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New Host Record of *Trichothecium roseum* on *Yucca aloifolia* from Gujranwala, Pakistan

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Abstract

During a disease survey of infected plants in Gujranwala, Punjab, Pakistan, untypical, irregular, dark brown to black necrotic patches on *Yucca aloifolia* were observed, causing leaf tip die back. Stereomicroscopic examination shows white to pinkish white mass giving powdery mildew appearance over the surface. Morpho-anatomical characterization identified the pathogen belong to the genus *Trichothecium*, as *Trichohecium roseum*. Description and illustration of the identified species are presented. Previously no work has been done on *Y. aloifolia* from Pakistan. To the best of our knowledge, this is the first report of *T. roseum* causing leaf die-back on an ornamental, *Yucca aloifolia* in Pakistan. It is a new host report of *T. roseum* for Pakistan and worldwide. A key to Pakistani species of genus *Trichothecium* is given in this paper.

Keywords: Fungal Host; Gujranwala; Leaf die-back; Necrosis; Ornamental Plants; *Trichothecium*; *Yuca aloifolia*

1. Introduction:

Yucca aloifolia L. of the family Agavaceae, is the second-largest genus of perennials, shrubs and trees, including about 50 species that are primarily found in Mexico and southern North America (Clary, 1997; Verhoek, 1998; Thiede, 2001). *Y. aloifolia* (Spanish Bayonet) is a broadleaf, evergreen ornamental shrub with simple or dense branches, bearing terminal rosettes of sword-shaped, digger-like deep green leaves. The 2 ft. long (60 cm) evergreen leaves are thick, rigid as well as small, sharp serrations along the margins and a very pointy tip. Reddish brown, thin and wiry roots are featured by this plant. The stems are 3-5 m tall, usually cylindrical to slightly expanded at the base, simple or sparsely branched higher up, fibrous, and covered with the remains of dead leaves. Its inflorescence features a heavy branching, upright panicle that grows to a height of 60 cm within the rosette and barely surpasses it (Smith *et al.,* 2012). The height and width of this plant, which can reach heights of 5–10 ft (150–300 cm) and 9–150 cm respectively, offer architectural height to the garden. In the spring or summer, they are topped with tall clusters of up to 2 feet (60 cm) long and large nodding bell-shaped creamy white blooms that are occasionally tinged violet. The blossoms captivate moths, hummingbirds and butterflies. Untroubled, long-lasting, and resistant. Spanish bayonet can withstand frost, drought, salt and heat stresses (Kishor *et al.,* 1992; Smith *et al.,* 2012).

It is widely grown and a striking landscape element. It fits well in either tropical, mediterranean, or dry landscapes and is excellent for seaside plantings. It is also a superb specimen plant for xeriscape gardening (Smith *et al.,* 2012).

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Yucca aloifolia is not only a rich plant for garden landscape ideas but offers indoor decoration as well. The rhizomes are used as a detergent, while the fruits are utilized as a purgative. *Yucca*'s significance is growing as a result of the isolation of several significant steroidal saponins from its leaves and rhizomes (Baghuguna & Sati, 1990; Kishor *et al.,* 1992). It is almost free of pests and diseases, but is vulnerable to aphids and possible leaf spots (Smith *et al.,* 2012). This economically significant plant is thought to be stressed by a variety of fungal pathogens. In this study, the presence of mycelia with conidiophores and conidia on the surface of the *Y. aloifolia* give it white, powdery mildew appearance observed during field sampling in Canal view society of Gujranwala, Punjab, Pakistan.

Previously reported fungal species on this host plant include, *Sphaerodothis pringlei* (Peck) Theiss. & Syd. (*Apodothina pringlei* (Peck) Petr.) from California (French, 1989); *Coniothyrium concentricum* (Desm.) Sacc. *(Microsphaeropsis concentrica* (Desm.) Morgan-Jones) from China (Tai, 1979), Puerto Rico (Stevenson, 1975) and Virgin Islands (Stevenson, 1975); *Anthostomella nigroannulata* Sacc.*, Colletotrichum yuccae* Pollacci and *Coniothyrium concentricum* from Cuba (Arnold, 1986; Yip, 1989); *Leptodothiorella notabilis* Petr. & Cif. (*Phyllosticta yuccae* Bissett) and *Pleospora thuemeniana* Sacc. (*Montagnula thuemeniana* (Sacc.) Crivelli) from Dominican Republic (Ciferri, 1961); *Alternaria* sp., *Botryodiplodia circinans* (Berk. & Broome) Petr., *Botryosphaeria* sp., *Cercospora* sp., *Coniothyrium concentricum, Cytosporina* sp*., Diplodia circinans* Berk. & Broome, *Fusarium lateritium* Nees, *Hendersonia* sp., *Kellermania anomala* (Cooke) Höhn., *Leptosphaeria obtusispora* Speg. (*Phaeosphaeriopsis obtusispora* (Speg.) M.P.S. Câmara, M.E. Palm & A.W. Ramaley), *Macrophoma* sp., *Phyllosticta yuccaegena* Ellis & Everh*., Pythium splendens* Hans Braun (*Globisporangium splendens* (Hans Braun) Uzuhashi, Tojo & Kakish.), *Physalospora* sp. and *Sphaerodothis pringlei* (*Apodothina pringlei*) from Florida (Petrak, 1953; Anonymous, 1960; Sobers, 1967; Morgan-Jones *et al.,* 1972; Alfieri *et al.,* 1984); *Coniothyrium concentricum* and *Microsphaeropsis concentrica* from Georgia (Anonymous, 1960; Hanlin, 1963; Dzhalagonia, 1965); *Cytospora yuccae* Politis and *Glomerella cingulata* (G.F. Atk.) Spauld. & H. Schrenk (*Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.) from Greece (Pantidou, 1973); *Alternaria alternata* (Fr.) Keissl., *Diplodia agaves* Niessl (*Striodiplodia agaves* (Niessl) Zambett.), *Microdiplodia agaves* (Niessl) Tassi and *Phomopsis yuccae* V.P. Sahni from India (Sahni, 1966; Mathur, 1979); *Microsphaeropsis concentrica* (Desm.) Morgan-Jones from Japan (Kobayashi, 2007) and Mississippi (Anonymous, 1960); *Torula herbarum* (Pers.) Link from Louisiana (Anonymous, 1960); *Alternaria* sp., *Asteromella* sp., *Colletotrichum* sp., *Capnodium* sp., *Dialonectria depauperata* (Cooke) Cooke (*Clonostachys rosea* f. rosea (Link) Schroers, Samuels, Seifert & W. Gams), *Gloeosporium* sp., *Rhizoctonia* sp., *Rosellinia* sp. and *Phytophthora* sp. from Mexico (Alvarez, 1976); *Cylindrosporium* sp., *Coniothyrium concentricum (Microsphaeropsis concentrica*) and *Leptosphaeria filamentosa* Ellis & Everh. (*Neophaeosphaeria filamentosa* (Ellis & Everh.) M.P.S. Câmara, M.E. Palm & A.W. Ramaley) from North Carolina (Grand, 1985); *Diplodia circinans* Berk. & Broome (*Botryodiplodia circinans*) from Philippines (Teodoro, 1937); *Anthostomella nigroannulata* Sacc. from Portugal (Unamuno, 1941); *Anthostomella nigroannulata* Sacc., *Cladosporium atriellum* Cooke*, Pleospora thuemeniana* Sacc. (*Montagnula thuemeniana* (Sacc.) Crivelli) from South Carolina (Cooke, 1878; Anonymous, 1960; Dugan *et al.*, 2004); *Capnodium* sp. and *Microsphaeropsis concentrica* from Venezuela (Urtiaga, 2004); and *Colletotrichum yuccae* from West Indies (Minter *et al.,* 2001).

From Pakistan, previously no work has been done on this ornamental plant. It is first time to report any fungal pathogen on this economically important plant species from Pakistan.

Since ornamental plants are cultivated primarily for their aesthetic value, any pathogenic attack can cause serious problems for the cultivars. To accomplish which, infected plants are never tolerated in flower gardens and commercial cultivation (Teodoro, 1937). In order to manage plant diseases and inhibit pathogenic growth and progression, identification of the leaf spot agents that affect ornamental plants can be proved as a useful tool. Therefore, the aim of this study was to identify and characterize the fungal pathogen isolated from *Yucca* plant in Gujranwala using morpho-anatomical characters.

1.1. Objectives:

- To identify and characterize pathogenic species like *T. roseum,* on an economically important plant of Pakistan.
- To provide a basic guideline for fungal species identification, as a preliminary step in plant disease management.

2. Materials and Methods:

2.1. Field Sampling and Infected Sample Collection:

A study survey was conducted in district Gujranwala, Punjab, Pakistan from April 2021 to June

2021 to document the fungal infection on plants. Diseased *Yucca aloifolia* leaves were collected from Canal view society, Gujranwala, Punjab. The field notes were prepared that comprised of plant name, location, date, pathogenic symptoms. The plant specimens were photographed from different angles. The presence of mycelia on the surface of the *Yucca aloifolia* gives it a white to rosy pink and powdery mildew appearance. Symptoms of disease were noted i.e., brown to black, oval to irregular circular necrotic spots of 4-6 mm size with an average of 2-4 spots per leaf. Spots were primarily observed only on leaves. Approximately 30% leaves (both young and old) were infected with this disease. For the isolation of causal organism, one spot per leaf and a total of ten symptomatic leaves were selected randomly.

The samples were pressed through plant presser then dried and preserved in brown bags and taken to the laboratory for further studies. The study area, Gujranwala, lies between 32° 09′ 42′′ north latitudes and 74° 11′ 17′′ east latitudes. The region typically undergoes hot, dry weather. The area experiences 872 mm of rain on average per year (Iqbal *et al.,* 2020) (Gujranwala Development Authority).

2.2. Microscopic Analysis:

For the morphological characterization of fungal isolate, the infected plant sample was first observed under stereomicroscope (LABOMED America, 7GA9, USA). To study under compound microscope, a slight amount of the powdered material from the infected leaf surface was scratched with the needle and mounted carefully in one drop of 5% aqueous KOH, over a glass slide. Details of microscopic examination were recorded at different magnifications of 4×, 10× and 40×. Different optical characters of mycelium, conidiophore and conidia and other hyphae were observed. Measurements were made with an ocular micrometer at 40×. Twenty readings of conidia and five for other characters were noted and illustrations were made. Light micrographs of microscopic characters of fungal pathogen were also captured.

3. Results:

3.1. Taxonomy:

Trichothecium roseum (Pers.) Link, Mag. Gesell. Naturf. Ferunde, Berlin 3(1-2): 18 (1809) (Figs. 1-3)

The fungus produced abundant septate mycelia, hyaline at first, then pale pink as the conidia developed. Conidiophores 130-254 × 2-4.5 µm, hyaline, septate, branched, long, slender, produces meristem arthrospore conidia apically and singly at young, then successively in a zigzag manner, sometimes slightly swollen at their tips (Fig. 2; A-C & M-O). Conidia 12-22 x 7-11 µm, hyaline or brightly colored, thick-walled, single septate, bicelled, each with a flattened protuberance at the base, ovoid, ellipsoidal to pyriform, characteristically held together in zigzag chains, produced in clusters, aleurioconidia, appearing in basipetal succession from the tip of the conidiophore (Fig. 2; A-L). Hyphae present as basal support, hyaline, non-septate, narrow, smooth-surfaced, thick-walled, 4-5.5 µm in diam.

These characters are consistant with the characteristics of *Trichothecium roseum* as reported by Dal Bello, 2008; Shamsi & Sultana, 2008; Kwon *et al.,* 2010; Inácio *et al.,* 2011; Hamid *et al.,* 2014; Oh *et al.,* 2014; Kidd *et al.,* 2016; Firouzianbandpey *et al.,* 2021).

3.2. Material Examined:

(Infected plant: *Y. aloifolia*): Pakistan: Province Punjab, district Gujrawala, Canal View Society, Gujranwala, from a local garden, along the road side, 21st April 2021 (Collector: Maryam Nawaz).

Figure 1: A-E. Different views of fungal infection on host plant, *Yucca aloifolia*. A. Infected host plant, *Y. aloifolia*. B-D. Leaves of *Y. aloifolia* showing fungal infection. E. Stereomicrograph of infected leaf. Scale bars: A= 5 cm, B-D-E= 0.8 cm, C= 1.7 cm

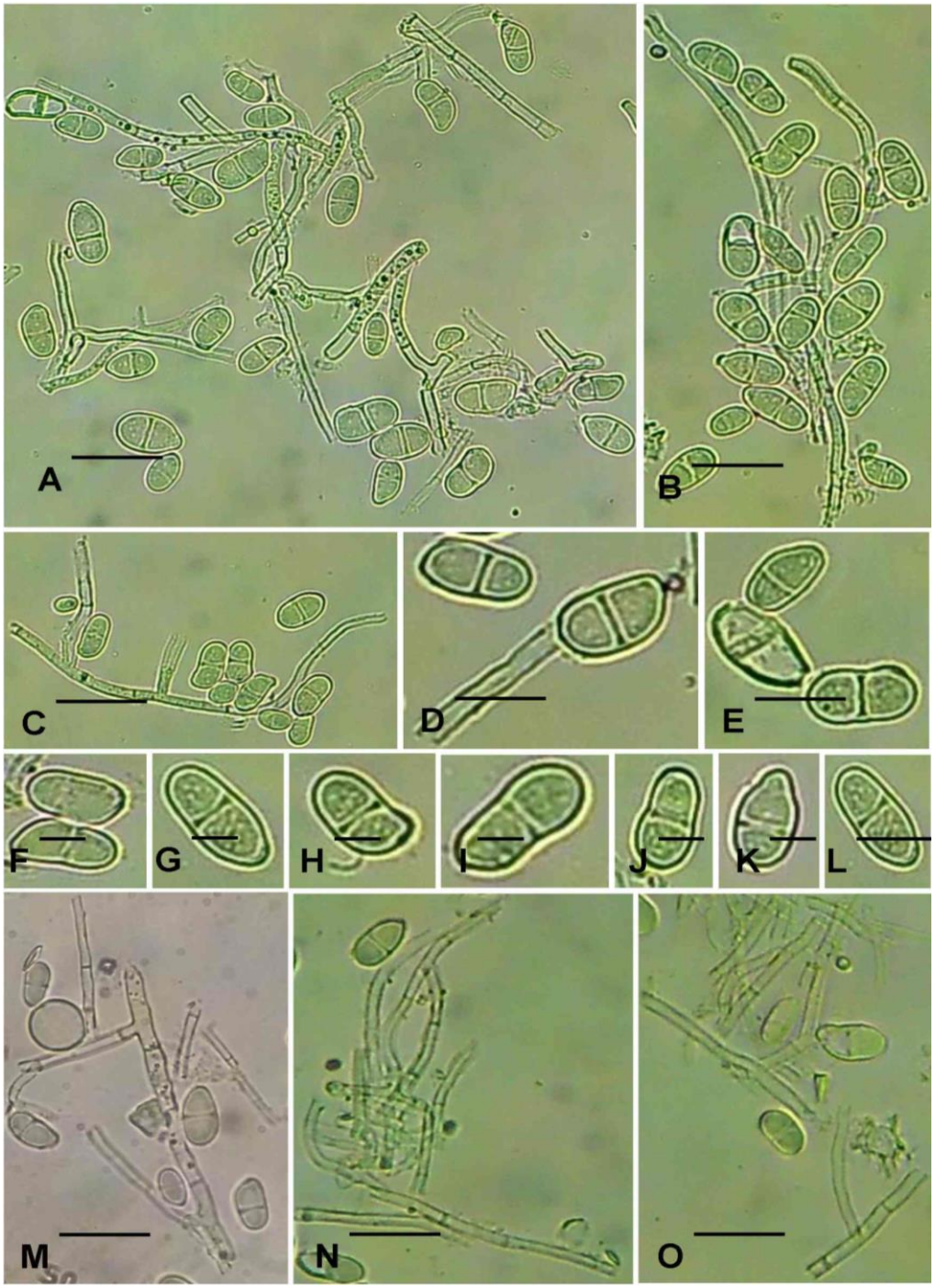

Figure 2: A-O. Light micrographs of microscopic features of *Trichothecium roseum*. A-D. Light micrographs of *T. roseum* mycelia. E-L. Light micrographs of conidia. M-O. Light micrographs of conidiophores and hyphae. Scale bars: A= 2 µm, B-C= 1.5 µm, D-E= 1 µm, F-L= 0.5 µm, M-O= 1.8 µm.

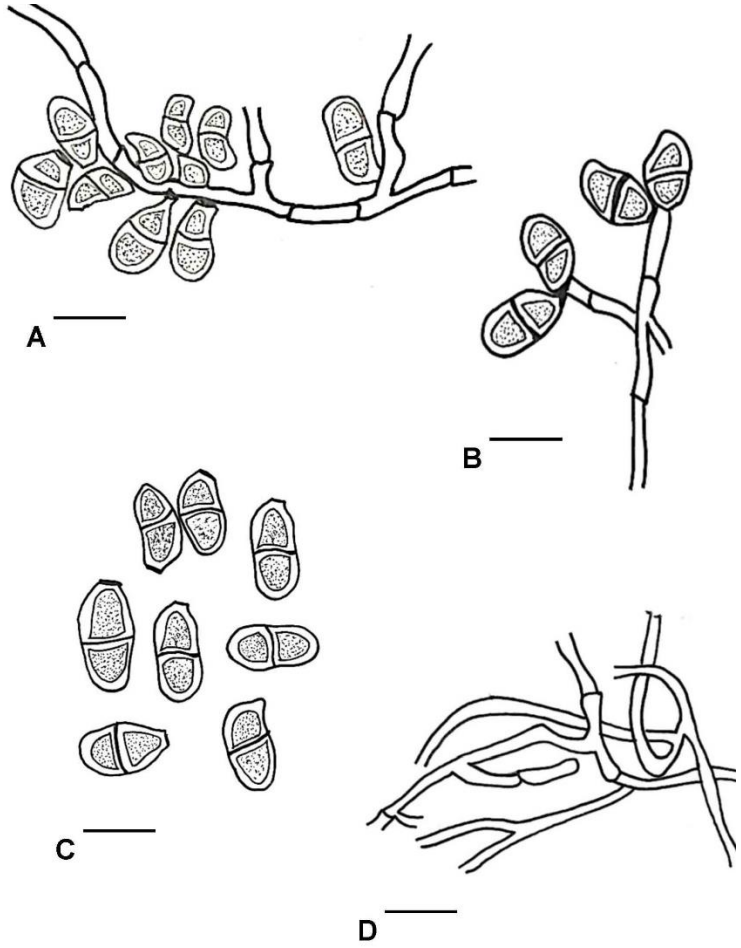

Figure 3: A-D. Illustrations of microscopic features of *Trichothecium roseum*. A&B. Conidiophores and conidia. C. Conidia. D. Hyphae. Scale bar: A= 2.5 µm, B= 4.6 µm, C=4.4 µm, D= 1.2 µm.

3.3. Key to Pakistani Species of Genus Trichothecium:

- Conidial septum constricted, upper cell larger than the lower; Conidia acute or minutely pointed at base, 24-30 µm × 15-20 µm; Conidiophores 150 µm × 4-5 µm.

  T. inaequale
- Conidial septum not constricted, both cells are equally divided; Conidia protrudely flattened at base, 12-22µm long, 7-11µm wide; Conidiophores 130-254 µm × 2-4.5µm.

  T. roseum

4. Discussion:

As member of the phylum Ascomycota, class Sordariomycetes, *Trichothecium* fungi are closely linked to *Acremonium* Link species (Summerbell *et al.,* 2011). The *Trichothecium* genus consists mostly of plant pathogenic species (Jones *et al.,* 1987) and is a small and heterogeneous genus of fungi having worldwide distribution. *Trichothecium* is often isolated from plant substrates, foodstuffs, soil and seeds of corn (Sharma, 1999; Sharma & Mishra, 2014). In Mycobank, 73 different species are listed under this genus (Sharma *et al.,* 2014). From Pakistan, reported species of *Trichothecium* are two i.e., *T. inaequale* Massee & E.S. Salmon and *T. roseum* from different hosts as described in Table 1 (Ahmad *et al.,* 1997; Hamid *et al.,* 2014). A key to identification of Pakistani species of *Trichothecium* is also presented. This time isolated species is *T.* roseum reported from a new host, *Y. aloifolia* from Pakistan.

Trichothecium species produce clusters of two-celled or single-septate conidia that are elliptical to pear-shaped (Sharma *et al.,* 2014). *T. roseum*, isolated from the leaf of *Y. aloifolia*, possess thick-walled, bicelled conidia that are hyaline and ovoid to pyriform in shape along with slanting and truncate

basal mark along with hyaline, septat, branched, progressively shortened conidiophore (Summerbell *et al.,* 2011).

In USDA ARS database, *T. roseum* showed about 222 different plant hosts found in different parts of the world (http://nt.ars-grin.gov/fungaldatabases/). Worldwide reported hosts of *T. roseum* includes *Abies alba* Mill., *Cucumis melo* L., *Citrullus lanatus* L., *Malus domestica* Borkh., *Polygonatum cyrtonema* Mill., *Prunus persica* L. Batsch, *P. persica* L. Batsch var. *nectarine*, *Solanum melongena* L., *Prunus salicina* Lindl., *Phaseolus vulgaris* L., *Musa acuminata* Colla from China, India, Japan, South America, United Kingdom and United States of America. *T. roseum* has also been reported to cause pink rot in vegetables, such as cucurbits and tomato, also infects many forest trees and tea leaves (Sharma *et al.,* 2014; Liu *et al.,* 2016)(USDA fungal host database).

From Pakistan, *T. roseum* was first reported on *Pyrus malus* L. from Quetta, from the soil of Faisalabad, on the bark of *Populus euramericana* (Dode) Guinier in Hyderabad, from the soil of *Pinus* L. Forest, in Azad Jammu and Kashmir (Chaudhari & Sacchar, 1934; Kheswala, 1936; Ahmad, 1956; Malik & Virk, 1968; Qureshi & Jamal, 1971; Matsushima, 1993; Ahmad *et al.,* 1997); from Almond seeds (Bilgrami & Choudhary, 1998); from wheat, sorghum and barley seeds (Fakhrunnisa & Ghaffar, 2006); from tomato, orange and apple in the vicinity of Sargodha (Hamid *et al.,* 2014). During current study, it is reported from an ornamental host plant for the first time i.e., from the leaf of *Yucca aloifolia* collected from Gujranwala, Pakistan and recorded as a new host report worldwide.

The findings of this study, in conjunction with the cases of fungal opportunistic pathogens that cause serious threats to ornamental plants, suggested that this type of plantation without proper antifungal treatment may pose serious economic threats to these plants.

Table1. Comparison of Pakistani species of genus *Trichothecium*

Sr. No.	*Trichothecium* species	Conidial Morphology				Conidiophores morphology		Reported sites	References
		septal position	septal constrictions	shape	size	shape	Size		
	T. inaequale	eccentric; upper cell 4-5 fold longer than the lower one	septum constricted slighltly inwards	ovate or subpyriform, at apex rounded, at base acute or more or less minutely pointed	24-30 × 15-20 µm	apex often nodular-denticulate	150µm × 4-5µm	On dead branches of *Gossypium* sp. on dung from Ladhar, Punjab	(Masse & Salmon, 1902; Ahmad, 1956)
	T. roseum	centric; both the cells are equally spaced	septum not constricted	ovoid, ellipsoidal to pyriform, each with a flattened protuberance at the bas, characteristically held together in zigzag chains	12-22 x 7-11 µm	sometimes slightly swollen at their tips	130–254 × 2– 4.5 µm	from apple, almond seeds, barley seeds, orange, sorghum seeds, tomato and wheat seeds, on *Pyrus malus,* bark of *Populus euramericana,* soil, from *Pinus* forest soil from Faisalabad, Hyderabad, Kotli (AJ&K), Punjab, Quetta, Sargodha	(Ahmad *et al.,* 1997; Bilgrami & Choudhary, 1998; Fakhrunnisa & Ghaffar, 2006; Shamsi & Sultana, 2008; Hamid *et al.,* 2014)

5. Conclusion:

Due to rich population of Gujranwala district, people are removing vegetation to cope up their needs and eradicating the natural flora. At the same time, these plants are also stressed by such type of fungal pathogens and getting destroyed. To rescue such economic plants, in this industrial and populous city of Gujranwala, it is the need of hour to accurately identify and timely report new fungal-host associations for the introduction of suitable fungicides and finding their efficient concentration for the control of pathogenic attacks on plants.

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