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Published in:

Physiological and Molecular Plant Pathology

DOI:

[10.1016/j.pmpp.2018.02.001](https://doi.org/10.1016/j.pmpp.2018.02.001)

Publication date:

2018

Citation for published version (APA):

Nayyar, B. G., Woodward, S., Mur, L., Akram, A., Arshad, M., Saqlan Naqvi, S. M., & Akhund, S. (2018). Identification and pathogenicity of *Fusarium* species associated with sesame (*Sesamum indicum* L.) seeds from the Punjab, Pakistan. *Physiological and Molecular Plant Pathology*, 102, 128-135.
<https://doi.org/10.1016/j.pmpp.2018.02.001>

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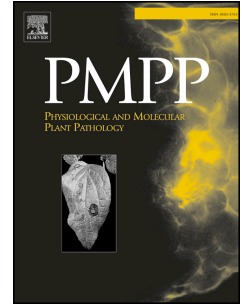
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Accepted Manuscript

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PII: S0885-5765(17)30103-0

DOI: [10.1016/j.pmpp.2018.02.001](https://doi.org/10.1016/j.pmpp.2018.02.001)

Reference: YPMPP 1315

To appear in: *Physiological and Molecular Plant Pathology*

Received Date: 1 April 2017

Revised Date: 29 January 2018

Accepted Date: 2 February 2018

Please cite this article as: Nayyar BG, Woodward S, Mur LAJ, Akram A, Arshad M, Naqvi SMS, Akhund S, Identification and pathogenicity of *Fusarium* species associated with sesame (*Sesamum indicum* L.) seeds from the Punjab, Pakistan, *Physiological and Molecular Plant Pathology* (2018), doi: 10.1016/j.pmpp.2018.02.001.

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1 **Identification and Pathogenicity of *Fusarium* species associated with**
2 **Sesame (*Sesamum indicum* L.) seeds from the Punjab, Pakistan**

3

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16

1 ABSTRACT

2 Sesame (*Sesamum indicum*) is an oil-crop in Asia and Africa and is widely grown in the
3 Punjab region of Pakistan. A total of 105 sesame seed samples were collected from different
4 locations in the Punjab from which 520 isolates of *Fusarium* spp. were recovered. These
5 isolates were initially grouped and identified based on morphological characteristics. The
6 identities of representatives of the three most frequently isolated groups (strains designated
7 F01, F98, F153) were identified as *Fusarium proliferatum*, on the basis of the sequencing of
8 ITS of rDNA and translation elongation factor (TEF-1 α) gene regions. Phylogenetic trees
9 generated using the maximum likelihood method showed that these three isolates and a *F.*
10 *proliferatum* reference sequence grouped in the same clade with *F. phyllophilum*, the most
11 closely related species. Pathogenicity tests demonstrated that these three isolates caused
12 disease on sesame plants. Disease Incidence (DI) and Disease Severity Index (DSI) data
13 indicated that F01 was the most virulent isolate, with DI and DSI of approximately 70%.
14 Culture filtrates of F01 reduced sesame seed germination (to 40%) and vigor (to 16.5%) of
15 sesame seedlings. This baseline study suggests that *F. proliferatum* infection of sesame seeds
16 could be a major source of yield loss in the Punjab, Pakistan which requires further attention.

17
18 **Keywords:** Sesame, *Fusarium*, Blotter Paper Method, ITS, TEF-1 α

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1 INTRODUCTION

2 Sesame (*Sesamum indicum* L.; Pedaliaceae) is one of the most important and oldest oil crops
3 used by humans (Noorka et al. 2011). Sesame seed contains 50-60 percent oil and 22 percent
4 protein. The oil is a semi-drying type and is mostly used in confectionary items; good quality
5 oil is also used for medicinal purposes (Smith and Salerno 1992). A particularly useful
6 characteristic of sesame oil is the long shelf life due to the presence of the antioxidant,
7 sesamol (Mohamed and Awatif 1998). This quality makes it applicable for use in the
8 manufacturing of margarine in various parts of the world where there is inadequate
9 refrigeration. Sesame oil is also used in paints, soaps, cosmetics, perfumes, bath oils,
10 insecticides, and pharmaceuticals (as a vehicle for drug delivery). Poppy seed, cottonseed,
11 and rape oils are frequently added to sesame oil. Sesame seeds and young leaves are eaten as
12 stews and used in soaps in Asia (Grubben and Denton 2004).

13 Sesame probably evolved in Africa and was spread early in human migrations through West
14 Asia, China, and Japan, regions which then became secondary centers of diversity. Sesame
15 was introduced into India by the earliest human migrants from Africa. Charred remains of
16 sesame at Harappa excavations (3600-1750 B.C) indicate that sesame was cultivated by the
17 Indus Valley civilization (Ashri 2007). Today, sesame (locally called 'til') is grown in 70
18 countries of the world, including 26 and 24 countries in Africa and Asia, respectively, with
19 average global production close to 3 million tons per annum. The top five producers account
20 for approximately 70% of global production. Sesame is typically grown in dry tropical and
21 subtropical regions. In Pakistan, sesame is grown in 65 districts as an irrigated or rain-fed
22 crop, particularly in the Punjab (Amjad 2014). Crucially, Pakistan represents a typical
23 climatic growing area for sesame and, therefore, yield constraints in that country are relevant
24 to other sesame producing regions (Fitzpatrick and Rene De Baaij 2013).

1 Diseases, particularly those caused by fungi, are major yield constraints in both sesame seed
2 production and in seed storage (Mbah and Akueshi 2000, 2001). Sesame production faces
3 numerous problems such as wilt, root rot and damping off, all due to attack by soil-borne
4 fungi. In Pakistan, charcoal rot, *Alternaria* leaf blight, bacterial leaf spot, bacterial blight,
5 phyllody, and wilting are major diseases affecting this crop. The most common and
6 destructive diseases are wilts caused by several species of *Fusarium* and *Verticillium*
7 (Thomson and Ockey, 1993). Infection of *Fusarium* species may also result in the
8 accumulation of toxic secondary metabolites in the seed, damping-off of seedlings and even
9 the death of the whole plant at the time of flowering, and thereby reduce yields (Farhan et al.
10 2010; Salleh and Mushitah 1991).

11 Accurate detection and diagnosis in the genus *Fusarium*, however, is complicated due to lack
12 of an accurate taxonomy. A number of factors, particularly a lack of clear morphological
13 characters separating species, had led to the broad species definitions, which, together with
14 observed variations and mutations in culture, have led to taxonomic systems that poorly
15 reflect species diversity. A result of this confusion, there is an inconsistent application of
16 species names to toxigenic and pathogenic isolates (Taylor et al. 2000). Accurate methods for
17 detecting infections of sesame seeds are required to develop robust pathogen and disease
18 management strategies.

19 Monitoring of plant pathogenic microorganisms can be carried out qualitatively by following
20 disease symptoms appearing on infected plants, or more quantitatively using molecular
21 methods, for example, PCR amplification and sequencing of loci that are indicative of the
22 fungal species; often referred to as the “barcode”. In this current research, we sought to apply
23 molecular barcoding approaches to identify *Fusarium* spp. isolated from infected sesame
24 seeds. The aim of the work was to determine the prevalence of *Fusarium* species associated

1 with sesame seeds in the Punjab province. Accurate identification of *Fusarium* spp. using
2 molecular techniques and pathogenicity tests will aid in the development of improved disease
3 management methods.

4

5 **MATERIALS AND METHODS**

6 **Pathogen Isolation from Seeds and Identification**

7 A total of 105 samples of sesame seeds were collected from the major sesame producing
8 areas of the Punjab, Pakistan, as indicated in Figure 1. Seeds were brought to the laboratory
9 and surface sterilized with NaOCl for 2 min. Surface sterilized and unsterilized seeds from
10 each sample were placed on three layers of moistened filter paper (Whatman™ 1001-090
11 Grade 1) discs, with 25 seeds per Petri dish. The Petri dishes were incubated at 22 ± 2 °C for
12 seven days in an alternating cycle of light and darkness (12 hours each) in a Versatile
13 Environmental Test Chamber (Sanyo, Japan) with illumination provided by 55 W fluorescent
14 tubes, giving a light intensity of 125-130 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The experiment was performed in
15 triplicate. After incubation, fungal colonies emerging from the seeds were counted and
16 isolated on potato dextrose agar (PDA; Oxoid, UK). The morphological characters were
17 noted and isolation frequency (Fr) and relative density (RD) of fungi were recorded as
18 follows:

$$19 \quad \text{Fr (\%)} = \frac{(\text{ns})}{\text{N}} \times 100$$

$$20 \quad \text{RD (\%)} = \frac{(\text{ni})}{\text{Ni}} \times 100$$

21 Where, ns is the number of samples on which a fungus occurred; N is the total number of
22 samples; ni is the number of isolates of a given fungal genus/species; and Ni is the total
23 number of fungal isolates obtained.

24 Isolates were identified on the basis of morphological characteristics with reference to
25 published keys (Booth 1977; Domsch et al. 1980; Leslie and Summerell 2006).

1 DNA extraction, PCR, and DNA Sequencing

2 Genomic DNA was extracted using the phenol-chloroform extraction method (González-
3 Mendoza et al. 2010). For DNA extraction, fresh cultures of *Fusarium* species were prepared
4 by using dilution method (Cha et al. 2007). After 7 days of incubation, 50 mg mycelium of
5 each isolate was harvested using a sterile surgical blade, and ground in liquid nitrogen using a
6 mortar and pestle, and transferred to 1.5 ml microcentrifuge tubes. The resulting powder was
7 suspended in 500 µl phenol and 1000 µl extraction buffer (1% sodium dodecyl sulphate, 1 M
8 Trizma base, 100 mM NaCl, 10 mM Na₂EDTA, pH 8.0), vortexed and centrifuged at 10,000
9 rpm for 10 min. The supernatant was transferred to a new microcentrifuge tube and 500 µl
10 chilled isopropanol and 50 µl 3 M, chilled sodium acetate (pH 4.8) added. Following gentle
11 mixing, the preparation was centrifuged at 10,000 rpm for 10 min. The supernatant was
12 discarded and the pellet was washed twice with 500 µl 70% ethanol with centrifuging, before
13 final re-suspension in 200 µl of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0).

14 For molecular identification, the internal transcribed spacer (ITS) and translation elongation
15 factor (TEF-1 α) regions were amplified, based on ITS1 / ITS4 primers and EF1-983F / EF1-
16 2212R primers, respectively (Table 1). PCR was conducted in a 25 µl reaction mixture
17 containing 2.5 µl PCR buffer (200 mM Tris HCl (pH 8.4), 500 mM KCl), 0.5 µl dNTPs, 1.5
18 µl MgCl₂, 0.5 µl DNA polymerase, 2.5 µl each primer, 14 µl DEPC H₂O and 1 µl template
19 DNA. The PCR reaction was performed in a MyCycler™ Thermal cycler (Bio-Rad, USA)
20 with initial denaturation at 95 °C for 5 min followed by 35 cycles of 95 °C for 30s, annealing
21 at 64 °C for 1 min (for ITS primers) and 72 °C for 1 min, and a final elongation step at 72 °C
22 for 1 min. For the TEF-1 α primers, a touchdown PCR was run with an annealing temperature
23 of 66 °C in the first cycle, successively reducing the temperature by 1 °C per cycle over the
24 next 9 cycles to reach a final temperature of 56 °C, which was used in the remaining 30-36

1 cycles. An extension time of 1 min 30 sec per cycle at 72 °C was applied. Amplified
2 fragments were analyzed on 1% agarose (Melford, UK) gel, purified and sequenced in both
3 directions by Macrogen, Korea. Sequences were analyzed using Mega 7 software and blasted
4 against the NCBI database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

5

6 **Pathogenicity Test**

7 Three most frequent *Fusarium* isolates were grown in PDA and conidia were harvested by
8 flooding cultures with 2% Tween 20. The suspension was collected and filtered through two
9 layers of sterilized cheesecloth. The spores were then adjusted to 1×10^6 conidia mL⁻¹ by
10 repeated hemocytometer counts (Garibaldi et al. 2004).

11 Sesame plants were grown in 13 × 11 cm plastic pots, with 3 plants per pot, containing
12 sterilized potting medium (soil, sand, farmyard manure; 1:1:1 w/w/w). For inoculation, a
13 channel, about 1 cm long and 3 cm deep, was created in the soil near each seedling and filled
14 with 10 mL spore suspension. Controls were treated with sterile distilled water. Five replicate
15 pots per treatment were prepared. Inoculated plants were covered with clear plastic bags for
16 24 h to maintain high humidity and placed in the glasshouse at 25/20 °C day/night
17 temperature and irrigated with distilled water on alternate days.

18 Symptoms were recorded after 30 days of treatment. Disease symptoms on roots and stems
19 were scored using a four-point categorical disease severity scale: 0 = no wilting symptoms, 1
20 = 25% wilting, 2 = 50% wilting and 3 = 100% wilting and plant death. The disease severity
21 index (DSI) was calculated as \sum (disease severity scale points × number of plants at each
22 scale point)/ (total number of seedlings assessed × disease severity scale of the highest scale
23 point observed) × 100 (Zhao et al. 2014).

1 To verify that the inoculated *Fusarium* isolates caused the symptoms on the sesame
2 seedlings, re-isolations were made from symptomatic parts of inoculated plants. After seven
3 days of inoculation, symptomatic tissues were surface sterilized in 5% NaOCl twice for 2 min
4 followed by rinsing with autoclaved distilled water for 2 min. Three pieces (3-6 mm long) of
5 roots or stems per plant were excised under aseptic conditions and transferred to PDA in Petri
6 dishes. Dishes were incubated at 28°C until the appearance of typical *Fusarium* colonies,
7 approx. 6 days after plating.

8

9 **Effect of Culture Filtrate on Seed Germination and Seedling Growth**

10 Erlenmeyer flasks containing 50 ml of potato dextrose broth were inoculated with the test
11 fungi (1×10^6 conidia mL⁻¹) and incubated for 14 days at 28 °C. Then, the culture broth and
12 fungal mycelia were carefully separated. A 50 ml volume of 3:2:1 (v/v/v) ethyl acetate:
13 chloroform: methanol was added to each flask containing culture broth, followed by shaking
14 overnight on a rotary shaker. Extracts were centrifuged at 5000 rpm for 30 min and the
15 supernatant was incubated in a water bath at 45 °C for 8-10 h to concentrate the extract to a
16 volume of 10 ml (Jaiswal et al. 2012).

17 Sesame seeds were surface sterilized in 5% NaOCl for 2 min followed by 3 rinses in sterile
18 distilled water and then suspended in culture filtrates (10 ml). Following incubation at 28 ±
19 2°C for 24 h, seeds were removed from the culture filtrates and washed in sterile distilled
20 water. Treated seeds were plated on 1.5 % water agar, with 10 seeds per Petri dish. Control
21 seeds were treated with distilled water. After 7 days of incubation, shoot and root lengths
22 were recorded. In addition, a vigor index was calculated (Jalander and Gachande 2012)
23 following the formulae:

24
$$\text{Germination \%} = \frac{\text{Germinated seeds of treatment}}{\text{Germinated seeds of control}} \times 100$$

1 Vigor index = Seed germination (%) × Seedling Length (Shoot + Root Length)

2

3 **Statistical Analysis**

4 Analysis of variance (ANOVA) was performed on the data of DI, DSI, germination percent,
5 and vigor index to determine the effects of representative *Fusarium* isolates on sesame seeds
6 and seedlings. The mean differences were compared by Duncan's multiple range test ($P <$
7 0.05). All calculations were made using SPSS (version 16.0; SPSS Inc, Chicago, IL).

8

9 **RESULTS**

10 **Morphological characterization of *Fusarium* isolates**

11 A total of 520 isolates of *Fusarium* were recovered from sesame seeds, separated into 23
12 morphological groups and identified on the basis of colony characters and conidial structure.
13 Three isolates (F01, F98, F153) as representative of the most frequent morphological groups
14 were identified as *F. sacchari* (39%), *F. phyllophilum* (40%) and *F. culmorum* (30%),
15 respectively (Table 2). Initially, these isolates had hyaline aerial mycelium which turned to
16 white or purple as colonies aged, and various pigmentations from white to light purple,
17 through deep reddish purple to dark reddish purple were observed. The growth rate ranged 4-
18 8 cm in 7 days. Most macroconidia were slender with a curved apical cell and a notched basal
19 cell. They were 3-5 septate with size ranged as 25-55 x 3-5µm. Microconidia from all isolates
20 were 0- septate, 7-12 x 2.2-3.0µm in size, oval with flattened bases and were mostly formed
21 in a false head (small, mucoid, adherent balls of conidia) (Figure 2). However,
22 chlamydospores were not found in any of these cultures. All the identified isolates were
23 submitted in the First Fungal Culture Bank of Pakistan (FCBP), University of the Punjab,

1 Lahore, Pakistan. These three representative isolates were selected for molecular
2 characterization and pathogenicity tests.

3

4 **Molecular characterization of *Fusarium* Isolates**

5 Selected isolates were identified using the ITS region of rDNA and TEF-1 α gene. Separation
6 of the PCR products of ITS amplification on an agarose gel revealed amplicons of ~650 bp in
7 length. All sequences exhibited 100% identity with *Fusarium proliferatum* ITS sequences in
8 GenBank (Table 3) (Figure 3). Analysis of the TEF-1 α sequences showed > 90% sequence
9 identity to *Fusarium proliferatum* although, some sequence variation between isolates was
10 observed (Table 3; Figure 4). Phylogenetic analysis suggested that F98, compared to F01 and
11 F153, was most closely related to the *Fusarium proliferatum* sequences present in GenBank.
12 TEF-1 α sequences from all three isolates, however, fall in the same clade.

13

14 **Pathogenicity Tests**

15 Inoculation of sesame plants with isolates F01, F98 and F153 resulted in the browning of
16 foliage by approximately 14 days after treatment, after which plants continued to decline,
17 becoming dark brown and necrotic. Roots became semi-transparent, shrunken, water-soaked
18 and eventually disintegrated and whole plants wilted after 30 days (Figure 5). The statistical
19 data revealed that there was a significant difference ($P < 0.05$) among the treatments.
20 Although, the mean difference was non-significant ($P > 0.05$) between F01 and F98 for disease
21 incidence but the results of DSI showed a significant difference ($P < 0.05$) among three
22 isolates. Hence F01 (70) was considered more pathogenic than F98 (53.33) and F153 (23.33)
23 based on disease severity assessments (Table 4). In control plants, 100% green leaves were
24 maintained over the assessment period. Re-isolations of the same three isolates were

1 successful: all re-isolated strains were morphologically identical to the fungus used in the
2 inoculations of the sesame plants.

3

4 **Effect of *Fusarium* Culture Filtrates on Germination of Sesame**

5 All three isolates showed the highly significant difference ($P < 0.05$) as compared to control.
6 Seed germination and vigor of sesame plants were adversely affected by culture filtrates of
7 isolate F01, with a 40-66% reduction in seed germination, and a 16.5% reduction in vigor,
8 when compared with control plants (Table 5; Figure 6). Germination rates of 66 and 63%
9 occurred in seeds treated with isolates F153 and F98, respectively. Overall, F01 showed a
10 highly significant reduction in germination percentage.

11

12 **DISCUSSION**

13 *Fusarium* is a major cause of wilt in sesame but the range of species causing the disease in
14 this host remains poorly defined. In this study, we surveyed the major sesame growing areas
15 of the Punjab (Pakistan) for wilt symptoms in order to determine the *Fusarium* species
16 causing the disease. Problems using morphological characteristics alone to identify *Fusarium*
17 species have been reported frequently (O'Donnell et al. 1998, 2000; Marasas et al. 2001;
18 Steenkamp et al. 1999). However, initial identification and characterization using
19 morphological features is important to separate isolates into smaller groups before applying
20 other methods of identification (Leslie and Summerell 2006). Distinguishing species within
21 the genus *Fusarium* using morphological characters is difficult even for specialists
22 (Summerell et al. 2003; Leslie and Summerell 2006) and it is now accepted that DNA
23 sequence-based identifications and PCR assays are needed to accurately identify species
24 within the complex genus *Fusarium*. The three most frequently isolated *Fusarium* species

1 identified initially as *F. sacchari* (39%), *F. phyllophilum* (40%) and *F. culmorum* (30%),
2 therefore, were subjected to “DNA bar-coding” approaches to obtain more stringent
3 identifications.

4 PCR and sequencing of the ITS regions have become a routine method for the detection,
5 identification, classification and phylogenetic analysis of many fungi at the species level
6 (Taylor et al. 2000). Sequences of the ITS regions can distinguish *Fusarium* from other fungi,
7 and taxon-selective ITS amplification has been used to detect *Fusarium* spp. (e.g. Pearson et
8 al. 2016; O’Donnell 1992). In the present work, ITS sequencing confirmed isolates obtained
9 from sesame were *Fusarium* species, but further discrimination required the use of translation
10 elongation factor-based primers. Translational elongation factor 1 α is a highly conserved
11 protein encoding region, which can be used to resolve between closely related species, and
12 has proved useful for phylogenetic and taxonomic analyses of *Fusarium* spp. (Geiser et al.
13 2004). Use of TEF-1 α primers demonstrated that the three *Fusarium* isolates tested here were
14 all *F. proliferatum*, providing a platform on to which to define virulence mechanisms in
15 sesame.

16 Previous reports on the occurrence and pathogenicity of *Fusarium* spp. were solely based on
17 samples isolated from infected, symptomatic plants. For example, various *Fusarium* species
18 including *F. oxysporum*, *F. proliferatum* and *F. redolens* proved highly virulent on onion,
19 causing the death of many inoculated seedlings. Among the isolates tested, *F. proliferatum*
20 proved highly pathogenic to onion in inoculation tests (Haapalainen et al. 2016). It is likely
21 that *F. proliferatum* is a problematic pathogen on a range of host plants, as this fungal species
22 is also reported causing serious problems on corn and wheat as well as onion (Conner et al.
23 1996; Logrieco et al. 2002).

1 There is little information available in the literature on pathogens attacking sesame during
2 germination. Work in Pakistan demonstrated that culture filtrates of *Xanthomonas campestris*
3 pv. *sesami* (Xcs) reduced germination progressively with increasing concentrations of the
4 filtrates (Firdous et al. 2013). According to another study conducted in the Punjab (Pakistan),
5 seeds and seedlings health of sesame were greatly affected by the spore suspension and
6 culture filtrates of *Alternaria alternata* (Nayyar et al. 2017). In the present work, both
7 inoculations with spore suspension and treatment with culture filtrates of *Fusarium* isolate
8 F01 suppressed germination and growth of sesame. It was assumed that toxic metabolites
9 produced into the culture filtrates by *Fusarium* were responsible for these symptoms. Toxins
10 produced by *Fusarium oxysporum* f. sp. *ciceris* (FOC) were shown to affect root growth in
11 chickpea and were thought to impact negatively on seed germination of chickpea (Khan et al.
12 2004). Deoxynivalenol, T-2 toxin, fumonisin B1, and nivalenol produced by *Fusarium* sp. are
13 known to suppress seed germination (Zonno and Vurro 1999). The production of secondary
14 metabolites by fungi is known to reduce seed quality and viability (Gopinath and Shetty
15 1988). Similar results were also observed on seeds of pigeon pea, chickpea and tomato
16 varieties (Raithak and Gachande 2013; Arya and Mathew 1991).

17

18 CONCLUSION

19 The most frequent *Fusarium* isolates obtained from sesame seed collected in the Punjab,
20 Pakistan were identified as *F. proliferatum* on the basis of molecular analyses. One isolate of
21 *F. proliferatum*, F01, was more virulent than the other two isolates tested, causing damping-
22 off, and reductions in growth and vigor of sesame seedlings.

23

24 Acknowledgements

1 This work is the part of a Ph.D. project of the first author and financially supported by the
2 Higher Education Commission (HEC) of Pakistan under the National Research Program for
3 Universities (NRPU) project (No. 20-5166). Moreover, HEC is also acknowledged for
4 providing funds to the first author (IRSIP No. 1-8/HEC/HRD/20143411) to visit the
5 Department of Plant and Soil Science, Institute of Biological and Environmental Sciences,
6 University of Aberdeen, Scotland, UK. The first two authors are thankful to the Govt. of
7 Scotland for providing the import license for three *Fusarium* isolates under Directive
8 2008/61/EC and Scottish License No. PH/9/2015.

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Table 1. Primers used for molecular identification of selected *Fusarium* isolates

Code	Primer Sequence (5'-3')	Target	Reference
ITS 1 (F)	TCCGTAGGTGAACCTGCGG	18S rDNA	White et al. (1990)
ITS 4 (R)	TCCTCCGCTTATTGATATGC	28S rDNA	White et al. (1990)
EF1-983 (F)	GCYCCYGGHCAYCGTGAYTTYAT	TEF-1 α	Rehner and Buckley (2005)
EF1-2212 (R)	CCRACRGCACRGTYYGTCTCAT	TEF-1 α	Rehner and Buckley (2005)

Table 2. Morphological characterization, isolation frequency and relative density of *Fusarium* isolates in sesame seeds

Isolate Code	Name of Fungi	Origin (city)	Surface Non Sterilized Seeds			Surface Sterilized Seeds			FCBP accession no.
			No. of isolates	Fr	RD	No. of isolates	Fr	RD	
F01	<i>Fusarium sacchari</i>	Sialkot	63	39	16.45	18	12	13.14	1416
F04	<i>Fusarium oxysporum</i>	Sialkot	1	2	0.26	0	0	0.00	1432
F12	<i>Fusarium torulosum</i>	Sialkot	9	12	2.35	0	0	0.00	1424
F14	<i>Fusarium phyllophilum</i>	Sialkot	11	22	2.87	8	12	5.84	1423
F15	<i>Fusarium oxysporum</i>	Sialkot	13	16	3.39	5	12	3.65	1442
F22	<i>Fusarium</i> sp.	Gujranwala	3	2	0.78	0	0	0.00	1428
F27	<i>Fusarium subglutinans</i>	Gujranwala	4	14	1.04	4	12	2.92	1444
F30	<i>Fusarium polyphialidicum</i>	Gujranwala	6	14	1.57	4	12	2.92	1445
F31	<i>Fusarium subglutinans</i>	Gujranwala	5	0	1.31	9	8	6.57	1446
F40	<i>Fusarium sacchari</i>	Gujranwala	1	2	0.26	1	2	0.73	1462
F52	<i>Fusarium oxysporum</i>	Gujranwala	12	0	3.13	10	2	7.30	1458
F74	<i>Fusarium solani</i>	Gujranwala	3	6	0.78	0	0	0.00	1460
F80	<i>Fusarium culmorum</i>	Gujranwala	12	4	3.13	2	0	1.46	1459
F84	<i>Fusarium lateritium</i>	Gujranwala	26	2	6.79	0	0	0.00	1455
F98	<i>Fusarium phyllophilum</i>	Gujranwala	55	40	14.36	12	26	8.76	1441
F153	<i>Fusarium culmorum</i>	Gujranwala	66	30	17.23	43	12	31.39	1418
F174	<i>Fusarium venenatum</i>	Gujranwala	26	26	6.79	10	4	7.30	1471
F190	<i>Fusarium redolens</i>	Gujranwala	37	4	9.66	0	2	0.00	1469
F269	<i>Fusarium semitectum</i>	Mandi Bahuddin	11	2	2.87	2	0	1.46	1464
F286	<i>Fusarium semitectum</i>	Mandi Bahuddin	14	2	3.66	2	2	1.46	1464
F311	<i>Fusarium scirpi</i>	Chakwal	4	2	1.04	5	2	3.65	1472
F346	<i>Fusarium equiseti</i>	Bahawalnagar	0	2	0.00	1	2	0.73	1468
F349	<i>Fusarium</i> sp.	Bahawalnagar	1	2	0.26	1	2	0.73	1473

Fr= Isolation frequency; RD= Relative Density; FCBP= Fungal Culture Bank of Pakistan

Table 3. Molecular Identification of three most frequent *Fusarium* isolates from sesame seeds.

Isolate Code	Origin (city)	Morphological Identification	Molecular Identification	NCBI accession no.	
				ITS	TEF-1 α
F01	Sialkot	<i>Fusarium sacchari</i>	<i>Fusarium proliferatum</i>	KX901460	KY247083
F98	Gujranwala	<i>Fusarium phyllophilum</i>	<i>Fusarium proliferatum</i>	KX901461	KY247084
F153	Gujranwala	<i>Fusarium culmorum</i>	<i>Fusarium proliferatum</i>	KX901462	KY247085

Table 4. Effects of spore suspension of three *Fusarium* isolates on disease incidence and disease severity in sesame seedlings.

Treatment	Total Seedlings	Wilted seedlings	Disease Incidence (%)	Disease Severity (DSI)	Rating Scale
F01	10	7	70b	70.00c	3
F98	10	8	80b	53.33b	2
F153	10	3	30a	23.33a	1

Values with same letter are not significantly different based on Duncan's multiple range test ($P < 0.05$).

Table 5. Effects of culture filtrates of *Fusarium* isolates on seed germination and seedling growth of sesame.

Treatment	Germination %	Root length (cm)	Shoot Length (cm)	Vigour Index
F01	40.00a	0.40a	0.50a	16.50a
F98	63.33b	0.40a	0.80a	26.13a
F153	66.67b	0.40a	1.00ab	27.67a
Control	86.67c	0.70b	1.50b	62.17b

Values with same letter are not significantly different based on Duncan's multiple range test ($P < 0.05$)

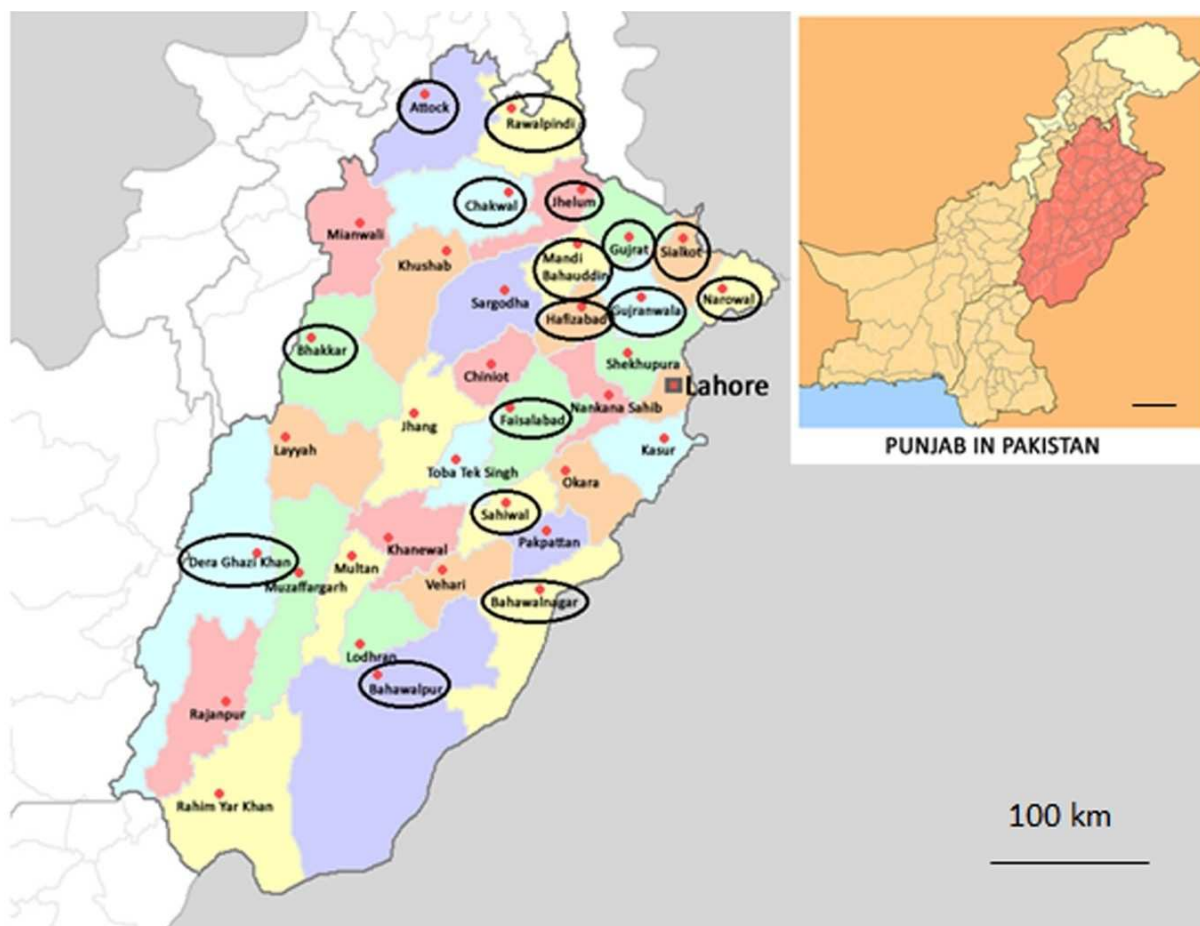


Figure 1. Map of the Punjab (Pakistan) indicating the major sesame growing districts where the sesame seeds were collected.

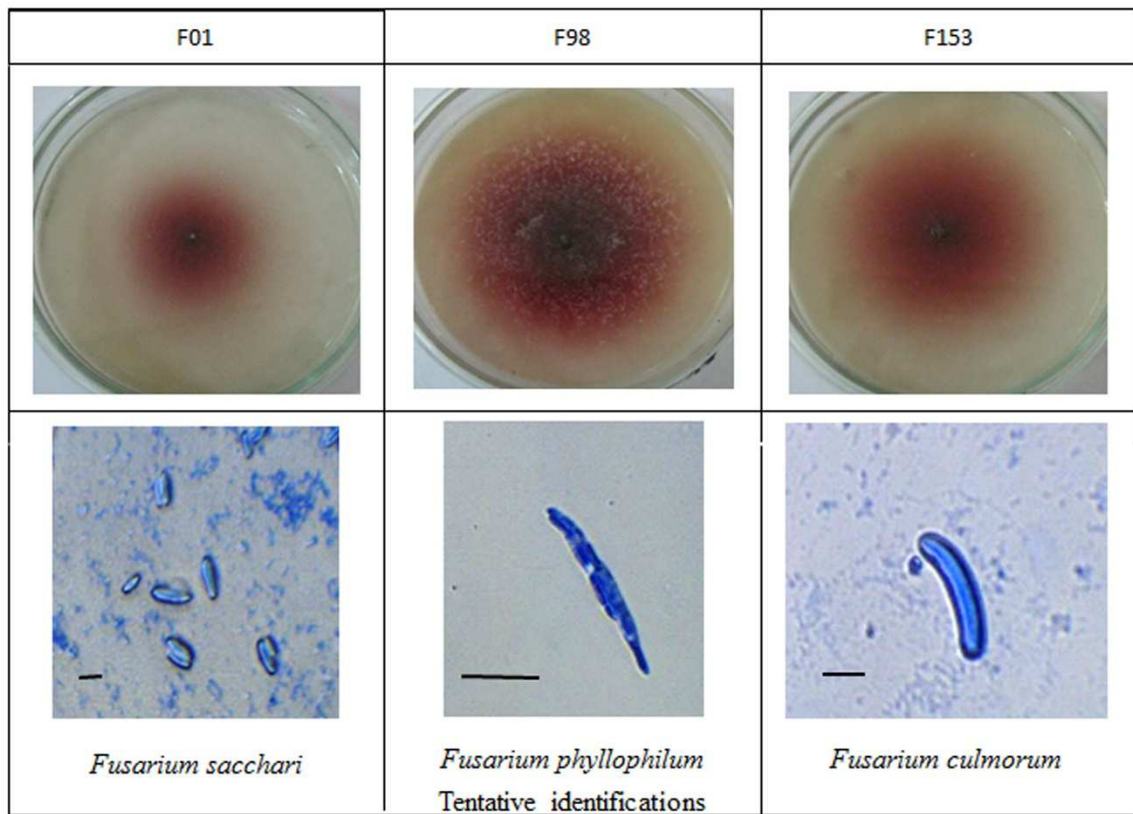


Figure 2. Colony and microscopic characters of three most frequent representative *Fusarium* isolates (F01, F98, F153) isolated from sesame seeds (bar = 20 μ m).

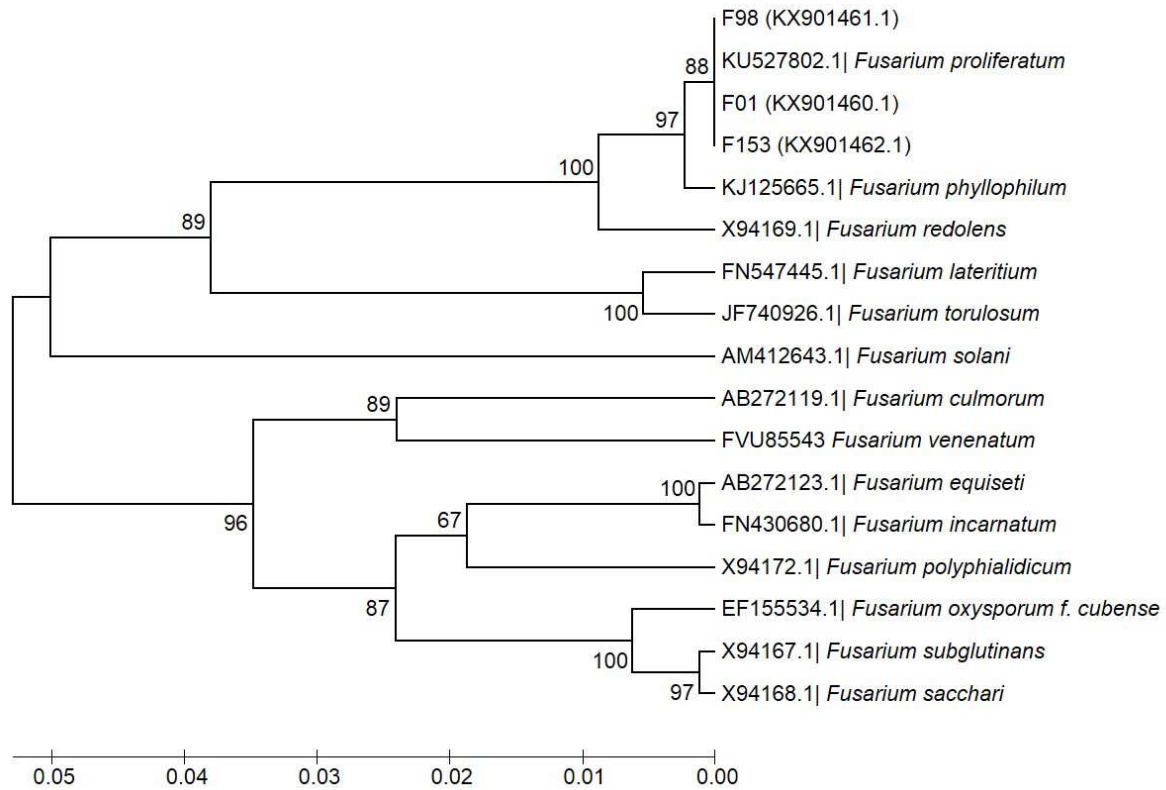


Figure 3. Maximum likelihood phylogenetic tree obtained from consensus sequences of *Fusarium* isolates (F01, F98, F153) from sesame seeds using ITS primers. Numbers above the branches are bootstrap values from 500 replicates. The scale indicates the genetic distance between the species.

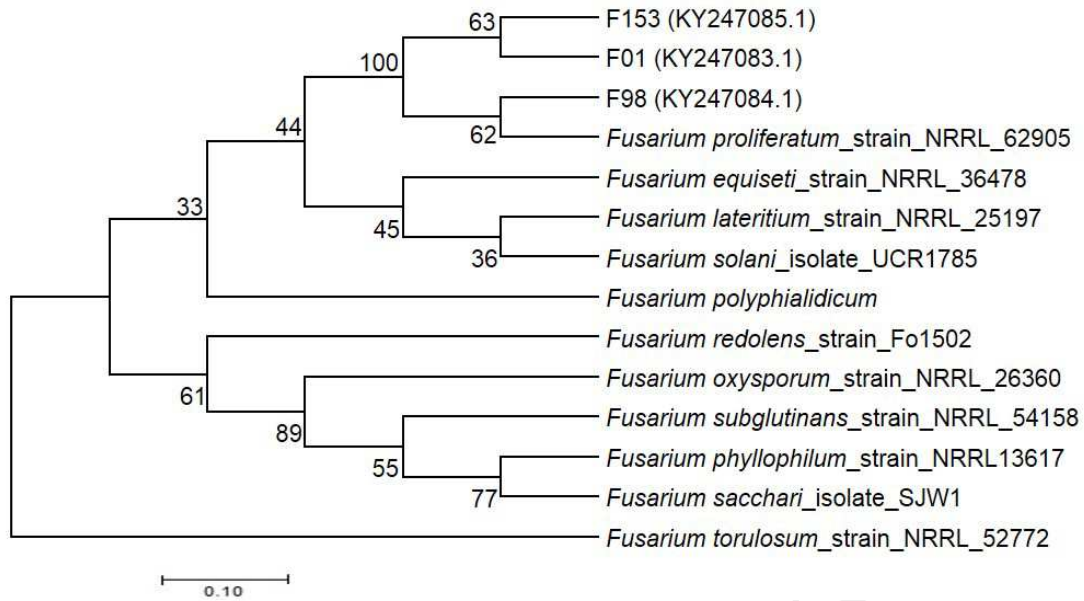


Figure 4. Maximum likelihood phylogenetic tree obtained from consensus sequences of *Fusarium* isolates (F01, F98, F153) from sesame seeds using translation elongation factor (TEF-1 α) primers. Numbers above the branches are bootstrap values from 500 replicates. The scale indicates the genetic distance between the species.



Figure 5. Thirty days post inoculation symptoms of three *Fusarium* isolates on sesame plants. (A) Control; (B) F01; (C) F98; (D) F153



Figure 6. Seven days post treatment effects of culture filtrates of *Fusarium* isolates on germination of sesame seeds. (A) Control; (B) F01; (C) F98; (D) F153

Identification and Pathogenicity of *Fusarium* species associated with Sesame (*Sesamum indicum* L.) seeds from Punjab, Pakistan

Highlights

- Sesame yielded *Fusarium* species as dominant pathogen.
- Three most frequent isolates were accurately identified as *Fusarium proliferatum* through DNA barcoding.
- These isolates were found as virulent with 70% disease severity index and reduced seed germination and vigor index of sesame plants.
- It can be suggested that *Fusarium proliferatum* infection might be a major source of sesame yield loss in the Punjab, Pakistan which requires further confirmation.