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Research Article

Mycoflora associated with cucurbits

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Abstract

Cucurbits are one of the most important groups of vegetables and fruits. Soil samples and fruits of cucurbits were collected and analyzed for the presence of mycofloara. Seven different fungi were isolated using agar plate method. Isolations were made from the soil and fruits of family Cucurbitaceae viz., *Cucumis sativus, Cucumis melo, Cucurbita pepo* and *Luffa cylindrica*. Fungal genera isolated were *Alternaria alternata, Aspergillus flavus, A.fumigatus, A.niger, A.nidulans, A. unguis* and *Fusarium oxysporum*.

Keywords: *Alternaria alternata*, *Aspergillus*, Cucurbitaceae, *Fusarium oxysporum*, mycoflora.

Introduction

Fruit crops are attacked by a wide range of microorganisms in the postharvest phase (Fatima *et al.,* 2009). Fungi are the most prevalent pathogens, which infects wide range of host plants and causing destructive and economically important losses of most fresh fruits and vegetables during storage and transportation (Sommer, 1985). Fruit, due to their low pH, higher moisture content and nutrient composition are very susceptible to attack by pathogenic fungi, which in addition to causing rots may make them unfit for consumption by producing mycotoxins (Philips, 1984; Moss, 2002). International agencies that monitor world food resources have acknowledged that one of the most feasible options for meeting future food needs is reduction of postharvest losses (Kelman, 1984). Careful post-harvest handling is the major but often neglected step towards offering a greater volume of nutritious food to planet and to prevent loss between harvesting and consumption.

Post-harvest diseases destroy 10-30% of the total yield of crops and in some perishable crops especially in developing countries; they destroy more than 30% of the crop yield (Kader, 2002; Agrios, 2005). Fruits and vegetables are highly perishable products; the quality is affected by post-harvest handling, transportation, storage and marketing. The improper handling, packaging, storage and transportation may result in decay and production of microorganisms, which become activated because of the changing physiological state of the fruits and vegetables (Wilson *et al*., 1991). In the present study, attempts have been made to investigate the spectrum of fungal flora associated with cucurbits.

Materials and Methods

Samples with prominent symptoms were collected from different vegetable farms. For further experimentation small pieces of about 1-2 cm from affected parts were cut. These pieces were then surface sterilized with 1% sodium hypochlorite ($NaOCl_2$) solution for about one minute followed by thorough washing with distilled sterilized water. Surface sterilized pieces were plated on malt extract agar (MEA) plates. Cultures were then incubated at 25±2˚C for six to seven days. For further purification and identification appeared fungal colonies were re-cultured on the respective media. Microscopic studies were done after maturation for the sake of identification. Further identification was done by First Fungal Culture Bank of Pakistan, Institute of Agricultural Sciences, University of the Punjab. Lahore. Pure cultures were stored at 4˚C in refrigerator.

Results and discussion

***Alternaria alternata*:** (Fr.) Keissler 1912

Colony of *A. alternata* was grey to black in colour, flat and powdery at maturity, the diameter of colony on MEA after 4 days is 3 cm and after 7 day 5 cm. its growth rate was moderate. Its reverse was pure black. Conidiophore pale brown to olive brown, their size is 25-60 × 3-3.5 µm, Straight or flexuous. Individual conidiophores arise directly from substrate forming bushy heads consisting of 4-8 large catenate conidia chains. Secondary conidiophores are generally short and 1-celled.Conidiophores individual conidiophores arise directly from substrate forming bushy heads consisting of 4-8 large catenate conidia chains; secondary conidiophores are generally short and 1-

celled (Fig. 1A). Conidia were pale brown to light brown, obclavate to obpyriform or ellipsoid, short conical beak at the tip, broad at the base, Surface smooth to slightly rough some times ,mature conidia typically 10-30 × 5-12 µm . There are longitudinal and transverse septation present in alternaria conidia which are 3-7 transepta, 1-5 longisepta .The conidia produce in chains often branched, long chain more than 5 to 15 conidia but in case of complex branching the conidia may be up to 50 in numbers (Fig. 1B).
Source: Field soil of *Luffa cylindrica*, *Cucumis sativus*. Fruits of *Cucurbita pepo*, *Cucumis sativus*, *Citrullus vulgaris*.

***Fusarium oxysporum*:** Schlecht 1824, Hans 1940

Macroscopic morphology vary significantly on different media, and descriptions here were based upon growth on ME agar 30±2 °C moderate growth rate. Colonies were initially white, becoming peach color at maturity. The reverse color of colony is brown (Fig. 2A). Hyphae were septate and hyaline. Conidiophores are and simple. Macroconidia usually produced abundantly, slightly sickle-shaped, narrow ended, thin-walled, with an attenuated apical cell and a foot-shaped basal cell. They were three to 5-septate measuring 23-54 × 3-4.5 µm. Microconidia were abundant, mostly non-septate, ellipsoidal to cylindrical, slightly curved, 5-12 × 2.3-3.5 µm (Fig. 2B).
Source: It is isolated from soil of *Luffa cylindrical* and *Cucumis sativus* and fruits of *Cucumis sativus*, *Luffa cylindrica*.

Aspergillus flavus: Link ex Gray 1821

Colonies on ME agar were olive to yellowish green with a cream/pale yellow reverse, rapid growth. Texture was cottony to granular. Sclerotia were present, they were dark brown. The diameter of colony was 3.5 cm in 3 days and in 7 days it reaches up to 7 cm on MEA (Fig. 3A). Hyphae were septate and hyaline. Conidial heads are radiate to loosely columnar with age. Conidiophores are clumsily roughened, uncolored, up to 800 µm long × 15–20 µm wide, vesicles globose to subglobose (20–40 µm), metulae (8–10 × 5–7 µm) covering nearly the entire vesicle in biseriate species. Some isolates may remain uniseriate, producing only phialides (8–12 × 3–4 µm) covering the vesicle. Conidia were smooth to very finely roughen or echinulate, globose to subglobose, 3–4 µm in diameter and olive green in colour (Fig. 3B).
Source: Soil of *Luffa cylindrica*, *Cucumis sativus*. Fruits of *Cucurbita pepo*, *Cucumis melo*.

Aspergillus fumigatus: Fresen 1863

A. fumigatus is a saprotroph that is widespread in nature, typically found in soil and decaying organic matter such as compost heaps, where it plays an essential role in carbon and nitrogen recycling. Colonies of the fungus produce from conidiophores thousands of minute grey-green conidia that readily become airborne.

Colonies on MEA agar were smoky gray-green with a slight yellow reverse. Very mature colonies turn slate gray. Texture is wooly to somewhat granular .Diameter of colony was 4.5 cm in 3 days and in 7 days it's up to 7 cm (Fig. 4A). Hyphae were septate and hyaline. Conidial heads are strongly columnar. Conidiophores are smooth-walled, uncolored, up to 300 µm long, and terminate in a dome-shaped vesicle that is 20–30 µm in diameter. This species is uniseriate with closely compacted phialides (5–10 ×2–3 µm) occurring only on the upper portion of the vesicle. Conidia were smooth to finely roughened, subglobose, 2-3.5 µm in diameter (Fig. 4B).
Source: Soil of *Luffa cylindrica*, *Cucumis sativus*. Fruits of *Cucurbita pepo*, *Cucumis melo*.

Aspergillus nidulans: (Eidam) Vuill

Colonies on MEA agar were dark green. Reverse is purplish to olive. Growth rate was moderate in comparison with other. In 4 days its diameter is 3.5 cm and in 7 days it's up to 7 cm. the colony texture is granular and dense (Fig. 5A). Hyphae were septate and hyaline. Conidial heads were columnar. Conidiophores were brown, short, 60-150 µm in length, and smooth-walled. Vesicles were hemispherical, small, 8–12 µm in diameter, with metulae and phialides occurring on the upper portion. Conidia were globose, 3–4 µm diameter and rough olive green in colour (Fig. 5B).
Source: Soil of *Cucumis sativus*.

Aspergillus niger: van Tieghem 1867

Colonies on malt extract agar were initially white, quickly becoming black with conidial production. Reverse is pale yellow and the texture of colony was powdery with 3.5 cm diameter in 3 days and in 7 days it may be reaches up to 8 cm (Fig. 6A).Hyphae are septate and hyaline. Conidial heads are radiate. The species is biseriate, metulae present. Conidiophores were long (>500 µm), smooth, and hyaline, becoming darker at the apex and terminating in a globose vesicle (30–75 µm in diameter). Metulae and phialides cover the entire vesicle. Conidia were brown to black, very rough, globose, and measure 4–5 µm in diameter (Fig. 6B).
Source: Fruit of *Cucurbita pepo*, *Cucumis melo*.

***Aspergillus unguis*: (**Emile-Wiel & L. Gaudin) Thom And Raper

Initially colonies are white on ME agar, yellowish-brown, reverse is off white, flat granular texture. Its diameter 2.5 in 3 days and in 7days it reaches up to 5 cm (Fig. 7A).

Conidial heads radiate to loosely columnar on MEA. Conidiophore dull brown, thick and rough walled, seta-like hyphae rising from foot cells usually present. Vesicles spathulate/ hemispherical, 20 µm diameter, biseriate. Metulae covering the upper part of the vesicle. Phialides were 6µm × 3µm. Conidia smooth walled or somewhat rough walled, dull green, spherical, 2.5–3.5 µm diameter (Fig. 7B).

Source: *Cucumis sativus*.

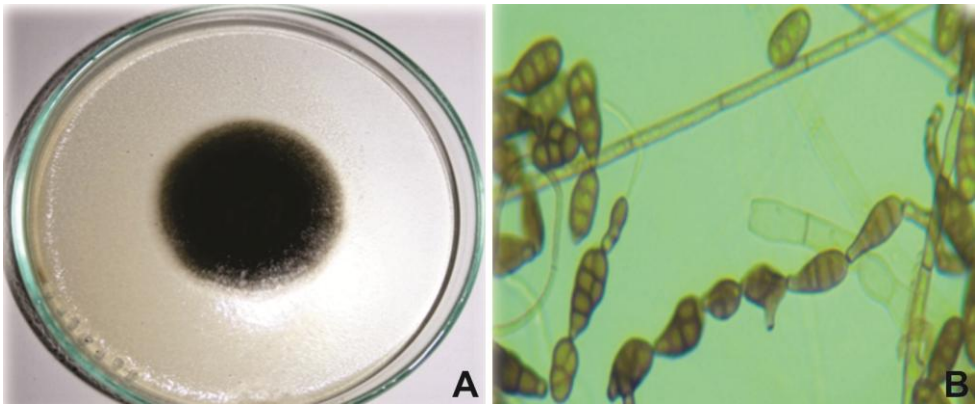

Fig. 1: A) Single spore colony of *A. alternata* (**B)** Conidia of *A. alternata* (microscopic image).

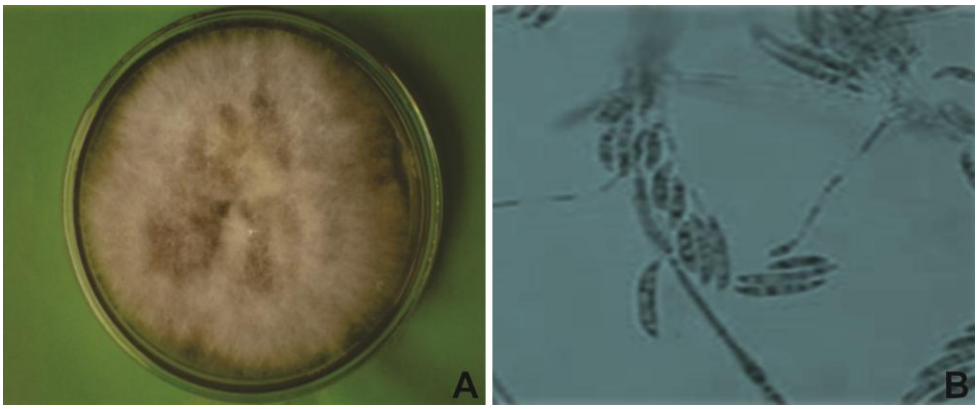

Fig. 2: (A) Single spore colony of *F. oxysporum* **(B)** *M*icro and macro conidia of *F. oxysporum* (microscopic image).

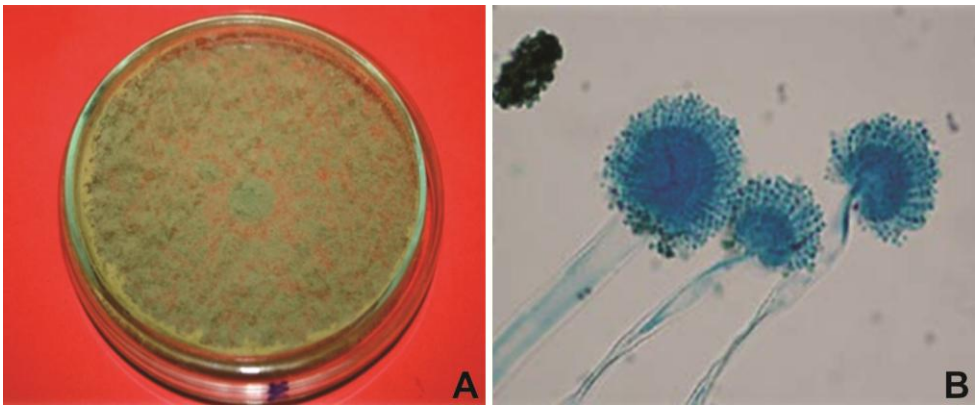

Fig. 3: (A) Single spore colony of *A. flavus* **(B)** Fruiting body: conidia, vesicle, and conidial head, of *A. flavus* (microscopic image).

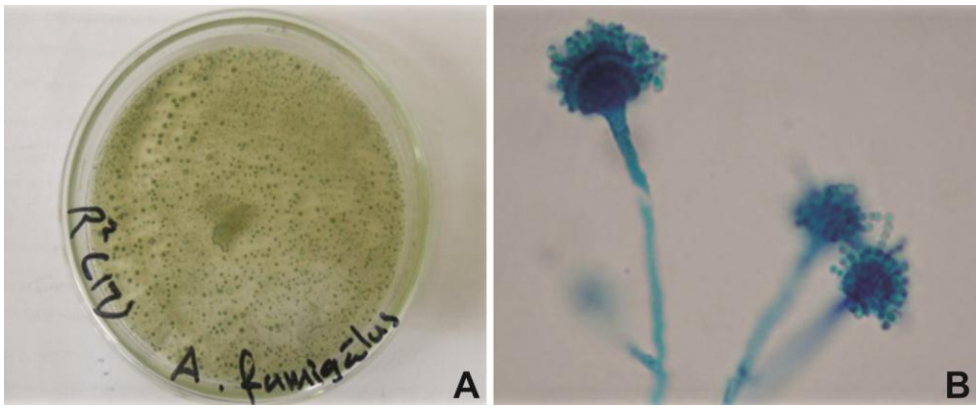

Fig. 4: (A) Single spore colony of *A. fumigatus* **(B)** Fruiting body: conidia, vesicle, and conidial head, of *A. fumigatus* (microscopic image).

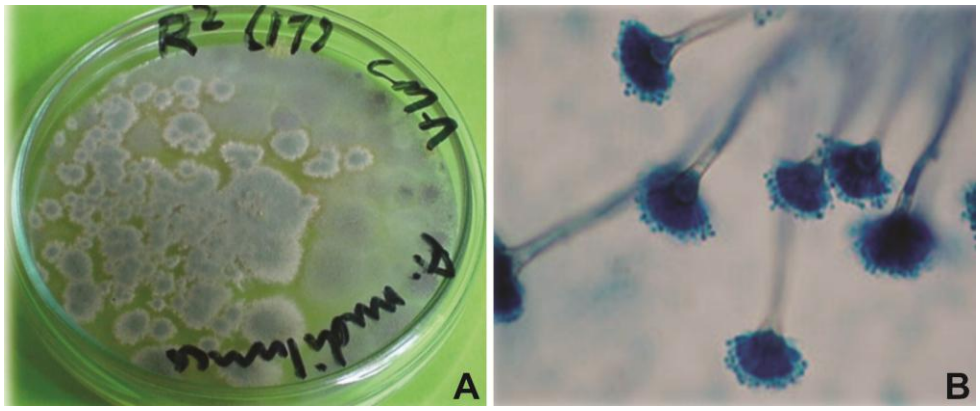

Fig. 5: (A) Single spore colony of *A. nidulans* **(B)** Fruiting body: conidia, vesicle, conidial head, of *A. nidulans* (microscopic image).

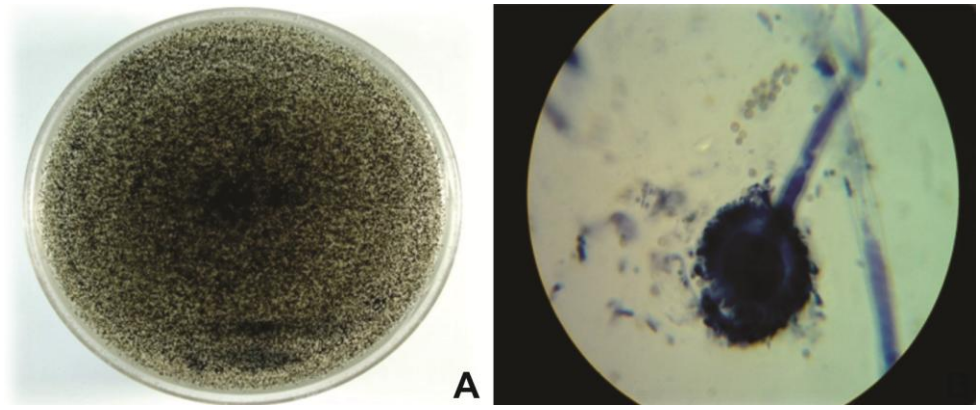

Fig. 6: (A) Single spore colony of *A. nigar* **(B)** Fruiting body: conidia, vesicle, conidial head, of *A. nigar* (microscopic image).

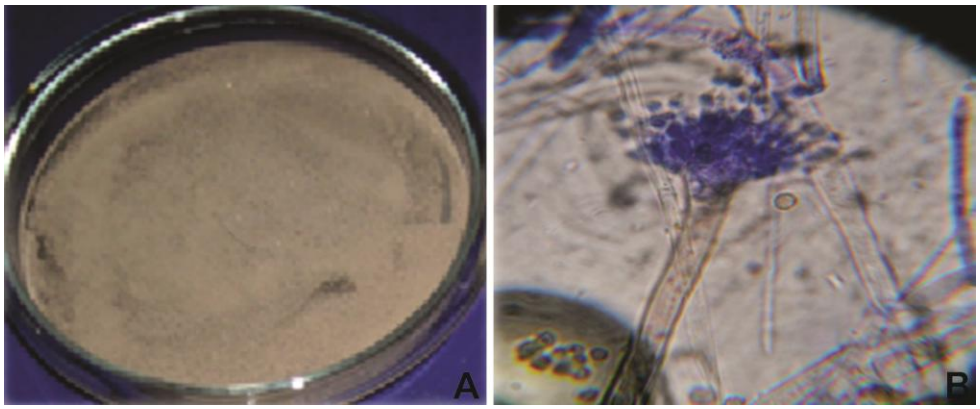

Fig. 7: (A) Single spore colony of *A. unguis* **(B)** Fruiting body: conidia, vesicle, and conidial head, of *A. unguis* (microscopic image).

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