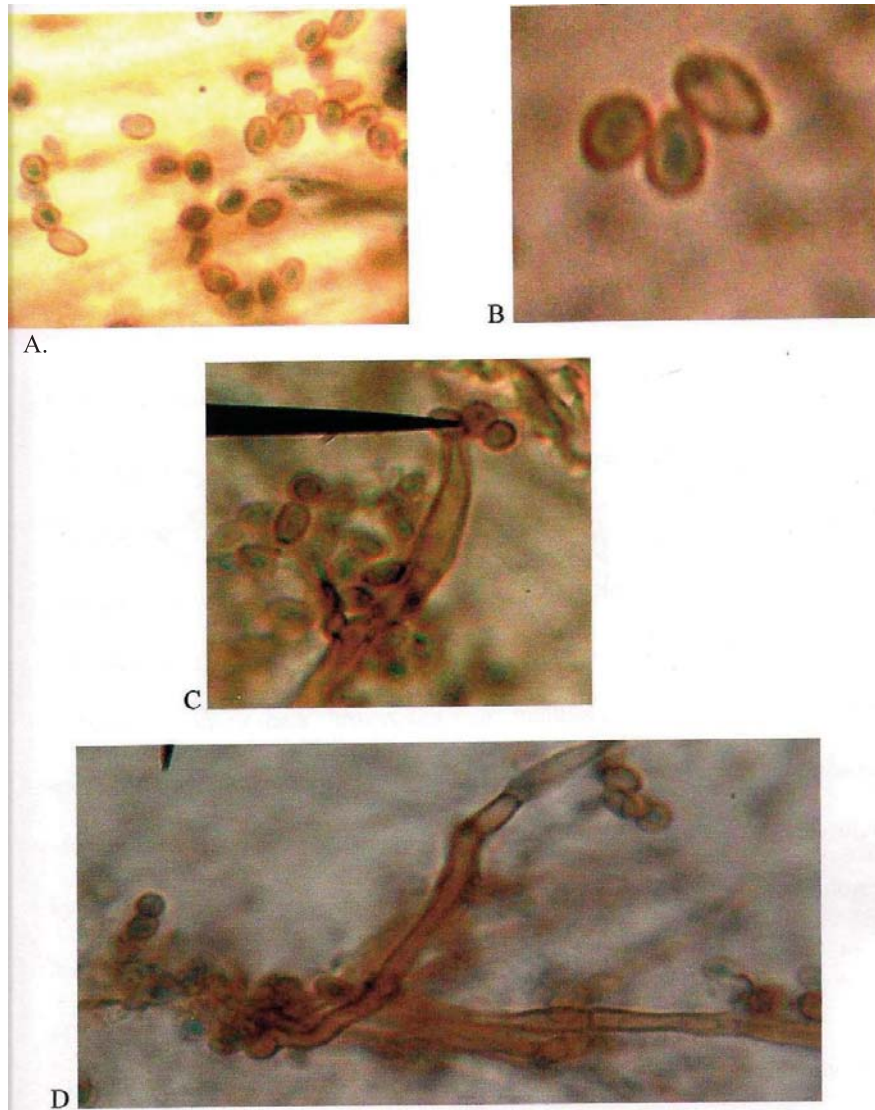


Fig. 3. *Gliomastix* state of *Wallrothiella subiculosa* (A-D) A. Conidia 400X, B. Conidia 1000X C. Conidia with conidiophore 400 x. D. septate Conidiophore 400x.

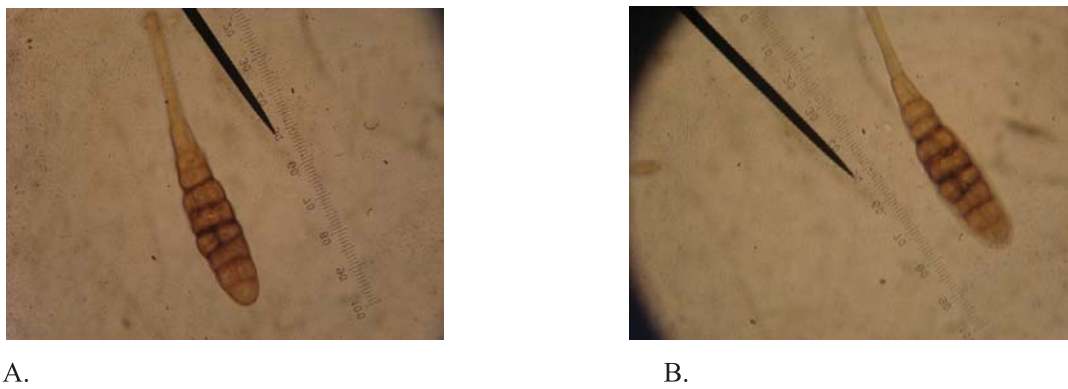


Fig. 4. (A-B): *Alternaria tenuissima* A. Conidia 1000X, B. Conidia 400X.

coniferous soil; Mansehra (Swat) [23]; and (as *G. convoluta* (Harz) Mason) on *Polyporus sp.*, Kaghan vally, Sarhan [4]; 3) *G. luzulae* (Fucekl) Mason ex Hughes from bush soil; Margalla (Islamabad) [23].

Gliomastix state of *Wallrothiella subiculosa* was not reported from Pakistan [8]; However it was reported on bamboos, oil palm, *Phormium*, *Solanum*, *Theobroma* etc. and isolated from the soils of Europe, Java, Hong Kong, New Zealand and West Africa [16]. In the present study, *Gliomastix* state of *Wallrothiella subiculosa* is reported on *Psidium guajava*, from Pakistan (Faisalabad) and is an addition to mycoflora of Pakistan.

Specimen Examined

Gliomastix state of *Wallrothiella subiculosa* on branches of *Psidium guajava*; Jhang Road garden, Faisalabad; September 4, 2007: S.Q. Abbas and Abida Perveen. G. C. U. M. H. # 20.

- 4) The fungus on *Psidium guajava* specimen No. 16 belongs to *Alternaria tenuissima* (Kunze ex pers) Wiltshire.

Alternaria tenuissima (Kunze ex pers) Wiltshire, [as (Fr.) Wiltshir] *Trans. Br. Mycol. Soc.* **18**: 157 (1933.) Fig. 4 (A-B)

=*Helminthosporium tenuissimum* Kunze in C.G. & T.F.L. Nees., *Nova Acta Acad. Caesar. Leop. Carol.* **9**: 242, (1818); Persoon, *Mycol. Eur.*; **1**: 18. (1822).

=*Macrosporium tenuissimum* Fr; *Syst. Mycol.*; **3**: 374. (1832)

Description of the Identified Fungus

Mycelium well developed septate branched pale brown. Conidiophore solitary or in groups, simple and branched, septate and thick walled, smooth, brown; 84 - 117 x 4.2 - 5.9 μm thick. Conidia usually smooth, sometimes minutely verruculose, generally with 3-7 transverse and 0-4 longitudinal septa, brown in colour. Sometimes slightly constricted at the position of septa. 22.75 - 97 x 10.5 - 17.5 μm thick in the broadest part, beak of conidia 2.8 - 4.2 μm thick, swollen at apex 4.2 - 4.9 μm wide.

The fungus under study is identified as *Alternaria tenuissima*; (Kunze ex pers) Wiltshire

Fungus under study can easily be distinguished from *A. dianthicolla*, *A. longissima*, *A. brassicae*, *A. solani*, *A. crassa*, *A. porri*, *A. carthami*, *A. dauci*, *A. passiflorae*, *A. cucumerina* by having very long transversely septate beak.

Furthermore this species is also compared with *Alternaria sonchi* Davis. Conidiophores of *Alternaria sonchi* are up to 80 x 5-9 μm , while the conidiophores of *Alternaria tenuissima* are up to 115 x 4-6 μm . Similarly conidia of *Alternaria sonchi* are 60-130 (77) x 15-26 μm (20) thick in the broader part; beak 4- 10 μm wide, while conidial length of the under study fungus is 23 - 98 x 10.5-17.5 μm thick in broader part; beak is 2.8-4.2 μm clearly differed from *Alternaria sonchi*.

The fungus under study differs from *Alternaria raphani* Groves & Skolko. The conidia in *A. raphani* are more longer and more wider (50 - 130 (70) x 14 -30 μm (22) μm .) than the conidia of under study fungus (22.75- 97 x 10.5-17.5 μm .) Both species are different from each other. The fungus under study closely resembled with *Alternaria tenuissima* in number of septa, in conidia, their Length and thickness. Conidia in *Alternaria tenuissima* are (22.75- 97 x 10.5-17.5 μm), and have 3-7 transverse septa resembled with *Alternaria tenuissima* where as conidia are (22-95 x 8-19 μm and 4-7 transverse septa. Similarly measurements of conidiophores also resembled with the fungus under study. Conidiophores in fungus under study are (84-117 x 4.2-5.9 μm) and are 115 x 4-6 μm in *Alternaria tenuissima*.

Alternaria tenuissima is a common fungus in Pakistan and found on more than 54 different plant belonging to different families including *Psidium guajava*, but not reported on *Psidium guajava* from Faisalabad [8]

In the present report, *Alternaria tenuissima* is for the first time reported on *Psidium guajava* from Faisalabad, Pakistan.

Specimen Examined

Alternaria tenuissima; on fruit of *Psidium guajava*; G.C. University, Faisalabad; July 27, 2007; S.Q. Abbas and Abida Perveen, G. C. U. M. H. # 16.

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