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Research Article

New Fungal Records on *Psidium guajava* from Pakistan

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Abstract: *Aspergillus niger* Van Tiegh, *Chalara* state of *Ceratocystis fimbriata* Ellis & Halst., *Alternaria dianthicola* Neergaard and *Lasiodiplodia ricini* Sacc. are reported for the first time on *Psidium guajava* from Faisalabad, Pakistan.

Keywords: *Aspergillus niger*, *Chalara* state of *Ceratocystis fimbriata*, *Alternaria dianthicola*, *Lasiodiplodia ricini*, *Psidium guajava*

1. INTRODUCTION

In a continuing project on survey and surveillance of fungal associations to the flora of district Faisalabad, Pakistan, a detailed survey of the area was carried out. The present article reports on the fungi observed on *Psidium guajava* L. (Guava; Amrud) belonging to family *Myrtaceae*. It is a small tree or shrub, about 6 meters tall. Flowers are white, Immature fruit ate green in color, while mature fruit varies in form, shape and colors. Its distribution and economic importance was fully discussed by [1]. Guava is grown worldwide and is reported to be stressed by a number of diseases including fungal diseases. Which are the most serious and devastating diseases, destroying thousands of trees annually, and it is also attaining the status of the national problem in Pakistan and India [2, 3, 4, 5, 6, 7, 8, 9, 10, 11]. Among the various diseases which attack guava plant, wilt is very destructive. This disease is caused by *Fusarium solani*, *F. sp. Psidii, Fusarium oxysporum* [3, 4, 5, 6]. This disease is characterized by yellowing and browning of leaves and tips of the twigs. Another important disease that is reported from Karachi and Faizabad (Rawalpindi) Pakistan is Anthracnose of guava caused by *Gloeosporium psidii* that attack aerial parts of the plant resulting the death of branches [7, 11].

Twenty one (21) fungi have been reported from Pakistan [2, 3, 4, 5, 6, 7, 12, 13, 11]. Lodi et al. [14] have also reported. *Pythium aphanedematum* on *Psidium guajava* from Tando Fazal, District Hyderabad and Safari Park, Karachi. This is the first Oomycetous fungus, reported on *Psidium guajava* from Pakistan; thus, fungi reported on *Psidium guajava* from Pakistan become twenty two (22).

Recently, Abbas et al. [1] reported four (4) fungi from Faisalabad. Out of them 3 fungi viz., *Rutola graminis* (Desm.) Craneand Schokn.*, Cladosporium nigrellum* Ellis and Ever hand *Gliomastix* state of *Wallrotheilla subiculosa* were new records on it from Pakistan (Faisalabad). *Alternaria tenuissima* (Nees, ex Fr.) was previously reported from Pakistan, but not from Faisalabad, thus the total fungi recorded from Pakistan up to 2014 became twenty five (25).

In this article, four new fungi are reported from Faisalabad, Pakistan; thus the total number of fungi observed on *Psidium guajava* in Pakistan has been raised to twenty nine (29).

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2. MATERIALS AND METHODS

Samples of *Psidium guajava* were collected from the different areas of District Faisalabad and Jhang. Areas from District Faisalabad included GC University, Faisalabad, University of Agriculture, Faisalabad, Sheikh Colony Faisalabad and District Jhang include suburb area of Jhang city.

Materials and Methods used were the same as described by [15]. Identification up to species level were carried out after consulting [16, 2, 17, 18, 19, 20, 21].

3. RESULTS AND DISCUSSION

I) The fungus found on *Psidium guajava* specimen #.14, identified as *Aspergillus niger* Van Teigh., *Annales des Sciences Naturelles; Botanique*, 5, **8**: 240 (1867) Fig. 1, (A & B).

Description of the Fungus

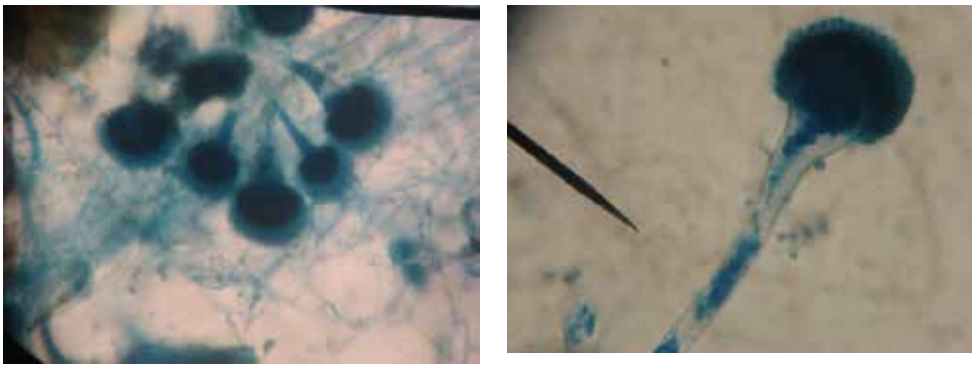
Mycelium immersed. Conidiophores erect, straight or flexuous, often up to 500 µm long, and 11-16 µm wide, hyaline or with the upper part brown, swollen at the apex into spherical vesicle which is usually 33-56 µm in diameter. Conidiogenous cells, hologenous stationary, flaskshaped. Conidia basipetal, catenate, dry, usually globose, brown, verruculose or echinulate, sometimes with the warts or spines arranged in discontinuous bands, 2.4-4.6 µm in diameter.

In the present studies the fungus identified as *Aspergillus niger* Van Teigh. This fungus is very common air contaminant and reported from all over the Pakistan. However it is not recorded on *Psidium*

Table 1. Total species of *Lasiodiplodia* spp. with reference to conidial measurement.

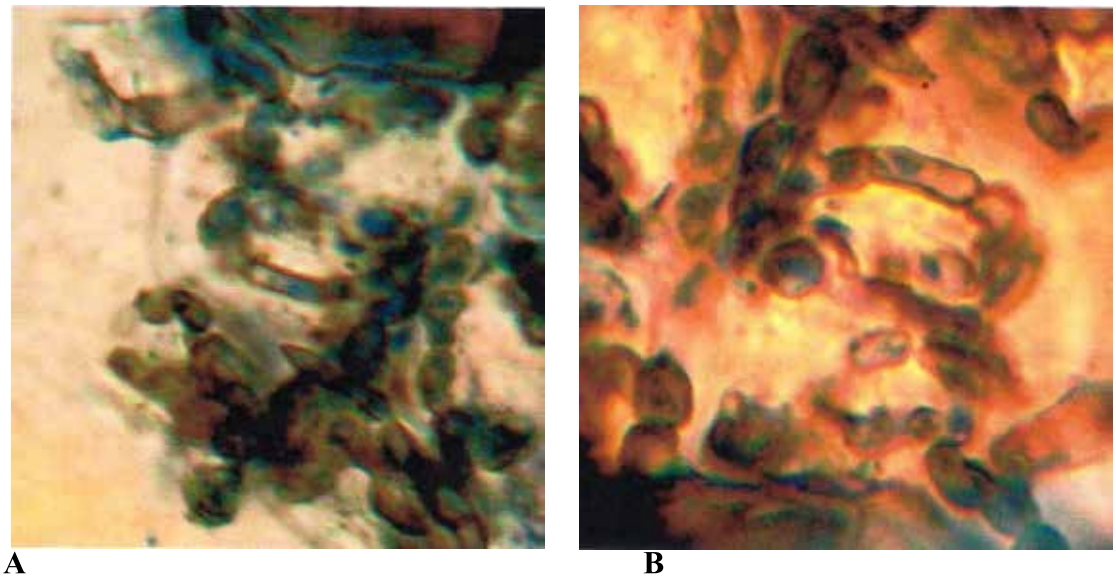
Name of species	Conidial measurement (µm)	Reference
L. abnormis	25–28 × 13–15	[39]
L. citricola	22.5–26.6 × 13.6–17.2	[16]
L. crassispora	27–30 × 14–17	[34]
L. fiorii	24–26 × 12–15	[23]
L .gilanensis	28.6 – 33.4 × 15.6 – 17.6	[16]
L. gonubiensis	32–36 × 16–18.5	[36]
L. hormozganensis	19.6–23.4 × 11.7–13.3	[16]
L. iraniensis	18.7–22.7 × 12.1–13.9	[16]
L. margaritacea	14–17 × 11–12	[36]
L. paraphysaria	30–32 × 15–16	[39]
The fungus investigated in this study	16-18× 10-11	This publication
L. parva	18.3–22.1 × 10.7–12.3	Alves et al. (20 [33]
L. plurivora	26.7–32.5 × 14.4–16.7	[35]
L. pseudotheobromae	25.5–30.5 × 14.8–17.2	[33]
	21.7–26.3 □□13.4–14.8	Abdollahzade [16]
L. ricini	16–19 × 10–11	[39]
L. rubropurpurea	24–33 × 13–17	[34]
L. theobromae	25.5–30.5 × 14.8–17.2	[33]
L. theobromae	22.4–24.2 × 12.9–14.3	[16]
L. thomasiana	28–30 × 11–12	[39]
L. undulata	20–32 × 13.5–19.2	[26]
L. venezuelensis	26–33 × 12–15	[34]

Abdollahzadehet al.[16]



A **B**

Fig. 1. *Aspergillus niger* A, B); A. Conidiophores, vesicles and conidiogenous cells 400X, B. single conidiophore with vesicle 1000X.



A **B**

Fig. 2 (A&B). *Chalara state of Ceratocystis fimbriata*. A. Immature conidia and conidiophores 400X. B. Mature conidia and conidiophores 1000X.

guajava from (Faisalabad) Pakistan.

The Specimen Examined

Aspergillus niger; on twigs of *Psidium guajava*; from G.C. University, Faisalabad; July, 22, 2007; S.Q. Abbas and Abida Perveen, G.C.U.F.M.H #14.

II).Fungus on *Psidium guajava* specimen #15 is identified as the *Chalara* state of *Ceratocystis fimbriata* Ellis & Halst. In *Bull. New Jers. Agric. Exp. Stn*.**7**6:14(1890) *J. Mycol*., **7**:1. (1891) Fig. 2, (A & B).

Description of the Fungus

Conidiophore hologenous stationary, straight or flexuous, septate, hyaline to pale, brown, smooth, conidiophores 4.2- 5.95 µm wide. Conidia of two types:

1) Cylindrical both ends truncate, hyaline or very pale brown, smooth, 3 - 4.4 µm wide.
2) Ellipsoidal, pryiform or obpyriform, truncate at the base, golden brown, thick walled, smooth, 5.5- 9.9 µm wide.

In the present study the fungus identified as *Chalara* state of *Ceratocystis fimbriata* Ellis & Halst.

The fungus being reported in this article resembled with *Scopulariopsis brevicaulis*

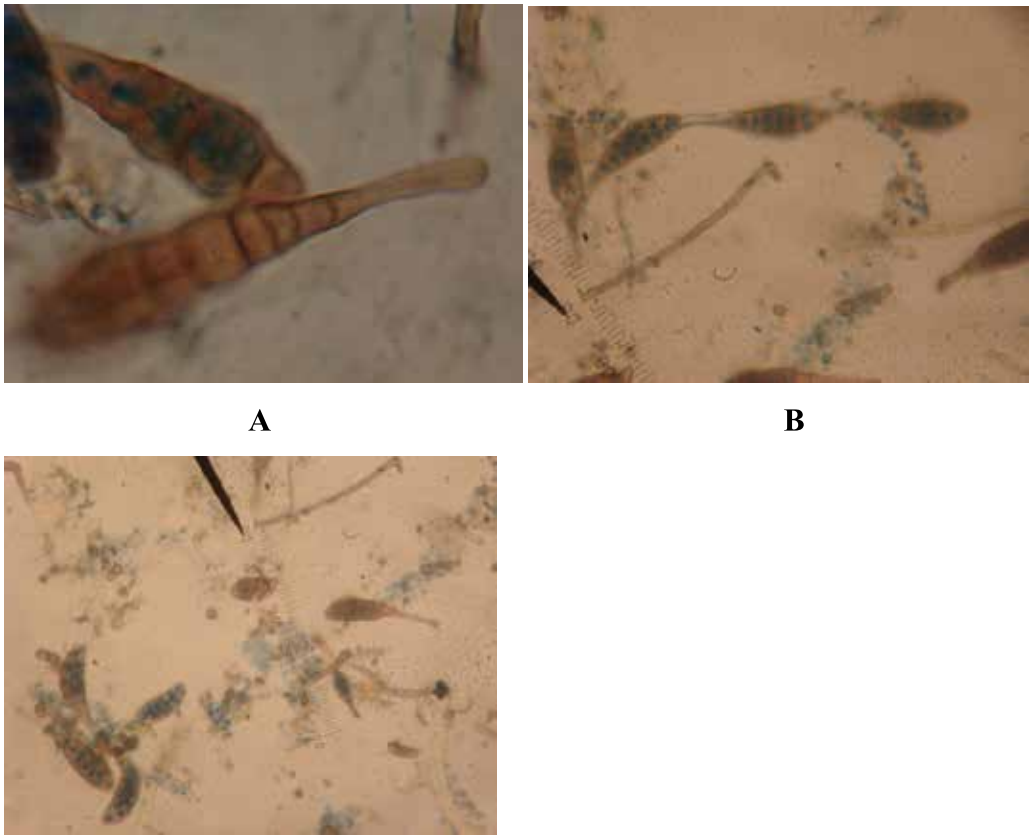


Fig. 3. *Alternaria dianthicola* (A-C) A. Conidia 1000X. B Conidia attached in a chain (400X), C. Mycelium with conidia (100X).

Bainier, The similarity of both fungi lies in that the conidiophore of both are hyaline and septate. Conidia of both fungi are truncate at the base; however they differ in the way that in the fungus under study the conidia are not in chain, while in *Scopulariopsis brevicaulis* conidia are in chain.

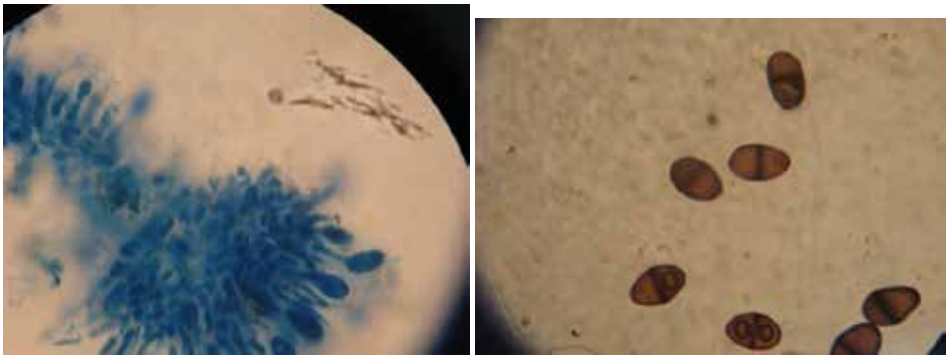
The fungus is also compared with *Chalara* state of *Ceratocystis adipose* Moreau. The conidiophore of both species were hologenous, stationary, brown, smooth and septate. However the conidia present in *Chalara* state of *Ceratocystis adipose* are some times in the form of long chains and verrucose to echinulate with often flattened spines, but the conidia in *Chalara* state of *Ceratocystis fimbriata* are smooth and not in the form of chain.

It is also compared with *Chalara* state of *Ceratocystis fimbriata*. The fungus under study completely resembled with *Chalara* state of *Ceratocystis fimbriata,* because conidiophore of both species are hyaline to pale brown, smooth, septate, hologenous, stationary, straight or flexuous and thickness of conidiophore of both species are also same. Furthermore conidia present in both taxa are smooth and of two types:

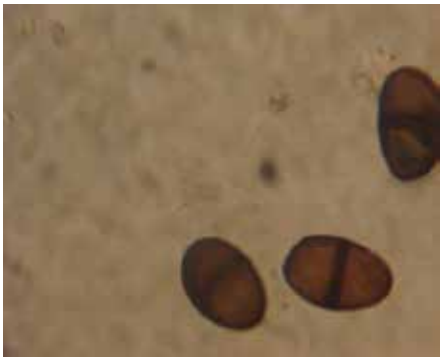
1) Some conidia are cylindrical, truncate at the ends.
2) Some conidia are ellipsoidal, pyriform, truncate at the base. Therefore, the fungus was identified as *Chalara* state of *Ceratocystis fimbriata*.

Chalara state of *Ceratocystis fimbriata* is very common throughout the world. [CMI Distribution Map 91] It isolated from a wide variety of plants; It causes moldy rot of rubber, black rot of sweet potato, trunk and branch canker of almond, apricot, coffee, blight of mango, canker and wilt of pimento, etc.[18, 22].

Rheman et al. [23] described *Ceratocystis mangiferum* from mangoes from Faisalabad, and



A **B**



C

Fig. 4(A-C). *Lasiodiplodia ricini.* A. Immature conidia and conidiogenous cells 400x, B. Mature conidia 400x. C. Mature conidia 1000.

the fungus on *Psidium guajava*is also reported from Faisalabad, therefore acritical study of the type of *Ceratocystis mangiferum* is necessary. Previously, there was no record of this fungus found from Pakistan[2]. In the present study, the fungus under study is a new report for Pakistan. Moreover *Psidium guajava* is also a new host for this fungus, from Pakistan.

The Specimen Examined

Chalara state of *Ceratocystis fimbriata*; on bark of *Psidium guajva*; Jhang Road, Faisalabad; August 24, 2007; S.Q. Abbas and AbidaPerveen, G.C.U.F.M.H.# 15.

III).A fungus found on *Psidium guajava* G.C.U.F.M.H # 17, identified as *Alternaria dianthicola* Neergaard, Neergaard, Danish species of *Alternaria* and *Stemphylium*: 190 (1945). Fig. 3, (A-C).

Description of the Fungus

Conidiophores pale olivaceous brown, septate, arising singly or in branched, straight or flexuous. The conidiophore 151 x 3.84-6.52 µm. Conidia straight or curved, obclavate or almost cylindrical, rostrate, pale olivaceous brown, smooth, with 4-12 transverse and up to 3 longitudinal or oblique septa, constricted at the septa, 56-136 x 10.5- 17.5 µm. The colour of beak and conidial body is same, sometimes inflated at the tip.

The fungus is identified as *Alternaria dianthicola* is compared with related species of *Alternaria.*

Alternaria ricini Hansford, resembles with the fungus under study. Colonies of both species are brown and conidiophore of both taxa arising singly or in groups, erect, simple, straight or flexuous. However they differ from each other in conidiophores measurement. Conidiophores of

Alternaria ricini are 80 x 5-9 µm, and 150 x 4-6 µm in fungus under study. Number of transverse septa in conidia of *Alternaria ricini* are 5-10 and4-12 µm in the fungus under study. Fungus under study also differs from *A. solani* and *A. longipes*, because conidia in *A. solani* are 150-300 x 15-19 µm and in *A. longipes* conidia are 35-110(69) x 11-21(14) µm, whereas in fungus under study the conidia are of 56-136 x 10.5- 17.5 µm and smaller than *A. solani* and *A. longipes*.

Alternaria pedwickii Ellis., resemble closely with the fungus under study in shape of conidia, however both differ due to the size of conidiophores *i.e.* 180 x 3-4 µm in the *Alternaria pedwickii* and 151 x 3.84-6.52 µm in the fungus under study. Similarly they also differ in conidial measurement. Conidia in *Alternaria pedwickii* are 95-170 x 11-20 µm and 56- 136 x 10.5- 17.5 µm in the fungus under study.

Alternaria cucumerina also differs from fungus under study as conidia are more longer and wider in *Alternaria cucumerina* 130-220 (180) x 15-24 (20) µm, than in the fungus under study 56- 136 x 10.5-17.5 µm. Similarly conidiophores in *Alternaria cucumerina* are wider *i.e.* of 110 x 6-10 µm than the fungus under study 3.84- 6.52 µm. *Alternaria sonchi* Davis, can also be differentiated from fungus under study. Conidia in *Alternariasonchi* are smaller but wider, 60- 130(77) x 15-26 µm and conidia are longer and less wider5 6- 136 x 10.5- 17.5 µm in the fungus under study, Similarly, conidiophores of *Alternaria sonchi* are smaller but wider 80 x 5-9 µm than the fungus under study 151 x 3.84- 6.52 µm.

The fungus completely resembles with *Alternaria dianthicola,* because the fungus under study shares all the characteristics with *Alternaria dianthicola.* The conidiophores measurement in *Alternaria dianthicola* are 150 x 4-6 µm and in the fungus under study are 151 x 3.84- 6.52 µm. Furthermore in both taxa conidiophore are brown, similarly conidial measurement also coincide with each other, conidia in *Alternaria dianthicola* are 55-130 (93) x 10-16 (13) µm thick in the broadest part and 56- 136 x 10.5- 17.5 µm in the fungus under study. Therefore it is identified as *Alternaria dianthicola*.

Twenty nine species of genus *Alternaria* are already reported from Pakistan [2]. This fungus was reported from Australia, Chile, Denmark, France, Germany, Italy, Jamaica, Malawi, Malaya, Netherlands, New Zealand, U.S.A. [18, 19]. However it is not reported from Pakistan [2].

In the present study *Alternaria dianthicola* observed for the first time on *Psidium guajava* from (Faisalabad), Pakistan.

The Specimen Examined

Alternaria dianthicola on leaves of *Psidium guajava,* GC University, Faisalabad; 5 August, 2007; S.Q. Abbas and Abida Perveen, G.C.U.F.M.H# 17.

Fungus on *Psidium guajava* specimen No. G.C.U.M.H No. 21 is identified as *Lasiodiplodia ricini* Sacc. *Nuovo G. bot. ital.* 22(1): 61 (1915). Fig. 4, A-C.

Description of the Fungus

Mycelium immersed, conidiomatastromatic. Ostiole absent. Conidiophore absent. Conidiogenous cells hologenous, no proliferation, hyaline. Immature conidia hyaline and thin walled. Mature conidia brown, oval both ends obtuse, uniseptate, euseptate and present in the middle of the conidia. Thick walled with many longitudinal striations.

Sutton [24]was of the opinion that *Lasiodiplodia theobromae* is the correct name of *Botrydiplodia theobromae*. However, Punithalingum (1980) [25] retained it as *Botryodiplodia theobromae* in his monograph.

Abbas et al.[26] when they were assessing the *Sphaeropsis undulata* Berk. &Curt., they pointed out that *Sphaeropsis undulata* is an earlier name for *Lasiodiplodia theobromae* (as *Botryodiplodia theobromae*), therefore a new combination *Lasiodiplodia undulata* (Berk. & Curt.) Abbas, Sutton, Ghaffar & Abbas was proposed.

Lasiodiplodia undulata (as *Botryodiplodia theobromae*) was reported on 41 plants belonging to different families from Pakistan, This fungus cause diseases on *Albizia lebbeck, Aloevera* (as *Aloe barbadensis*), *Althurium andraeanum, Arachis hypogaea, Argyreia speciosa, Bauhinia veriegata, Bignonia* sp., *Broussonetia papyrifera,*

Borassus flabellifer, Cappris decidua as *Cappris aphylla*), *Citrus auratium, Citrus aurantifolia, Cosmos sulphureus, Dalbergia sissoo, Erythrina indica, Euphorbia tirucalli, Ficus palmata, Ficus retusa, Gossypium neglectum, Gossypium sp. Helianthus annus, Ipomoea carnea. Ipomoea gossypiodes, Lagenaria siciraria* (as *Lagenaria vulgaris*), *Mangifera indica, Mimosa subicaulis, Maniho tesculenta* (as *Manihot utilissima*), *Melia azedarach, Moringa oleifera, Morus alba, Nerium oleander* (as *Nerium indicum*), *Pandanus tectorius* (as *Pandanus odaratissimus*), *Pedilanthus tithymaloides, Prosopis julifora, Prosopis spicigera, Psidium guajava, Withania somnifera, Catharanthus roseus* (as *Vincarosea*), *Znniasp, Ziziphus mauritiana* on dead branches of from Lahore, Changa Manga, Ladhar (Sheikhupura); Faisalabad, Tondo Jam, Bimber, Karachi [15, 27, 28, 29, 30, 2, 6, 31, 32, 13].

In recent years morphological as well on DNA finger printing and sequence work was carried out (9, 16, 33, 34, 35, 36.]. Abdollahzadeh et al. [16] carried out a detail studies on *Lasiodiplodia theobromae* described from different part of world using morphological as well on DNA finger printing and sequenceand accepted 14 species of *Lasiodiplodia*. Abdollahzadeh et al [16] were of the opinion that conidial dimension of *Botryodiplodia theobromae* never exceed 30 µm. in length and 16 µm. in width, while the conidial length in *Lasiodiplodia undulata* are up to 32 µm. and width is up to 19.2 µm. Therefore they consider that both species are separate taxa. Fungus under study is identified as *Lasiodiplodia ricini* Sacc., due to conidial and pycnidial morphology and dimensional characters.

Lasiodiplodia ricini Sacc. can easily can be differentiated by *Botryodiplodia ricinicola* (Sacc.) Petr.[37], which has bigger and wider conidia (22-30 x 12-16 µm). *Botryodiplodia ricinincola* Ahmad, Nom. rej. [38] also differs from *Lasiodiplodia ricini* Sacc. in having bigger and slightly wider conidia 17-28 X 11-12 µm.

Botryodiplodia theobromae was also reported on *Psidium guajava* from Tando Jam Sindh, Pakistan Khan and Kamal (1968) [8]. However it is not reported from Faisalabad, Punjab, Pakistan [2].

The fungus under study (conidia 16-18× 10-11 µm) differs from the following *Lasiodiplodia* spp. In having bigger conidia viz.;- *L. abnormis* (25 - 28 × 13 - 15 µm); *L. citricola* (22.5 - 26.6 × 13.6 - 17.2 µm); *L. crassispora* (27 - 30 × 14 - 17 µm); *L. fioriii* (24 - 26 × 12 - 15 µm); *L. hormozganensis* (19.6 - 23.4 × 11.7 - 13.3 µm); *L. iraniensis* (18.7 - 22.7 × 12.1- 13.9 µm); *L. parva* (18.3 - 22.1 × 10.7 - 12.3 µm); *L. pseudotheobromae* [; (25.5 - 30.5× 14.8 - 17.2 µm [33]; (21.7 - 26.3 × 13.4 - 14.8 µm;) [16]; *L. theobromae* [(23.6–28.8 × 13–15.4 µm) [33];`(22.4 - 24.2 × 12.9 - 14.3 µm) [16]; *L. gonubiensis* (32 - 36 × 1 6 - 18.5 µm) ; *L. gilanensis* (28.6 - 33.4 × 15.6 - 17.6 µm); *L. thomasiana* (28 - 30 × 11 - 12 µm) and *L. undulata* (20 - 32 × 13.5 - 19.2 µm); *L. venezuelensis* (26–33 × 12–15 µm). It differs from *L. margaritacea* (14 - 17 × 11 - 12 µm) which has smaller conidia. Fungus under study completely resembles with *L. ricini* (16–19 × 10–11) in conidial morphology and dimensions. In the present studies, *Lasiodiplodia ricini* is a new report on *Psidium guajava* from Pakistan (Faisalabad).

Work of Abdollahzadeh et al. (2010) [16] is very important therefore, it is necessary that all the fungi described as *Lasiodiplodia undulata* (*Lasiodiplodia theobromae*, or *Botryodiplodia theobromae*) on different hosts from Pakistan needs an urgent revision in the light of morphological and DNA finger printing and sequence.

Specimen Examined

Lasiodiplodia ricini identified from the bark of *Psidium guajava*; Jhang Road garden; October 2, 2007: S.Q. Abbas and Abida Perveen, G.C.U.M.H # 21.

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