First report of *Geotrichum candidum* causing post-harvest rot of Ber (*Ziziphus mauritiana*) & Guava (*Psidium guajava*) from Pakistan

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Citation

Received: 20/06/2023 Revised: 10/08/2023 Accepted: 15/08/2023 Online First: 22/08/2023

Abstract

The current research involved the isolation of fungal species from spoiled fruit samples gathered from markets in Chichawatni. The method used for isolating filamentous fungi from infected parts of the fruits involved culturing on a Potato Dextrose Agar (PDA) medium following surface sterilization. The isolated fungal species were found belonging to the *Geotrichum* genus based on morpho-anatomical analysis. This study was aimed to isolate, characterize and identify the filamentous fungi associated with post-harvest spoilage of ber and guava being sold in Chichawatni. Findings of this work could lead to the development and recommendation of appropriate strategies for post-harvest disease control.

Keywords: Filamentous fungi; Post-harvest diseases; Spoilt fruits; Taxonomy

Introduction

Although many filamentous fungi are a beneficial source of industrial chemicals and metabolites [1], however, many are destructive pathogens as well. Microbial pathogens such as bacteria, fungi and viruses cause diseases, result in loss of post-harvest crop production of fruits. The disease primarily affects mature fruits, although it may also develop on damaged premature fruits. Signs of the diseases include soft, watery, brown decay on the fruit surface, often accompanied by a thin layer of white mycelial growth [2, 3]. Post-harvest diseases cause destruction of fresh fruits and vegetables in many ways [4]. The diseases that affect fruit’s morphology and taste cause significant economic losses every year. Some famous fungal rots during post-harvest were generally due to black mold (*Aspergillus niger*), green mold (*Penicillium digitatum*) and *Geotrichum candidum* etc. [5]. Main objectives of current study were to isolate filamentous fungi associated with post-harvest spoilage of ber and guava and to identify the filamentous fungi using colony morphology and microscopic features.

Materials and Methods

Sample collection and selection

Fruit samples were gathered from the local fruit markets in Chichawatni, which mainly operates as an open-air marketplace offering seasonal fruits from both local growers and other regions. The collected fruit samples of different types were initially stored in polythene bags, properly labeled with unique codes, and transported to the laboratory for further analysis.
Sample culturing
Petri plates were prepared by pouring approximately 20-25 ml of molten and cooled PDA media into each plate, which was then allowed to solidify for 15 minutes. A small piece of spoiled fruit was taken and applied to the surface of the solidified media using a disinfected needle after surface sterilization. To prevent impurity, the Petri plates were sealed with parafilm, particularly around the edges, and were then incubated at 25°C for several days until the fungal cultures had grown. The growing cultures were identified using microscopy. For microscopic analysis, a small amount of the fungal strain was transferred to a glass slide for slide preparation, again using a disinfected needle. A drop of 5% KOH/ Lactic acid/ Trypan blue or Congo red (for hyaline structures) was used as mounting medium. A cover slip was carefully placed over the sample to prevent air bubbles from getting trapped.

Morphological & anatomical study
On PDA media, fungal colonies' morphological and anatomical characteristics were recorded, and drawings and micro-graphs were created. To identify the isolated filamentous fungal taxa, relevant literature, and identification keys were consulted, such as "Fungi of Pakistan" [6] and "Illustrated Genera of Imperfect Fungi" [7], and other literature [8, 9].

Results

Macroscopic characterization
Colony morphology
Impure culture white, raised, small colonies, uneven, reverse of colony brown (Fig. 1 & 2: A & B). Pure culture white on PDA, uneven, spread on whole plate, with central umbo, uneven, like woolly threads, easily detachable. Texture rough. Margin irregular, colony covering the sides of plate. Reverse of colony white, small colonies (Figs. 1 & 2: C & D).

Microscopic characterization
Conidiophore hyaline, septate, thick-walled, dichotomously branched, cytoplasmic contents visible, septa present at joints, wavy, flexuous and straight, basal ends not visible, bulging at some points, undulate at some points, 5.7-8.5 μm (Fig. 3: A & B; Fig. 4: B). Arthroconidia hyaline, cylindrical to sub-globose, irregular size, texture smooth, thick walled, form chains, 5.7-11.5 × 3-6 μm (Fig. 3: C & D; Fig. 4: A). Mycelial hyphae not significantly different from conidiophore.

Figure 1. A-D: Colony morphology of Geotrichum candidum. A&B. Impure cultures from Ber (Ziziphus mauritiana). C&D. Pure cultures. Scale bar: A-D= 1.4 cm
Figure 2. A-D: Colony morphology of *Geotrichum candidum* from Guava (*Psidium guajava*). A&B. Impure cultures from Guava. C&D. Pure cultures. Scale bar: A-D= 1.3 cm

Figure 3. A-D: Light micrographs of microscopic features of *Geotrichum candidum*. A & B. Septate hyphae with conidia. C&D. Conidia. Scale bar: A-B= 35 μm. C= 15 μm. D= 23 μm
Figure 4. A-B: Illustrations of *Geotrichum candidum*. A. Conidia. B. Septate, branched hyphae. Scale bar: A = 22 μm. B = 35 μm

Figure 5. Graphical overview of isolation and identification of *G. candidum* causing post-harvest rot of fruit samples in this study

**Material examined**
GC#1 was isolated from the Ber (*Ziziphus mauritiana* Lam.) fruit sample taken from the market of Chichawatni.
GC#2 was isolated from the Guava (*Psidium guajava* L.) fruit sample taken from the market of Chichawatni.

**Discussion**
*Geotrichum* is an extensively studied genus from taxonomic point of view. It belongs to family Dipodascaceae of order Saccharomycetales and division Ascomycota. It is comprised of a group of filamentous fungi that resemble yeast in morphology and forms arthroconidia. This
can be confused with *Trichosporon* Behrend, a genus that forms blastoconidia, a distinguishing character not found in the *Geotrichum*. In this study, one species of this genus, i.e., *G. candidum* is isolated from spoilt Ber and Guava fruit samples causing post-harvest rot of these fruits. *G. candidum* is macroscopically characterized by fast growing, whitish colony on PDA [8] and microscopically by cylindrical to sub-globose shaped conidia and also by septate, thick-walled, dichotomously branched hyphae (5.7-8.5μm). Our species also produced white colony, spread on the whole plate with central umbo, form chains of arthroconidia (up to 3-6 × 5.7-11.5μm) as described by Carmichael (1957) in his study. Morphological descriptions and light micro-graps of both the species are same (Carmichael, 1957 vs this study), therefore both strains of *Geotrichum* (GC#1 & GC#2) were identified as *G. candidum*.

**Commentary:** *G. candidum* is an acid-tolerant fungus with yeast-like morphology. As far as its distribution is concerned, it is cosmopolitan and worldwide. It is commonly found in air, soil, water, and living tissues of plants, animals and humans [10]. It is also famous for its use in fermented milks, therefore, a very significant component of fermentation industry [11]. It serves as a culture for cheese making because it adds aroma, taste to cheese and brings a characteristic appearance to it. *G. candidum* is a ripening agent that has been used in France for at least thirty years. It naturally occurs in dairy products and fermented milk. The European Food and Feed Culture Association and the International Dairy Federation both enlisted it as a microbe that has previously been used in dairy products. [12]. The dairy industry has a particular interest in the various metabolic pathways of *G. candidum*. This fungal species plays a crucial role in cheese maturation, and its contribution to cheese ripening and flavor development is well-known. *G. candidum* is crucial for the formation of various soft and semi-hard cheeses and can have a good effect on the development of flavor and aroma, thanks to its wide range of metabolic capacity. Additionally, it might have an impact on the development of other microbes, both good and bad. The type of production and the presence of biotypes that possess particular metabolic traits influence the significance of *G. candidum* in cheese manufacturing [13].

*G. candidum* is also regarded as an opportunistic pathogen and can cause several diseases causing low to high impact on host. It is the most famous for causing post-harvest rot of vegetables, fruits and causing the spoilage of dairy products as well. The presence of this taxon is indicative of poor sanitary conditions. Previously, it has been isolated from seeds and fruits of *Solanum lycopersicum* L. (= *Lycopersicon esculentum*) in Pakistan [6]. Current study brings the first report of *G. candidum* causing the post-harvest rot of spoilt fruit samples of Ber and Guava in local markets of Chichawatni, Punjab, Pakistan.

**Conclusion**

In the current study, *Geotrichum candidum* is reported first time causing the spoilage of Ber and Guava from Pakistan.

**Authors’ contributions**

Conceived and designed the experiment: N Yousaf. Performed the experiment: F Zahid. Analyzed the data: F Zahid & M Abbas. Contributed materials/ analysis/tools: F Zahid, M Abbas & S Rasheed, Wrote the paper: F Zahid & N Yousaf.

**References**

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