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Diversity of fungi causing fruit rot of cucurbits (pumpkin, cucumber and watermelon) in Kashmir Valley

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A preliminary investigation on the diversity of fungi causing fruit rot in cucurbits (pumpkin, cucumber and watermelon) was conducted in 2021-2022, at different locations in Kashmir Valley. The results revealed that eight different species of fungi were causing fruit rot in cucurbits (pumpkin, cucumber and watermelon) in Kashmir Valley. These fungi include *Alternaria alternata,Fusarium solani*, *Aspergillus niger, A. flavus*, *Curvularia lunata*, *Penicillium* sp., *Fusarium* sp., and *Rhizopus* sp. The most frequent isolated pathogenic fungi was *Fusarium* spp.which accounted for 80–90% of all rots.

Keywords : Cucurbitaceae, cultural characteristic, microscopic characteristics, potato dextrose agar

INTRODUCTION

The family Cucurbitaceae, or “cucurbits” or “gourds,”consists of 118 genera and 825 species of plants that are often grown as summer vegetables (Avinash *et al.* 2021). It ranks highest among the plant families as many cucurbit plant species are used as human food.They are native to most countries of the globe, paticularly the tropics and temperate areas. India features as the second major vegetables producing countries in the world with 2.8% of the total cropped area and 13.38% of total vegetable production (Kundu 2012).Out of the whole production of vegetables ,cucurbits comprise of 5.6% of total vegetables in india and as per the FAO estimate, cucurbit crops in India are cultivated on about 4.29 million ha with a productivity of 10.52 t/ha and are widely grown in all states, including Jammu and Kashmir (Yousuf and Dar, 2016). Besides economic values,the vegetables of Cucurbitaceae are important due to their nutritional values (Avinash and Rai, 2013). Cucurbits are sources of carbohydrates and water; their seeds contain large amount of oil and protein (Neeraj and Verma, 2010).

Some species, like cucumber, muskmelon, and bitter melon, are considered to have medicinal properties due to cucurbitac in which is used to treat malaria, cholera,anemia, and jaundice (Kaushik *et al.* 2015).*Cucumis sativa* fruits possess depurative, diuretic, and purgative properties and are also effective for the treatment of blemished skin and heat rash (Afari *et al.* 2012). In the cucurbitaceae family, annual vines make up the majority of the species, but some are also woody lianas, prickly shrubs, and trees. Their fruits are large, fleshy, and typically covered in hard skin; they are modified dry berries known as pepo.

Cucurbit production and its nutritional and economic value is under threat due to various biotic and abiotic factors. Among biotic factors fruit rot caused by *Fusarium* spp.,*Phytophthora* spp., *Acremonium* spp., and *Pythium* spp.,is the most common cause resulting up to 70% yield loss (Hopkins and Thomas 1996).A large diversity of fungi affects cucurbits by causing fruit rot

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diseases including *Alternaria alternata* (*Alternaria* Rot), *Penicillum* spp., (Blue Mold), *Myrothecium roridum* (Crater Rot), *Pythium* spp.,*(Pythium* Cottony Leak), *Rhizopus stolonifer (Rhizopus* Soft Rot), *Didymella bryoniae* (Black Rot), *Colletrotrichum or biculare* (Anthracnose), *Cladosporium cucumerinum* (Scab).Some of the most prevalent pre- and postharvest diseases of cucurbits are fruit rots caused by various *Fusarium* spp. (Aktaruzzaman *et al.* 2018). On pumpkins, fruit rot is evident by everything from small, pitted, corky spots to big, sunken areas coated in a white or grey mold. Most infections occur in areas that are in contact with the soil. Phytophthora rot, which is caused by the oomycete *Phytophthora capsici* Leonian, is one of the greatest threats to the world's supply of pumpkins and other cucurbits (Babadoost, 2000).The first sign of Phytophthora infection is typically a water-soaked or depressed patch on fruit that has come into touch with the soil.The pathogen causes fruit to become completely coated with a white, yeast-like growth with numerous fruiting bodies (sporangia) and affected fruit may be covered entirely. Several reports suggest that fungal rot caused considerable losses to vegetables worldwide, including in India (Mitcham and Mitchell, 2002). In Jammu and Kashmir, fungal rot also causes severe production problems at both the pre-and postharvest stages of the vegetables (Nisa*et al.*2011, Jan *et al.*2022, Nisa *et al.* 2022). However, very little work has been done on the fungal rot of cucurbits in Kashmir Valley. Hence the present study entitled "Diversity of fungi causing fruit rot of cucurbits (pumpkin, cucumber and watermelon) in Kashmir Valley" aimed to isolate, culture, and identify the fungi causing fruit rot of cucurbits in Kashmir Valley. The study will help to unravel and document the fungi causing fruitrot diseases of cucurbits and will make newer additions to the mycoflora of India.

MATERIALS AND METHODS

Sample collection

A survey was conducted in various kitchen gardens, local markets, and storage godowns of Kupwara, Baramulla, Bandipora, Ganderbal and Srinagar districts in the Kashmir Valley (Fig. 1). The rotten fruits of some cucurbits (pumpkin, cucumber and watermelon) were collected in air-tight polythene bags and were brought to the Plant Pathology and Mycology Laboratory, Department of Botany, University of Kashmir. These fruits were then stored in the refrigeratorat at 4 ^{0}C for further analysis.

Isolation and Identification of fungi

Isolation of fungi causing fruit rot in cucurbits was performed by taking small pieces of diseased fruit samples and then surface sterilizing by immersing the diseased fruit portion in 1% sodium hypochlorite solution for one minute, then washing with double distilled water and finally transferring to petriplates containing PDA (potato dextrose agar). The petri plates were incubated at 28^{o}C till the growth of fungal mycelium appeared. After the seventh-day of incubation period, the isolated fungi were identified based on morphological, cultural, and microscopic characteristics such as mycelium, sporangiophore, conidiophores, conidia, and acervuliby staining with lactophenol cotton blue and were observed under a light microscope (Leica DM 750) at 100×, 400× and 1000× magnifications.The final identification was done by using relevant literature, monographs and fungal key (Watanabe, 2002;Gilman, 2008).

RESULTS AND DISCUSSION

The current investigation led to the identification of eight fungal species causing fruit rot disease from three different cucurbits (pumpkin, cucumber, watermelon) in Kashmir Valley. The symptoms, cultural and microscopic charac teristics of these identified fungi are described as under:

Alternaria rot of pumpkin and watermelon

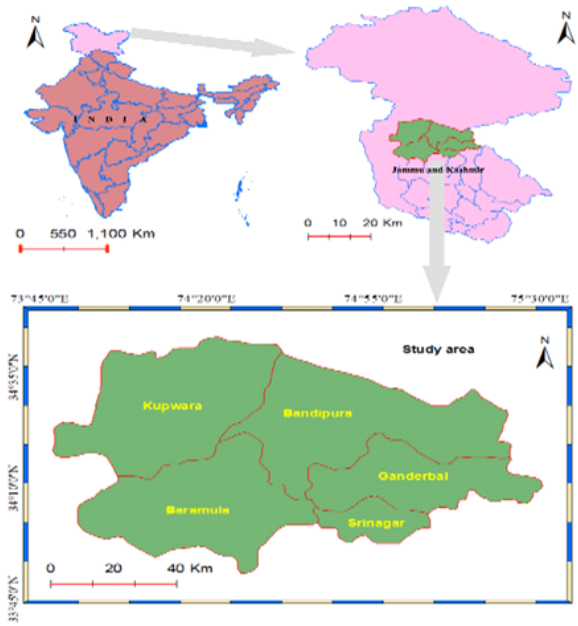
***Causal agent**: Alternaria alternate (Fr.) Keissl. 1992*

Symptoms

Young fruits, foliage, and twigs are the targets of this attack, which results in brown to black lesions encircled by a yellow halo**.** On the peel, fruits possess legions that range in size from tiny spots to enormous pockmarks. As the fruit ages, the

Table 1: Fungal species isolated from cucurbits (Pumpkin, Cucumber and Watermelon) along with the host plant

Fungal species	Host plant	Division
Alternaria alternate (Fr.) Keissl. 1992	Pumpkin, Watermelon	Ascomycota
Fusarium solani (Mart.) Sacc. 1881	Pumpkin, Cucumber	Ascomycota
Fusarium sp.	Cucumber,Watermelon	Ascomycota
Aspergillus niger Tiegh. 1867	Pumpkin, Cucumber	Ascomycota
Aspergillus flavus Link 1809	Pumpkin, Watermelon	Ascomycota
Curvularialunata (Wakker) Boedigin	Cucumber	Ascomycota
Penicillum sp.	Pumpkin, Cucumber	Ascomycota
Rhizopus sp.	Cucumber,Watermelon	Zygomycota



young lesions can become dislodged and leave a light tan pockmark.The fungus penetrates the fruit rind and causes localized necrosis (Fig. 2a,b).

Cultural characters

On PDA,colonies grew very fastand were black to olivaceous-black in colour. The reverse was dark blackish at the center, and the edges were orange(Fig. 2c,d).

Microscopic characters

Mycelium brown, septate, and *produced* branched conidiophores measuring 18–52 × 2.5–4.1 µm, conidia brown measuring 15–37 × 4.7–13.5 µm with a small cylindrical beak and up to five transverse septa (Fig. 2e).

Fusarium rot of pumpkin and cucumber

Causal agent: *Fusarium solani (Mart.) Sacc. 1881*

Symptoms

Water-soaked lesions developed on the fruits at the point of contact with soil. As the condition worsened, whole fruit began to collapse and develop a soft rot with leakage. Additionally, a soft, dark-brown decay appeared at the base of the main veins (Fig. 3a,b).

Cultural characters

On PDA media colonies appears white, cottony with fluffy mycelial growth.The reverse of the culture appears somewhat pinkish orange because of the release of specific metabolites into the culture media by fungus (Fig. 3c, d).

Microscopic characters

Both microconidia and macroconidia were present. Macroconidia hyaline, linear, 2-3 septate measuring 12-35 × 2.6-3.8 µm with slightly curved and blunt ends. Microconidia round to oval,

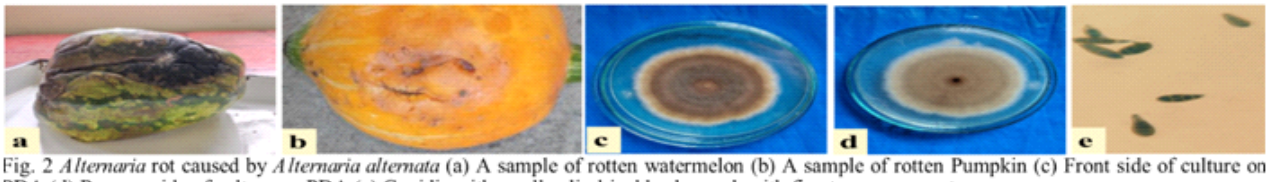


Fig. 2 *Alternaria* rot caused by *Alternaria alternata* (a) A sample of rotten watermelon (b) A sample of rotten Pumpkin (c) Front side of culture on PDA (d) Reverse side of culture on PDA (e) Conidia with small cylindrical beaks, each with five transverse septa

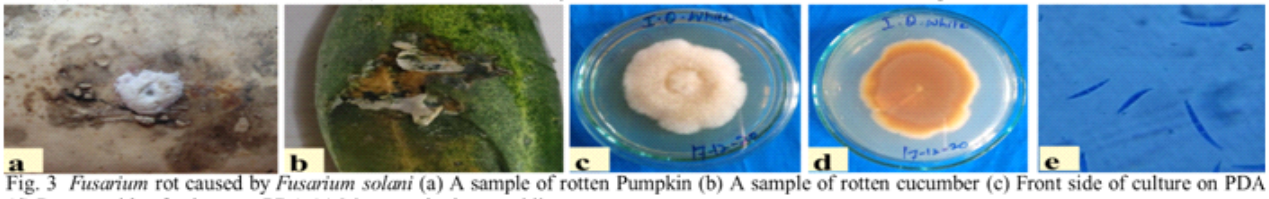


Fig. 3 *Fusarium* rot caused by *Fusarium solani* (a) A sample of rotten Pumpkin (b) A sample of rotten cucumber (c) Front side of culture on PDA (d) Reverse side of culture on PDA (e) Macro and micro conidia

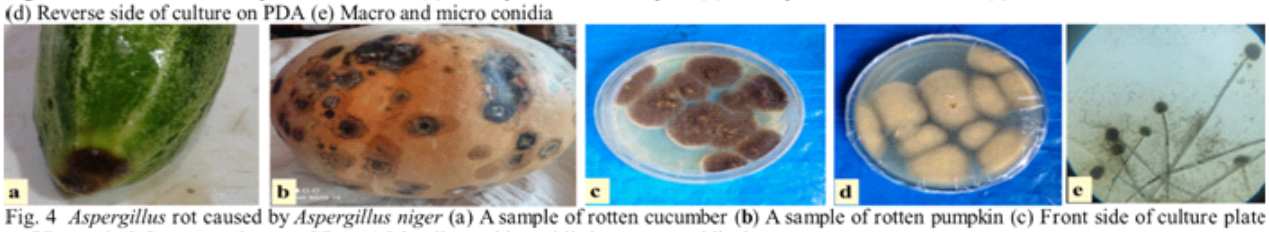


Fig. 4 *Aspergillus* rot caused by *Aspergillus niger* (a) A sample of rotten cucumber (b) A sample of rotten pumpkin (c) Front side of culture plate on PDA and (d) Reverse culture on PDA (e) Mycelium with conidia borne on conidiophores

Fig. 5 *Aspergillus* rot caused by *Aspergillus flavus* (a) A sample of rotten watermelon (b) A sample of rotten pumpkin (c) Front side of culture on PDA and (d) Reverse culture on PDA (e) Conidiophores and conidia

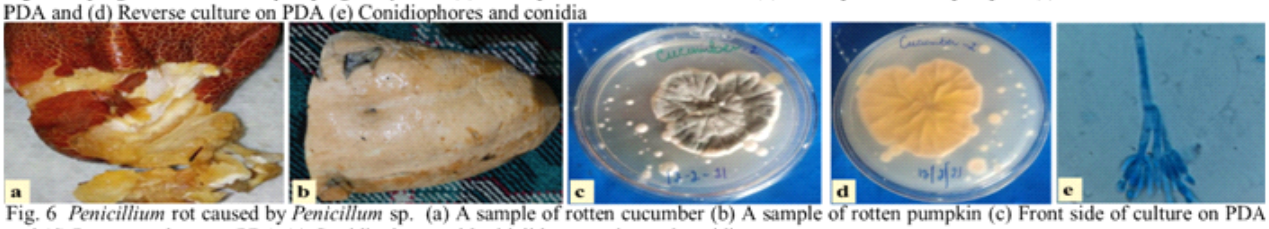


Fig. 6 *Penicillium* rot caused by *Penicillum* sp. (a) A sample of rotten cucumber (b) A sample of rotten pumpkin (c) Front side of culture on PDA and (d) Reverse culture on PDA (e) Conidiophores with phialides, metulae and conidia

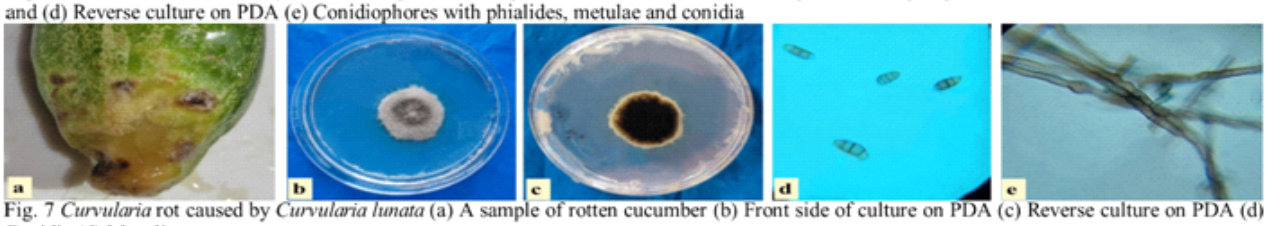


Fig. 7 *Curvularia* rot caused by *Curvularia lunata* (a) A sample of rotten cucumber (b) Front side of culture on PDA (c) Reverse culture on PDA (d) Conidia (d) Mycelium

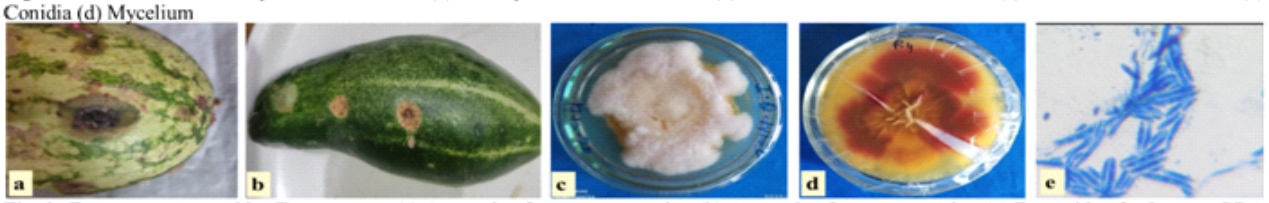


Fig. 8 *Fusarium* rot caused by *Fusarium* sp. (a) A sample of rotten watermelon (b) A sample of rotten cucumber (c) Front side of culture on PDA (d) Reverse culture on PDA (e) Macro and microconidia

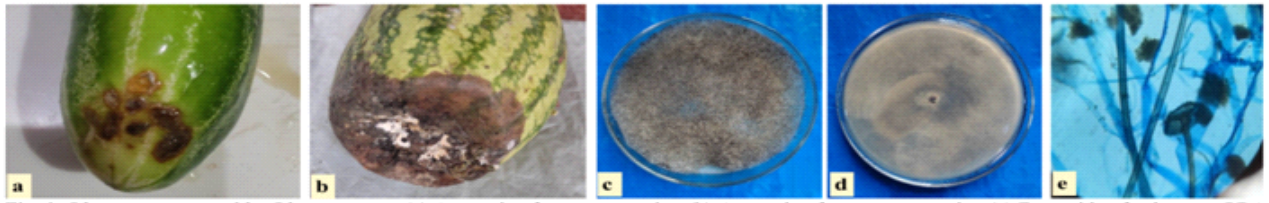


Fig. 9 *Rhizopus* rot caused by *Rhizopus* sp. . (a) A sample of rotten cucumber (b) A sample of rotten watermelon (c) Front side of culture on PDA (d) Reverse culture on PDA (e) Mycelium with rhizoids, stolons, sporangiophores and sporangium

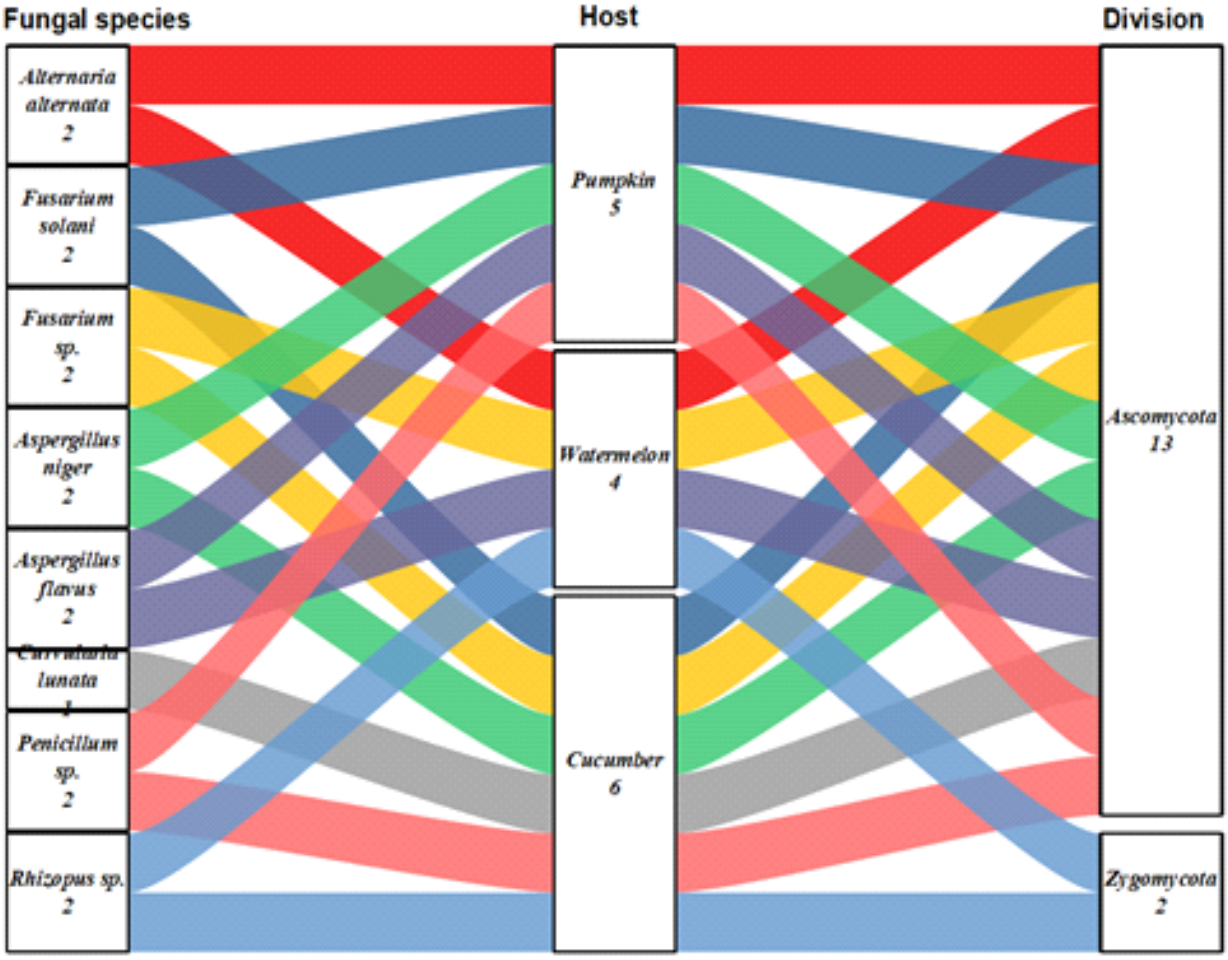


Fig 9: Alluvial diagram showing the relation between fungal species and their hosts

hyaline, 0-1 septate measuring 3-7× 1.9-2.4 μm (Fig. 3e).

***Aspergillus* rot of cucumber and pumpkin**
Causal agent: *Aspergillus niger Tiegh. 1867*

Symptom

The lesions initially appeared as sunken, light to dark, water-soaked patches that leaked a watery ooze. More mature lesions were cracked and dried. Symptoms developed inside the fruit, in the inner space between the pulp and the seed (Fig. 4a,b).

Cultural characters

On PDA media,colonies appeared black and mostly had a dense felt of erect conidiophores (Fig. 4c,d).

Microscopic characters

Mycelium was septate and well-developed., and contained an interwoven hyphal mass, conidiophores unbranched, aseptate, and smooth-walled with lengths ranging from 300 to 400 μm long and darkened at the top before ending in the globose vesicle. Sterigmata, which resemble finger-like projections, covered the entire surface of the vesicle which bears spherical black conidia measuring 2.1-4.5 μm in diameter (Fig. 4e).

***Aspergillus* rot of watermelon and pumpkin**
Causal agent: *Aspergillus flavus Link 1809*

Symptoms

The infection began during flowering and early fruit development.The fungus grew inside the fruit

without any external symptom. External decay was close to the calyx of the fruit and showed some yellowish to brownish- red discoloration (Fig. 5a,b).

Cultural characters

On PDA media colonies grew rapidly at 25±2^{0}C and were olive to lime green in colour.The texture was woolly to cottony to somewhat granular (Fig. 5c,d).

Microscopic characters

Hyphae thread-like, septate, and hyaline branches that form the mycelium. Conidiophores coarsely roughened, hyaline, and up to 800 µm × 15-20 µm, conidial heads radiated.Conidia smooth, globose, and 3-6 µm in diameter (Fig. 5e).

Penicillium rot of cucumber and pumpkin
Causal agent: *Penicillium sp.*

Symptoms

Lesions began as light brown and then turned tan brown , the tissue turned soft and watery. The fruit that was gathered showed various states of deterioration, from minor lesions without obvious sporulation to entirely rotten fruit covered in blue-green spores. The fruit had a musty, earthy smell and showed signs of blue mould(Fig. 6a,b).

Cultural Characters

On PDA media colonies grew rapidly and appeared white first and then turned bluish-green, mostly consisting of a dense felt of conidiophores and conidia (Fig. 6c,d).

Microscopic characters

Hyphae slender, tubular, branched, hyaline,septate and were created from several mycelium threads that entwined to form a hyphal network.. Conidiophores were long, thick tubes with vesicles at the top which give rise to phialides which in turns produced metulae. Conidia globose measuring 2.1–3.0 µm in diameter (Fig. 6e).

Curvularia rot of cucumber
Causal Agent: *Curvularia lunata (Wakker) Boedigin*

Symptoms

The rot began as a dark brown to black spot on the fruit's surface and subsequently grew larger, causing furthur rot and disposal of infected fruit (Fig. 7a,b).

Cultural Characters

On PDA media colonies were flat, woolly to cottony and brownish on top side and dark black on the back side, woolly to cottony (Fig. 7c,d).

Microscopic Characters

Conidiophore septate, branched, bent at the points where the conidia originated,occurring singly or in clusters, Conidia boat shaped mostly 3-4 celled measuring 16.5–24.5 × 5.5–12.5 µm (Fig. 7e).

Fusarium rot of watermelon and cucumber
Causal agent: *Fusarium* sp.

Symptoms

The initial infection began when the fruit touched the soil. Infected fruit developed circular lesions.The tissue beneath was discolored and hadacorky texture.As lesions developed, the coloration progressed to whitish pink, and rot formed **(**Fig. 8a,b).

Cultural characters

On PDA media **c**olonies were white, cottony or fibrous in texture with areal mycelium and at later stages, the colour of colonies varied from pink to light or dark violet (Fig. 8c-d).

Microscopic Characters

Both macroconidia and microconidia were present. Microconidia ovate, hyaline and non-septate, measuring 5-6 × 2-3 µm. Macroconidia 2-5 septate measuring 20-34×4-5 µm (Fig. 8e).

Rhizopus rot of watermelon
Casual agent: *Rhizopus sp.*

Symptoms

The affected tissues were at the end of the fruit and were moist and soft. Wet soft lesions spread on fruit often from their stylar, stalk ends, or even wounds. Affected tissues took a translucent appearance.Numerous aerial fruiting bodies, in black pinheads,were visible on the damaged tissue (Fig. 9a,b).

Cultural Characters

On PDA media colonies grew fast and faded from white to dark as they produced spores and were similar to cotton candy. The back side of colony was creamish white (Fig. 9c,d).

Microscopic Characters

Mycelium branched and composed of three types of hyphae: stolons, rhizoids, and unbranched sporangiophores measuring upto 1.5mm. Sporangia terminal, circular, smooth and measuring 145-215 μm in diameter, sporang iospores globose measuring 5.5–10.5 × 4.4–6.5 μm **(**Fig. 9e).

The results revealed that eight different species of fungi were isolated from rotten samples of pumpkin, cucumber, and watermelon in Kashmir Valley. These fungi include *Alternaria alternata, Fusarium solani*, *Aspergillus niger, A.flavus*, *Curvularia lunata*, *Penicillium* sp., *Fusarium* sp*.,* and *Rhizopus* sp. Five fungi, *Alternaria alternata*, *Fusarium solani,Aspergillus niger*, *A. flavus*, and *Penicillium* sp., were isolated from pumpkin. Six fungi namely *Fusarium solani*, *Aspergillus niger, Rhizopus* sp.,*Curvularia lunata*, *Penicillium* sp., *Fusarium* sp. were isolated from cucumber. While as four fungi namely*, Rhizopus* sp., *Alternaria alternata,Fusarium* sp., and *Aspergillus flavus* were isolated from watermelon. *Fusarium* species, particularly *Fusarium solani* and *Fusarium* sp. accounted for 80–90% of all rots, and they were the most frequently isolated fungi. The lowest isolated pathogen, *Curvularia lunata*, was only found in cucumbers.

Our results are in conformity with Zitter (1996), McGrath (2004), Aktaruzzaman *et al*. (2018) and Naungmek *et al.* (2019) who also have isolated these pathogenic fungi from fruit rot of cucurbits. The *Fusarium* species cause several diseases, including crown rot, wilt, fruit rot, stem rot,and seedling blight among pumpkin, cucumber, and watermelon worldwide (Naungmek *et al.* 2019; McGrath 2004). *Fusarium* fruit rot of cucurbits was first described in detail in South Africa in 1932 (Martyn 1996). Besides *Fusarium solani* and other *Fusarium* species that causes fruit rot in pumpkin,cucumber and watermelon, a number of other fusarium species including *F. graminearum, F. acuminatium, F. culmorum, F. moniliforme, F. semitectum, F. equiseti* and *F. scirpi* have been reported as causal agents of cucurbit fruit rot (Bruton and Duthie 1996). In a recent investigation,Elmer *et al.*(2007) determined *Fusarium solani* as the main causal agent of fruit rot of pumpkins in the united states.The presence of several *Fusarium* species suggested that vegetable fruits are prone to *Fusarium* contamination. *Fusarium solani*, *Aspergillus niger* and *Curvularia* sp. that we isolated from diseased cucumber were also isolated from the diseased cucumber fruits collected from green houses in Oman (Al-Sadi *et al.* 2011). *Aspergillus niger* and *A. flavus* isolated from our diseased pumpkin were also isolated from the same fruit in Hong Adamawa state (Kazi *et al.* 2019).

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DECLARATION

Conflict of Interest. Authors declare no conflict of interest.

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