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Isolation and Identification of Different Fungal Species from Major *Kharif* Vegetables of Sindh Province, Pakistan

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ABSTRACT

Background: *Altenaria* species are posing major threat to vegetable crops nowadays, thus it is of utmost importance to identify its different species, so that potential control measures may be explored.

Methods: The isolation and identification of different fungi was conducted from major *Kharif* vegetables, tomato, chilli and eggplant. Samples showing typical symptoms of fruit rot and leaf spot were collected from fields and then pathogens were isolated and identified at laboratory using standard procedures.

Result: The total of 07 fungal species, *Alternaria alternata, A. solani, Aspergillus flavus, A. niger, Fusarium oxysporum, Pencillium* sp., and *Rhizopus stolonifer* isolated from tomato fruit rot. Similarly, 07 fungi *viz*; *A. alternata, A. tenuissima, A. flavus, A. niger, Colletotrichum capisi, Penicillium* sp. and *R. stolonifer* from chilli fruit rot and 06 fungi *viz*; *A. alternata, A. flavus, A. niger, F. solani, Penicillium* sp., and *R. stolonifer* from eggplant leaf spots were isolated. Significantly highest infection frequency was recorded for *A. solani* (48.83%); and *A. tenuissima* (44%) from tomato and chilli fruit rot, respectively. From eggplant leaf spot it was significantly highest for *A. alternata* (34.5%). Study concludes that 03 species, *A. solani, A. tenuissima* and *A. alternata*, dominantly damaged tomato, chilli fruits and eggplant leaves.

Key words: *Alternaria* species, Identification, Isolation, *Kharif* vegetables.

INTRODUCTION

Vegetables are the main part of human diet worldwide. They play significant role particularly as a source of phyto-nutriceuticals: vitamins (C, A, B1, B6, B9, E), minerals, dietary fibre and phytochemicals (Dias and Ryder, 2011). Vegetables contained strong antioxidants and some of phyto-chemicals of vegetables origin are able to diminish the hazard of disease by detoxification of carcinogens and modifying metabolic activation (Kaur and Aggarwal, 2007). Pakistan's agriculture is increasingly becoming an important component of vegetable production. The species of family Solanaceae such as eggplant, potato, chilli peppers and tomato are considered most important vegetables of Pakistan. Tomato, *Lycopersicon esculentum* L. is the world's well-liked vegetable. The by-products of tomatoes are widely used in kitchens in the form of paste, juice and ketchup (Tewari and Vishunavat, 2012). Chilli pepper, *Capsicum annuum* L. is a versatile crop in which fruits are picked either at green mature or red mature stage. Eggplant (*Solanum melongena* L.) is a summer vegetable and is considered important due to low price (Zacharia and Philip, 2010).

Alternaria species are considered to be most important plant pathogens. Genus *Alternaria* is a large group of fungi. The taxonomy of these fungi is based on conidial characteristics like color, form, patterns of secondary sporulation and septation, biochemical properties and host relationship (Lawrence *et al*., 2016). The *Alternaria* genus associated with Phylum Ascomycota, subdivision Pezizomycotina, class Dothediomycetes. *Alternaria* species produce multicellular pigmented spores in chains or in branching fashions. When *Alternaria* attacks host leaf, it produces a sequence of concentric rings at the infection site (Mamgain *et al.,* 2013). *Alternaria* diseases occur on several crop plants and cause huge yield and economic losses. The *Alternaria* cause blight disease which is one of the main diseases, causing heavy yield loss of 32 to 57% (Jayapradh and Raja, 2016). Alternaria leaf spot affecting chilli plants, is a widespread and highly destructive disease under favorable environment. This disease causes a yield loss up to 100%. *Alternaria* can also live through spores

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and mycelium in crop residues of diseased plants or within/ on seeds (Boedo *et al*., 2011).

World-wide a great number of *Alternaria* species have been recorded which cause economic loss by infecting various crops. However, study on *Alternaria* with particular focus on Kharif crop is still a gap in the international scientific society. Specifically in Pakistan, such kind of research have not been reported. Current research project was thus planned whereby the main objective of the study was to isolate and identify different *Alternaria* species affecting the commonly consumed *kharif* vegetables so as to plan their management strategies.

MATERIALS AND METHODS

Place of study

The present study was conducted in the laboratories of Department of Plant Protection, Sindh Agriculture University, Tandojam, Pakistan from February to October 2017.

Isolation and identification of *Alternaria* species

The samples of major Kharif vegetables *viz*; chilli, tomato fruit and eggplant leaves showing typical symptoms of leaf spot and fruit rot were collected from chilli, eggplant and tomato growing fields and placed in perforated polythene bags. Samples were brought to the laboratory for further analysis (Fig 1).

Isolation of pathogen (s)

The collected samples were thoroughly washed with tap water. Small pieces of infected portion about 2-3 mm in length were cut at the junction of diseased and healthy tissues with the help of alcohol sterilized sharp blade. These pieces were surface sterilized in 0.1 per cent mercuric chloride solution ($HgCl_2$) for one minute followed by three washing with sterilized distilled water in beakers under aseptic conditions using laminar air flow. The pieces were then completely dried by placing on sterilized blotting paper. Five bits were transferred aseptically to the petriplates containing sterile potato dextrose agar (PDA) medium amended with an antibacterial agent and filled up to quarter strength. The inoculated plates were incubated at 25±2°C.

All the plates were monitored regularly, and growing colonies were subjected to different laboratory codes for calculating frequency percentage and subjecting to further analysis. About 3-5 isolations were made throughout the experiment. The frequency of the fungi in the collected specimens from each locality was recorded by using the following formula:

$$\text{Frequency (\%)} = \frac{\text{Number of pieces colonized}}{\text{Total number of pieces studied}} \times 100$$

The culture thus obtained was subjected to purification. A single spore culture technique was used to purify the isolates. Sub-culturing of isolates was made time to time to maintain the fresh culture for further analysis until the end of experiments.

Identification of pathogens

Temporary slides of fungal isolates from pure cultures were made and observed under light microscope. Morphological and cultural characters of isolated fungi were recorded and compared with standard keys for establishing their identity (Barnett and Hunter, 1972; Brayford, 1993). In addition, internet databases were also used to compare the morphological characteristics of isolates.

Statistical analysis

The data obtained in present study were statistically analyzed by using the standard procedures for analysis of variance (linear model) and mean separation (least significant difference, LSD) of all parameters were analyzed by using the computer software Statistix 8.1 (Analytical Software, 2005). All differences described in the text were significant at the 5% level of probability.

RESULTS AND DISCUSSION

Association of *Alternaria* spp. with major *kharif* vegetables

The results with reference to isolation and identification of *Alternaria* spp. and other associated fungi from major *Kharif* vegetables *viz*; chilli, eggplant and tomato revealed the occurrence of different fungal species with leaf spot and

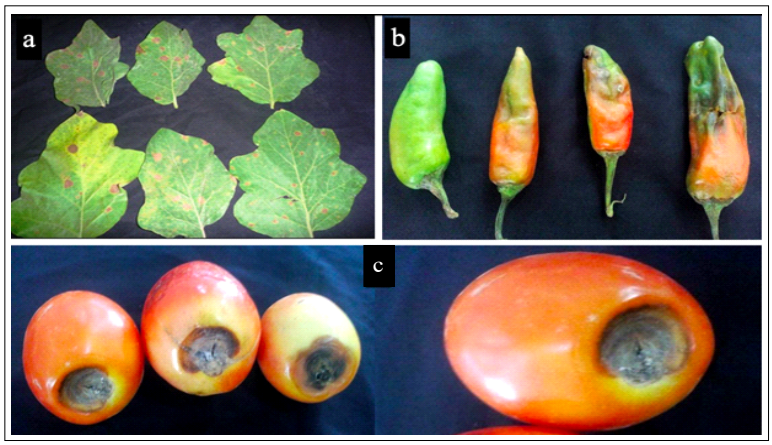


Fig 1: Samples collected from affected a) eggplant plant (leaves), b) chilli fruit and c) tomato fruit for the isolation of *Alternaria* spp.

fruit rot disease that may cause severe losses to vegetables. A total of 07 fungal species *viz; A. alternata, A. solani, A. flavus, A. niger, F.oxysporum, Pencillium* sp., and *R. stolonifer* were isolated from the tomato fruit rot. Similarly, 07 fungi *viz; A. alternata, A. tenuissima, A. flavus, A. niger, C. capisi, Penicillium* sp. and *R. stolonifera* were isolated from chilli fruit rot. Whereas, 06 fungi *viz; A. alternata, A.s flavus, A. niger, F. solani, Penicillium* sp., and *R. stolonifer* were isolated from egg plant leaf spots (Table 1).

Morphological characteristics of *Alternaria* species

Alternaria species were identified using their specified features. The characteristics which were used for the identification are given below in the respective section.

***Alternaria solani* Sorauer**

The *A. solani* was indentified based on the morphological characteristics from tomato fruit rot. The purified culture of the *A. solani* on PDA produced aerial mycelium, yellowish to reddish diffusible pigments later changed to greyish black with black reverse. Microscopic examination revealed septate brown hyphae, with septate and brown conidiophores bearing conidia in chains. The conidia were 12-20 × 120-296 µm and are found singly or in chains of two. Conidia were with 9-11 transverse septa (cross walls) and long beaks. Conidiophores were pale brown, simple and branched, bearing catenulate conidia at the apex and apical fertile parts (Fig 2).

***Alternaria tenuissima* Samuel paul wiltshire**

The *A. tenuissima* was indentified based on the morphological characteristics from chilli leaf spots. The isolates developed conidial chains of 6 to 18 conidia in length and the uncommon secondary chains of 1 to 4 conidia in length. Conidial chains were typically branched by the lateral growth of secondary conidiophores from distal terminal conidial cells and subsequently formed conidia. Conidia were typically ovate to obclavate in shape. The conidia size of *A. tenuissima* (13.9 to 43.0 × 5.8 to 13.7 µm) was similar to that of *A. alternata* (Fig 3).

***Alternaria alternata* (Fr.) keissler**

The *A. alternata* was indentified based on the morphological characteristics from eggplant leaf spots. Microscopic examination revealed septate brown hyphae, with septate and brown conidiophores bearing conidia in chains. Conidiophores were pale brown, simple and branched, bearing catenulate conidia at the apex and apical fertile parts. Conidia catenulate, mostly up to 9 in a chain, were often branched. Conidia were prosperous, acropetally developed, dark brown, spindle-shaped, often with cylindrical beaks, muriform composed of 3-4 transverse walls and 1-2 longitudinal walls (Fig 4).

Infection frequency of fungi associated with major kharif vegetables

The infection frequency of different fungi isolated from tomato fruit rot revealed significant ($P<0.05 = 0.00000$) difference among the different isolates. Singnificantly highest infection frequency was recorded for *A. solani* (48.83%) followed by *A. alternata* (13.417%), *R. stolonifer* (11.417%) and *A. flavus* (10.67%). Whereas, the lowest infection frequency was recorded for *F. oxisporum* (1.917%) followed by *A. niger* (3.167%) and *Pencillium* sp. (7.167%) from

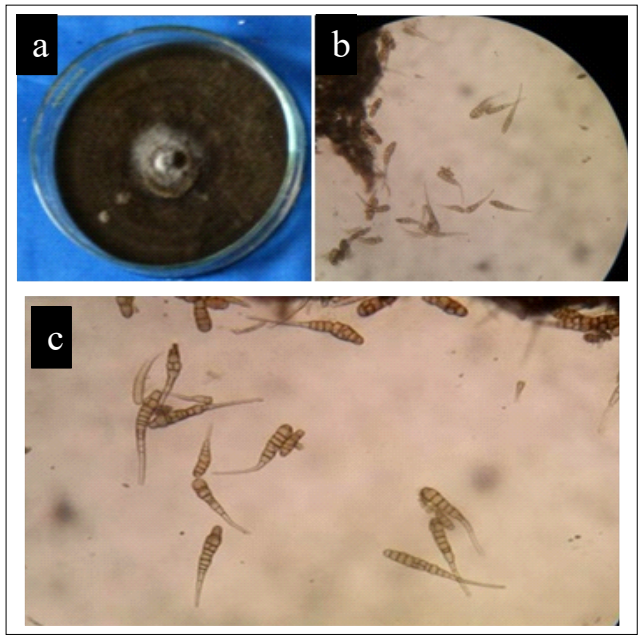


Fig 2: Morphological characteristics of *Alternaria solani.* (a = Colony Culture; b = Microscopic view of conidia at 10x lens; c = Microscopic view of conidia at 40x lens).

Table 1: Different fungal species isolated from major Kharif vegetables.

S. No	Fungal species		
	Tomato fruit	Chilli fruit	Eggplant leaves
1	*Alternaria alternata*	*Alternaria alternate*	*Alternaria alternata*
2	*Alternaria solani*	*Alternaria tenuissima*	*Aspergillus flavus*
3	*Aspergillus flavus*	*Aspergillus flavus*	*Aspergillus niger*
4	*Aspergillus niger*	*Aspergillus niger*	*Fusarium solani*
5	*Fusarium oxisporum*	*Colletotrichum capisi*	*Penicillium* sp
6	*Pencillium* sp.	*Penecillium* sp.	*Rhizopus stolonifer*
7	*Rhizopus stolonifer*	*Rhizopus stolonifer*	-
Total	07	07	06

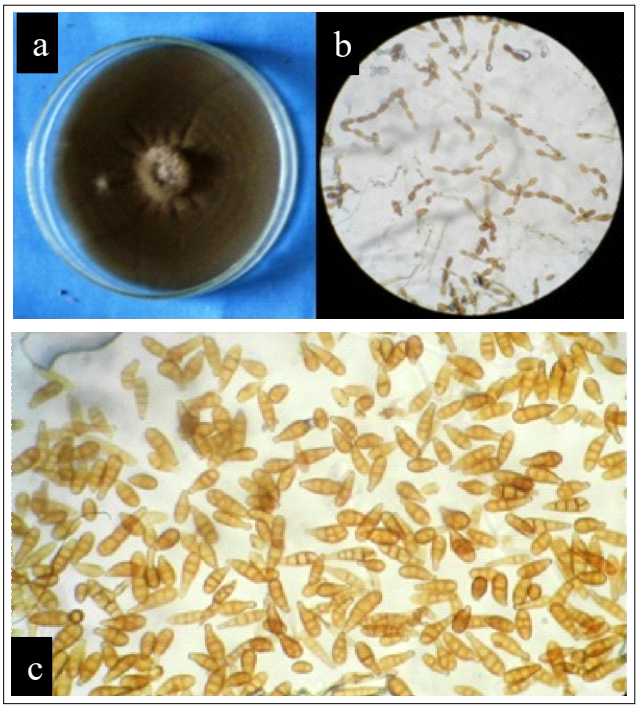


Fig 3: Morphological characteristics of *Alternaria tenuissima.* (a = Colony Culture; b = Microscopic view of conidia at 10x lens; c = Microscopic view of conidia at 40x lens).



Fig 4: Morphological characteristics of *Alternaria alternata.* (a = Colony Culture; b = Microscopic view of conidia; c = Microscopic view of conidia at 40x lens).

tomato fruit rot (Fig 5). The infection frequency of various fungal isolates from the chilli fruit rot showed singificant (P<0.05 = 0.00000) difference among different isolates. Significantly maximum infection frequency was noticed for *A. tenuissima* (44%) followed by *A. alternata* (14.67%), *Pencillium* sp. (13.33%) and *A. flavus* (13.33%). Whereas, minimum infection frequency was recorded for *Colletotrichum capisi* (2%) followed by *R. stolonifer* (6%) and *A. niger* (7.33%) from chilli leaf spots (Fig 6). In case of eggplant, the infection frequecny of different fungal species revealed significant difference (P<0.05 = 0.00000) among eachother. Significantly highest infection was recorded for *A. alternata* (34.5%) followed by *F. solani* (17.5%) and *A. flavus* (12.25%). Whereas, lowest infection frequencu was recorded for *Penicillium* sp. (3%) followed by *A. niger* (4.25%) and *R. stolonifer* (11%) from the leaf spots of eggplant (Fig 7). The species of genus *Alternaria* are always remained an increasing threat to diverse crops globally and causing several economically important diseases. The diseases caused by *Alternaria* species pose huge yield losses and reduce the economic value and quality of the crop plants (Gaya Karunasinghe *et al*., 2020). The blight disease in tomato and *Alternaria* leaf spot in chilli caused by *Alternaria* sp. are considered economically important diseases. The pathogen has been reported to cause seed, seedling, leaf, fruit diseases as well. Post-harvest decay of fruit and seed has also been reported due to this pathogen (El-Garhy *et al*., 2020). It is obvious that several *Alternaria* spp. exist to cause infections in the economically important crops. Therefore, it is essential to manage the pathogen effectively using various methods applicable to reduce the intensity. In addition, the pathogen evolution is a continuous process and has been accelerated by modern plant trade. The climate change may have mixed effects on disease establishment that results due to failure of disease control (Meena *et al*., 2017). Isolation and identification of actual disease pathogen could be used as basic tool for understanding the progression of disease and exploring the curative agents. As genus *Altenaria* is posing major threat to the vegetables nowadays, thus it is of utmost importance to identify its different species, so that potential control measures may be explored. In this regards, present study was conducted on the isolation and identification of different *Alternaria* species from major *Kharif* vegetables in the Laboratory of Department of Plant Protection, Sindh Agriculture University, Tando jam, Pakistan.

In the current study, three species, *A. solani, A. tenuissima* and *A. alternata* were predominantly isolated from tomato, chilli fruit rot and eggplant infected leaves, respectively, that may cause severe losses to these vegetables. The infection frequency of fungi isolated from tomato fruit rot revealed significant difference among the isolates. Singnificantly highest infection frequency was recorded for *A. solani* (48.83%); whereas, the lowest was recorded for *F. oxisporum* (1.917 %) from tomato fruit rot. In chilli fruit, significantly maximum infection frequency was

noticed for *A. tenuissima* (44%) and minimum was recorded for *C. capisi* (2%). In case of eggplant leaf spot, significantly highest percent infection was recorded for *A. alternata* (34.5%), whereas, lowest was recorded for *Penicillium* sp. (3%). In previous studies a great number of species were recorded for the genus *Alternaria* infecting different crops causing world-wide economic loss (Kirk, 2008). *A. alternata* caused early blight of potato leaf spot disease (Pati *et al.*, 2008). *A. solani* causing early blight of tomato (*L. esculentum*) crop is the most destructive (Reni and Roeland, 2006) . It has been reported that fungal diseases, particularly early blight caused by *A. solani* is most common and destructive one causing great reduction in the quantity and quality of fruit yield wherever tomato is grown (Tewari and Vishunavat, 2012). In chilli, several fungi were isolated by different workers such as *Cercospora capsici, A. solani*, *C. capsici, Phytophthora* spp., *Erysiphe cichoracearum* and *Leveillula taurica*, *A. solani*, *F. oxysporum*, *A. terreus, A. candidus,*

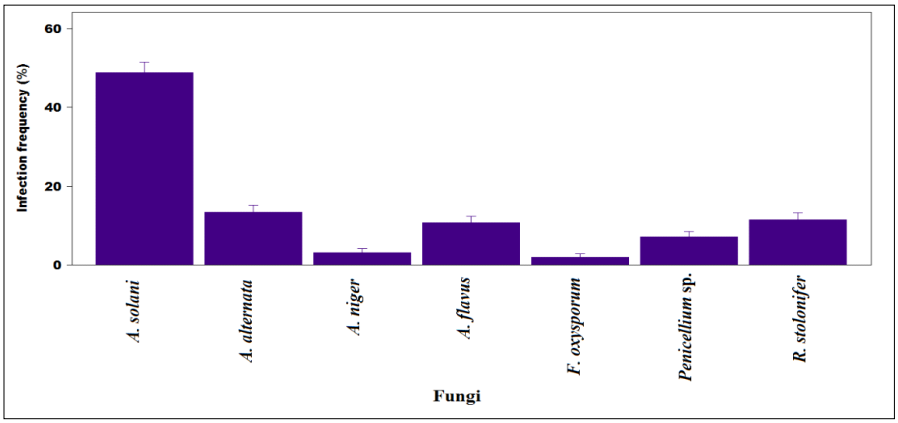


Fig 5: Infection frequency of different fungi isolated from tomato fruit rot.

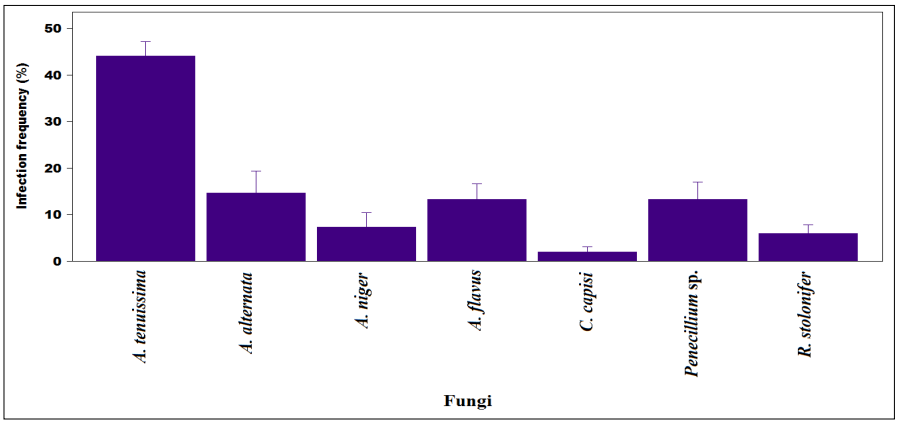


Fig 6: Infection frequency of different fungi isolated from chilli fruit rot.

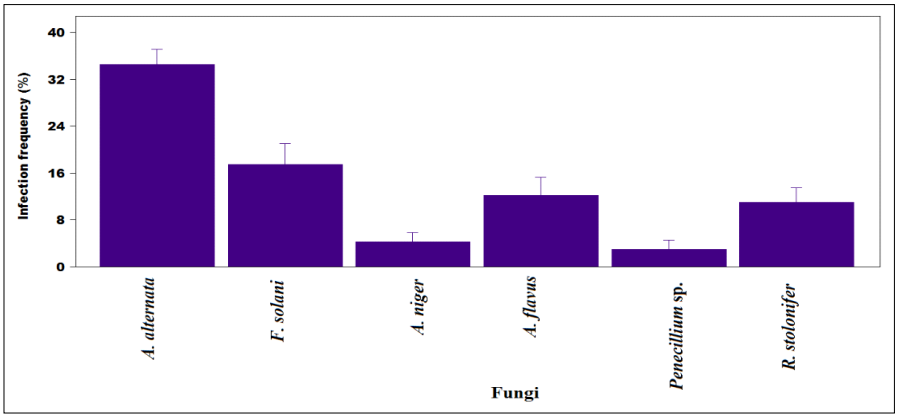


Fig 7: Infection frequency of different fungi isolated from eggplant leaf spots.

A. niger, F. moniliforme, F. sporotrichioides, Paecilomyces variotii, P. corylophilum (Błaszczyk *et al*., 2011; Guo-Yin *et al*., 2013; Jidda and Musa, 2016). Similarly, in eggplant *A. solani, A. tenuissima, F. solani, C.gloeosporioides, B. cinerea, Penicillium* sp., *R. nigricans, Curvularia lunata, Botryodiplodia theobromae, Mucor* sp., *Rhizoctonia solani* and *A. niger* were observed in rotten fruits. *A. solani*, *A. alternata*, *A. flavus*, *M. hiemalis* and *R. stolonifer* were identified from vgetables like as eggplant, tomto and chilli (Kuc'mierz and Sumera 2009; Naureen *et al*., 2009; Das and Sharma, 2012; Gambari *et al.,* 2013; Akwaji *et al*., 2016). Our studies are also in agreement with above mentioned reports regarding the association of different fungal species with fruit rot and leaf spot diseases of tomato, chilli and eggplant, respectively.

CONCLUSION

In conclusion, a total of 07 fungal species each from the tomato, and chilli fruit rots and 06 from eggplant leaf spots were isolated during present study. However, three species of genus *Alternaria viz*; *A. solani, A. tenuissima* and *A. alternata* were predominantly isolated from tomato, chilli infected fruits and eggplant infected leaves, respectively. The identified *Alternaria* species of current study affecting the commonly consumed kharif vegetables would be helpful to plan their management strategies.

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