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<https://esciencepress.net/journals/phytopath>**FIRST REPORT OF *RHIZOPUS STOLONIFER* CAUSING *RHIZOPUS* BUNCH ROT ON GRAPES IN PAKISTAN****<sup>a</sup>Salman Ghuffar\*, <sup>a</sup>Gulshan Irshad, <sup>a</sup>Amjad S. Gondal, <sup>b</sup>Raees Ahmed, <sup>c</sup>Hafizi Rosli, <sup>d</sup>Xiaoyu Zhang, <sup>e</sup>Hafiz M. U. Aslam, <sup>a</sup>Muhammad A. Iqbal, <sup>d</sup>Mark L. Gleason**<sup>a</sup> Department of Plant Pathology, PMAS-Arid Agriculture University Rawalpindi, Pakistan.<sup>b</sup> Department of Plant Pathology, University of the Poonch Rawalakot, Pakistan.<sup>c</sup> School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia.<sup>d</sup> Department of Plant Pathology & Microbiology Iowa State University, Ames, Iowa, USA.<sup>e</sup> Department of Plant Pathology, University of Agriculture Faisalabad, Pakistan.\*Corresponding Author Email: [mominsalman2610@gmail.com](mailto:mominsalman2610@gmail.com)**ABSTRACT**

During June 2016, a postharvest survey of table grapes was carried out in the main fruit markets of Attock (33°46'07.9"N 72°21'43.0"E) and Jehlum (32°56'22.3"N 73°43'31.4"E) districts of Punjab Province. At the time of sampling, two cultivars (King's Ruby and Perlette) were sampled at five different locations of both districts. Disease incidence percentage of bunches averaged 03 to 04%. Infected fruits appeared water-soaked, light brown and were covered by fluffy mycelium consisting of erect sporangiophores with black sporangia on the top (Figure 1). Symptomatic tissue pieces were surface-sterilized with 0.1% NaOCl for 30 sec, rinsed three times with sterile distilled water, dried on filter paper for 45 sec and incubated on potato dextrose agar (PDA) at 25°C. After 1 day, mycelium on PDA was transferred to a fresh PDA plate and incubated at 25°C with a 12-h photoperiod. Within 3 days of incubation, white to yellow colonies with black aerial sporangia were formed (Figure 2). A total of 64 isolates were examined morphologically. Sporangiophores were erect, light brown and 623 to 3800 µm long. One to three rhizoids were observed opposite to each sporangiophore. Sporangia were black, globose to sub-globose, 91 to 124 µm in diameter. Columellae were conical to cylindrical and 86 to 187 µm long × 72 to 205 µm wide. Sporangiospores were hyaline to light dark grey, globose, ellipsoidal in shape and 6.8 to 12.4 × 3.6 to 12.5 µm (Figure 3). These features were identical to the description of *Rhizopus stolonifer* (Ehrenb.) Vuill (Liou *et al.*, 2007). For molecular identification, the ITS1-5.8S-ITS2 region of two representative isolates (Rizo 05 and Rizo 07) was amplified with primers ITS1/ITS4 (White *et al.*, 1990). Sequence comparison of both isolates (Accession no. MH348205 and MH356272) revealed 100% identity with previously reported isolates of *Rhizopus stolonifer* (Accession no. MG865992, KU729185, HM051076, and MF374842). To confirm Koch's postulates, 10-µl aliquots of spore suspensions (10<sup>6</sup> spores/ml) of Rizo 05 and Rizo 07 were pipetted onto three non-wounded and four wounded asymptomatic grape berries (seven berries per isolate), Sterile distilled water was applied to asymptomatic berries to serve as a negative control. Berries were incubated at 25 ± 2°C in sterile moist chamber (Ghuffar *et al.*, 2018). The experiment was conducted twice. Three days after inoculation, black to light brown, fluffy mycelium with the symptoms of previous infections was observed on both wounded and non-wounded inoculated berries, whereas no symptoms were recorded on the negative control (Figure 4). Morphological descriptions of the fungus re-isolated from each of the inoculated berries was identical to that of the original cultures. Previously, *Rhizopus stolonifer* has been reported as a pathogen on grapes in Chile (Latorre *et al.*, 2002). To best of our knowledge, this is the first report of *Rhizopus stolonifer* causing bunch rot of grapes in Pakistan. Findings of the present studies will help to devise effective disease management strategies against *Rhizopus* rot of grapes in Pakistan.

**Keywords:** *Rhizopus stolonifer*, grapes, *Rhizopus bunch rot*, ITS region, pathogenicity.Running Title: *Rhizopus stolonifer* causing *Rhizopus* bunch rot of grapes

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Figure 1. Infected fruit with water-soaked, black to light brown fluffy mycelial growth.

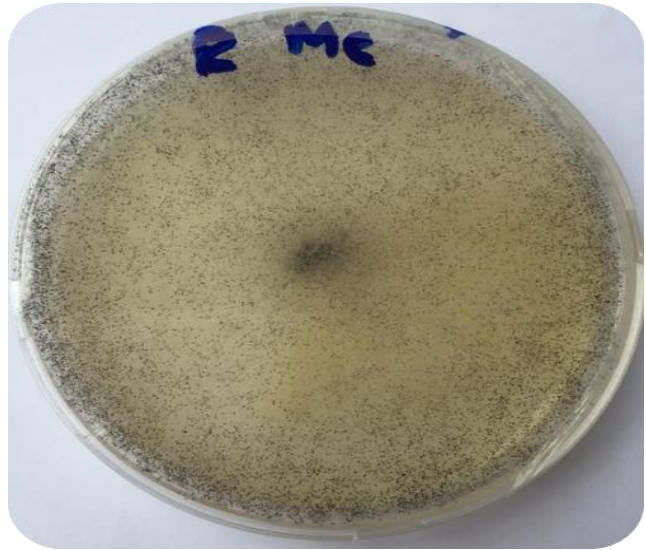


Figure 2. White to yellow colony with black aerial sporangia on PDA.

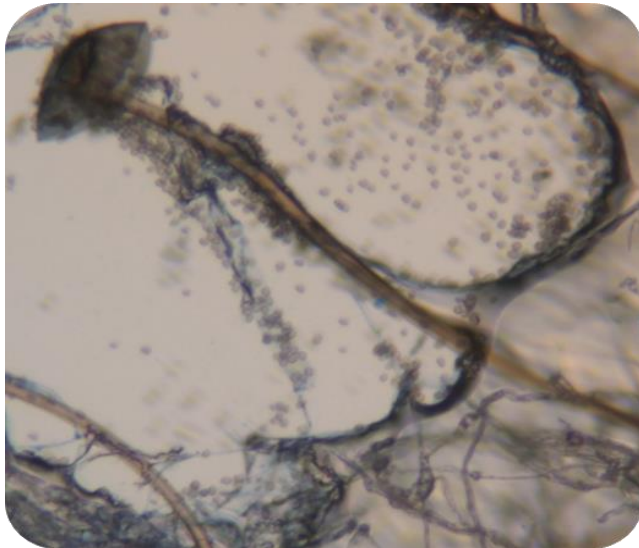


Figure 3. Microscopic observation of *Rhizopus stolonifer*.



Figure 4. Pathogenicity test showing black to light brown, fluffy mycelium.

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